



#4259 Review

- Summary
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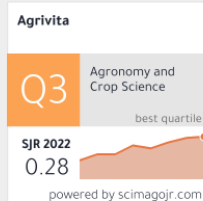
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COVER PAGE

I. The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

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The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

ABSTRACT

1
2 *Pepper Yellow Leaf Curl Virus* (PYLCV) has been increasingly threatening to most red chili cultivation and
3 many efforts to control the disease has been applied but the results is less satisfying except when involving
4 insecticides. Non insecticide control measures could be better alternative to minimize the unwanted side
5 impact of the chemical. Experiments of using cultural methods to suppress the disease initiation in the field
6 has been conducted in the area where the disease has been an endemic and the vector of the disease was
7 abundant. The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical
8 barrier. Seeds harvested from infected plants were used for seed treatment experiment, and commercial
9 seeds mostly used by local farmers were used for the other experiments. The results showed that hot water
10 treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric could reduce
11 incidence and severity of the disease. Tomato was a better intercrop compared to eggplant, mungbean and
12 soybean in reducing disease incidence but their effects on disease severity and yield reduction were not
13 significantly different. Basil and marigold were better trap crops compare to cosmos and zinnia, even though
14 the effect on yield reduction was not significant. Side net barrier using 50 mesh cheese cloth with 125 cm
15 high could reduce the initiation of the disease because *Bemisia tabaci* as the main vector of the disease
16 tended to fly close to the ground when they came from short distance. Even though all of the experiments
17 showed the potential of cultural technique in reducing incoming vector to bring in and transmit PYLCV,
18 roguing is still required to prevent secondary and subsequent transmissions in the field

KEYWORDS

19
20 *Bemisia tabaci*, hot water treatment, intercropping, physical barrier, trap cropping,

INTRODUCTION

21
22 Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country,
23 with some provinces have become the production centres of this commodity from where the majority of nation
24 demand is fulfilled. The eleven biggest provinces as the red chili production centres are West Java, North
25 Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South
26 Sumatra with total production of 1229262 tons in 2022 (Statistik 2022) which contribute approximately 75% of
27 national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered
28 all over the country.

29 Production of red chilli has been fluctuating due to several factors eventually causing significant fluctuation in
30 chilli price which finally made farmers and traders under inconvenience situations. Plant diseases has been
31 the main factor causing production reduction of chilli in the country. The diseases include bacterial leaf spot
32 which causes yield reduction up to 66% (Utami et al. 2022), anthracnose which under severe infection might

33 cause yield losses up to 80% (Suprpta 2022), fusarium which had a record to cause 50% yield losses (Sutarini
34 et al. 2015), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a
35 begomovirus, which under favourable condition might cause yield losses up to 70% (Novrianty et al. 2013).
36 Symptoms of viral diseases caused by begomovirus are obvious and dominated by yellowing or yellow
37 mosaic appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling,
38 smaller fruit, flower abortion and fruit discoloration (Lavanya and Arun 2021)

39 PYLCV often reach its high incidence and intensity during dry season when the weather is favourable to
40 the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn (Hemiptera:
41 Aleyrodidae) with incubation period ranges from 2 to 4 weeks) (Czosnek et al. 2017). The disease is
42 increasingly frightening because it has spread to all chilli production centres in the country since its first
43 appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in
44 Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV)
45 belongs to begomovirus (Sakata et al. 2008). Begomovirus is a member of Family Geminiviridae, a big plant
46 virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini
47 (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4
48 genera based on their vector and genome organization. Members of the family transmitted persistently by
49 white fly (*B. tabaci*) are grouped into Genus Begomovirus, a bipartite virus having 2 components of single
50 stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al.
51 2017). In Indonesia, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two
52 strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum.
53 Both strains have bipartite genome (Sharma et al. 2010). Instead of infecting chilli and tomato, begomovirus
54 was reported to infect other crop species and cause similar symptoms and damages. The other crops found
55 to be infected by begomovirus included melon, water melon, pepper and eggplant (Subiastuti et al. 2019).

56 AS the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
57 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
58 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal Singh et al.
59 2020). Furthermore, the insect also reported to infest more than 500 plant species include tomato, water
60 melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose,
61 poinsettia, lantana, and lily (Schuster et al. 2009). The insect is known to reproduce with high fecundity. A
62 female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host
63 plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid
64 appear from unfertilized eggs (Brown et al. 1995)

65 *B. tabaci* has at least 39 biotypes, feeds on more than 361 species from 89 families (Li et al. 2011) and is able
66 to transmit more than 200 plant viruses (Lu et al. 2019; MacLeod et al. 2022), and 90% of plant viruses
67 transmitted by the vector belong to *begomovirus* (Kanakala and Ghanim 2016). To transmit PYLCV
68 successfully, *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8
69 hours (Czosnek et al. 2017). The vector remains viruliferous until 13 days after virus acquisition or until the
70 vector dies (Chen et al. 2011; Horowitz et al. 2011)

71 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
72 controlling whitefly with synthetic insecticide is not economical (Hema et al. 2014) and generated resistant
73 genotypes and reduced natural enemies (Wang et al. 2020), cultural control should be better alternative to

74 solve the problems. Cultural control is to conserve natural enemies and increase biological diversity through
75 management of biotic and abiotic environment. Abiotic environment of the crop can be manipulated by
76 modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be manipulated by
77 applying crop rotation, intercropping, trap cropping, and crop spacing (Gabryś and Kordan 2012; Zaefarian
78 and Rezvani 2016). Cultural control has become better alternative since the insect has strong ability to evolve
79 pesticide resistance (Basit 2019).

80 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
81 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques such as
82 mulching and physical barrier were reported to be effective to reduce whitefly invasion into the protected plots
83 (Capinera 2005). The degree of whitefly exclusion might be not too significant, but it should be considered that
84 the techniques should have enough contribution to the implementation of integrated pest management (Lapidot
85 et al. 2014).

86 Cultural practices have been implemented as parts of integrated pest management with various level of
87 success and failure depended on the pest and the crops in concern (Kenyon et al. 2014), and there have been
88 no report on the effects of cultural control on the appearance and development of PYLCV on red chilli.
89 Research on the effects of seed treatment, intercropping, trap cropping, and crop barrier as parts of integrated
90 management of the pest in concern was strongly merited.

91 MATERIALS AND METHODS

92 Study area

93 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
94 insect proof screenhouse and experimental field of Sriwijaya University, located in District Indralaya, Ogan Ilir
95 South Sumatra. The location was surrounded by local farmers fields where some of them cultivated red chili
96 and PYLCV has been an endemic. The research consisted of four (4) experiments, one experiment was
97 conducted in the screenhouse and the others were conducted in the field. Some preparation works were done
98 in the Insectarium of Department of Plant Pest ad Disease Faculty of Agriculture Sriwijaya University.

99 Procedures

100 Seed transmission of PYLCV

101 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds
102 were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds
103 then underwent fresh water test and only the sunk seeds were used for the experiment, while the floated seeds
104 were not used because they were obviously not viable. The experiment was arranged in a completely
105 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
106 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
107 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
108 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh et
109 al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of their
110 rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to
111 obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
112 (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, and

113 the trays were then placed in an insect proof screen box for seed germination. The seedlings germinated in
114 each treatment unit were used to calculate the viability of the treated seeds. To observe the seed born
115 PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and
116 all polybags were then placed in insect proof house and were arranged accordingly to completely randomized
117 design.

118 **Intercropping effect on PYLCV**

119 Red chili plants were planted experimentally under intercropping pattern with mungbean (*Vigna radiata*),
120 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
121 as the control. The experiment was arranged in a randomized block design with five treatments and five
122 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were
123 planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure
124 that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV
125 were infected chili plants that spread in farmers' fields around the research site. Additionally, some infected
126 chili plants were deliberately planted around the experimental plots. The vector of PYLCV was abundant in the
127 area since the insect is polyphagous and good flyer especially from 06.00 to 13.00 hours (Blackmer and Byrne
128 1993). Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity
129 and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult
130 to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

131 **Trap crop effect on PYLCV**

132 An experiment to verify the effects of trap crops on the PYLCV infection was conducted using basil (*Ocimum*
133 *basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and no trap
134 crop as control. The trap crops selected were those belonged to refugia which normally attract crop invader
135 insect (Capinera 2005). The experiment was arranged in a randomized block design with 5 treatments and 5
136 replications resulting in 25 experimental units. The experimental unit was 4 x 2 m plot on which red chilli was
137 planted at 60 x 40 cm spacing. Trap crops were planted surrounding experimental plots at 25 cm spacing,
138 positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi et al.
139 2020), three (3) weeks before transplanting red chilli seedling to the plots. Chilli seedlings used in this
140 experiment were prepared in insect proof boxes together with those used in other experiments, but seed
141 transmission experiment. The parameters observed and the method of observation were also similar to
142 those observed and applied in intercropping experiment.

143 **Effect of physical barrier on PYLCV infection**

144 An experiment to verify the effect of physical barrier on the invasion of PYLCV vectors was conducted using
145 cheesecloth as physical net barrier treatment. Nets were previously used as an antihail device in fruit
146 production. However, having a lot of beneficial impacts on agriculture, especially in controlling pests and
147 diseases, net had been considered to be used as a nonaggressive pest and diseases control device
148 (Grasswitz 2019). The experiment was conducted in experimental garden where 4 x 2 m plots were made as
149 experiment units. Physical barrier covering crop cultivation with insect net has been used in tobacco cultivation
150 to prevent the invasion of *B. tabaci* (Aji et al. 2015). In this experiment, physical barrier using insect net was
151 used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The
152 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width

153 less than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm, (Harish et al. 2016). The
154 experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots
155 were prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no barrier
156 as control. The barriers were put in place before seedling transplanting. Seedlings and virus resources used
157 and data collection in this experiment were similar to those applied in intercropping and trap crop experiments.

158 **Crop maintenance and observation**

159 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
160 mechanical technique was applied to control unwanted weeds, pests and diseases. For the experiment of seed
161 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
162 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
163 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
164 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
165 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
166 entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop
167 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
168 calculated according to disease scores described by Lapidot et al. (2006) as follow: 0 = no visible symptoms;
169 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends;
170 3 = a wide range of leaf yellowing, curling and cupping; and 4 = very severe leaf yellowing, and pronounced
171 leaf curling. The severity was calculated using the following formula:

$$172 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

173 Note: DS = disease severity

174 v = disease score (0 to 4)

175 n = number of plants showing disease score v

176 Z = the highest disease score

177 N = total number of plants observed

178 Yield reduction was calculated using the following formula

$$179 \quad YR = \frac{w}{W} \times 100\%$$

180 YR = yield reduction

181 w = weight of first three harvests of infected plant

182 W = average weight of first three harvests of healthy plants in the same plot

183 **Data analysis**

184 Results of PYLCV infections were expressed as mean \pm standard deviation. ANOVA was used to analyze all
185 collected data, and significant differences between means were determined using Honestly Significant
186 Difference (HSD) test at 95% degree of significance.

187 **RESULTS AND DISCUSSION**

188 **Seed transmission of PYLCV**

189 Yellow Leaf Curl Virus (PYLCV) is a member of begomovirus which was suspected to be transmissible
190 through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds,

191 ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the
192 seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might
193 be direct since infection of virus could make seeds more sensitive to deterioration as reported by Bueso et al.
194 (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis
195 seeds up to 65%. Ali and Kobayashi (2010) also reported that virus infection could cause premature aging of
196 the seeds which eventually affected their viability.

197 Some of the seedling also produced PYLCV infection symptoms indicating that PYLCV was a seed borne
198 virus. The seed transmission of begomovirus had previously been reported by Kothandaraman et al. (2016)
199 who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus
200 was seed-borne. A similar finding had also been reported by Kil et al. (2020) who worked with *Tomato yellow*
201 *leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another
202 begomovirus showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV) as
203 reported by Pérez-Padilla et al. (2020) who worked with the virus on more than 3000 tomato plants and
204 concluded that TYLCV was seed borne, but seed transmission was not a general property of the virus. Fadhila
205 et al. (2020) who worked with PepYLCIV, could detect DNA of the virus in 67-100% of seedlings grown from
206 seeds harvested from infected plants.

207 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
208 PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water
209 treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds
210 and weakened parts of them resulted the longer incubation period. According to Paylan et al. (2014), heated
211 water at 65°C, together with HCl and ozon, were very effective treatment to reduce virus concentration in the
212 seeds and had no negative effect on the seeds. Even though the seed treatments affected the virus infection,
213 seed borne infection of the virus still occurred indicated that the effect could not reach the embryo where the
214 virus normally present. According to Ojuederie et al. (2010), seed borne viruses present in seed coat,
215 endosperm, nucleus or embryo, but only viruses present in the seed embryo can be transmitted to the next
216 generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the
217 dipping time. Longer dipping time might have better effect to seed borne virus infection, but it might also affect
218 the seed germination. According to Nega et al. (2003), hot water treatment at temperature 53° for 30 minutes
219 did not affect seed germination and provided a good phytosanitary. Instead of reducing seed borne virus
220 infection, hot water treatment was also reported to be effective to eradicate bacterial seed borne pathogens
221 and was highly recommended for pepper, eggplant and tomato seeds (Miller and Ivey 2005).

222 Seed borne virus infection in this research were relatively high because the seeds used were harvested from
223 infected plants. Seed borne virus infection with the level as presented in Table 1 could be categorized as
224 dangerous and threatening, because under the presence of the virus vectors, infected seed as low as 0.1%
225 was enough to start an endemic (Pagán 2022). Such high seed borne virus infection has also been reported
226 by Bashir et al. (2002) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*.
227 Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

228 All treatments in the experiment resulted in PYLCV infection which confirmed that the virus is transmitted
229 vertically from generation to generation of the host plant. The seed borne nature of begomovirus had also been
230 reported by (Kothandaraman et al. 2016) who studied the seed transmission of *Mungbean yellow mosaic virus*,
231 by Kil et al. (2020) who reported that *Tomato yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al.

232 (2020) who worked with more than 3000 tomato plants and concluded that the virus was seed borne but seed
233 transmission was not a general property of the virus.

234 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
235 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
236 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
237 virus and zika virus (Al Hadhrami et al. 2022; Jennings and Parks 2020), and plant virus such as *Cucumber*
238 *mosaic virus* (Hamidson et al. 2018).

239 **Intercropping effect on PYLCV**

240 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
241 different family from the main crop. The use of mungbean and soybean (Family Leguminosae) and tomato and
242 eggplant (Family Solanaceae) did not affect disease severity and yield reduction but significantly affected
243 disease incidence, of which only tomato caused significant reduction of PYLCV infections (Table 2). The
244 reduction of viral disease under intercropping was a direct effect of the reduction of incoming vector, as
245 reported by Li et al. (2011) that intercropping could effectively control insect pest. The effect of intercropping
246 to insect vector might be by reducing the vector invasion to the crop due to the presence of intercrop as
247 alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant disease by
248 causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence could be
249 caused by its effect on incoming vector, since tomato has insect repellent effect, especially against mosquito
250 (University 2022) and aphid (Vanderlinden 2012)

251 Researches on the use of Leguminous crops as intercrops has been frequent but mostly for the intention of
252 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
253 on plant disease was reported. Most of the research were combination between leguminous and food crops
254 such as maize-mungbean (Syafruddin and Suwardi 2020), cotton-mungbean (Liang et al. 2020), sorghum-
255 mungbean (Temeche et al. 2022), sorghum-soybean (AR 2018), maize-soybean (Berdjour et al. 2020), and
256 rice-soybean (Putra and Sas 2023).

257 **Trap crop effect on PYLCV.**

258 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However,
259 only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos,
260 tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests
261 (Sarjan et al. 2023). Trap crop could also affect the main crops by intercepting, arresting or retaining pest
262 thereby limiting the number of insect pest reaching the main crop (Shelton and Badenes 2006). Furthermore,
263 the significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to
264 Maia and Moore (2011), basil and tagetes had trait of repellent against thrips and variety of flies. Husna et al.
265 (2020) that crude extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes*
266 *aegypti* larvae, and according to Farindira (2015), at higher concentration the crude extract could be used as
267 repellent against the mosquito. Chokeychajaroenporn et al. (1994) also reported that the extract of basil leaves
268 was a strong larvicidal against mosquito with $EC_{50} = 81$ ppm and $EC_{90} = 113$ ppm. Marigold also displayed
269 strong repellent against mosquito (Ponkiya et al. 2018), and contained extractable toxicants which effective as
270 repellent and larvicidal against *B. tabaci* (Fabrick et al. 2020) and against eggplant fruit and shoot borer

271 *Leucinodes orbonali* (Calumpang and Ohsawa 2015). When a trap crop has no insect repellent trait, it works
272 by intercepting, arresting or retaining insects through its colour or odor. According to Junker et al. (2010),
273 insects are attracted to colour and odour and all Homopteran insect, but Family Aphididae, are attracted to
274 colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil)
275 produced green flower but with strong odour.

276 **Physical barrier effect on PYLCV Infection**

277 Physical barrier using cheese cloth 50 mesh could reduce the infection of PYLCV indicated by lower disease
278 incidence and severity, only barriers that are more than 100 cm high have a significant effect on disease
279 incidence (Table 4). This result confirmed that a barrier of 50 mesh could effectively prevent *B. tabaci* to the
280 protected plot (Berlinger et al. 1991; Harish et al. 2016). It is shown in Table 4 that the higher the barrier the
281 lower the disease incidence caused by PYLCV, an indication that *B. tabaci*, the only vector of PYLCV, could
282 fly higher than 100 cm which had no effect on disease incidence. According to (Berlinger et al. 1991) *B. tabaci*
283 could undergo effective long-distance flying, and the longer they flew the higher they climbed ((Blackmer and
284 Byrne 1993). According to Fereres (2000), height of barrier was very important to effectively blocked insect to
285 infest the protected crop. The height of 125 cm could significantly reduce the number of *B. tabaci* but not
286 totally, indicated that the insect still flew above 125 cm, even though 70% of the insect flew close to the ground
287 (Isaacs and Byrne 1998). *B. tabaci* actually has two type of flight behavior, foraging flight and migratory flight.
288 In migratory flight, the insect could be picked up and carried by air currents (Byrne et al. 1996) and the insect
289 containing mature eggs has been trapped at 150 m above ground and this could be among the 30% of vertically
290 distributed *B. tabaci* in the air. The net actually not only controlled *B. tabaci*, but also other flying insect. Net
291 barrier was reported to reduce the population of winged aphid by more than 40 times. White net was much
292 more effective than the yellow ones (Cohen 1981).

293 **CONCLUSIONS AND SUGGESTION (Arial 10)**

294 The first experiment of seed borne transmission of PYLCV concluded that PYLCV infecting red chili was a
295 seed borne virus and the rate of seed borne infection could be partially reduced by hot water treatment and
296 turmeric crude extract. In the intercropping experiment, tomato was good enough as intercrops for red chili in
297 term of reducing PYLCV infections, but it would be better to somewhat shorten the chili spacing and the ratio
298 of chili/tomato should not be 1:1 to compensate for the lost red chili population due to intercropping. Basil and
299 marigold are functional as trap crops to protect chili from incoming *B. tabaci* which might bring in and transmit
300 PYLCV. Marigold is more recommended since the plant can grow higher and more protective than basil. In
301 the experiment of physical barrier, the use of 50 mesh cheese cloth as side barrier could reduce PYLCV
302 infection frequency only at the height of 125 cm. The entry of viruliferous *B. tabaci* into the plot crossed the
303 barrier can potentially be reduce by using denser and higher net. Even though the cultural techniques studied
304 can partially reduce PYLCV infection frequency through their effect on the infestation of *B. tabaci*, disease
305 monitoring is very important. Since *B. tabaci* is very actively mobile and efficiently transmit the virus, roguing
306 the infected plant is a must to prevent further transmission.

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Tables

519 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and

520 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58,90±1,68	29,45±0,54 b	6,80±0,77 ab	4,10±0,60 ab
Ginger crude extract	54,00±1,87	30,86±1,09 b	8,40±1,30 ab	4,90±0,82 ab
Turmeric crude extract	51,17±2,29	21,40±1,02 a	3,20±0,37 a	1,80±0,26 a
Javanese ginger crude extract	51,74±3,03	23,84±1,61 a	5,60±0,74 ab	4,00±0,49 ab
Control	43,66±3,11	22,84±1,06 a	9,60±0,57b	6,70±0,41 b
F Calculated	2,76 ^{ns}	12,12*	1,94*	2,14*
P Value	0,08	0,001	0,04	0,02
HSD 5%		5,15	5,61	3,56

521 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty

522 Significant Difference Test.

523 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and

524 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16,18±2,35 ab	10,95±1,04	47,19±2,29
Mung bean	15,23±2,39 ab	9,52±1,58	49,22±2,20
Soybean	14,58±1,97 ab	8,57±1,25	46,41±3,27
Tomato	7,62±0,88 a	4,52±0,66	40,00±1,47
Control	26,66±0,88 b	14,28±1,10	46,26±2,04
F Calculated	5,74*	3,44 ^{ns}	0,82 ^{ns}
P Value	0,01	0,05	0,54

HSD 5%

12,18

-

-

525 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 526 Significant Difference Test.

527 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
 528 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8,14±1,36 a	5,85±0,86 a	50,21±2,41
Cosmos	10,28±1,29 ab	7,12±0,57 ab	48,41±1,54
Marigold	4,57±0,53 a	3,57±0,38 a	42,80±1,22
Zinnia	13,71±1,59 ab	11,28±1,31 ab	47,32±1,35
control	16,57±0,81 b	13,14±1,00 b	52,01±1,62
F Calculated	5,51*	7,70*	1,50 ^{ns}
P Value	0,01	0,003	0,27
HSD 5%	8,41	6,06	-

529 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 530 Significant Difference Test.

531 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 532 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11,42±1,53 ab	8,71±0,86 bc	49,87±0,85
Side barrier 75 cm	9,71±0,52 ab	8,00±0,47 abc	47,42±1,41
Side barrier 100 cm	8,00±1,26 ab	6,57±0,71 ab	49,00±1,36
Side barrier 125 cm	5,14±0,81 a	3,57±0,74 a	43,40±1,08
Control	14,28±0,68 b	12,14±0,92 c	52,32±2,11
F Calculated	5,28*	8,456*	1,73 ^{ns}
P Value	0,01	0,002	0,21
HSD 5%	6,40	4,58	

533 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 534 Significant Difference Test.

COVER PAGE

I. The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

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Add international candidates from abroad. May have high h index

The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

1 **ABSTRACT (maximum 200 words, it must : the objective, method, research products)**

2 *Pepper Yellow Leaf Curl Virus* (PYLCV) has been increasingly threatening to most red chili cultivation and
3 many efforts to control the disease has been applied but the results is less satisfying except when involving
4 insecticides. Non insecticide control measures could be better alternative to minimize the unwanted side
5 impact of the chemical. Experiments of using cultural methods to suppress the disease initiation in the field
6 has been conducted in the area where the disease has been an endemic and the vector of the disease was
7 abundant. The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical
8 barrier. Seeds harvested from infected plants were used for seed treatment experiment, and commercial
9 seeds mostly used by local farmers were used for the other experiments. The results showed that hot water
10 treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric could reduce
11 incidence and severity of the disease. Tomato was a better intercrop compared to eggplant, mungbean and
12 soybean in reducing disease incidence but their effects on disease severity and yield reduction were not
13 significantly different. Basil and marigold were better trap crops compare to cosmos and zinnia, even though
14 the effect on yield reduction was not significant. Side net barrier using 50 mesh cheese cloth with 125 cm
15 high could reduce the initiation of the disease because *Bemisia tabaci* as the main vector of the disease
16 tended to fly close to the ground when they came from short distance. Even though all of the experiments
17 showed the potential of cultural technique in reducing incoming vector to bring in and transmit PYLCV,
18 roguing is still required to prevent secondary and subsequent transmissions in the field

19 **KEYWORDS**

20 *Bemisia tabaci*, hot water treatment, intercropping, physical barrier, trap cropping,

21 **INTRODUCTION (just primer supporting references, less than 10 years)**

22 Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country,
23 with some provinces have become the production centres of this commodity from where the majority of nation
24 demand is fulfilled. The eleven biggest provinces as the red chili production centres are West Java, North
25 Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South
26 Sumatra with total production of 1229262 tons in 2022 (Statistik 2022) which contribute approximately 75% of
27 national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered
28 all over the country.

29 Production of red chilli has been fluctuating due to several factors eventually causing significant fluctuation in
30 chilli price which finally made farmers and traders under inconvenience situations. Plant diseases has been
31 the main factor causing production reduction of chilli in the country. The diseases include bacterial leaf spot
32 which causes yield reduction up to 66% (Utami et al. 2022), anthracnose which under severe infection might
33 cause yield losses up to 80% (Suprpta 2022), fusarium which had a record to cause 50% yield losses (Sutarini
34 et al. 2015), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a
35 begomovirus, which under favourable condition might cause yield losses up to 70% (Novrianty et al. 2013).
36 Symptoms of viral diseases caused by begomovirus are obvious and dominated by yellowing or yellow
37 mosaic appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling,
38 smaller fruit, flower abortion and fruit discoloration (Lavanya and Arun 2021)

39 PYLCV often reach its high incidence and intensity during dry season when the weather is favourable to
40 the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn (Hemiptera:
41 Aleyrodidae) with incubation period ranges from 2 to 4 weeks) (Czosnek et al. 2017). The disease is
42 increasingly frightening because it has spread to all chilli production centres in the country since its first
43 appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in
44 Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV)
45 belongs to begomovirus (Sakata et al. 2008). Begomovirus is a member of Family Geminiviridae, a big plant
46 virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini
47 (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4
48 genera based on their vector and genome organization. Members of the family transmitted persistently by
49 white fly (*B. tabaci*) are grouped into Genus Begomovirus, a bipartite virus having 2 components of single
50 stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al.
51 2017). In Indonesia, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two
52 strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum.
53 Both strains have bipartite genome (Sharma et al. 2010). Instead of infecting chilli and tomato, begomovirus
54 was reported to infect other crop species and cause similar symptoms and damages. The other crops found
55 to be infected by begomovirus included melon, water melon, pepper and eggplant (Subiastuti et al. 2019).

56 AS the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
57 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
58 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal Singh et al.
59 2020). Furthermore, the insect also reported to infest more than 500 plant species include tomato, water
60 melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose,
61 poinsettia, lantana, and lily (Schuster et al. 2009). The insect is known to reproduce with high fecundity. A
62 female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host
63 plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid
64 appear from unfertilized eggs (Brown et al. 1995)

65 *B. tabaci* has at least 39 biotypes, feeds on more than 361 species from 89 families (Li et al. 2011) and is able
66 to transmit more than 200 plant viruses (Lu et al. 2019; MacLeod et al. 2022), and 90% of plant viruses
67 transmitted by the vector belong to *begomovirus* (Kanakala and Ghanim 2016). To transmit PYLCV
68 successfully, *B tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8

69 hours (Czosnek et al. 2017). The vector remains viruliferous until 13 days after virus acquisition or until the
70 vector dies (Chen et al. 2011; Horowitz et al. 2011)

71 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
72 controlling whitefly with synthetic insecticide is not economical (Hema et al. 2014) and generated resistant
73 genotypes and reduced natural enemies (Wang et al. 2020), cultural control should be better alternative to
74 solve the problems. Cultural control is to conserve natural enemies and increase biological diversity through
75 management of biotic and abiotic environment. Abiotic environment of the crop can be manipulated by
76 modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be manipulated by
77 applying crop rotation, intercropping, trap cropping, and crop spacing (Gabryś and Kordan 2012; Zaefarian
78 and Rezvani 2016). Cultural control has become better alternative since the insect has strong ability to evolve
79 pesticide resistance (Basit 2019).

80 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
81 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques such as
82 mulching and physical barrier were reported to be effective to reduce whitefly invasion into the protected plots
83 (Capinera 2005). The degree of whitefly exclusion might be not too significant, but it should be considered that
84 the techniques should have enough contribution to the implementation of integrated pest management (Lapidot
85 et al. 2014).

86 Cultural practices have been implemented as parts of integrated pest management with various level of
87 success and failure depended on the pest and the crops in concern (Kenyon et al. 2014), and there have been
88 no report on the effects of cultural control on the appearance and development of PYLCV on red chilli.
89 Research on the effects of seed treatment, intercropping, trap cropping, and crop barrier as parts of integrated
90 management of the pest in concern was strongly merited.

91 **The objective of the research is**

92 **MATERIALS AND METHODS (when and where?)**

93 **Study area**

94 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
95 insect proof screenhouse and experimental field of Sriwijaya University, located in District Indralaya, Ogan Ilir
96 South Sumatra. The location was surrounded by local farmers fields where some of them cultivated red chili
97 and PYLCV has been an endemic. The research consisted of four (4) experiments, one experiment was
98 conducted in the screenhouse and the others were conducted in the field. Some preparation works were done
99 in the Insectarium of Department of Plant Pest ad Disease Faculty of Agriculture Sriwijaya University.

100 **Procedures**

101 **Seed transmission of PYLCV**

102 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds
103 were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds
104 then underwent fresh water test and only the sunk seeds were used for the experiment, while the floated seeds
105 were not used because they were obviously not viable. The experiment was arranged in a completely
106 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of

107 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
108 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
109 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh et
110 al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of their
111 rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to
112 obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
113 (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, and
114 the trays were then placed in an insect proof screen box for seed germination. The seedlings germinated in
115 each treatment unit were used to calculated the viability of the treated seeds. To observed the seed born
116 PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and
117 all polybags were then placed in insect proof house and were arranged accordingly to completely randomized
118 design.

119 **Intercropping effect on PYLCV**

120 Red chili plants were planted experimentally under intercropping pattern with mungbean (*Vigna radiata*),
121 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
122 as the control. The experiment was arranged in a randomized block design with five treatments and five
123 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were
124 planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure
125 that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV
126 were infected chili plants that spread in farmers' fields around the research site. Additionally, some infected
127 chili plants were deliberately planted around the experimental plots. The vector of PYLCV was abundant in the
128 area since the insect is polyphagous and good flyer especially from 06.00 to 13.00 hours (Blackmer and Byrne
129 1993). Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity
130 and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult
131 to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

132 **Trap crop effect on PYLCV**

133 An experiment to verify the effects of trap crops on the PYLCV infection was conducted using basil (*Ocimum*
134 *basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and no trap
135 crop as control. The trap crops selected were those belonged to refugia which normally attract crop invader
136 insect (Capinera 2005). The experiment was arranged in a randomized block design with 5 treatments and 5
137 replications resulting in 25 experimental units. The experimental unit was 4 x 2 m plot on which red chilli was
138 planted at 60 x 40 cm spacing. Trap crops were planted surrounding experimental plots at 25 cm spacing,
139 positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi et al.
140 2020), three (3) weeks before transplanting red chilli seedling to the plots. Chilli seedlings used in this
141 experiment were prepared in insect proof boxes together with those used in other experiments, but seed
142 transmission experiment. The parameters observed and the method of observation were also similar to
143 those observed and applied in intercropping experiment.

144 **Effect of physical barrier on PYLCV infection**

145 An experiment to verify the effect of physical barrier on the invasion of PYLCV vectors was conducted using
146 cheesecloth as physical net barrier treatment. Nets were previously used as an antihail device in fruit

147 production. However, having a lot of beneficial impacts on agriculture, especially in controlling pests and
148 diseases, net had been considered to be used as a nonaggressive pest and diseases control device
149 (Grasswitz 2019). The experiment was conducted in experimental garden where 4 x 2 m plots were made as
150 experiment units. Physical barrier covering crop cultivation with insect net has been used in tobacco cultivation
151 to prevent the invasion of *B. tabaci* (Aji et al. 2015). In this experiment, physical barrier using insect net was
152 used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The
153 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width
154 less than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm, (Harish et al. 2016). The
155 experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots
156 were prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no barrier
157 as control. The barriers were put in place before seedling transplanting. Seedlings and virus resources used
158 and data collection in this experiment were similar to those applied in intercropping and trap crop experiments.

159 **Crop maintenance and observation**

160 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
161 mechanical technique was applied to control unwanted weeds, pests and diseases. For the experiment of seed
162 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
163 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
164 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
165 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
166 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
167 entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop
168 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
169 calculated according to disease scores described by Lapidot et al. (2006) as follow: 0 = no visible symptoms;
170 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends;
171 3 = a wide range of leaf yellowing, curling and cupping; and 4 = very severe leaf yellowing, and pronounced
172 leaf curling. The severity was calculated using the following formula:

$$173 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

174 Note: DS = disease severity

175 v = disease score (0 to 4)

176 n = number of plants showing disease score v

177 Z = the highest disease score

178 N = total number of plants observed

179 Yield reduction was calculated using the following formula

$$180 \quad YR = \frac{w}{W} \times 100\%$$

181 YR = yield reduction

182 w = weight of first three harvests of infected plant

183 W = average weight of first three harvests of healthy plants in the same plot

184 **Data analysis**

185 Results of PYLCV infections were expressed as mean \pm standard deviation. ANOVA was used to analyze all
186 collected data, and significant differences between means were determined using Honestly Significant
187 Difference (HSD) test at 95% degree of significance.

188 **RESULTS AND DISCUSSION (just primer supporting references, less than 10 years)**

189 **Seed transmission of PYLCV**

190 Yellow Leaf Curl Virus (PYLCV) is a member of begomovirus which was suspected to be transmissible
191 through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds,
192 ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the
193 seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might
194 be direct since infection of virus could make seeds more sensitive to deterioration as reported by Bueso et al.
195 (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis
196 seeds up to 65%. Ali and Kobayashi (2010) also reported that virus infection could cause premature aging of
197 the seeds which eventually affected their viability.

198 Some of the seedling also produced PYLCV infection symptoms indicating that PYLCV was a seed borne
199 virus. The seed transmission of begomovirus had previously been reported by Kothandaraman et al. (2016)
200 who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus
201 was seed-borne. A similar finding had also been reported by Kil et al. (2020) who worked with *Tomato yellow*
202 *leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another
203 begomovirus showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV) as
204 reported by Pérez-Padilla et al. (2020) who worked with the virus on more than 3000 tomato plants and
205 concluded that TYLCV was seed borne, but seed transmission was not a general property of the virus. Fadhila
206 et al. (2020) who worked with PepYLCIV, could detect DNA of the virus in 67-100% of seedlings grown from
207 seeds harvested from infected plants.

208 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
209 PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water
210 treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds
211 and weakened parts of them resulted the longer incubation period. According to Paylan et al. (2014), heated
212 water at 65°C, together with HCl and ozon, were very effective treatment to reduce virus concentration in the
213 seeds and had no negative effect on the seeds. Even though the seed treatments affected the virus infection,
214 seed borne infection of the virus still occurred indicated that the effect could not reach the embryo where the
215 virus normally present. According to Ojuederie et al. (2010), seed borne viruses present in seed coat,
216 endosperm, nucleus or embryo, but only viruses present in the seed embryo can be transmitted to the next
217 generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the
218 dipping time. Longer dipping time might have better effect to seed borne virus infection, but it might also affect
219 the seed germination. According to Nega et al. (2003), hot water treatment at temperature 53° for 30 minutes
220 did not affect seed germination and provided a good phytosanitary. Instead of reducing seed borne virus
221 infection, hot water treatment was also reported to be effective to eradicate bacterial seed borne pathogens
222 and was highly recommended for pepper, eggplant and tomato seeds (Miller and Ivey 2005).

223 Seed borne virus infection in this research were relatively high because the seeds used were harvested from
224 infected plants. Seed borne virus infection with the level as presented in Table 1 could be categorized as
225 dangerous and threatening, because under the presence of the virus vectors, infected seed as low as 0.1%
226 was enough to start an endemic (Pagán 2022). Such high seed borne virus infection has also been reported
227 by Bashir et al. (2002) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*.
228 Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

229 All treatments in the experiment resulted in PYLCV infection which confirmed that the virus is transmitted
230 vertically from generation to generation of the host plant. The seed borne nature of begomovirus had also been
231 reported by (Kothandaraman et al. 2016) who studied the seed transmission of *Mungbean yellow mosaic virus*,
232 by Kil et al. (2020) who reported that *Tomato yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al.
233 (2020) who worked with more than 3000 tomato plants and concluded that the virus was seed borne but seed
234 transmission was not a general property of the virus.

235 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
236 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
237 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
238 virus and zika virus (Al Hadhrami et al. 2022; Jennings and Parks 2020), and plant virus such as *Cucumber*
239 *mosaic virus* (Hamidson et al. 2018).

240 **Intercropping effect on PYLCV**

241 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
242 different family from the main crop. The use of mungbean and soybean (Family Leguminosae) and tomato and
243 eggplant (Family Solanaceae) did not affect disease severity and yield reduction but significantly affected
244 disease incidence, of which only tomato caused significant reduction of PYLCV infections (Table 2). The
245 reduction of viral disease under intercropping was a direct effect of the reduction of incoming vector, as
246 reported by Li et al. (2011) that intercropping could effectively control insect pest. The effect of intercropping
247 to insect vector might be by reducing the vector invasion to the crop due to the presence of intercrop as
248 alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant disease by
249 causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence could be
250 caused by its effect on incoming vector, since tomato has insect repellent effect, especially against mosquito
251 (University 2022) and aphid (Vanderlinden 2012)

252 Researches on the use of Leguminous crops as intercrops has been frequent but mostly for the intention of
253 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
254 on plant disease was reported. Most of the research were combination between leguminous and food crops
255 such as maize-mungbean (Syafuruddin and Suwardi 2020), cotton-mungbean (Liang et al. 2020), sorghum-
256 mungbean (Temeche et al. 2022), sorghum-soybean (AR 2018), maize-soybean (Berdjour et al. 2020), and
257 rice-soybean (Putra and Sas 2023).

258 **Trap crop effect on PYLCV.**

259 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However,
260 only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos,

261 tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests
262 (Sarjan et al. 2023). Trap crop could also affect the main crops by intercepting, arresting or retaining pest
263 thereby limiting the number of insect pest reaching the main crop (Shelton and Badenes 2006). Furthermore,
264 the significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to
265 Maia and Moore (2011), basil and tagetes had trait of repellent against thrips and variety of flies. Husna et al.
266 (2020) that crude extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes*
267 *aegypti* larvae, and according to Farindira (2015), at higher concentration the crude extract could be used as
268 repellent against the mosquito. Chokechaiaroenporn et al. (1994) also reported that the extract of basil leaves
269 was a strong larvicidal against mosquito with $EC_{50} = 81$ ppm and $EC_{90} = 113$ ppm. Marigold also displayed
270 strong repellent against mosquito (Ponkiya et al. 2018), and contained extractable toxicants which effective as
271 repellent and larvicidal against *B. tabaci* (Fabrick et al. 2020) and against eggplant fruit and shoot borer
272 *Leucinodes orbonali* (Calumpang and Ohsawa 2015). When a trap crop has no insect repellent trait, it works
273 by intercepting, arresting or retaining insects through its colour or odour. According to Junker et al. (2010),
274 insects are attracted to colour and odour and all Homopteran insect, but Family Aphididae, are attracted to
275 colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil)
276 produced green flower but with strong odour.

277 **Physical barrier effect on PYLCV Infection**

278 Physical barrier using cheese cloth 50 mesh could reduce the infection of PYLCV indicated by lower disease
279 incidence and severity, only barriers that are more than 100 cm high have a significant effect on disease
280 incidence (Table 4). This result confirmed that a barrier of 50 mesh could effectively prevent *B. tabaci* to the
281 protected plot (Berlinger et al. 1991; Harish et al. 2016). It is shown in Table 4 that the higher the barrier the
282 lower the disease incidence caused by PYLCV, an indication that *B. tabaci*, the only vector of PYLCV, could
283 fly higher than 100 cm which had no effect on disease incidence. According to (Berlinger et al. 1991) *B. tabaci*
284 could undergo effective long-distance flying, and the longer they flew the higher they climbed ((Blackmer and
285 Byrne 1993). According to Fereres (2000), height of barrier was very important to effectively blocked insect to
286 infest the protected crop. The height of 125 cm could significantly reduce the number of *B. tabaci* but not
287 totally, indicated that the insect still flew above 125 cm, even though 70% of the insect flew close to the ground
288 (Isaacs and Byrne 1998). *B. tabaci* actually has two type of flight behavior, foraging flight and migratory flight.
289 In migratory flight, the insect could be picked up and carried by air currents (Byrne et al. 1996) and the insect
290 containing mature eggs has been trapped at 150 m above ground and this could be among the 30% of vertically
291 distributed *B. tabaci* in the air. The net actually not only controlled *B. tabaci*, but also other flying insect. Net
292 barrier was reported to reduce the population of winged aphid by more than 40 times. White net was much
293 more effective than the yellow ones (Cohen 1981).

294 **CONCLUSIONS AND SUGGESTION (Arial 10) (No discussion, here)**

295 The first experiment of seed borne transmission of PYLCV concluded that PYLCV infecting red chili was a
296 seed borne virus and the rate of seed borne infection could be partially reduced by hot water treatment and
297 turmeric crude extract. In the intercropping experiment, tomato was good enough as intercrops for red chili in
298 term of reducing PYLCV infections, but it would be better to somewhat shorten the chili spacing and the ratio
299 of chili/tomato should not be 1:1 to compensate for the lost red chili population due to intercropping. Basil and
300 marigold are functional as trap crops to protect chili from incoming *B. tabaci* which might bring in and transmit

301 PYLCV. Marigold is more recommended since the plant can grow higher and more protective than basil. In
302 the experiment of physical barrier, the use of 50 mesh cheese cloth as side barrier could reduce PYLCV
303 infection frequency only at the height of 125 cm. The entry of viruliferous *B. tabaci* into the plot crossed the
304 barrier can potentially be reduce by using denser and higher net. Even though the cultural techniques studied
305 can partially reduce PYLCV infection frequency through their effect on the infestation of *B.tabaci*, disease
306 monitoring is very important. Since *B. tabaci* is very actively mobile and efficiently transmit the virus, roguing
307 the infected plant is a must to prevent further transmission.

308 **REFERENCES (Arial 10) → consistent, DOI, less than 10 years, check guidelines**

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Tables

520 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
521 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58,90±1,68	29,45±0,54 b	6,80±0,77 ab	4,10±0,60 ab
Ginger crude extract	54,00±1,87	30,86±1,09 b	8,40±1,30 ab	4,90±0,82 ab
Turmeric crude extract	51,17±2,29	21,40±1,02 a	3,20±0,37 a	1,80±0,26 a
Javanese ginger crude extract	51,74±3,03	23,84±1,61 a	5,60±0,74 ab	4,00±0,49 ab
Control	43,66±3,11	22,84±1,06 a	9,60±0,57b	6,70±0,41 b
F Calculated	2,76 ^{ns}	12,12*	1,94*	2,14*
P Value	0,08	0,001	0,04	0,02
HSD 5%		5,15	5,61	3,56

522 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
523 Significant Difference Test.

524 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
 525 yield reduction caused by the disease

Intercrop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16,18±2,35 ab	10,95±1,04	47,19±2,29
Mung bean	15,23±2,39 ab	9,52±1,58	49,22±2,20
Soybean	14,58±1,97 ab	8,57±1,25	46,41±3,27
Tomato	7,62±0,88 a	4,52±0,66	40,00±1,47
Control	26,66±0,88 b	14,28±1,10	46,26±2,04
F Calculated	5,74*	3,44 ^{ns}	0,82 ^{ns}
P Value	0,01	0,05	0,54
HSD 5%	12,18	-	-

526 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 527 Significant Difference Test.

528 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
 529 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8,14±1,36 a	5,85±0,86 a	50,21±2,41
Cosmos	10,28±1,29 ab	7,12±0,57 ab	48,41±1,54
Marigold	4,57±0,53 a	3,57±0,38 a	42,80±1,22
Zinnia	13,71±1,59 ab	11,28±1,31 ab	47,32±1,35
control	16,57±0,81 b	13,14±1,00 b	52,01±1,62
F Calculated	5,51*	7,70*	1,50 ^{ns}
P Value	0,01	0,003	0,27
HSD 5%	8,41	6,06	-

530 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 531 Significant Difference Test.

532 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 533 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11,42±1,53 ab	8,71±0,86 bc	49,87±0,85
Side barrier 75 cm	9,71±0,52 ab	8,00±0,47 abc	47,42±1,41
Side barrier 100 cm	8,00±1,26 ab	6,57±0,71 ab	49,00±1,36
Side barrier 125 cm	5,14±0,81 a	3,57±0,74 a	43,40±1,08
Control	14,28±0,68 b	12,14±0,92 c	52,32±2,11
F Calculated	5,28*	8,456*	1,73 ^{ns}
P Value	0,01	0,002	0,21
HSD 5%	6,40	4,58	-

534 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 535 Significant Difference Test.

COVER PAGE

I. The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

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The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

1 **ABSTRACT (maximum 200 words, it must : the objective, method, research products)**

2 An experiment was conducted to investigate the effects of cultural techniques to prevent the infection of yellow
3 leaf curl disease of chili caused by *Pepper Yellow Leaf Curl Virus* (PYLCV). The experiment was conducted in
4 the area where the disease has been an endemic and *Bemisia tabaci*, the vector of the disease, was abundant.
5 The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds
6 harvested from infected plants were used for seed treatment experiment, and commercial seeds mostly used
7 by local farmers were used for the other experiments. The results showed that PYLCV was a seedborne virus
8 and hot water treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric
9 could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant,
10 mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield
11 reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and
12 zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but
13 not the lower ones. Under various cultural technique, PYLCV caused chili yield reduction of 40 – 53%.

14 **KEYWORDS**

15 *Bemisia tabaci*, hot water treatment, intercropping, physical barrier, trap cropping.

16 **INTRODUCTION (just primer supporting references, less than 10 years)**

17 Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country,
18 with some provinces have become the production centres of this commodity from where the majority of nation
19 demand is fulfilled. The eleven biggest chili producing provinces in 2022 were West Java, North Sumatra,
20 Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra
21 with total production of 1229262 tons in 2022 (Statistics Indonesia 2022) which contribute approximately 75%
22 of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered
23 all over the country.

24 Production of red chilli fluctuated due to several factors which eventually caused significant fluctuation in chilli
25 price which finally made farmers and traders under inconvenience situations. Plant diseases has been the
26 main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which
27 causes yield reduction up to 66% (Utami et al. 2022), anthracnose which under severe infection might cause
28 yield losses up to 80% (Suprpta 2022), fusarium which had a record to cause 40% yield losses (Parihar et al.
29 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a begomovirus,
30 which under favourable condition caused yield losses up to 70% (Novrianty et al. 2013). Symptoms of
31 diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic appears on
32 infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower
33 abortion and fruit discoloration (Lavanya and Arun 2021).

34 Disease caused by PYLCV often reached its high incidence and intensity during dry season when the weather
35 is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn
36 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek et al. 2017). The disease
37 is increasingly frightening because it has spread to all chilli production centres in the country since its first
38 appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in
39 Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV),
40 belongs to begomovirus (Fadhila et al. 2020). Begomovirus is a member of Family Geminiviridae, a big plant
41 virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini
42 (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4
43 genera based on their vector and genome organization. Members of the family transmitted persistently by
44 white fly (*B. tabaci*) are grouped into Genus Begomovirus, a bipartite virus having 2 components of single
45 stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al.
46 2017). In Indonesia, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two
47 strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum.
48 Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar
49 symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon,
50 pepper and eggplant (Subiastuti et al. 2019).

51 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
52 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
53 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal et al. 2020).
54 Furthermore, the insect also reported to infest more than 600 plant species include tomato, water melon,
55 pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia,
56 lantana, and lily (Li et al. 2021). The insect is known to reproduce with high fecundity. A female can produce
57 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves.
58 Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from
59 unfertilized eggs (Xie et al. 2014).

60 *B. tabaci* has at least 43 species complex, (Shah and Liu 2013) and is able to transmit more than 200 plant
61 viruses (Lu et al. 2019; MacLeod et al. 2022), and 90% of plant viruses transmitted by the vector belong to
62 *begomovirus* (Kanakala and Ghanim 2016). To transmit PYLCV successfully, *B tabaci* requires an acquisition
63 period of at least 90 minutes and a latent period of at least 8 hours (Czosnek et al. 2017). The vector remains
64 viruliferous until 13 days after virus acquisition or until the vector dies (Roy et al. 2021).

65 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
66 controlling whitefly with synthetic insecticide is not economical (Hema et al. 2014) and generated resistant
67 genotypes and reduced natural enemies (Wang et al. 2020). Under such conditions, cultural control should be
68 better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological
69 diversity through management of biotic and abiotic environment. Abiotic environment of the crop can be
70 manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be
71 manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian and Rezvani
72 2016). Cultural control of insects has become better alternative sincemost insect has strong ability to evolve
73 pesticide resistance (Basit 2019).

74 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
75 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques by
76 modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
77 were reported to be effective to reduce whitefly invasion into the protected areas (Simmons and Shapiro-Ilan
78 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
79 techniques have enough contribution to the implementation of integrated pest management (Lapidot et al.
80 2014).

81 Cultural practices have been implemented as parts of integrated pest management with various level of
82 success and failure depended on the pest and the crops in concern (Kenyon et al. 2014), and there have been
83 no report on the effects of cultural control on the appearance and development of PYLCV on red chilli.

84 The objective of the research is to verify the seed transmission trait of *Pepper yellow leaf curl virus* and to
85 observe the effects of intercropping, crop barrier and physical barrier on the natural transmission of PYLCV by
86 its vector, *B. tabaci*.

87 **MATERIALS AND METHODS (when and where?)**

88 **Study area**

89 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
90 2022. The research was experimental research consisted of four different experiments. The first experiment
91 was a seed treatment of PYLCV experiment, conducted in Insectarium and insect-proof greenhouse of
92 Department of Plant Pest and Disease, Faculty of Agriculture, Sriwijaya University. The other three
93 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
94 natural transmission and infection of PLCV. The field experiments were conducted in experimental garden of
95 Sriwijaya University located in District Indralaya, Ogan Ilir South Sumatra, surrounded by local farmers' fields
96 where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

97 **Procedures**

98 **Seed treatment effect on PYLCV experiment**

99 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
100 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
101 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
102 seeds were not used because they were obviously not viable. The experiment was arranged in a completely
103 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
104 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
105 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
106 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh et
107 al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of their
108 rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to
109 obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
110 (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, and
111 the trays were then placed in an insect proof screen box for seed germination. The seedlings germinated in
112 each treatment unit were used to calculated the viability of the treated seeds. To observed the seed born

113 PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and
114 all polybags were then placed in insect proof greenhouse and were arranged accordingly to completely
115 randomized design.

116 **Intercropping effect on PYLCV experiment**

117 Red chili plants were planted experimentally under intercropping pattern with mungbean (*Vigna radiata*),
118 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
119 as the control. The experiment was arranged in a randomized block design with five treatments and five
120 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were
121 planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure
122 that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV
123 were infected chili plants that spread in farmers' fields around the research site. Additionally, some infected
124 chili plants were deliberately planted around the experimental plots. The vector of PYLCV was abundant in the
125 area since the insect is polyphagous (Kumar et al. 2023) and most of vegetation in the area were host of the
126 vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity
127 and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult
128 to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

129 **Trap cropping effect on PYLCV experiment**

130 An experiment to verify the effects of trap crops on the PYLCV infection was conducted using basil (*Ocimum*
131 *basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and with no
132 trap crop as control. The trap crops selected were those belonged to refugia which normally attract and trap
133 invader insect (Hardiansyah et al. 2021). The experiment was arranged in a randomized block design with 5
134 treatments and 5 replications resulting in 25 experimental units. The experimental unit was 4 x 2 m plot on
135 which red chilli was planted at 60 x 40 cm spacing. Trap crops were planted surrounding experimental plots at
136 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the
137 plots. The crop were positioned as border crop so that they could intercept insect before they attack the main
138 crops (Priyadi et al. 2020).. Chilli seedlings used in this experiment were prepared in insect proof boxes
139 together with those used in other field experiments. The parameters observed and the method of
140 observation were also similar to those observed and applied in intercropping experiment.

141 **Effect of physical barrier on PYLCV experiment**

142 An experiment to verify the effect of physical barrier on the invasion of PYLCV vectors was conducted using
143 cheesecloth as physical net barrier. Nets were previously used as an antihail device in fruit production.
144 However, having a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had
145 been considered to be used as a nonaggressive pest and diseases control device (Grasswitz 2019). The
146 experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units.
147 Physical barrier covering crop cultivation with insect net has been used in tobacco cultivation to prevent the
148 invasion of *B. tabaci* (Aji et al. 2015). In this experiment, physical barrier using insect net was used only as
149 side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh
150 cheesecloth was used in this experiment because it could prevent the entry of insect with body width less than
151 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish et al. 2016). The experiment
152 was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were

153 prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no barrier as
154 control. The net barriers were put in place before seedling transplanting. Seedlings and virus resources used
155 and data collection in this experiment were similar to those applied in intercropping and trap crop experiments.

156 **Crop maintenance and observation**

157 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
158 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
159 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
160 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
161 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
162 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
163 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
164 entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop
165 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
166 calculated according to disease scores described by Sharma et al. (2018) as follow: 0 = no visible symptoms;
167 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends;
168 3 = a wide range of leaf yellowing, curling and cupping; and 4 = very severe leaf yellowing, and pronounced
169 leaf curling. The severity was calculated using the following formula:

$$170 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

171 Note: DS = disease severity

172 v = disease score (0 to 4)

173 n = number of plants showing disease score v

174 Z = the highest disease score

175 N = total number of plants observed

176 Yield reduction was calculated using the following formula

$$177 \quad YR = \frac{w}{W} \times 100\%$$

178 YR = yield reduction

179 w = weight of first three harvests of infected plant

180 W = average weight of first three harvests of healthy plants in the same plot

181 **Data analysis**

182 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
183 collected data, and significant differences between means were determined using Honestly Significant
184 Difference (HSD) test a 95% degree of significance.

185 **RESULTS AND DISCUSSION (just primer supporting references, less than 10 years)**

186 **Seed transmission of PYLCV**

187 *Pepper yellow leaf curl virus* (PYLCV) is a member of begomovirus which was suspected to be transmissible
188 through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds,
189 ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the
190 seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might

191 be directly since infection of virus could make seeds more sensitive to deterioration as reported by Bueso et
192 al. (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis
193 seeds up to 65%. Nallathambi et al. (2020) also reported that virus infection could cause abnormal physical
194 function of seeds and establish itself in any part of the seed which eventually affected their viability and
195 potentially initiates seedborne disease.

196 Some of the seedling also produced PYLCV infection symptoms indicating that PYLCV was a seed borne virus.
197 The seed transmission of begomovirus had previously been reported by Kothandaraman et al. (2016) who
198 studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus was
199 seed-borne. A similar finding was also reported by Kil et al. (2020) who worked with *Tomato yellow leaf curl*
200 *New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus
201 showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV) as reported by Pérez-
202 Padilla et al. (2020) who worked with the virus on more than 3000 tomato plants and concluded that TYLCV
203 was seed borne, but seed transmission was not a general property of the virus. In this experiment, the
204 seedborne infection of PYLCV was relatively high because the seed was harvested from infected plants which
205 potentially brought the virus, as reported by Fadhila et al. (2020) who worked with PepYLCIV and could detect
206 DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

207 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
208 PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water
209 treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds
210 and weakened parts of them resulted the longer incubation period. According to Paylan et al. (2014), heated
211 water at 65°C, together with HCl and ozon, were very effective treatment to reduce virus concentration in the
212 seeds and had no negative effect on the seeds. Even though the seed treatments affected the virus infection,
213 seed borne infection of the virus still occurred indicated that the effect could not reach the embryo where the
214 virus normally present. Seed borne viruses present in seed coat, endosperm, nucleus or embryo, but only
215 viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the
216 effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might
217 have better effect to seed borne virus infection, but it might also affect the seed germination. According to
218 Farajollahi et al. (2014), hot water treatment could induce seed germination but duration of drenching the seeds
219 in hot water could reduce seed viability. Instead of reducing seed borne virus infection, hot water treatment
220 was also reported to be effective to eradicate bacterial seed borne pathogens and was highly recommended
221 for pepper, eggplant and tomato seeds (Kim et al. 2022).

222 Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and
223 threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to
224 start an endemic (Pagán 2022). High seed borne tobamovirus infection has also been reported by
225 Dombrovsky and Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
226 *virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

227 All treatments in the experiment resulted in PYLCV infection which confirmed that, as a member of
228 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
229 nature of begomovirus had also been reported by (Kothandaraman et al. 2016) who studied the seed
230 transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato yellow leaf curl*

231 *New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato plants and
232 concluded that the virus was seed borne but seed transmission was not a general property of the virus.

233 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
234 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
235 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
236 virus and zika virus (Al Hadhrami et al. 2022; Jennings and Parks 2020), and plant virus such as *Cucumber*
237 *mosaic virus* (Hamidson et al. 2018).

238 **Intercropping effect on PYLCV**

239 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
240 different family from the main crop. The use of mung bean and soybean (Family Leguminosae) and tomato
241 and eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but significantly affected
242 disease incidence, of which only tomato caused significant reduction of PYLCV infections (Table 2). The
243 reduction of viral disease under intercropping was a direct effect of the reduction of incoming vector, as
244 reported by Mir et al. (2022) that intercropping could effectively control insect pest. The effect of intercropping
245 to insect vector might be by reducing the vector invasion to the crop due to the presence of intercrop as
246 alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant disease by
247 causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence could be
248 caused by its effect on incoming vector, since tomato has insect repellent effect, especially against mosquito
249 and aphid (University 2022).

250 Researches on the use of Leguminous crops as intercrops has been frequent but mostly for the intention of
251 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
252 on plant disease was reported. Most of the research were combination between leguminous and food crops
253 such as maize-mungbean (Syafruddin and Suwardi 2020), cotton-mungbean (Liang et al. 2020), sorghum-
254 mungbean (Temeche et al. 2022), sorghum-soybean (Saber 2018), maize-soybean (Berdjour et al. 2020),
255 and rice-soybean (Putra and Sas 2023).

256 **Trap crop effect on PYLCV.**

257 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However,
258 only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos,
259 tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests
260 (Sarjan et al. 2023). Barrier crop could also affect the main crops by intercepting, arresting or retaining pest
261 thereby limiting the number of insect pest and insect vector reaching the main crop which eventually reduce
262 incidence of viral disease (Waweru et al. 2021). Furthermore, the significant effect of basil and marigold on
263 PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait
264 of repellent against *B. tabaci* and prevented the insect oviposition. Husna et al. (2020) reported that crude
265 extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes aegypti* larvae, and
266 according to Farindira (2015), at higher concentration the crude extract could be used as repellent against the
267 mosquito. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against
268 mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong repellent against mosquito
269 (Ponkiya et al. 2018), and contained extractable toxicants which effective as repellent and larvicidal against *B.*

270 *tabaci* (Fabrick et al. 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Calumpang and
271 Ohsawa 2015), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al. 2019). When a trap crop
272 has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour (Acharya
273 et al. 2021) or odor (Shao et al. 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour.
274 Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced
275 green flower but with strong odor.

276 **Physical barrier effect on PYLCV**

277 Physical barrier using cheese cloth 50 mesh could reduce the infection of PYLCV indicated by lower disease
278 incidence and severity, only barriers that are more than 100 cm high have a significant effect on disease
279 incidence (Table 4). This result confirmed that a barrier of 50 mesh could effectively prevent *B. tabaci* to the
280 protected plot (Harish et al. 2016). It is shown in Table 4 that the higher the barrier the lower the disease
281 incidence caused by PYLCV, an indication that *B. tabaci*, the only vector of PYLCV, could fly higher than 100
282 cm and transmitting the virus. However, *B. tabaci* is not a good flyer even though the insect might spread to
283 long distance carried by wind or transported materials. The maximum distance covered by single flying of the
284 whitefly is 17 m (Maruthi et al. 2017), but in a dense plant population the insect can move from plant to plant
285 easily, and if the insect is viruliferous, massive virus spread is inevitable. According to Tillman (2014), height
286 of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the
287 height of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicated that the insect still
288 flew above 125 cm to enter the plot and lower side barrier could not effectively block the insect. The
289 cheesecloth net could only work mechanically in blocking flying insect and had no effect on the insect behavior.
290 This was different from barrier crop which not only block the flying insects but also attract or repel the insect,
291 depended on the crop species. Flying insect such as *B. tabaci* could not easily differentiate host and nonhost
292 plants which made barrier crop more effective than net barrier (Udiarto et al. 2023). *B. tabaci* actually has two
293 type of flight behavior, foraging flight and migratory flight. Foraging flight is close to earth surface or within flight
294 boundary, while migratory flight is above the boundary where the insect could be picked up and carried by air
295 currents (Reynolds et al. 2017), and the insect containing mature eggs has been trapped at 150 m above
296 ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier could
297 actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect bigger than mesh
298 size of the net.

299 **CONCLUSIONS AND SUGGESTION (Arial 10) (No discussion, here)**

300 The first experiment of seed borne transmission of PYLCV concluded that PYLCV infecting red chili was a
301 seed borne virus and the rate of seed borne infection could be partially reduced by hot water treatment and
302 turmeric crude extract. In the intercropping experiment, tomato was good enough as intercrops for red chili in
303 term of reducing PYLCV infections. Basil and marigold were functional as trap crops to protect chili from
304 incoming *B. tabaci* and from PYLCV infection. Marigold is more recommended since the plant can grow higher
305 and more protective than basil. In the experiment of physical barrier, the use of 50 mesh cheese cloth as side
306 barrier could reduce PYLCV infection frequency only at the height of 125 cm. The entry of viruliferous *B. tabaci*
307 into the plot crossed the barrier can potentially be reduce by using denser and higher net. In all cultural
308 techniques applied in the field experiments, PYLCV infection occurred and cause yield reduction ranged from
309 40 to 53%.

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516 **Tables**

517

518 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
519 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58,90±1,68	29,45±0,54 b	6,80±0,77 ab	4,10±0,60 ab
Ginger crude extract	54,00±1,87	30,86±1,09 b	8,40±1,30 ab	4,90±0,82 ab
Turmeric crude extract	51,17±2,29	21,40±1,02 a	3,20±0,37 a	1,80±0,26 a
Javanese ginger crude extract	51,74±3,03	23,84±1,61 a	5,60±0,74 ab	4,00±0,49 ab
Control	43,66±3,11	22,84±1,06 a	9,60±0,57b	6,70±0,41 b
F Calculated	2,76 ^{ns}	12,12*	1,94*	2,14*
P Value	0,08	0,001	0,04	0,02
HSD 5%		5,15	5,61	3,56

520 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
521 Significant Difference Test.522 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
523 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16,18±2,35 ab	10,95±1,04	47,19±2,29
Mung bean	15,23±2,39 ab	9,52±1,58	49,22±2,20
Soybean	14,58±1,97 ab	8,57±1,25	46,41±3,27
Tomato	7,62±0,88 a	4,52±0,66	40,00±1,47
Control	26,66±0,88 b	14,28±1,10	46,26±2,04
F Calculated	5,74*	3,44 ^{ns}	0,82 ^{ns}
P Value	0,01	0,05	0,54
HSD 5%	12,18	-	-

524 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
525 Significant Difference Test.526 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
527 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8,14±1,36 a	5,85±0,86 a	50,21±2,41
Cosmos	10,28±1,29 ab	7,12±0,57 ab	48,41±1,54
Marigold	4,57±0,53 a	3,57±0,38 a	42,80±1,22
Zinnia	13,71±1,59 ab	11,28±1,31 ab	47,32±1,35
control	16,57±0,81 b	13,14±1,00 b	52,01±1,62
F Calculated	5,51*	7,70*	1,50 ^{ns}
P Value	0,01	0,003	0,27
HSD 5%	8,41	6,06	-

528 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
529 Significant Difference Test.

530 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 531 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11,42±1,53 ab	8,71±0,86 bc	49,87±0,85
Side barrier 75 cm	9,71±0,52 ab	8,00±0,47 abc	47,42±1,41
Side barrier 100 cm	8,00±1,26 ab	6,57±0,71 ab	49,00±1,36
Side barrier 125 cm	5,14±0,81 a	3,57±0,74 a	43,40±1,08
Control	14,28±0,68 b	12,14±0,92 c	52,32±2,11
F Calculated	5,28*	8,456*	1,73 ^{ns}
P Value	0,01	0,002	0,21
HSD 5%	6,40	4,58	

532 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 533 Significant Difference Test.

COVER PAGE

I. The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

Running title: Effects of cultural techniques on Pepper yellow leaf curl virus

Suparman ¹⁾, Arsi²⁾ a Yulia Pujiastuti¹⁾ and Rahmat Pratama¹⁾

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The effects of various cultural techniques on the transmission and infectious development of *Pepper yellow leaf curl virus* on red chili

(Running title: Effects of cultural techniques on *Pepper yellow leaf curl virus*)

1 **ABSTRACT (maximum 200 words, it must : the objective, method, research products)**

2 An experiment was conducted to investigate the effects of cultural techniques to prevent the infection of yellow
3 leaf curl disease of chili caused by *Pepper yellow leaf curl virus* (PYLCV). The experiment was conducted in
4 the area where the disease has been an endemic and *Bemisia tabaci*, the vector of the disease, was abundant.
5 The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds
6 harvested from infected plants were used for seed treatment experiment, and commercial seeds mostly used
7 by local farmers were used for the other experiments. The results showed that PYLCV was a seedborne virus
8 and hot water treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric
9 could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant,
10 mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield
11 reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and
12 zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but
13 not the lower ones. Under various cultural technique, PYLCV caused chili yield reduction of 40 – 53%.

14 **KEYWORDS**
15 *Bemisia tabaci*, cultural technique, *Pepper yellow leaf curl virus*, seed treatment

16 **INTRODUCTION (just primer supporting references, less than 10 years)**

17 Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country,
18 with some provinces have become the production centres of this commodity from where the majority of nation
19 demand is fulfilled. The eleven biggest red chili producing provinces in 2022 were West Java, North Sumatra,
20 Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra
21 with total production of 1229262 tons. The chili production varied among different provinces and the three
22 provinces in Java contributed to 58.3% to the national production (Siregar & Suroso, 2021).

23 Production of red chilli fluctuated due to several factors which inevitably caused significant fluctuation in chilli
24 price which eventually made farmers and traders under inconvenience situations. Plant diseases has been the
25 main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which
26 causes yield reduction up to 66% (Utami, Meale & Young, 2022), anthracnose which under severe infection
27 might cause yield losses up to 80% (Suprpta, 2022), fusarium which had a record to cause 40% yield losses
28 (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a
29 begomovirus, which under favourable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al,
30 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow

31 mosaic appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling,
32 smaller fruit, flower abortion and fruit discoloration (Lavanya & Arun 2021).

33 Disease caused by PYLCV often reached its high incidence and intensity during dry season when the weather
34 is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn
35 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek, Hariton-Shalev, Sobol,
36 Gorovits, & Ghanim, 2017). The disease is increasingly frightening because it has spread to all chilli production
37 centres in the country since its first appearance detected in 1999 (Gaswanto, Syukur, Hidayat, & Gunaeni,
38 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed
39 as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila, Lal, Vo, Ho, Hidayat,
40 Lee, Kil, & Lee, 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of
41 members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2
42 components of single stranded DNA genome inside. Geminiviridae comprises of 4 genera based on their
43 vector and genome organization. Members of the family transmitted persistently by white fly (*B. tabaci*) are
44 grouped into Genus Begomovirus, a bipartite virus having 2 components of single stranded DNA recognized
45 as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek, Hariton-Shalev, Sobol, Gorovits,
46 & Ghanim, 2017). In Indonesia, *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) was recognized to have
47 two strains i.e. PeYLCIV-Tomato which also infects tomato and PeYLCIV-Ageratum which also infects
48 ageratum. Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and
49 cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon,
50 water melon, pepper and eggplant (Subiastuti, Hartono, Daryono, 2019).

51 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
52 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
53 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal, Singh, &
54 Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included
55 tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant,
56 chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is
57 known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm.
58 The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from
59 fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al., 2014).

60 *B. tabaci* has at least 43 species complex, (Shah & Liu, 2013) and is able to transmit more than 200 plant
61 viruses (Lu, Chen, Li, Shi, Gu, & Yan, 2019; MacLeod, Canty, & Polaszek, 2022), and 90% of plant viruses
62 transmitted by the vector belong to *begomovirus* (Kanakala & Ghanim, 2016). To transmit PYLCV successfully,
63 *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8 hours (Czosnek,
64 Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). The vector remains viruliferous until 13 days after virus
65 acquisition or until the vector dies (Roy, Chakraborty, & Ghosh, 2021).

66 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
67 controlling whitefly with synthetic insecticide is not economical (Patra & Hath, 2022) and generated resistant
68 genotypes and reduced natural enemies (Wang et al., 2020). Under such conditions, cultural control should
69 be better alternative to solve the problems. Cultural control is to conserve natural enemies and increase
70 biological diversity through management of biotic and abiotic environment. Abiotic environment of the crop can
71 be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be

72 manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian & Rezvani,
73 2016). Cultural control of insects has become better alternative since most insect has strong ability to evolve
74 pesticide resistance (Basit, 2019).

75 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
76 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques by
77 modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
78 were reported to be effective to reduce whitefly invasion into the protected areas (Simmons & Shapiro-Ilan,
79 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
80 techniques have enough contribution to the implementation of integrated pest management (Lapidot, Legg,
81 Wintermantel, & Polston, 2014).

82 Cultural practices have been implemented as parts of integrated pest management with various level of
83 success and failure depended on the pest and the crops in concern (Kenyon, Kumar, Tsai, & Hughes, 2014),
84 and there have been no report on the effects of cultural control on the appearance and development of PYLCV
85 on red chilli.

86 The objective of the research is to verify the seed transmission trait of *Pepper yellow leaf curl virus* and to
87 observe the effects of seed treatments, intercropping, trap cropping, and physical barrier on the natural
88 transmission and infection development of PYLCV by its vector, *B. tabaci*.

89 **MATERIALS AND METHODS (when and where?)**

90 **Study area**

91 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
92 2022. The research was experimental research consisted of four different experiments. The first experiment
93 was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof screenhouse
94 of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three
95 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
96 natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden
97 of Universitas Sriwijaya located in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers'
98 fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

99 **Procedures**

100 **Seed treatment effect on PYLCV experiment**

101 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
102 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
103 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
104 seeds were not used because they were obviously not viable. The experiment was arranged in a completely
105 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
106 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
107 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
108 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh,
109 Awasthi, Jangre, & Nirmalkar, 2020). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of
110 their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth

111 to obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
112 (Kabede, Ayalew, & Yeesuf, 2013); John, Ihum, Olusolape, & Janfa, 2018). The treated seeds were sown
113 accordingly in each double trays, and the trays were then placed in an insect proof screen box for seed
114 germination. The seedlings germinated in each treatment unit were used to calculated the viability of the
115 treated seeds. To observed the seed borned PYLCV infection, 50 four-leaf seedlings were then transferred
116 individually to a 20 cm diameter polybag, and all polybags were then placed in insect proof greenhouse and
117 were arranged accordingly to completely randomized design.

118 **Intercropping effect on PYLCV experiment**

119 Red chili plants were planted experimentally under intercropping pattern with mung bean (*Vigna radiata*),
120 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
121 as the control. The experiment was arranged in a randomized block design with five treatments and five
122 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red
123 chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All
124 seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field
125 and brought in by its vector, *B. tabaci*. Sources of PYLCV were infected chili plants that spread in farmers'
126 fields around the research site. Additionally, some infected chili plants were deliberately planted around the
127 experimental plots. The vector of PYLCV was abundant in the area since the insect is polyphagous with
128 hundreds number of host species (Pym et al., 2019) and most of vegetation in the area were host of the vector.
129 Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield
130 reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect
131 when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

132 **Trap cropping effect on PYLCV experiment**

133 An experiment to investigate the effects of trap crops on the PYLCV infection was conducted using basil
134 (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and
135 with no trap crop as control. The trap crops selected were those belonged to refugia which normally attract
136 and trap invader insect (Hardiansyah, Hartini, & Musa, 2021). The experiment was arranged in a randomized
137 block design with 5 treatments and 5 replications resulting in 25 experimental units. The experimental unit was
138 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing. Trap crops were planted surrounding the
139 experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red
140 chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before
141 they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chilli seedlings used in this experiment
142 were prepared in insect proof boxes, together with those used in other field experiments. The parameters
143 observed and the method of observation were similar to those observed and applied in intercropping
144 experiment.

145 **Effect of physical barrier on PYLCV experiment**

146 An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using
147 cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device in
148 fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and
149 diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical
150 barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent

151 the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental
152 garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect
153 net was used only as side barrier and let the top opened for the access of pollinator and other beneficial
154 insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect
155 with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish,
156 Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design
157 with 5 treatments and 5 replications for which 25 plots were prepared. Four level of physical barrier height
158 were applied i.e. 50, 75, 100 and 125 cm and no side barrier as control. The net barriers were put in place
159 before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were
160 similar to those applied in intercropping and trap crop experiments.

161 **Crop maintenance and observation**

162 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
163 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
164 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
165 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
166 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
167 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
168 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
169 entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop
170 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
171 calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy,
172 (2022) as follow: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some
173 yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping; and 4 =
174 very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following
175 formula:

$$176 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

177 Note: DS = disease severity

178 v = disease score (0 to 4)

179 n = number of plants showing disease score v

180 Z = the highest disease score

181 N = total number of plants observed

182 Yield reduction was calculated using the following formula

$$183 \quad YR = \frac{w}{W} \times 100\%$$

184 YR = yield reduction

185 w = weight of first three harvests of infected plant

186 W = average weight of first three harvests of healthy plants in the same plot

187 **Data analysis**

188 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
189 collected data, and significant differences between means were determined using Honestly Significant
190 Difference (HSD) test a 95% degree of significance.

191 RESULTS AND DISCUSSION (just primer supporting references, less than 10 years)

192 Seed transmission of PYLCV

193 *Pepper yellow leaf curl virus* (PYLCV) is a member of begomovirus which was suspected to be transmissible
194 through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds,
195 ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the
196 seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might
197 be directly since infection of virus could make seeds more sensitive to deterioration as reported by Bueso,
198 Serrano, Pallás, & Sánchez-Navarro, (2017) who found that infection of *Cucumber mosaic virus* (CMV) could
199 reduce the viability of *Arabidopsis* seeds up to 65%. Nallathambi, Umamaheswari, Lal, Manjunatha, & Berliner
200 (2020) also reported that virus infection could cause abnormal physical function of seeds and establish itself
201 in any part of the seed which eventually affected their viability and potentially initiates seedborne disease.

202 Some of the seedling also produced PYLCV infection symptoms, confirming that PYLCV was a seed borne
203 virus. The seed transmission of begomovirus had previously been reported by Kothandaraman, Devadason,
204 & Ganesan (2016) who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and
205 concluded that the virus was seed-borne. A similar finding was also reported by Kil et al. (2020) who worked
206 with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible.
207 Another begomovirus showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV)
208 as reported by Pérez-Padilla et al., (2020), who worked with the virus on more than 3000 tomato plants and
209 concluded that TYLCV was seed borne, but seed transmission was not a general property of the virus. In this
210 experiment, the seedborne infection of PYLCV was relatively high because the seed was harvested from
211 infected plants which potentially brought the virus, as reported by Fadhila et al. (2020) who worked with
212 PepYLCIV and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested from
213 infected plants.

214 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
215 PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water
216 treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds
217 and weakened parts of them resulted the longer incubation period. According to Paylan, Erkan, Cetinkaya,
218 Ergun, & Pazarlar (2014), heated water at 65°C, together with HCl and ozon, were very effective treatment to
219 reduce virus concentration in the seeds and had no negative effect on the seeds. Even though the seed
220 treatments affected the virus infection, seed borne infection of the virus still occurred indicated that the effect
221 could not reach the embryo where the virus normally present. Seed borne viruses present in seed coat,
222 endosperm, nucleus or embryo, but only viruses present in the seed embryo can be transmitted to the next
223 generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the
224 dipping time. Longer dipping time might have better effect to seed borne virus infection, but it might also affect
225 the seed germination. According to Farajollahi, Gholinejad, & Jafari (2014), hot water treatment could induce
226 seed germination but duration of drenching the seeds in hot water could reduce seed viability. Instead of
227 reducing seed borne virus infection, hot water treatment was also reported to be effective to eradicate bacterial
228 seed borne pathogens and was highly recommended for pepper, eggplant and tomato seeds (Kim, Shim, Lee,
229 & Wangchuk, 2022).

230 Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and
231 threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to

232 start an endemic (Pagán, 2022). High seed borne tobamovirus infection has also been reported by
233 Dombrovsky & Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
234 *virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

235 All treatments in the experiment resulted in PYLCV infection which confirmed that, as a member of
236 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
237 nature of begomovirus had also been reported by (Kothandaraman, Devadason, & Ganesan, 2016) who
238 studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato*
239 *yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato
240 plants and concluded that the virus was seed borne but seed transmission was not a general property of the
241 virus.

242 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
243 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
244 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
245 virus and zika virus (Al Hadhrami, Battashi, & Al Hashami, 2022); Jennings & Parks 2020), and plant virus
246 such as *Cucumber mosaic virus* (Hamidson, Damiri, & Angraini, 2018).

247 **Intercropping effect on PYLCV**

248 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
249 different family from the main crop. The use of mung bean and soybean (Family Leguminosae), tomato and
250 eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but affected disease
251 incidence in the range of 39.30 to 71.41%, with only tomato caused significant reduction of PYLCV infections
252 (Table 2). The reduction of viral disease under intercropping was a direct effect of the reduction of incoming
253 vector, as reported by Mir et al. (2022) that intercropping could effectively control insect pest. The effect of
254 intercropping to insect vector might be by reducing the vector invasion to the crop due to the presence of
255 intercrop as alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant
256 disease by causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence
257 could be caused by its effect on incoming vector, since tomato has insect repellent effect, especially against
258 mosquito and aphid (Setyaningrum, Unih, Pratami, & Kanedi, 2023).

259 Research on the use of leguminous crops as intercrops has been frequent but mostly for the intention of
260 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
261 on plant disease was reported. Most of the research were combinations between leguminous and food crops
262 such as maize-mung bean (Syafuruddin & Suwardi, 2020), cotton-mung bean (Liang, He, & Shi, 2020),
263 sorghum-mungbean (Temeche, Getachew, Hailu, & Abebe, 2022), sorghum-soybean (Saber 2018), maize-
264 soybean (Berdjour, Dugje, Dzomeku, & Rahman, 2020), and rice-soybean (Putra & Sas, 2023).

265 **Trap crop effect on PYLCV.**

266 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. Basil,
267 cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect
268 pests (Sarjan, Haryanto, Supeno, & Jihadi, 2023), but the effects of the four crops were different when being
269 used barrier crops. Barrier crop could affect the main crops by intercepting, arresting or retaining pest thereby
270 limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence

271 of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could
272 significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The
273 significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to
274 Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the insect
275 oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5%
276 effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman, Srinivasan, Suganthi,
277 Ranganathan, Gimbin, & Shanmugam (2018), the extract was also effective against *Culex tritaeniorhynchus*,
278 *Aedesal bopictus* and *Anopheles subpictuat*. Husna et al. (2020) also reported that the extract of basil leaves
279 was a strong larvicidal against mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong
280 repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal
281 against *B. tabaci* (Fabrck, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali*
282 (Dikr & Belete, 2021), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap
283 crop has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour
284 (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but
285 Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured
286 flower and one species (basil) produced green flower but with strong odor.

287 **Physical barrier effect on PYLCV**

288 Physical barrier using cheese cloth 50 mesh could reduce the infestation of PYLCV vectors indicated by the
289 reduction of disease incidence and severity up to 64.00% and 70.59% respectively, but only physical barriers
290 at 125 cm high could significantly reduce incidence and severity of pepper yellow leaf curl disease (Table 4).
291 This result was in accordance to that reported by (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016)
292 that a barrier of fine mesh could effectively prevent *B. tabaci* to the protected plot. It is shown in the table that
293 the higher the barrier the lower the disease incidence caused by PYLCV, an indication that *B. tabaci*, the only
294 vector of PYLCV, could fly higher than 100 cm and transmitting the virus.. According to Tillman (2014), height
295 of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the
296 height of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicated that the insect
297 could fly above such altitude even though the whitefly is not a good flyer even though it might spread to long
298 distance carried by wind or transported materials. The maximum distance covered by single flying of the
299 whitefly is 17 m (Maruthi, Jeremiah, Mohammed, & Legg, 2017), but in a dense plant population the insect
300 can move from plant to plant easily, and if the insect is viruliferous, massive virus spread is inevitable.

301 The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in
302 blocking flying insect and had no effect on the insect behavior. This was different from barrier crop which not
303 only block the flying insects but also attract or repel the insect, depended on the crop species. Flying insect
304 such as *B. tabaci* could not easily differentiate host and nonhost plants which made barrier crop more effective
305 than net barrier (Udiarto, Setiawati, Muharam, & Dadi, 2023). *B. tabaci* actually has two type of flight behavior,
306 foraging flight and migratory flight. Foraging flight is close to earth surface or within flight boundary, while
307 migratory flight is above the boundary where the insect could be picked up and carried by air currents
308 (Reynolds, Chapman, & Drake, 2017), and the insect containing mature eggs has been trapped at 150 m
309 above ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier
310 could actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect with body
311 width wider than mesh size of the net.

312

CONCLUSIONS AND SUGGESTION (Arial 10) (No discussion, here)

313 The first experiment of seed borne transmission of PYLCV concluded and verified that the virus infecting red
 314 chili was a seed borne virus. Hot water treatment and crude extract of red ginger could lengthen the average
 315 incubation period of PYLCV up to 6.61 days to 8.02 days respectively, and crude extract red ginger reduced
 316 the infection frequency and disease severity up to 66.67 % and 73.13% respectively, compared to control. In
 317 the intercropping experiment, tomato was good enough as intercrops for red chili in term of reducing PYLCV
 318 infections which could reduce disease incidence up to 71.41%. Basil and marigold were functional as trap
 319 crops to protect chili from incoming *B. tabaci* and could reduce incidence and severity of disease transmitted
 320 by the insect up to 72.42% and 72.83% respectively. In the experiment of physical barrier, the use of 50 mesh
 321 cheese cloth as side barrier at the height of 125 cm could reduce PYLCV infection frequency up to 70.59%. In
 322 all cultural techniques applied in the field experiments, PYLCV infection on red chili caused yield reduction of
 323 40 to 53%.

324 Infection of PYLCV on red chili is easily spread by *B. tabaci* and the disease is very damaging to the crop yield.
 325 Even though some cultural techniques are effective to reduce PYLCV transmission by each vector, regular
 326 disease monitoring is important and destroy infected plants is necessary to eliminate virus inoculum.

327 **REFERENCES (Arial 10) → consistent, DOI, less than 10 years, check guidelines**

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536 Tables

537 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
538 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

539 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
540 Significant Difference Test.

541 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
542 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54

HSD 5%

12.18

-

-

543 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 544 Significant Difference Test.

545 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
 546 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

547 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 548 Significant Difference Test.

549 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 550 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

551 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 552 Significant Difference Test.

COVER PAGE

I. The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

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The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

1 **ABSTRACT (maximum 200 words, it must : the objective, method, research products)**

2 An experiment was conducted to investigate the effects of cultural techniques to prevent the infection of yellow
3 leaf curl disease of chili caused by *Pepper Yellow Leaf Curl Virus* (PYLCV). The experiment was conducted in
4 the area where the disease has been an endemic and *Bemisia tabaci*, the vector of the disease, was abundant.
5 The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds
6 harvested from infected plants were used for seed treatment experiment, and commercial seeds mostly used
7 by local farmers were used for the other experiments. The results showed that PYLCV was a seedborne virus
8 and hot water treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric
9 could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant,
10 mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield
11 reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and
12 zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but
13 not the lower ones. Under various cultural technique, PYLCV caused chili yield reduction of 40 – 53%.

14 **KEYWORDS**

15 *Bemisia tabaci*, hot water treatment, intercropping, physical barrier, trap cropping.

16 **INTRODUCTION (just primer supporting references, less than 10 years)**

17 Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country,
18 with some provinces have become the production centres of this commodity from where the majority of nation
19 demand is fulfilled. The eleven biggest chili producing provinces in 2022 were West Java, North Sumatra,
20 Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra
21 with total production of 1229262 tons in 2022 (Statistics Indonesia 2022) which contribute approximately 75%
22 of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered
23 all over the country.

24 Production of red chilli fluctuated due to several factors which eventually caused significant fluctuation in chilli
25 price which finally made farmers and traders under inconvenience situations. Plant diseases has been the
26 main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which
27 causes yield reduction up to 66% (Utami et al. 2022), anthracnose which under severe infection might cause
28 yield losses up to 80% (Suprpta 2022), fusarium which had a record to cause 40% yield losses (Parihar et al.
29 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a begomovirus,
30 which under favourable condition caused yield losses up to 70% (Novrianty et al. 2013). Symptoms of
31 diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic appears on
32 infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower
33 abortion and fruit discoloration (Lavanya and Arun 2021).

34 Disease caused by PYLCV often reached its high incidence and intensity during dry season when the weather
35 is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn
36 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek et al. 2017). The disease
37 is increasingly frightening because it has spread to all chilli production centres in the country since its first
38 appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in
39 Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV),
40 belongs to begomovirus (Fadhila et al. 2020). Begomovirus is a member of Family Geminiviridae, a big plant
41 virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini
42 (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4
43 genera based on their vector and genome organization. Members of the family transmitted persistently by
44 white fly (*B. tabaci*) are grouped into Genus Begomovirus, a bipartite virus having 2 components of single
45 stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al.
46 2017). In Indonesia, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two
47 strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum.
48 Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar
49 symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon,
50 pepper and eggplant (Subiastuti et al. 2019).

51 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
52 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
53 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal et al. 2020).
54 Furthermore, the insect also reported to infest more than 600 plant species include tomato, water melon,
55 pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia,
56 lantana, and lily (Li et al. 2021). The insect is known to reproduce with high fecundity. A female can produce
57 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves.
58 Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from
59 unfertilized eggs (Xie et al. 2014).

60 *B. tabaci* has at least 43 species complex, (Shah and Liu 2013) and is able to transmit more than 200 plant
61 viruses (Lu et al. 2019; MacLeod et al. 2022), and 90% of plant viruses transmitted by the vector belong to
62 *begomovirus* (Kanakala and Ghanim 2016). To transmit PYLCV successfully, *B. tabaci* requires an acquisition
63 period of at least 90 minutes and a latent period of at least 8 hours (Czosnek et al. 2017). The vector remains
64 viruliferous until 13 days after virus acquisition or until the vector dies (Roy et al. 2021).

65 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
66 controlling whitefly with synthetic insecticide is not economical (Hema et al. 2014) and generated resistant
67 genotypes and reduced natural enemies (Wang et al. 2020). Under such conditions, cultural control should be
68 better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological
69 diversity through management of biotic and abiotic environment. Abiotic environment of the crop can be
70 manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be
71 manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian and Rezvani
72 2016). Cultural control of insects has become better alternative sincemost insect has strong ability to evolve
73 pesticide resistance (Basit 2019).

74 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
75 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques by
76 modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
77 were reported to be effective to reduce whitefly invasion into the protected areas (Simmons and Shapiro-Ilan
78 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
79 techniques have enough contribution to the implementation of integrated pest management (Lapidot et al.
80 2014).

81 Cultural practices have been implemented as parts of integrated pest management with various level of
82 success and failure depended on the pest and the crops in concern (Kenyon et al. 2014), and there have been
83 no report on the effects of cultural control on the appearance and development of PYLCV on red chilli.

84 The objective of the research is to verify the seed transmission trait of *Pepper yellow leaf curl virus* and to
85 observe the effects of intercropping, crop barrier and physical barrier on the natural transmission of PYLCV by
86 its vector, *B. tabaci*.

87 **MATERIALS AND METHODS (when and where?)**

88 **Study area**

89 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
90 2022. The research was experimental research consisted of four different experiments. The first experiment
91 was a seed treatment of PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of
92 Department of Plant Pest and Disease, Faculty of Agriculture, Sriwijaya University. The other three
93 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
94 natural transmission and infection of PLCV. The field experiments were conducted in experimental garden of
95 Sriwijaya University located in District Indralaya, Ogan Ilir South Sumatra, surrounded by local farmers' fields
96 where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

97 **Procedures**

98 **Seed treatment effect on PYLCV experiment**

99 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
100 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
101 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
102 seeds were not used because they were obviously not viable. The experiment was arranged in a completely
103 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
104 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
105 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
106 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh et
107 al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of their
108 rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to
109 obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
110 (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, and
111 the trays were then placed in an insect proof screen box for seed germination. The seedlings germinated in
112 each treatment unit were used to calculated the viability of the treated seeds. To observed the seed born

113 PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and
114 all polybags were then placed in insect proof greenhouse and were arranged accordingly to completely
115 randomized design.

116 **Intercropping effect on PYLCV experiment**

117 Red chili plants were planted experimentally under intercropping pattern with mungbean (*Vigna radiata*),
118 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
119 as the control. The experiment was arranged in a randomized block design with five treatments and five
120 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were
121 planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure
122 that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV
123 were infected chili plants that spread in farmers' fields around the research site. Additionally, some infected
124 chili plants were deliberately planted around the experimental plots. The vector of PYLCV was abundant in the
125 area since the insect is polyphagous (Kumar et al. 2023) and most of vegetation in the area were host of the
126 vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity
127 and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult
128 to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

129 **Trap cropping effect on PYLCV experiment**

130 An experiment to verify the effects of trap crops on the PYLCV infection was conducted using basil (*Ocimum*
131 *basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and with no
132 trap crop as control. The trap crops selected were those belonged to refugia which normally attract and trap
133 invader insect (Hardiansyah et al. 2021). The experiment was arranged in a randomized block design with 5
134 treatments and 5 replications resulting in 25 experimental units. The experimental unit was 4 x 2 m plot on
135 which red chilli was planted at 60 x 40 cm spacing. Trap crops were planted surrounding experimental plots at
136 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the
137 plots. The crop were positioned as border crop so that they could intercept insect before they attack the main
138 crops (Priyadi et al. 2020).. Chilli seedlings used in this experiment were prepared in insect proof boxes
139 together with those used in other field experiments. The parameters observed and the method of
140 observation were also similar to those observed and applied in intercropping experiment.

141 **Effect of physical barrier on PYLCV experiment**

142 An experiment to verify the effect of physical barrier on the invasion of PYLCV vectors was conducted using
143 cheesecloth as physical net barrier. Nets were previously used as an antihail device in fruit production.
144 However, having a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had
145 been considered to be used as a nonaggressive pest and diseases control device (Grasswitz 2019). The
146 experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units.
147 Physical barrier covering crop cultivation with insect net has been used in tobacco cultivation to prevent the
148 invasion of *B. tabaci* (Aji et al. 2015). In this experiment, physical barrier using insect net was used only as
149 side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh
150 cheesecloth was used in this experiment because it could prevent the entry of insect with body width less than
151 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish et al. 2016). The experiment
152 was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were

153 prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no barrier as
154 control. The net barriers were put in place before seedling transplanting. Seedlings and virus resources used
155 and data collection in this experiment were similar to those applied in intercropping and trap crop experiments.

156 **Crop maintenance and observation**

157 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
158 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
159 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
160 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
161 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
162 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
163 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
164 entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop
165 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
166 calculated according to disease scores described by Sharma et al. (2018) as follow: 0 = no visible symptoms;
167 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends;
168 3 = a wide range of leaf yellowing, curling and cupping; and 4 = very severe leaf yellowing, and pronounced
169 leaf curling. The severity was calculated using the following formula:

$$170 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

171 Note: DS = disease severity

172 v = disease score (0 to 4)

173 n = number of plants showing disease score v

174 Z = the highest disease score

175 N = total number of plants observed

176 Yield reduction was calculated using the following formula

$$177 \quad YR = \frac{w}{W} \times 100\%$$

178 YR = yield reduction

179 w = weight of first three harvests of infected plant

180 W = average weight of first three harvests of healthy plants in the same plot

181 **Data analysis**

182 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
183 collected data, and significant differences between means were determined using Honestly Significant
184 Difference (HSD) test a 95% degree of significance.

185 **RESULTS AND DISCUSSION (just primer supporting references, less than 10 years)**

186 **Seed transmission of PYLCV**

187 *Pepper yellow leaf curl virus* (PYLCV) is a member of begomovirus which was suspected to be transmissible
188 through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds,
189 ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the
190 seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might

191 be directly since infection of virus could make seeds more sensitive to deterioration as reported by Bueso et
192 al. (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis
193 seeds up to 65%. Nallathambi et al. (2020) also reported that virus infection could cause abnormal physical
194 function of seeds and establish itself in any part of the seed which eventually affected their viability and
195 potentially initiates seedborne disease.

196 Some of the seedling also produced PYLCV infection symptoms indicating that PYLCV was a seed borne virus.
197 The seed transmission of begomovirus had previously been reported by Kothandaraman et al. (2016) who
198 studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus was
199 seed-borne. A similar finding was also reported by Kil et al. (2020) who worked with *Tomato yellow leaf curl*
200 *New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus
201 showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV) as reported by Pérez-
202 Padilla et al. (2020) who worked with the virus on more than 3000 tomato plants and concluded that TYLCV
203 was seed borne, but seed transmission was not a general property of the virus. In this experiment, the
204 seedborne infection of PYLCV was relatively high because the seed was harvested from infected plants which
205 potentially brought the virus, as reported by Fadhila et al. (2020) who worked with PepYLCIV and could detect
206 DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

207 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
208 PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water
209 treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds
210 and weakened parts of them resulted the longer incubation period. According to Paylan et al. (2014), heated
211 water at 65°C, together with HCl and ozon, were very effective treatment to reduce virus concentration in the
212 seeds and had no negative effect on the seeds. Even though the seed treatments affected the virus infection,
213 seed borne infection of the virus still occurred indicated that the effect could not reach the embryo where the
214 virus normally present. Seed borne viruses present in seed coat, endosperm, nucleus or embryo, but only
215 viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the
216 effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might
217 have better effect to seed borne virus infection, but it might also affect the seed germination. According to
218 Farajollahi et al. (2014), hot water treatment could induce seed germination but duration of drenching the seeds
219 in hot water could reduce seed viability. Instead of reducing seed borne virus infection, hot water treatment
220 was also reported to be effective to eradicate bacterial seed borne pathogens and was highly recommended
221 for pepper, eggplant and tomato seeds (Kim et al. 2022).

222 Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and
223 threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to
224 start an endemic (Pagán 2022). High seed borne tobamovirus infection has also been reported by
225 Dombrovsky and Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
226 *virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

227 All treatments in the experiment resulted in PYLCV infection which confirmed that, as a member of
228 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
229 nature of begomovirus had also been reported by (Kothandaraman et al. 2016) who studied the seed
230 transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato yellow leaf curl*

231 *New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato plants and
232 concluded that the virus was seed borne but seed transmission was not a general property of the virus.

233 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
234 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
235 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
236 virus and zika virus (Al Hadhrami et al. 2022; Jennings and Parks 2020), and plant virus such as *Cucumber*
237 *mosaic virus* (Hamidson et al. 2018).

238 **Intercropping effect on PYLCV**

239 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
240 different family from the main crop. The use of mung bean and soybean (Family Leguminosae) and tomato
241 and eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but significantly affected
242 disease incidence, of which only tomato caused significant reduction of PYLCV infections (Table 2). The
243 reduction of viral disease under intercropping was a direct effect of the reduction of incoming vector, as
244 reported by Mir et al. (2022) that intercropping could effectively control insect pest. The effect of intercropping
245 to insect vector might be by reducing the vector invasion to the crop due to the presence of intercrop as
246 alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant disease by
247 causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence could be
248 caused by its effect on incoming vector, since tomato has insect repellent effect, especially against mosquito
249 and aphid (University 2022).

250 Researches on the use of Leguminous crops as intercrops has been frequent but mostly for the intention of
251 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
252 on plant disease was reported. Most of the research were combination between leguminous and food crops
253 such as maize-mungbean (Syafruddin and Suwardi 2020), cotton-mungbean (Liang et al. 2020), sorghum-
254 mungbean (Temeche et al. 2022), sorghum-soybean (Saber 2018), maize-soybean (Berdjour et al. 2020),
255 and rice-soybean (Putra and Sas 2023).

256 **Trap crop effect on PYLCV.**

257 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However,
258 only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos,
259 tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests
260 (Sarjan et al. 2023). Barrier crop could also affect the main crops by intercepting, arresting or retaining pest
261 thereby limiting the number of insect pest and insect vector reaching the main crop which eventually reduce
262 incidence of viral disease (Waweru et al. 2021). Furthermore, the significant effect of basil and marigold on
263 PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait
264 of repellent against *B. tabaci* and prevented the insect oviposition. Husna et al. (2020) reported that crude
265 extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes aegypti* larvae, and
266 according to Farindira (2015), at higher concentration the crude extract could be used as repellent against the
267 mosquito. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against
268 mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong repellent against mosquito
269 (Ponkiya et al. 2018), and contained extractable toxicants which effective as repellent and larvicidal against *B.*

270 *tabaci* (Fabrick et al. 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Calumpang and
271 Ohsawa 2015), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al. 2019). When a trap crop
272 has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour (Acharya
273 et al. 2021) or odor (Shao et al. 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour.
274 Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced
275 green flower but with strong odor.

276 **Physical barrier effect on PYLCV**

277 Physical barrier using cheese cloth 50 mesh could reduce the infection of PYLCV indicated by lower disease
278 incidence and severity, only barriers that are more than 100 cm high have a significant effect on disease
279 incidence (Table 4). This result confirmed that a barrier of 50 mesh could effectively prevent *B. tabaci* to the
280 protected plot (Harish et al. 2016). It is shown in Table 4 that the higher the barrier the lower the disease
281 incidence caused by PYLCV, an indication that *B. tabaci*, the only vector of PYLCV, could fly higher than 100
282 cm and transmitting the virus. However, *B. tabaci* is not a good flyer even though the insect might spread to
283 long distance carried by wind or transported materials. The maximum distance covered by single flying of the
284 whitefly is 17 m (Maruthi et al. 2017), but in a dense plant population the insect can move from plant to plant
285 easily, and if the insect is viruliferous, massive virus spread is inevitable. According to Tillman (2014), height
286 of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the
287 height of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicated that the insect still
288 flew above 125 cm to enter the plot and lower side barrier could not effectively block the insect. The
289 cheesecloth net could only work mechanically in blocking flying insect and had no effect on the insect behavior.
290 This was different from barrier crop which not only block the flying insects but also attract or repel the insect,
291 depended on the crop species. Flying insect such as *B. tabaci* could not easily differentiate host and nonhost
292 plants which made barrier crop more effective than net barrier (Udiarto et al. 2023). *B. tabaci* actually has two
293 type of flight behavior, foraging flight and migratory flight. Foraging flight is close to earth surface or within flight
294 boundary, while migratory flight is above the boundary where the insect could be picked up and carried by air
295 currents (Reynolds et al. 2017), and the insect containing mature eggs has been trapped at 150 m above
296 ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier could
297 actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect bigger than mesh
298 size of the net.

299 **CONCLUSIONS AND SUGGESTION (Arial 10) (No discussion, here)**

300 The first experiment of seed borne transmission of PYLCV concluded that PYLCV infecting red chili was a
301 seed borne virus and the rate of seed borne infection could be partially reduced by hot water treatment and
302 turmeric crude extract. In the intercropping experiment, tomato was good enough as intercrops for red chili in
303 term of reducing PYLCV infections. Basil and marigold were functional as trap crops to protect chili from
304 incoming *B. tabaci* and from PYLCV infection. Marigold is more recommended since the plant can grow higher
305 and more protective than basil. In the experiment of physical barrier, the use of 50 mesh cheese cloth as side
306 barrier could reduce PYLCV infection frequency only at the height of 125 cm. The entry of viruliferous *B. tabaci*
307 into the plot crossed the barrier can potentially be reduce by using denser and higher net. In all cultural
308 techniques applied in the field experiments, PYLCV infection occurred and cause yield reduction ranged from
309 40 to 53%.

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516 **Tables**

517

518 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
519 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58,90±1,68	29,45±0,54 b	6,80±0,77 ab	4,10±0,60 ab
Ginger crude extract	54,00±1,87	30,86±1,09 b	8,40±1,30 ab	4,90±0,82 ab
Turmeric crude extract	51,17±2,29	21,40±1,02 a	3,20±0,37 a	1,80±0,26 a
Javanese ginger crude extract	51,74±3,03	23,84±1,61 a	5,60±0,74 ab	4,00±0,49 ab
Control	43,66±3,11	22,84±1,06 a	9,60±0,57b	6,70±0,41 b
F Calculated	2,76 ^{ns}	12,12*	1,94*	2,14*
P Value	0,08	0,001	0,04	0,02
HSD 5%		5,15	5,61	3,56

520 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
521 Significant Difference Test.522 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
523 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16,18±2,35 ab	10,95±1,04	47,19±2,29
Mung bean	15,23±2,39 ab	9,52±1,58	49,22±2,20
Soybean	14,58±1,97 ab	8,57±1,25	46,41±3,27
Tomato	7,62±0,88 a	4,52±0,66	40,00±1,47
Control	26,66±0,88 b	14,28±1,10	46,26±2,04
F Calculated	5,74*	3,44 ^{ns}	0,82 ^{ns}
P Value	0,01	0,05	0,54
HSD 5%	12,18	-	-

524 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
525 Significant Difference Test.526 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
527 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8,14±1,36 a	5,85±0,86 a	50,21±2,41
Cosmos	10,28±1,29 ab	7,12±0,57 ab	48,41±1,54
Marigold	4,57±0,53 a	3,57±0,38 a	42,80±1,22
Zinnia	13,71±1,59 ab	11,28±1,31 ab	47,32±1,35
control	16,57±0,81 b	13,14±1,00 b	52,01±1,62
F Calculated	5,51*	7,70*	1,50 ^{ns}
P Value	0,01	0,003	0,27
HSD 5%	8,41	6,06	-

528 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
529 Significant Difference Test.

530 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 531 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11,42±1,53 ab	8,71±0,86 bc	49,87±0,85
Side barrier 75 cm	9,71±0,52 ab	8,00±0,47 abc	47,42±1,41
Side barrier 100 cm	8,00±1,26 ab	6,57±0,71 ab	49,00±1,36
Side barrier 125 cm	5,14±0,81 a	3,57±0,74 a	43,40±1,08
Control	14,28±0,68 b	12,14±0,92 c	52,32±2,11
F Calculated	5,28*	8,456*	1,73 ^{ns}
P Value	0,01	0,002	0,21
HSD 5%	6,40	4,58	

532 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 533 Significant Difference Test.

COVER PAGE

I. The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

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The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

1 **ABSTRACT (maximum 200 words, it must : the objective, method, research products)**

2 An experiment was conducted to investigate the effects of cultural techniques to prevent the infection of yellow
3 leaf curl disease of chili caused by *Pepper Yellow Leaf Curl Virus* (PYLCV). The experiment was conducted in
4 the area where the disease has been an endemic and *Bemisia tabaci*, the vector of the disease, was abundant.
5 The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds
6 harvested from infected plants were used for seed treatment experiment, and commercial seeds mostly used
7 by local farmers were used for the other experiments. The results showed that PYLCV was a seedborne virus
8 and hot water treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric
9 could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant,
10 mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield
11 reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and
12 zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but
13 not the lower ones. Under various cultural technique, PYLCV caused chili yield reduction of 40 – 53%.

14 **KEYWORDS**

15 *Bemisia tabaci*, hot water treatment, intercropping, physical barrier, trap cropping.

16 **INTRODUCTION (just primer supporting references, less than 10 years)**

17 Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country,
18 with some provinces have become the production centres of this commodity from where the majority of nation
19 demand is fulfilled. The eleven biggest chili producing provinces in 2022 were West Java, North Sumatra,
20 Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra
21 with total production of 1229262 tons in 2022 (Statistics Indonesia 2022) which contribute approximately 75%
22 of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered
23 all over the country.

24 Production of red chilli fluctuated due to several factors which eventually caused significant fluctuation in chilli
25 price which finally made farmers and traders under inconvenience situations. Plant diseases has been the
26 main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which
27 causes yield reduction up to 66% (Utami et al. 2022), anthracnose which under severe infection might cause
28 yield losses up to 80% (Suprpta 2022), fusarium which had a record to cause 40% yield losses (Parihar et al.
29 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a begomovirus,
30 which under favourable condition caused yield losses up to 70% (Novrianty et al. 2013). Symptoms of
31 diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic appears on
32 infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower
33 abortion and fruit discoloration (Lavanya and Arun 2021).

34 Disease caused by PYLCV often reached its high incidence and intensity during dry season when the weather
35 is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn
36 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek et al. 2017). The disease
37 is increasingly frightening because it has spread to all chilli production centres in the country since its first
38 appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in
39 Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV),
40 belongs to begomovirus (Fadhila et al. 2020). Begomovirus is a member of Family Geminiviridae, a big plant
41 virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini
42 (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4
43 genera based on their vector and genome organization. Members of the family transmitted persistently by
44 white fly (*B. tabaci*) are grouped into Genus Begomovirus, a bipartite virus having 2 components of single
45 stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al.
46 2017). In Indonesia, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two
47 strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum.
48 Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar
49 symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon,
50 pepper and eggplant (Subiastuti et al. 2019).

51 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
52 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
53 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal et al. 2020).
54 Furthermore, the insect also reported to infest more than 600 plant species include tomato, water melon,
55 pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia,
56 lantana, and lily (Li et al. 2021). The insect is known to reproduce with high fecundity. A female can produce
57 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves.
58 Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from
59 unfertilized eggs (Xie et al. 2014).

60 *B. tabaci* has at least 43 species complex, (Shah and Liu 2013) and is able to transmit more than 200 plant
61 viruses (Lu et al. 2019; MacLeod et al. 2022), and 90% of plant viruses transmitted by the vector belong to
62 *begomovirus* (Kanakala and Ghanim 2016). To transmit PYLCV successfully, *B tabaci* requires an acquisition
63 period of at least 90 minutes and a latent period of at least 8 hours (Czosnek et al. 2017). The vector remains
64 viruliferous until 13 days after virus acquisition or until the vector dies (Roy et al. 2021).

65 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
66 controlling whitefly with synthetic insecticide is not economical (Hema et al. 2014) and generated resistant
67 genotypes and reduced natural enemies (Wang et al. 2020). Under such conditions, cultural control should be
68 better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological
69 diversity through management of biotic and abiotic environment. Abiotic environment of the crop can be
70 manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be
71 manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian and Rezvani
72 2016). Cultural control of insects has become better alternative sincemost insect has strong ability to evolve
73 pesticide resistance (Basit 2019).

74 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
75 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques by
76 modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
77 were reported to be effective to reduce whitefly invasion into the protected areas (Simmons and Shapiro-Ilan
78 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
79 techniques have enough contribution to the implementation of integrated pest management (Lapidot et al.
80 2014).

81 Cultural practices have been implemented as parts of integrated pest management with various level of
82 success and failure depended on the pest and the crops in concern (Kenyon et al. 2014), and there have been
83 no report on the effects of cultural control on the appearance and development of PYLCV on red chilli.

84 The objective of the research is to verify the seed transmission trait of *Pepper yellow leaf curl virus* and to
85 observe the effects of intercropping, crop barrier and physical barrier on the natural transmission of PYLCV by
86 its vector, *B. tabaci*.

87 **MATERIALS AND METHODS (when and where?)**

88 **Study area**

89 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
90 2022. The research was experimental research consisted of four different experiments. The first experiment
91 was a seed treatment of PYLCV experiment, conducted in Insectarium and insect-proof greenhouse of
92 Department of Plant Pest and Disease, Faculty of Agriculture, Sriwijaya University. The other three
93 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
94 natural transmission and infection of PLCV. The field experiments were conducted in experimental garden of
95 Sriwijaya University located in District Indralaya, Ogan Ilir South Sumatra, surrounded by local farmers' fields
96 where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

97 **Procedures**

98 **Seed treatment effect on PYLCV experiment**

99 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
100 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
101 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
102 seeds were not used because they were obviously not viable. The experiment was arranged in a completely
103 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
104 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
105 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
106 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh et
107 al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of their
108 rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to
109 obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
110 (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, and
111 the trays were then placed in an insect proof screen box for seed germination. The seedlings germinated in
112 each treatment unit were used to calculated the viability of the treated seeds. To observed the seed born

113 PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and
114 all polybags were then placed in insect proof greenhouse and were arranged accordingly to completely
115 randomized design.

116 **Intercropping effect on PYLCV experiment**

117 Red chili plants were planted experimentally under intercropping pattern with mungbean (*Vigna radiata*),
118 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
119 as the control. The experiment was arranged in a randomized block design with five treatments and five
120 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were
121 planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure
122 that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV
123 were infected chili plants that spread in farmers' fields around the research site. Additionally, some infected
124 chili plants were deliberately planted around the experimental plots. The vector of PYLCV was abundant in the
125 area since the insect is polyphagous (Kumar et al. 2023) and most of vegetation in the area were host of the
126 vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity
127 and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult
128 to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

129 **Trap cropping effect on PYLCV experiment**

130 An experiment to verify the effects of trap crops on the PYLCV infection was conducted using basil (*Ocimum*
131 *basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and with no
132 trap crop as control. The trap crops selected were those belonged to refugia which normally attract and trap
133 invader insect (Hardiansyah et al. 2021). The experiment was arranged in a randomized block design with 5
134 treatments and 5 replications resulting in 25 experimental units. The experimental unit was 4 x 2 m plot on
135 which red chilli was planted at 60 x 40 cm spacing. Trap crops were planted surrounding experimental plots at
136 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the
137 plots. The crop were positioned as border crop so that they could intercept insect before they attack the main
138 crops (Priyadi et al. 2020).. Chilli seedlings used in this experiment were prepared in insect proof boxes
139 together with those used in other field experiments. The parameters observed and the method of
140 observation were also similar to those observed and applied in intercropping experiment.

141 **Effect of physical barrier on PYLCV experiment**

142 An experiment to verify the effect of physical barrier on the invasion of PYLCV vectors was conducted using
143 cheesecloth as physical net barrier. Nets were previously used as an antihail device in fruit production.
144 However, having a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had
145 been considered to be used as a nonaggressive pest and diseases control device (Grasswitz 2019). The
146 experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units.
147 Physical barrier covering crop cultivation with insect net has been used in tobacco cultivation to prevent the
148 invasion of *B. tabaci* (Aji et al. 2015). In this experiment, physical barrier using insect net was used only as
149 side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh
150 cheesecloth was used in this experiment because it could prevent the entry of insect with body width less than
151 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish et al. 2016). The experiment
152 was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were

153 prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no barrier as
154 control. The net barriers were put in place before seedling transplanting. Seedlings and virus resources used
155 and data collection in this experiment were similar to those applied in intercropping and trap crop experiments.

156 **Crop maintenance and observation**

157 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
158 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
159 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
160 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
161 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
162 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
163 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
164 entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop
165 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
166 calculated according to disease scores described by Sharma et al. (2018) as follow: 0 = no visible symptoms;
167 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends;
168 3 = a wide range of leaf yellowing, curling and cupping; and 4 = very severe leaf yellowing, and pronounced
169 leaf curling. The severity was calculated using the following formula:

$$170 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

171 Note: DS = disease severity

172 v = disease score (0 to 4)

173 n = number of plants showing disease score v

174 Z = the highest disease score

175 N = total number of plants observed

176 Yield reduction was calculated using the following formula

$$177 \quad YR = \frac{w}{W} \times 100\%$$

178 YR = yield reduction

179 w = weight of first three harvests of infected plant

180 W = average weight of first three harvests of healthy plants in the same plot

181 **Data analysis**

182 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
183 collected data, and significant differences between means were determined using Honestly Significant
184 Difference (HSD) test a 95% degree of significance.

185 **RESULTS AND DISCUSSION (just primer supporting references, less than 10 years)**

186 **Seed transmission of PYLCV**

187 *Pepper yellow leaf curl virus* (PYLCV) is a member of begomovirus which was suspected to be transmissible
188 through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds,
189 ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the
190 seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might

191 be directly since infection of virus could make seeds more sensitive to deterioration as reported by Bueso et
192 al. (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis
193 seeds up to 65%. Nallathambi et al. (2020) also reported that virus infection could cause abnormal physical
194 function of seeds and establish itself in any part of the seed which eventually affected their viability and
195 potentially initiates seedborne disease.

196 Some of the seedling also produced PYLCV infection symptoms indicating that PYLCV was a seed borne virus.
197 The seed transmission of begomovirus had previously been reported by Kothandaraman et al. (2016) who
198 studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus was
199 seed-borne. A similar finding was also reported by Kil et al. (2020) who worked with *Tomato yellow leaf curl*
200 *New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus
201 showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV) as reported by Pérez-
202 Padilla et al. (2020) who worked with the virus on more than 3000 tomato plants and concluded that TYLCV
203 was seed borne, but seed transmission was not a general property of the virus. In this experiment, the
204 seedborne infection of PYLCV was relatively high because the seed was harvested from infected plants which
205 potentially brought the virus, as reported by Fadhila et al. (2020) who worked with PepYLCIV and could detect
206 DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

207 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
208 PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water
209 treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds
210 and weakened parts of them resulted the longer incubation period. According to Paylan et al. (2014), heated
211 water at 65°C, together with HCl and ozon, were very effective treatment to reduce virus concentration in the
212 seeds and had no negative effect on the seeds. Even though the seed treatments affected the virus infection,
213 seed borne infection of the virus still occurred indicated that the effect could not reach the embryo where the
214 virus normally present. Seed borne viruses present in seed coat, endosperm, nucleus or embryo, but only
215 viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the
216 effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might
217 have better effect to seed borne virus infection, but it might also affect the seed germination. According to
218 Farajollahi et al. (2014), hot water treatment could induce seed germination but duration of drenching the seeds
219 in hot water could reduce seed viability. Instead of reducing seed borne virus infection, hot water treatment
220 was also reported to be effective to eradicate bacterial seed borne pathogens and was highly recommended
221 for pepper, eggplant and tomato seeds (Kim et al. 2022).

222 Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and
223 threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to
224 start an endemic (Pagán 2022). High seed borne tobamovirus infection has also been reported by
225 Dombrovsky and Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
226 *virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

227 All treatments in the experiment resulted in PYLCV infection which confirmed that, as a member of
228 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
229 nature of begomovirus had also been reported by (Kothandaraman et al. 2016) who studied the seed
230 transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato yellow leaf curl*

231 *New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato plants and
232 concluded that the virus was seed borne but seed transmission was not a general property of the virus.

233 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
234 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
235 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
236 virus and zika virus (Al Hadhrami et al. 2022; Jennings and Parks 2020), and plant virus such as *Cucumber*
237 *mosaic virus* (Hamidson et al. 2018).

238 **Intercropping effect on PYLCV**

239 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
240 different family from the main crop. The use of mung bean and soybean (Family Leguminosae) and tomato
241 and eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but significantly affected
242 disease incidence, of which only tomato caused significant reduction of PYLCV infections (Table 2). The
243 reduction of viral disease under intercropping was a direct effect of the reduction of incoming vector, as
244 reported by Mir et al. (2022) that intercropping could effectively control insect pest. The effect of intercropping
245 to insect vector might be by reducing the vector invasion to the crop due to the presence of intercrop as
246 alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant disease by
247 causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence could be
248 caused by its effect on incoming vector, since tomato has insect repellent effect, especially against mosquito
249 and aphid (University 2022).

250 Researches on the use of Leguminous crops as intercrops has been frequent but mostly for the intention of
251 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
252 on plant disease was reported. Most of the research were combination between leguminous and food crops
253 such as maize-mungbean (Syafruddin and Suwardi 2020), cotton-mungbean (Liang et al. 2020), sorghum-
254 mungbean (Temeche et al. 2022), sorghum-soybean (Saber 2018), maize-soybean (Berdjour et al. 2020),
255 and rice-soybean (Putra and Sas 2023).

256 **Trap crop effect on PYLCV.**

257 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However,
258 only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos,
259 tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests
260 (Sarjan et al. 2023). Barrier crop could also affect the main crops by intercepting, arresting or retaining pest
261 thereby limiting the number of insect pest and insect vector reaching the main crop which eventually reduce
262 incidence of viral disease (Waweru et al. 2021). Furthermore, the significant effect of basil and marigold on
263 PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait
264 of repellent against *B. tabaci* and prevented the insect oviposition. Husna et al. (2020) reported that crude
265 extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes aegypti* larvae, and
266 according to Farindira (2015), at higher concentration the crude extract could be used as repellent against the
267 mosquito. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against
268 mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong repellent against mosquito
269 (Ponkiya et al. 2018), and contained extractable toxicants which effective as repellent and larvicidal against *B.*

270 *tabaci* (Fabrick et al. 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Calumpang and
271 Ohsawa 2015), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al. 2019). When a trap crop
272 has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour (Acharya
273 et al. 2021) or odor (Shao et al. 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour.
274 Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced
275 green flower but with strong odor.

276 **Physical barrier effect on PYLCV**

277 Physical barrier using cheese cloth 50 mesh could reduce the infection of PYLCV indicated by lower disease
278 incidence and severity, only barriers that are more than 100 cm high have a significant effect on disease
279 incidence (Table 4). This result confirmed that a barrier of 50 mesh could effectively prevent *B. tabaci* to the
280 protected plot (Harish et al. 2016). It is shown in Table 4 that the higher the barrier the lower the disease
281 incidence caused by PYLCV, an indication that *B. tabaci*, the only vector of PYLCV, could fly higher than 100
282 cm and transmitting the virus. However, *B. tabaci* is not a good flyer even though the insect might spread to
283 long distance carried by wind or transported materials. The maximum distance covered by single flying of the
284 whitefly is 17 m (Maruthi et al. 2017), but in a dense plant population the insect can move from plant to plant
285 easily, and if the insect is viruliferous, massive virus spread is inevitable. According to Tillman (2014), height
286 of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the
287 height of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicated that the insect still
288 flew above 125 cm to enter the plot and lower side barrier could not effectively block the insect. The
289 cheesecloth net could only work mechanically in blocking flying insect and had no effect on the insect behavior.
290 This was different from barrier crop which not only block the flying insects but also attract or repel the insect,
291 depended on the crop species. Flying insect such as *B. tabaci* could not easily differentiate host and nonhost
292 plants which made barrier crop more effective than net barrier (Udiarto et al. 2023). *B. tabaci* actually has two
293 type of flight behavior, foraging flight and migratory flight. Foraging flight is close to earth surface or within flight
294 boundary, while migratory flight is above the boundary where the insect could be picked up and carried by air
295 currents (Reynolds et al. 2017), and the insect containing mature eggs has been trapped at 150 m above
296 ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier could
297 actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect bigger than mesh
298 size of the net.

299 **CONCLUSIONS AND SUGGESTION (Arial 10) (No discussion, here)**

300 The first experiment of seed borne transmission of PYLCV concluded that PYLCV infecting red chili was a
301 seed borne virus and the rate of seed borne infection could be partially reduced by hot water treatment and
302 turmeric crude extract. In the intercropping experiment, tomato was good enough as intercrops for red chili in
303 term of reducing PYLCV infections. Basil and marigold were functional as trap crops to protect chili from
304 incoming *B. tabaci* and from PYLCV infection. Marigold is more recommended since the plant can grow higher
305 and more protective than basil. In the experiment of physical barrier, the use of 50 mesh cheese cloth as side
306 barrier could reduce PYLCV infection frequency only at the height of 125 cm. The entry of viruliferous *B. tabaci*
307 into the plot crossed the barrier can potentially be reduce by using denser and higher net. In all cultural
308 techniques applied in the field experiments, PYLCV infection occurred and cause yield reduction ranged from
309 40 to 53%.

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516 **Tables**

517

518 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
519 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58,90±1,68	29,45±0,54 b	6,80±0,77 ab	4,10±0,60 ab
Ginger crude extract	54,00±1,87	30,86±1,09 b	8,40±1,30 ab	4,90±0,82 ab
Turmeric crude extract	51,17±2,29	21,40±1,02 a	3,20±0,37 a	1,80±0,26 a
Javanese ginger crude extract	51,74±3,03	23,84±1,61 a	5,60±0,74 ab	4,00±0,49 ab
Control	43,66±3,11	22,84±1,06 a	9,60±0,57b	6,70±0,41 b
F Calculated	2,76 ^{ns}	12,12*	1,94*	2,14*
P Value	0,08	0,001	0,04	0,02
HSD 5%		5,15	5,61	3,56

520 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
521 Significant Difference Test.522 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
523 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16,18±2,35 ab	10,95±1,04	47,19±2,29
Mung bean	15,23±2,39 ab	9,52±1,58	49,22±2,20
Soybean	14,58±1,97 ab	8,57±1,25	46,41±3,27
Tomato	7,62±0,88 a	4,52±0,66	40,00±1,47
Control	26,66±0,88 b	14,28±1,10	46,26±2,04
F Calculated	5,74*	3,44 ^{ns}	0,82 ^{ns}
P Value	0,01	0,05	0,54
HSD 5%	12,18	-	-

524 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
525 Significant Difference Test.526 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
527 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8,14±1,36 a	5,85±0,86 a	50,21±2,41
Cosmos	10,28±1,29 ab	7,12±0,57 ab	48,41±1,54
Marigold	4,57±0,53 a	3,57±0,38 a	42,80±1,22
Zinnia	13,71±1,59 ab	11,28±1,31 ab	47,32±1,35
control	16,57±0,81 b	13,14±1,00 b	52,01±1,62
F Calculated	5,51*	7,70*	1,50 ^{ns}
P Value	0,01	0,003	0,27
HSD 5%	8,41	6,06	-

528 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
529 Significant Difference Test.

530 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 531 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11,42±1,53 ab	8,71±0,86 bc	49,87±0,85
Side barrier 75 cm	9,71±0,52 ab	8,00±0,47 abc	47,42±1,41
Side barrier 100 cm	8,00±1,26 ab	6,57±0,71 ab	49,00±1,36
Side barrier 125 cm	5,14±0,81 a	3,57±0,74 a	43,40±1,08
Control	14,28±0,68 b	12,14±0,92 c	52,32±2,11
F Calculated	5,28*	8,456*	1,73 ^{ns}
P Value	0,01	0,002	0,21
HSD 5%	6,40	4,58	

532 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 533 Significant Difference Test.

COVER PAGE

I. The effects of various cultural techniques on the transmission and infectious development of Pepper yellow leaf curl virus on red chili

Running title: Effects of cultural techniques on Pepper yellow leaf curl virus

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1. The candidates should have speciality in authors' research topic

2. The candidates should come from different institutions with authors (*especially from different countries*)

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The effects of various cultural techniques on the transmission and infectious development of *Pepper yellow leaf curl virus* on red chili

(Running title: Effects of cultural techniques on *Pepper yellow leaf curl virus*)

ABSTRACT

1
2 An experiment was conducted to investigate the effects of cultural techniques to prevent the infection of yellow
3 leaf curl disease of chili caused by *Pepper yellow leaf curl virus* (PYLCV). The experiment was conducted in
4 the area where the disease has been an endemic and *Bemisia tabaci*, the vector of the disease, was abundant.
5 The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds
6 harvested from infected plants were used for seed treatment experiment, and commercial seeds mostly used
7 by local farmers were used for the other experiments. The results showed that PYLCV was a seedborne virus
8 and hot water treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric
9 could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant,
10 mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield
11 reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and
12 zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but
13 not the lower ones. Under various cultural technique, PYLCV caused chili yield reduction of 40 – 53%.

KEYWORDS

14
15 *Bemisia tabaci*, cultural technique, *Pepper yellow leaf curl virus*, seed treatment

INTRODUCTION (just primer supporting references, less than 10 years)

16
17 Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country,
18 with some provinces have become the production centres of this commodity from where the majority of nation
19 demand is fulfilled. The eleven biggest red chili producing provinces in 2022 were West Java, North Sumatra,
20 Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra
21 with total production of 1229262 tons. The chili production varied among different provinces and the three
22 provinces in Java contributed to 58.3% to the national production (Siregar & Suroso, 2021).

23 Production of red chilli fluctuated due to several factors which inevitably caused significant fluctuation in chilli
24 price which eventually made farmers and traders under inconvenience situations. Plant diseases has been the
25 main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which
26 causes yield reduction up to 66% (Utami, Meale & Young, 2022), anthracnose which under severe infection
27 might cause yield losses up to 80% (Suprpta, 2022), fusarium which had a record to cause 40% yield losses
28 (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a
29 begomovirus, which under favourable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al,
30 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow
31 mosaic appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling,
32 smaller fruit, flower abortion and fruit discoloration (Lavanya & Arun 2021).

33 Disease caused by PYLCV often reached its high incidence and intensity during dry season when the weather
34 is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn
35 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek, Hariton-Shalev, Sobol,
36 Gorovits, & Ghanim, 2017). The disease is increasingly frightening because it has spread to all chilli production
37 centres in the country since its first appearance detected in 1999 (Gaswanto, Syukur, Hidayat, & Gunaeni,
38 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed
39 as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila, Lal, Vo, Ho, Hidayat,
40 Lee, Kil, & Lee, 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of
41 members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2
42 components of single stranded DNA genome inside. Geminiviridae comprises of 4 genera based on their
43 vector and genome organization. Members of the family transmitted persistently by white fly (*B. tabaci*) are
44 grouped into Genus Begomovirus, a bipartite virus having 2 components of single stranded DNA recognized
45 as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek, Hariton-Shalev, Sobol, Gorovits,
46 & Ghanim, 2017). In Indonesia, *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) was recognized to have
47 two strains i.e. PeYLCIV-Tomato which also infects tomato and PeYLCIV-Ageratum which also infects
48 ageratum. Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and
49 cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon,
50 water melon, pepper and eggplant (Subiastuti, Hartono, Daryono, 2019).

51 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
52 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
53 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal, Singh, &
54 Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included
55 tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant,
56 chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is
57 known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm.
58 The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from
59 fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al., 2014).

60 *B. tabaci* has at least 43 species complex, (Shah & Liu, 2013) and is able to transmit more than 200 plant
61 viruses (Lu, Chen, Li, Shi, Gu, & Yan, 2019; MacLeod, Canty, & Polaszek, 2022), and 90% of plant viruses
62 transmitted by the vector belong to *begomovirus* (Kanakala & Ghanim, 2016). To transmit PYLCV successfully,
63 *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8 hours (Czosnek,
64 Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). The vector remains viruliferous until 13 days after virus
65 acquisition or until the vector dies (Roy, Chakraborty, & Ghosh, 2021).

66 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
67 controlling whitefly with synthetic insecticide is not economical (Patra & Hath, 2022) and generated resistant
68 genotypes and reduced natural enemies (Wang et al., 2020). Under such conditions, cultural control should
69 be better alternative to solve the problems. Cultural control is to conserve natural enemies and increase
70 biological diversity through management of biotic and abiotic environment. Abiotic environment of the crop can
71 be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be
72 manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian & Rezvani,

73 2016). Cultural control of insects has become better alternative since most insect has strong ability to evolve
74 pesticide resistance (Basit, 2019).

75 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
76 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques by
77 modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
78 were reported to be effective to reduce whitefly invasion into the protected areas (Simmons & Shapiro-Ilan,
79 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
80 techniques have enough contribution to the implementation of integrated pest management (Lapidot, Legg,
81 Wintermantel, & Polston, 2014).

82 Cultural practices have been implemented as parts of integrated pest management with various level of
83 success and failure depended on the pest and the crops in concern (Kenyon, Kumar, Tsai, & Hughes, 2014),
84 and there have been no report on the effects of cultural control on the appearance and development of PYLCV
85 on red chilli.

86 The objective of the research is to verify the seed transmission trait of *Pepper yellow leaf curl virus* and to
87 observe the effects of seed treatments, intercropping, trap cropping, and physical barrier on the natural
88 transmission and infection development of PYLCV by its vector, *B. tabaci*.

89 **MATERIALS AND METHODS (when and where?)**

90 **Study area**

91 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
92 2022. The research was experimental research consisted of four different experiments. The first experiment
93 was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof greenhouse
94 of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three
95 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
96 natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden
97 of Universitas Sriwijaya located in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers'
98 fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

99 **Procedures**

100 **Seed treatment effect on PYLCV experiment**

101 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
102 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
103 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
104 seeds were not used because they were obviously not viable. The experiment was arranged in a completely
105 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
106 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
107 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
108 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh,
109 Awasthi, Jangre, & Nirmalkar, 2020). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of
110 their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth
111 to obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes

112 (Kabede, Ayalew, & Yeesuf, 2013); John, Ihum, Olusolape, & Janfa, 2018). The treated seeds were sown
113 accordingly in each double trays, and the trays were then placed in an insect proof screen box for seed
114 germination. The seedlings germinated in each treatment unit were used to calculated the viability of the
115 treated seeds. To observed the seed borned PYLCV infection, 50 four-leaf seedlings were then transferred
116 individually to a 20 cm diameter polybag, and all polybags were then placed in insect proof screenhouse and
117 were arranged accordingly to completely randomized design.

118 **Intercropping effect on PYLCV experiment**

119 Red chili plants were planted experimentally under intercropping pattern with mung bean (*Vigna radiata*),
120 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
121 as the control. The experiment was arranged in a randomized block design with five treatments and five
122 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red
123 chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All
124 seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field
125 and brought in by its vector, *B. tabaci*. Sources of PYLCV were infected chili plants that spread in farmers'
126 fields around the research site. Additionally, some infected chili plants were deliberately planted around the
127 experimental plots. The vector of PYLCV was abundant in the area since the insect is polyphagous with
128 hundreds number of host species (Pym et al., 2019) and most of vegetation in the area were host of the vector.
129 Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield
130 reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect
131 when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

132 **Trap cropping effect on PYLCV experiment**

133 An experiment to investigate the effects of trap crops on the PYLCV infection was conducted using basil
134 (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and
135 with no trap crop as control. The trap crops selected were those belonged to refugia which normally attract
136 and trap invader insect (Hardiansyah, Hartini, & Musa, 2021). The experiment was arranged in a randomized
137 block design with 5 treatments and 5 replications resulting in 25 experimental units. The experimental unit was
138 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing. Trap crops were planted surrounding the
139 experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red
140 chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before
141 they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chilli seedlings used in this experiment
142 were prepared in insect proof boxes, together with those used in other field experiments. The parameters
143 observed and the method of observation were similar to those observed and applied in intercropping
144 experiment.

145 **Effect of physical barrier on PYLCV experiment**

146 An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using
147 cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device in
148 fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and
149 diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical
150 barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent
151 the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental

152 garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect
153 net was used only as side barrier and let the top opened for the access of pollinator and other beneficial
154 insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect
155 with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish,
156 Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design
157 with 5 treatments and 5 replications for which 25 plots were prepared. Four level of physical barrier height
158 were applied i.e. 50, 75, 100 and 125 cm and no side barrier as control. The net barriers were put in place
159 before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were
160 similar to those applied in intercropping and trap crop experiments.

161 **Crop maintenance and observation**

162 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
163 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
164 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
165 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
166 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
167 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
168 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
169 entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop
170 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
171 calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy,
172 (2022) as follow: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some
173 yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping; and 4 =
174 very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following
175 formula:

$$176 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

177 Note: DS = disease severity

178 v = disease score (0 to 4)

179 n = number of plants showing disease score v

180 Z = the highest disease score

181 N = total number of plants observed

182 Yield reduction was calculated using the following formula

$$183 \quad YR = \frac{w}{W} \times 100\%$$

184 YR = yield reduction

185 w = weight of first three harvests of infected plant

186 W = average weight of first three harvests of healthy plants in the same plot

187 **Data analysis**

188 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
189 collected data, and significant differences between means were determined using Honestly Significant
190 Difference (HSD) test a 95% degree of significance.

RESULTS AND DISCUSSION

191

192 **Seed transmission of PYLCV**

193 *Pepper yellow leaf curl virus* (PYLCV) is a member of begomovirus which was suspected to be transmissible
194 through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds,
195 ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the
196 seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might
197 be directly since infection of virus could make seeds more sensitive to deterioration as reported by Bueso,
198 Serrano, Pallás, & Sánchez-Navarro, (2017) who found that infection of *Cucumber mosaic virus* (CMV) could
199 reduce the viability of *Arabidopsis* seeds up to 65%. Nallathambi, Umamaheswari, Lal, Manjunatha, & Berliner
200 (2020) also reported that virus infection could cause abnormal physical function of seeds and establish itself
201 in any part of the seed which eventually affected their viability and potentially initiates seedborne disease.

202 Some of the seedling also produced PYLCV infection symptoms, confirming that PYLCV was a seed borne
203 virus. The seed transmission of begomovirus had previously been reported by Kothandaraman, Devadason,
204 & Ganesan (2016) who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and
205 concluded that the virus was seed-borne. A similar finding was also reported by Kil et al. (2020) who worked
206 with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible.
207 Another begomovirus showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV)
208 as reported by Pérez-Padilla et al., (2020), who worked with the virus on more than 3000 tomato plants and
209 concluded that TYLCV was seed borne, but seed transmission was not a general property of the virus. In this
210 experiment, the seedborne infection of PYLCV was relatively high because the seed was harvested from
211 infected plants which potentially brought the virus, as reported by Fadhila et al. (2020) who worked with
212 PepYLCIV and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested from
213 infected plants.

214 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
215 PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water
216 treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds
217 and weakened parts of them resulted the longer incubation period. According to Paylan, Erkan, Cetinkaya,
218 Ergun, & Pazarlar (2014), heated water at 65°C, together with HCl and ozon, were very effective treatment to
219 reduce virus concentration in the seeds and had no negative effect on the seeds. Even though the seed
220 treatments affected the virus infection, seed borne infection of the virus still occurred indicated that the effect
221 could not reach the embryo where the virus normally present. Seed borne viruses present in seed coat,
222 endosperm, nucleus or embryo, but only viruses present in the seed embryo can be transmitted to the next
223 generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the
224 dipping time. Longer dipping time might have better effect to seed borne virus infection, but it might also affect
225 the seed germination. According to Farajollahi, Gholinejad, & Jafari (2014), hot water treatment could induce
226 seed germination but duration of drenching the seeds in hot water could reduce seed viability. Instead of
227 reducing seed borne virus infection, hot water treatment was also reported to be effective to eradicate bacterial
228 seed borne pathogens and was highly recommended for pepper, eggplant and tomato seeds (Kim, Shim, Lee,
229 & Wangchuk, 2022).

230 Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and
231 threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to

232 start an endemic (Pagán, 2022). High seed borne tobamovirus infection has also been reported by
233 Dombrovsky & Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
234 *virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

235 All treatments in the experiment resulted in PYLCV infection which confirmed that, as a member of
236 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
237 nature of begomovirus had also been reported by (Kothandaraman, Devadason, & Ganesan, 2016) who
238 studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato*
239 *yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato
240 plants and concluded that the virus was seed borne but seed transmission was not a general property of the
241 virus.

242 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
243 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
244 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
245 virus and zika virus (Al Hadhrami, Battashi, & Al Hashami, 2022); Jennings & Parks 2020), and plant virus
246 such as *Cucumber mosaic virus* (Hamidson, Damiri, & Angraini, 2018).

247 **Intercropping effect on PYLCV**

248 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
249 different family from the main crop. The use of mung bean and soybean (Family Leguminosae), tomato and
250 eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but affected disease
251 incidence in the range of 39.30 to 71.41%, with only tomato caused significant reduction of PYLCV infections
252 (Table 2). The reduction of viral disease under intercropping was a direct effect of the reduction of incoming
253 vector, as reported by Mir et al. (2022) that intercropping could effectively control insect pest. The effect of
254 intercropping to insect vector might be by reducing the vector invasion to the crop due to the presence of
255 intercrop as alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant
256 disease by causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence
257 could be caused by its effect on incoming vector, since tomato has insect repellent effect, especially against
258 mosquito and aphid (Setyaningrum, Unih, Pratami, & Kanedi, 2023).

259 Research on the use of leguminous crops as intercrops has been frequent but mostly for the intention of
260 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
261 on plant disease was reported. Most of the research were combinations between leguminous and food crops
262 such as maize-mung bean (Syafuddin & Suwardi, 2020), cotton-mung bean (Liang, He, & Shi, 2020),
263 sorghum-mungbean (Temeche, Getachew, Hailu, & Abebe, 2022), sorghum-soybean (Saber 2018), maize-
264 soybean (Berdjour, Dugje, Dzomeku, & Rahman, 2020), and rice-soybean (Putra & Sas, 2023).

265 **Trap crop effect on PYLCV.**

266 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. Basil,
267 cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect
268 pests (Sarjan, Haryanto, Supeno, & Jihadi, 2023), but the effects of the four crops were different when being
269 used barrier crops. Barrier crop could affect the main crops by intercepting, arresting or retaining pest thereby
270 limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence

271 of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could
272 significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The
273 significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to
274 Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the insect
275 oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5%
276 effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman, Srinivasan, Suganthi,
277 Ranganathan, Gimbin, & Shanmugam (2018), the extract was also effective against *Culex tritaeniorhynchus*,
278 *Aedesal bopictus* and *Anopheles subpictuat*. Husna et al. (2020) also reported that the extract of basil leaves
279 was a strong larvicidal against mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong
280 repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal
281 against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali*
282 (Dikr & Belete, 2021), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap
283 crop has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour
284 (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but
285 Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured
286 flower and one species (basil) produced green flower but with strong odor.

287 **Physical barrier effect on PYLCV**

288 Physical barrier using cheese cloth 50 mesh could reduce the infestation of PYLCV vectors indicated by the
289 reduction of disease incidence and severity up to 64.00% and 70.59% respectively, but only physical barriers
290 at 125 cm high could significantly reduce incidence and severity of pepper yellow leaf curl disease (Table 4).
291 This result was in accordance to that reported by (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016)
292 that a barrier of fine mesh could effectively prevent *B. tabaci* to the protected plot. It is shown in the table that
293 the higher the barrier the lower the disease incidence caused by PYLCV, an indication that *B. tabaci*, the only
294 vector of PYLCV, could fly higher than 100 cm and transmitting the virus.. According to Tillman (2014), height
295 of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the
296 height of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicated that the insect
297 could fly above such altitude even though the whitefly is not a good flyer even though it might spread to long
298 distance carried by wind or transported materials. The maximum distance covered by single flying of the
299 whitefly is 17 m (Maruthi, Jeremiah, Mohammed, & Legg, 2017), but in a dense plant population the insect
300 can move from plant to plant easily, and if the insect is viruliferous, massive virus spread is inevitable.

301 The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in
302 blocking flying insect and had no effect on the insect behavior. This was different from barrier crop which not
303 only block the flying insects but also attract or repel the insect, depended on the crop species. Flying insect
304 such as *B. tabaci* could not easily differentiate host and nonhost plants which made barrier crop more effective
305 than net barrier (Udiarto, Setiawati, Muharam, & Dadi, 2023). *B. tabaci* actually has two type of flight behavior,
306 foraging flight and migratory flight. Foraging flight is close to earth surface or within flight boundary, while
307 migratory flight is above the boundary where the insect could be picked up and carried by air currents
308 (Reynolds, Chapman, & Drake, 2017), and the insect containing mature eggs has been trapped at 150 m
309 above ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier
310 could actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect with body
311 width wider than mesh size of the net.

CONCLUSIONS AND SUGGESTION

312

313 The first experiment of seed borne transmission of PYLCV concluded and verified that the virus infecting red
314 chili was a seed borne virus. Hot water treatment and crude extract of red ginger could lengthen the average
315 incubation period of PYLCV up to 6.61 days to 8.02 days respectively, and crude extract red ginger reduced
316 the infection frequency and disease severity up to 66.67 % and 73.13% respectively, compared to control. In
317 the intercropping experiment, tomato was good enough as intercrops for red chili in term of reducing PYLCV
318 infections which could reduce disease incidence up to 71.41%. Basil and marigold were functional as trap
319 crops to protect chili from incoming *B. tabaci* and could reduce incidence and severity of disease transmitted
320 by the insect up to 72.42% and 72.83% respectively. In the experiment of physical barrier, the use of 50 mesh
321 cheese cloth as side barrier at the height of 125 cm could reduce PYLCV infection frequency up to 70.59%. In
322 all cultural techniques applied in the field experiments, PYLCV infection on red chili caused yield reduction of
323 40 to 53%.

324 Infection of PYLCV on red chili is easily spread by *B. tabaci* and the disease is very damaging to the crop yield.
325 Even though some cultural techniques are effective to reduce PYLCV transmission by each vector, regular
326 disease monitoring is important and destroy infected plants is necessary to eliminate virus inoculum.

327

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536 Tables

537 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
538 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

539 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
540 Significant Difference Test.

541 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
542 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54

HSD 5%

12.18

-

-

543 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 544 Significant Difference Test.

545 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
 546 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

547 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 548 Significant Difference Test.

549 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 550 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

551 Note: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty
 552 Significant Difference Test.

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The effects of various cultural techniques on the transmission and infectious development of *Pepper yellow leaf curl virus* on red chili

(Running title: Effects of cultural techniques on *Pepper yellow leaf curl virus*)

ABSTRACT

An experiment was conducted to investigate the effects of cultural techniques to prevent the infection of yellow leaf curl disease of chili caused by *Pepper yellow leaf curl virus* (PYLCV). The experiment was conducted in the area where the disease has been an endemic and *Bemisia tabaci*, the vector of the disease, was abundant.

The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds harvested from infected plants were used for seed treatment experiment, and commercial seeds mostly used by local farmers were used for the other experiments. The results showed that PYLCV was a seedborne virus and hot water treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant, mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but not the lower ones. Under various cultural technique, PYLCV caused chili yield reduction of 40–53%.

KEYWORDS

Bemisia tabaci, cultural technique, *Pepper yellow leaf curl virus*, seed treatment

INTRODUCTION

Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country, with some provinces have become the production centres of this commodity from where the majority of nation demand is fulfilled. The eleven biggest red chili producing provinces in 2022 were West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra with total production of 1229262 tons. The chili production varied among different provinces and the three provinces in Java contributed to 58.3% to the national production (Siregar & Suroso, 2021).

Production of red chilli fluctuated due to several factors which inevitably caused significant fluctuation in chilli price which eventually made farmers and traders under inconvenience situations. Plant diseases has been the main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which causes yield reduction up to 66% (Utami, Meale & Young, 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprpta, 2022), fusarium which had a record to cause 40% yield losses (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a begomovirus, which under favourable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al, 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower abortion and fruit discoloration (Lavanya & Arun 2021).

Disease caused by PYLCV often reached its high incidence and intensity during dry season when the weather is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn

Commented [A1]: Some different

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1 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek, Hariton-Shalev, Sobol,
2 Gorovits, & Ghanim, 2017). The disease is increasingly frightening because it has spread to all chilli production
3 centres in the country since its first appearance detected in 1999 (Gaswanto, Syukur, Hidayat, & Gunaeni,
4 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed
5 as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila, Lal, Vo, Ho, Hidayat,
6 Lee, Kil, & Lee, 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of
7 members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2
8 components of single stranded DNA genome inside. Geminiviridae comprises of 4 genera based on their
9 vector and genome organization. Members of the family transmitted persistently by white fly (*B. tabaci*) are
10 grouped into Genus Begomovirus, a bipartite virus having 2 components of single stranded DNA recognized
11 as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek, Hariton-Shalev, Sobol, Gorovits,
12 & Ghanim, 2017). In Indonesia, *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) was recognized to have
13 two strains i.e. PeYLCIV-Tomato which also infects tomato and PeYLCIV-Ageratum which also infects
14 ageratum. Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and
15 cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon,
16 water melon, pepper and eggplant (Subiastuti, Hartono, Daryono, 2019).

17 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
18 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
19 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal, Singh, &
20 Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included
21 tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant,
22 chilli, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is
23 known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm.
24 The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from
25 fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al., 2014).

26 *B. tabaci* has at least 43 species complex, (Shah & Liu, 2013) and is able to transmit more than 200 plant
27 viruses (Lu, Chen, Li, Shi, Gu, & Yan, 2019; MacLeod, Canty, & Polaszek, 2022), and 90% of plant viruses
28 transmitted by the vector belong to *begomovirus* (Kanakala & Ghanim, 2016). To transmit PYLCV successfully,
29 *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8 hours (Czosnek,
30 Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). The vector remains viruliferous until 13 days after virus
31 acquisition or until the vector dies (Roy, Chakraborty, & Ghosh, 2021).

32 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
33 controlling whitefly with synthetic insecticide is not economical (Patra & Hath, 2022) and generated resistant
34 genotypes and reduced natural enemies (Wang et al., 2020). Under such conditions, cultural control should
35 be better alternative to solve the problems. Cultural control is to conserve natural enemies and increase
36 biological diversity through management of biotic and abiotic environment. Abiotic environment of the crop can
37 be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be
38 manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian & Rezvani,
39 2016). Cultural control of insects has become better alternative since most insect has strong ability to evolve
40 pesticide resistance (Basit, 2019).

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Commented [A7]: So, which one is used in this research? Why do you use PYLCV instead of PepYLCIV? Please explain

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1 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
2 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques by
3 modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
4 were reported to be effective to reduce whitefly invasion into the protected areas (Simmons & Shapiro-Ilan,
5 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
6 techniques have enough contribution to the implementation of integrated pest management (Lapidot, Legg,
7 Wintermantel, & Polston, 2014).

8 Cultural practices have been implemented as parts of integrated pest management with various level of
9 success and failure depended on the pest and the crops in concern (Kenyon, Kumar, Tsai, & Hughes, 2014),
10 and there have been no report on the effects of cultural control on the appearance and development of PYLCV
11 on red chilli.

12 The objective of the research is to verify the seed transmission trait of *Pepper yellow leaf curl virus* and to
13 observe the effects of seed treatments, intercropping, trap cropping, and physical barrier on the natural
14 transmission and infection development of PYLCV by its vector, *B. tabaci*.

15 MATERIALS AND METHODS

16 Study area

17 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
18 2022. The research was experimental research consisted of four different experiments. The first experiment
19 was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof screenhouse
20 of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three
21 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
22 natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden
23 of Universitas Sriwijaya located in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers'
24 fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

25 Procedures

26 Seed treatment effect on PYLCV experiment

27 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
28 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
29 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
30 seeds were not used because they were obviously not viable. The experiment was arranged in a completely
31 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
32 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
33 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
34 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh,
35 Awasthi, Jangre, & Nirmalkar, 2020). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of
36 their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth
37 to obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
38 (Kabede, Ayalew, & Yeesuf, 2013); John, Ihum, Olusolape, & Janfa, 2018). The treated seeds were sown
39 accordingly in each double trays, and the trays were then placed in an insect proof screen box for seed

Commented [A9]: This statement is confusing for readers. Revise it to make clear, what do you mean.

Commented [A10]: So what is your novelty?

Commented [A11]: There are many recent reports that begomovirus are seedborne, as well as PYLCV by Fadhila et al. This statement should also been supported in molecular detection. This statement is better to delete

Commented [A12]: Should be explained as well as in text, that the treatments here were applied & analysed independently and could not compare each other.

Commented [A13]: Why there is no negative control of certified seeds as healthy indicated seeds?

Commented [A14]: Please separate this as Seeds and Seedlings Preparation, because it will be used for all treatments, right?

1 germination. The seedlings germinated in each treatment unit were used to calculate the viability of the
2 treated seeds. To observe the seed-borne PYLCV infection, 50 four-leaf seedlings were then transferred
3 individually to a 20 cm diameter polybag, and all polybags were then placed in insect-proof screenhouse and
4 were arranged accordingly to a completely randomized design.

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5 Intercropping effect on PYLCV experiment

6 Red chili plants were planted experimentally under an intercropping pattern with mung bean (*Vigna radiata*),
7 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
8 as the control. The experiment was arranged in a randomized block design with five treatments and five
9 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red
10 chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All
11 seedlings were prepared in insect-proof boxes to ensure that all PYLCV infection was initiated in the field
12 and brought in by its vector, *B. tabaci*. Sources of PYLCV were infected chili plants that spread in farmers'
13 fields around the research site. Additionally, some infected chili plants were deliberately planted around the
14 experimental plots. The vector of PYLCV was abundant in the area since the insect is polyphagous with
15 hundreds of host species (Pym et al., 2019) and most of the vegetation in the area were hosts of the vector.
16 Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield
17 reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect
18 when the vector of PYLCV arrived and inoculated the virus to the experimental plants in the plots.

Commented [A16]: Seed prep?

Commented [A17]: How many blocks and reps per block? In RCBD should be has block and reps within block

19 Trap cropping effect on PYLCV experiment

20 An experiment to investigate the effects of trap crops on the PYLCV infection was conducted using basil
21 (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and
22 with no trap crop as control. The trap crops selected were those that belonged to refugia which normally attract
23 and trap the invader insect (Hardiansyah, Hartini, & Musa, 2021). The experiment was arranged in a randomized
24 block design with 5 treatments and 5 replications resulting in 25 experimental units. The experimental unit was
25 4 x 2 m plot on which red chili was planted at 60 x 40 cm spacing. Trap crops were planted surrounding the
26 experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red
27 chili seedling to the plots. The crop was positioned as border crop so that they could intercept the insect before
28 they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chili seedlings used in this experiment
29 were prepared in insect-proof boxes, together with those used in other field experiments. The parameters
30 observed and the method of observation were similar to those observed and applied in intercropping
31 experiment.

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32 Effect of physical barrier on PYLCV experiment

33 An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using
34 cheesecloth as physical net barrier. Cheese net was selected as it was previously used as an antihail device in
35 fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and
36 diseases, net had been used as a nonaggressive pest and disease control device (Grasswitz, 2019). Physical
37 barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent
38 the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in an experimental
39 garden where 4 x 2 m plots were made as experimental units. In this experiment, physical barrier using insect
40 net was used only as side barrier and the top was opened for the access of pollinators and other beneficial

1 insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect
2 with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish,
3 Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design
4 with 5 treatments and 5 replications for which 25 plots were prepared. Four level of physical barrier height
5 were applied i.e. 50, 75, 100 and 125 cm and no side barrier as control. The net barriers were put in place
6 before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were
7 similar to those applied in intercropping and trap crop experiments.

Commented [A20]: idem

8 **Crop maintenance and observation**

9 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
10 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
11 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
12 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
13 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
14 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
15 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
16 entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop
17 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
18 calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy,
19 (2022) as follow: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some
20 yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping; and 4 =
21 very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following
22 formula:

$$23 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

24 Note: DS = disease severity

25 v = disease score (0 to 4)

26 n = number of plants showing disease score v

27 Z = the highest disease score

28 N = total number of plants observed

29 Yield reduction was calculated using the following formula

$$30 \quad YR = \frac{w}{W} \times 100\%$$

31 YR = yield reduction

32 w = weight of first three harvests of infected plant

33 W = average weight of first three harvests of healthy plants in the same plot

Commented [A21]: Literature?

34 **Data analysis**

35 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
36 collected data, and significant differences between means were determined using Honestly Significant
37 Difference (HSD) test a 95% degree of significance.

38

RESULTS AND DISCUSSION

Seed transmission of PYLCV

Pepper yellow leaf curl virus (PYLCV) is a member of begomovirus which was suspected to be transmissible through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds, ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might be directly since infection of virus could make seeds more sensitive to deterioration as reported by Bueso, Serrano, Pallás, & Sánchez-Navarro, (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of *Arabidopsis* seeds up to 65%. Nallathambi, Umamaheswari, Lal, Manjunatha, & Berliner (2020) also reported that virus infection could cause abnormal physical function of seeds and establish itself in any part of the seed which eventually affected their viability and potentially initiates seedborne disease.

Some of the seedling also produced PYLCV infection symptoms, confirming that PYLCV was a seed borne virus. The seed transmission of begomovirus had previously been reported by Kothandaraman, Devadason, & Ganesan (2016) who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus was seed-borne. A similar finding was also reported by Kil et al. (2020) who worked with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV) as reported by Pérez-Padilla et al., (2020), who worked with the virus on more than 3000 tomato plants and concluded that TYLCV was seed borne, but seed transmission was not a general property of the virus. In this experiment, the seedborne infection of PYLCV was relatively high because the seed was harvested from infected plants which potentially brought the virus, as reported by Fadhila et al. (2020) who worked with *PepYLCIV* and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds and weakened parts of them resulted the longer incubation period. According to Paylan, Erkan, Cetinkaya, Ergun, & Pazarlar (2014), heated water at 65°C, together with HCl and ozon, were very effective treatment to reduce virus concentration in the seeds and had no negative effect on the seeds. Even though the seed treatments affected the virus infection, seed borne infection of the virus still occurred indicated that the effect could not reach the embryo where the virus normally present. Seed borne viruses present in seed coat, endosperm, nucleus or embryo, but only viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might have better effect to seed borne virus infection, but it might also affect the seed germination. According to Farajollahi, Gholinejad, & Jafari (2014), hot water treatment could induce seed germination but duration of drenching the seeds in hot water could reduce seed viability. Instead of reducing seed borne virus infection, hot water treatment was also reported to be effective to eradicate bacterial seed borne pathogens and was highly recommended for pepper, eggplant and tomato seeds (Kim, Shim, Lee, & Wangchuk, 2022).

Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to

Commented [A22]: Effect of seed treatment on

1 start an endemic (Pagán, 2022). High seed borne tobamovirus infection has also been reported by
2 Dombrovsky & Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
3 *virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

4 All treatments in the experiment resulted in PYLCV infection which confirmed that, as a member of
5 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
6 nature of begomovirus had also been reported by (Kothandaraman, Devadason, & Ganesan, 2016) who
7 studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato*
8 *yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato
9 plants and concluded that the virus was seed borne but seed transmission was not a general property of the
10 virus.

11 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
12 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
13 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
14 virus and zika virus (Al Hadhrami, Battashi, & Al Hashami, 2022); Jennings & Parks 2020), and plant virus
15 such as *Cucumber mosaic virus* (Hamidson, Damiri, & Angraini, 2018).

16 Intercropping effect on PYLCV

17 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
18 different family from the main crop. The use of mung bean and soybean (Family Leguminosae), tomato and
19 eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but affected disease
20 incidence in the range of 39.30 to 71.41%, with only tomato caused significant reduction of PYLCV infections
21 (Table 2). The reduction of viral disease under intercropping was a direct effect of the reduction of incoming
22 vector, as reported by Mir et al. (2022) that intercropping could effectively control insect pest. The effect of
23 intercropping to insect vector might be by reducing the vector invasion to the crop due to the presence of
24 intercrop as alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant
25 disease by causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence
26 could be caused by its effect on incoming vector, since tomato has insect repellent effect, especially against
27 mosquito and aphid (Setyaningrum, Unih, Pratami, & Kanedi, 2023).

28 Research on the use of leguminous crops as intercrops has been frequent but mostly for the intention of
29 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
30 on plant disease was reported. Most of the research were combinations between leguminous and food crops
31 such as maize-mung bean (Syafuruddin & Suwardi, 2020), cotton-mung bean (Liang, He, & Shi, 2020),
32 sorghum-mungbean (Temeche, Getachew, Hailu, & Abebe, 2022), sorghum-soybean (Saber 2018), maize-
33 soybean (Berdjour, Dugje, Dzomeku, & Rahman, 2020), and rice-soybean (Putra & Sas, 2023).

34 Trap crop effect on PYLCV.

35 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. Basil,
36 cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect
37 pests (Sarjan, Haryanto, Supeno, & Jihadi, 2023), but the effects of the four crops were different when being
38 used barrier crops. Barrier crop could affect the main crops by intercepting, arresting or retaining pest thereby
39 limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence

Commented [A23]: Please note that except turmeric the effect is not statistically significant. Therefore could not be concluded they reduce the disease. Please revise the sentence because it could be misleading

Commented [A24]: I think in the case of viral disease, the severity is important. If the disease severity is not significant compare to control, how do you explain that the method is effective? Why don't you explain that disease severity is only reducing incidence but not severity, therefore other method is needed?

1 of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could
2 significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The
3 significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to
4 Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the insect
5 oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5%
6 effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman, Srinivasan, Suganthi,
7 Ranganathan, Gimbut, & Shanmugam (2018), the extract was also effective against *Culex tritaeniorhynchus*,
8 *Aedes albopictus* and *Anopheles subpictus*. Husna et al. (2020) also reported that the extract of basil leaves
9 was a strong larvicide against mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong
10 repellent against mosquito and contained extractable toxicants which effective as repellent and larvicide
11 against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonalis*
12 (Dikr & Belete, 2021), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap
13 crop has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour
14 (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but
15 Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured
16 flower and one species (basil) produced green flower but with strong odor.

Commented [A25]: How about the marigold?

17 Physical barrier effect on PYLCV

18 Physical barrier using cheese cloth 50 mesh could reduce the infestation of PYLCV vectors indicated by the
19 reduction of disease incidence and severity up to 64.00% and 70.59% respectively, but only physical barriers
20 at 125 cm high could significantly reduce incidence and severity of pepper yellow leaf curl disease (Table 4).
21 This result was in accordance to that reported by (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016)
22 that a barrier of fine mesh could effectively prevent *B. tabaci* to the protected plot. It is shown in the table that
23 the higher the barrier the lower the disease incidence caused by PYLCV, an indication that *B. tabaci*, the only
24 vector of PYLCV, could fly higher than 100 cm and transmitting the virus. According to Tillman (2014), height
25 of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the
26 height of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicated that the insect
27 could fly above such altitude even though the whitefly is not a good flyer even though it might spread to long
28 distance carried by wind or transported materials. The maximum distance covered by single flying of the
29 whitefly is 17 m (Maruthi, Jeremiah, Mohammed, & Legg, 2017), but in a dense plant population the insect
30 can move from plant to plant easily, and if the insect is viruliferous, massive virus spread is inevitable.

Commented [A26]: Please elaborate also, why the reducing yield is not significant compare to control? Does the treatment effective then?

31 The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in
32 blocking flying insect and had no effect on the insect behavior. This was different from barrier crop which not
33 only block the flying insects but also attract or repel the insect, depended on the crop species. Flying insect
34 such as *B. tabaci* could not easily differentiate host and nonhost plants which made barrier crop more effective
35 than net barrier (Udiarto, Setiawati, Muharam, & Dadi, 2023). *B. tabaci* actually has two type of flight behavior,
36 foraging flight and migratory flight. Foraging flight is close to earth surface or within flight boundary, while
37 migratory flight is above the boundary where the insect could be picked up and carried by air currents
38 (Reynolds, Chapman, & Drake, 2017), and the insect containing mature eggs has been trapped at 150 m
39 above ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier
40 could actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect with body
41 width wider than mesh size of the net.

CONCLUSIONS AND SUGGESTION

The first experiment of seed borne transmission of PYLCV concluded and verified that the virus infecting red chili was a seed borne virus. Hot water treatment and crude extract of red ginger could lengthen the average incubation period of PYLCV up to 6.61 days to 8.02 days respectively, and crude extract red ginger reduced the infection frequency and disease severity up to 66.67% and 73.13% respectively, compared to control. In the intercropping experiment, tomato was good enough as intercrops for red chili in term of reducing PYLCV infections which could reduce disease incidence up to 71.41%. Basil and marigold were functional as trap crops to protect chili from incoming *B. tabaci* and could reduce incidence and severity of disease transmitted by the insect up to 72.42% and 72.83% respectively. In the experiment of physical barrier, the use of 50 mesh cheese cloth as side barrier at the height of 125 cm could reduce PYLCV infection frequency up to 70.59%. In all cultural techniques applied in the field experiments, PYLCV infection on red chili caused yield reduction of 40 to 53%.

Infection of PYLCV on red chili is easily spread by *B. tabaci* and the disease is very damaging to the crop yield. Even though some cultural techniques are effective to reduce PYLCV transmission by each vector, regular disease monitoring is important and destroy infected plants is necessary to eliminate virus inoculum.

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Commented [A27]: The numbers of results are misleading. Please re-count

Commented [A28]: Red?

Commented [A29]: No information about red ginger

Commented [A30]: How did you get this number? It should be compare to control. It could be misleading.

Commented [A31]: Should be result of control - result of tomato. Because the results have already been in percentage. Should be only subtraction of control - treatment.

Commented [A32]: Idem. Please re-count: control - treatment

Commented [A33]: idem

1 Related Traits and Yield of tomato at Wondo Genet, Southern Ethiopia. *Journal of Biology, Agriculture and*
2 *Healthcare* 11(6), 1-11. <http://doi.org/10.7176/JBAH/11-6-01>

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1 **Tables**

2 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
3 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

4 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
5 Significant Difference Test.

6 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
7 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54
HSD 5%	12.18	-	-

8 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
9 Significant Difference Test.

10 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
11 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

12 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
13 Significant Difference Test.

1 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 2 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

3 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 4 Significant Difference Test.

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The effects of various cultural techniques on the transmission and infectious development of *Pepper yellow leaf curl virus* on red chili

(Running title: Effects of cultural techniques on *Pepper yellow leaf curl virus*)

ABSTRACT

An experiment was conducted to investigate the effects of cultural techniques to prevent the infection of yellow leaf curl disease of chili caused by *Pepper yellow leaf curl virus* (PYLCV). The experiment was conducted in the area where the disease has been an endemic and *Bemisia tabaci*, the vector of the disease, was abundant.

The cultural techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds harvested from infected plants were used for seed treatment experiment, and commercial seeds mostly used by local farmers were used for the other experiments. The results showed that PYLCV was a seedborne virus and hot water treatment at 65°C for 30 minutes could lengthen incubation period and crude extract turmeric could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant, mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but not the lower ones. Under various cultural technique, PYLCV caused chili yield reduction of 40–53%.

KEYWORDS

Bemisia tabaci, cultural technique, *Pepper yellow leaf curl virus*, seed treatment

INTRODUCTION

Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country, with some provinces have become the production centres of this commodity from where the majority of nation demand is fulfilled. The eleven biggest red chili producing provinces in 2022 were West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra with total production of 1229262 tons. The chili production varied among different provinces and the three provinces in Java contributed to 58.3% to the national production (Siregar & Suroso, 2021).

Production of red chilli fluctuated due to several factors which inevitably caused significant fluctuation in chilli price which eventually made farmers and traders under inconvenience situations. Plant diseases has been the main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which causes yield reduction up to 66% (Utami, Meale & Young, 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprpta, 2022), fusarium which had a record to cause 40% yield losses (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a begomovirus, which under favourable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al, 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower abortion and fruit discoloration (Lavanya & Arun 2021).

Disease caused by PYLCV often reached its high incidence and intensity during dry season when the weather is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn

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1 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek, Hariton-Shalev, Sobol,
2 Gorovits, & Ghanim, 2017). The disease is increasingly frightening because it has spread to all chilli production
3 centres in the country since its first appearance detected in 1999 (Gaswanto, Syukur, Hidayat, & Gunaeni,
4 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed
5 as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila, Lal, Vo, Ho, Hidayat,
6 Lee, Kil, & Lee, 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of
7 members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2
8 components of single stranded DNA genome inside. Geminiviridae comprises of 4 genera based on their
9 vector and genome organization. Members of the family transmitted persistently by white fly (*B. tabaci*) are
10 grouped into Genus Begomovirus, a bipartite virus having 2 components of single stranded DNA recognized
11 as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek, Hariton-Shalev, Sobol, Gorovits,
12 & Ghanim, 2017). In Indonesia, *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) was recognized to have
13 two strains i.e. PeYLCIV-Tomato which also infects tomato and PeYLCIV-Ageratum which also infects
14 ageratum. Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and
15 cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon,
16 water melon, pepper and eggplant (Subiastuti, Hartono, Daryono, 2019).

17 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
18 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
19 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal, Singh, &
20 Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included
21 tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant,
22 chilli, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is
23 known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm.
24 The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from
25 fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al., 2014).

26 *B. tabaci* has at least 43 species complex, (Shah & Liu, 2013) and is able to transmit more than 200 plant
27 viruses (Lu, Chen, Li, Shi, Gu, & Yan, 2019; MacLeod, Canty, & Polaszek, 2022), and 90% of plant viruses
28 transmitted by the vector belong to *begomovirus* (Kanakala & Ghanim, 2016). To transmit PYLCV successfully,
29 *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8 hours (Czosnek,
30 Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). The vector remains viruliferous until 13 days after virus
31 acquisition or until the vector dies (Roy, Chakraborty, & Ghosh, 2021).

32 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
33 controlling whitefly with synthetic insecticide is not economical (Patra & Hath, 2022) and generated resistant
34 genotypes and reduced natural enemies (Wang et al., 2020). Under such conditions, cultural control should
35 be better alternative to solve the problems. Cultural control is to conserve natural enemies and increase
36 biological diversity through management of biotic and abiotic environment. Abiotic environment of the crop can
37 be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be
38 manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian & Rezvani,
39 2016). Cultural control of insects has become better alternative since most insect has strong ability to evolve
40 pesticide resistance (Basit, 2019).

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1 Cultural controls is also important in managing whitefly *B. tabaci* because inappropriate crop management
2 may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques by
3 modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
4 were reported to be effective to reduce whitefly invasion into the protected areas (Simmons & Shapiro-Ilan,
5 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
6 techniques have enough contribution to the implementation of integrated pest management (Lapidot, Legg,
7 Wintermantel, & Polston, 2014).

8 Cultural practices have been implemented as parts of integrated pest management with various level of
9 success and failure depended on the pest and the crops in concern (Kenyon, Kumar, Tsai, & Hughes, 2014),
10 and there have been no report on the effects of cultural control on the appearance and development of PYLCV
11 on red chilli.

12 The objective of the research is to verify the seed transmission trait of *Pepper yellow leaf curl virus* and to
13 observe the effects of seed treatments, intercropping, trap cropping, and physical barrier on the natural
14 transmission and infection development of PYLCV by its vector, *B. tabaci*.

15 MATERIALS AND METHODS

16 Study area

17 Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in
18 2022. The research was experimental research consisted of four different experiments. The first experiment
19 was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof screenhouse
20 of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three
21 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
22 natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden
23 of Universitas Sriwijaya located in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers'
24 fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

25 Procedures

26 Seed treatment effect on PYLCV experiment

27 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
28 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
29 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
30 seeds were not used because they were obviously not viable. The experiment was arranged in a completely
31 randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of
32 ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger
33 (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to
34 make 200 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh,
35 Awasthi, Jangre, & Nirmalkar, 2020). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr of
36 their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth
37 to obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for 30 minutes
38 (Kabede, Ayalew, & Yeesuf, 2013); John, Ihum, Olusolape, & Janfa, 2018). The treated seeds were sown
39 accordingly in each double trays, and the trays were then placed in an insect proof screen box for seed

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1 germination. The seedlings germinated in each treatment unit were used to calculate the viability of the
2 treated seeds. To observe the seed-borne PYLCV infection, 50 four-leaf seedlings were then transferred
3 individually to a 20 cm diameter polybag, and all polybags were then placed in insect-proof screenhouse and
4 were arranged accordingly to a completely randomized design.

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5 Intercropping effect on PYLCV experiment

6 Red chili plants were planted experimentally under an intercropping pattern with mung bean (*Vigna radiata*),
7 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
8 as the control. The experiment was arranged in a randomized block design with five treatments and five
9 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red
10 chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All
11 seedlings were prepared in insect-proof boxes to ensure that all PYLCV infection was initiated in the field
12 and brought in by its vector, *B. tabaci*. Sources of PYLCV were infected chili plants that spread in farmers'
13 fields around the research site. Additionally, some infected chili plants were deliberately planted around the
14 experimental plots. The vector of PYLCV was abundant in the area since the insect is polyphagous with
15 hundreds of host species (Pym et al., 2019) and most of the vegetation in the area were hosts of the vector.
16 Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield
17 reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect
18 when the vector of PYLCV arrived and inoculated the virus to the experimental plants in the plots.

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19 Trap cropping effect on PYLCV experiment

20 An experiment to investigate the effects of trap crops on the PYLCV infection was conducted using basil
21 (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and
22 with no trap crop as control. The trap crops selected were those that belonged to refugia which normally attract
23 and trap invader insects (Hardiansyah, Hartini, & Musa, 2021). The experiment was arranged in a randomized
24 block design with 5 treatments and 5 replications resulting in 25 experimental units. The experimental unit was
25 4 x 2 m plot on which red chili was planted at 60 x 40 cm spacing. Trap crops were planted surrounding the
26 experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red
27 chili seedling to the plots. The crops were positioned as border crops so that they could intercept insects before
28 they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chili seedlings used in this experiment
29 were prepared in insect-proof boxes, together with those used in other field experiments. The parameters
30 observed and the method of observation were similar to those observed and applied in intercropping
31 experiment.

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32 Effect of physical barrier on PYLCV experiment

33 An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using
34 cheesecloth as physical net barrier. Cheese net was selected as it was previously used as an antihail device in
35 fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and
36 diseases, net had been used as a nonaggressive pest and disease control device (Grasswitz, 2019). Physical
37 barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent
38 the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in an experimental
39 garden where 4 x 2 m plots were made as experimental units. In this experiment, physical barrier using insect
40 net was used only as a side barrier and the top was opened for the access of pollinators and other beneficial

1 insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect
2 with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish,
3 Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design
4 with 5 treatments and 5 replications for which 25 plots were prepared. Four level of physical barrier height
5 were applied i.e. 50, 75, 100 and 125 cm and no side barrier as control. The net barriers were put in place
6 before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were
7 similar to those applied in intercropping and trap crop experiments.

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8 **Crop maintenance and observation**

9 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
10 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
11 transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and
12 disease severity. Incubation period was described as the period from seed sowing to the appearance of the
13 first symptom of PYLCV. Data on chilli production was not collected since the experimental plants were not
14 grown from good seed and the plants were not under optimal cultivation conditions. For the other experiments,
15 where the PYLCV brought in by its vector, data on incubation period could not be measured because the
16 entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop
17 the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was
18 calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy,
19 (2022) as follow: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some
20 yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping; and 4 =
21 very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following
22 formula:

$$23 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

24 Note: DS = disease severity

25 v = disease score (0 to 4)

26 n = number of plants showing disease score v

27 Z = the highest disease score

28 N = total number of plants observed

29 Yield reduction was calculated using the following formula

$$30 \quad YR = \frac{w}{W} \times 100\%$$

31 YR = yield reduction

32 w = weight of first three harvests of infected plant

33 W = average weight of first three harvests of healthy plants in the same plot

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34 **Data analysis**

35 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
36 collected data, and significant differences between means were determined using Honestly Significant
37 Difference (HSD) test a 95% degree of significance.

38

RESULTS AND DISCUSSION

Seed transmission of PYLCV

Pepper yellow leaf curl virus (PYLCV) is a member of begomovirus which was suspected to be transmissible through seeds. The use of seeds harvested from infected plants has resulted in low viability of the seeds, ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all of the seed treatments could not significantly increase the viability. The effects of PYLCV on the seed viability might be directly since infection of virus could make seeds more sensitive to deterioration as reported by Bueso, Serrano, Pallás, & Sánchez-Navarro, (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of *Arabidopsis* seeds up to 65%. Nallathambi, Umamaheswari, Lal, Manjunatha, & Berliner (2020) also reported that virus infection could cause abnormal physical function of seeds and establish itself in any part of the seed which eventually affected their viability and potentially initiates seedborne disease.

Some of the seedling also produced PYLCV infection symptoms, confirming that PYLCV was a seed borne virus. The seed transmission of begomovirus had previously been reported by Kothandaraman, Devadason, & Ganesan (2016) who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus was seed-borne. A similar finding was also reported by Kil et al. (2020) who worked with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus showing ability to spread as seed borne virus was *Tomato yellow leaf curl virus* (TYLCV) as reported by Pérez-Padilla et al., (2020), who worked with the virus on more than 3000 tomato plants and concluded that TYLCV was seed borne, but seed transmission was not a general property of the virus. In this experiment, the seedborne infection of PYLCV was relatively high because the seed was harvested from infected plants which potentially brought the virus, as reported by Fadhila et al. (2020) who worked with *PepYLCIV* and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of PYLCV but had not significant effect on disease incidence and severity when compared to control. Hot water treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the seeds and weakened parts of them resulted the longer incubation period. According to Paylan, Erkan, Cetinkaya, Ergun, & Pazarlar (2014), heated water at 65°C, together with HCl and ozon, were very effective treatment to reduce virus concentration in the seeds and had no negative effect on the seeds. Even though the seed treatments affected the virus infection, seed borne infection of the virus still occurred indicated that the effect could not reach the embryo where the virus normally present. Seed borne viruses present in seed coat, endosperm, nucleus or embryo, but only viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might have better effect to seed borne virus infection, but it might also affect the seed germination. According to Farajollahi, Gholinejad, & Jafari (2014), hot water treatment could induce seed germination but duration of drenching the seeds in hot water could reduce seed viability. Instead of reducing seed borne virus infection, hot water treatment was also reported to be effective to eradicate bacterial seed borne pathogens and was highly recommended for pepper, eggplant and tomato seeds (Kim, Shim, Lee, & Wangchuk, 2022).

Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to

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1 start an endemic (Pagán, 2022). High seed borne tobamovirus infection has also been reported by
2 Dombrovsky & Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
3 *virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

4 All treatments in the experiment resulted in PYLCV infection which confirmed that, as a member of
5 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
6 nature of begomovirus had also been reported by (Kothandaraman, Devadason, & Ganesan, 2016) who
7 studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato*
8 *yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato
9 plants and concluded that the virus was seed borne but seed transmission was not a general property of the
10 virus.

11 All crude extracts used as seed treatment could reduce the incidence and severity of PYLCV but only turmeric
12 crude extract reduced significantly compared to control. Turmeric has been widely used as herbal medicine
13 and showed antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A
14 virus and zika virus (Al Hadhrami, Battashi, & Al Hashami, 2022); Jennings & Parks 2020), and plant virus
15 such as *Cucumber mosaic virus* (Hamidson, Damiri, & Angraini, 2018).

16 Intercropping effect on PYLCV

17 Intercropping affected the incidence of PYLCV on red chili, especially when the intercropping plants were of
18 different family from the main crop. The use of mung bean and soybean (Family Leguminosae), tomato and
19 eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but affected disease
20 incidence in the range of 39.30 to 71.41%, with only tomato caused significant reduction of PYLCV infections
21 (Table 2). The reduction of viral disease under intercropping was a direct effect of the reduction of incoming
22 vector, as reported by Mir et al. (2022) that intercropping could effectively control insect pest. The effect of
23 intercropping to insect vector might be by reducing the vector invasion to the crop due to the presence of
24 intercrop as alternative host in the plots, as suggested by Boudreau (2013) that intercrops affected a plant
25 disease by causing alteration of vector dispersal. The significant effect of tomato in reducing disease incidence
26 could be caused by its effect on incoming vector, since tomato has insect repellent effect, especially against
27 mosquito and aphid (Setyaningrum, Unih, Pratami, & Kanedi, 2023).

28 Research on the use of leguminous crops as intercrops has been frequent but mostly for the intention of
29 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
30 on plant disease was reported. Most of the research were combinations between leguminous and food crops
31 such as maize-mung bean (Syafuddin & Suwardi, 2020), cotton-mung bean (Liang, He, & Shi, 2020),
32 sorghum-mungbean (Temeche, Getachew, Hailu, & Abebe, 2022), sorghum-soybean (Saber 2018), maize-
33 soybean (Berdjour, Dugje, Dzomeku, & Rahman, 2020), and rice-soybean (Putra & Sas, 2023).

34 Trap crop effect on PYLCV.

35 Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. Basil,
36 cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect
37 pests (Sarjan, Haryanto, Supeno, & Jihadi, 2023), but the effects of the four crops were different when being
38 used barrier crops. Barrier crop could affect the main crops by intercepting, arresting or retaining pest thereby
39 limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence

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Commented [A24]: I think in the case of viral disease, the severity is important. If the disease severity is not significant compare to control, how do you explain that the method is effective? Why don't you explain that disease severity is only reducing incidence but not severity, therefore other method is needed?

1 of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could
2 significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The
3 significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to
4 Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the insect
5 oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5%
6 effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman, Srinivasan, Suganthi,
7 Ranganathan, Gimbut, & Shanmugam (2018), the extract was also effective against *Culex tritaeniorhynchus*,
8 *Aedes albopictus* and *Anopheles subpictus*. Husna et al. (2020) also reported that the extract of basil leaves
9 was a strong larvicide against mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong
10 repellent against mosquito and contained extractable toxicants which effective as repellent and larvicide
11 against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonalis*
12 (Dikr & Belete, 2021), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap
13 crop has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour
14 (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but
15 Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured
16 flower and one species (basil) produced green flower but with strong odor.

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17 Physical barrier effect on PYLCV

18 Physical barrier using cheese cloth 50 mesh could reduce the infestation of PYLCV vectors indicated by the
19 reduction of disease incidence and severity up to 64.00% and 70.59% respectively, but only physical barriers
20 at 125 cm high could significantly reduce incidence and severity of pepper yellow leaf curl disease (Table 4).
21 This result was in accordance to that reported by (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016)
22 that a barrier of fine mesh could effectively prevent *B. tabaci* to the protected plot. It is shown in the table that
23 the higher the barrier the lower the disease incidence caused by PYLCV, an indication that *B. tabaci*, the only
24 vector of PYLCV, could fly higher than 100 cm and transmitting the virus. According to Tillman (2014), height
25 of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the
26 height of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicated that the insect
27 could fly above such altitude even though the whitefly is not a good flyer even though it might spread to long
28 distance carried by wind or transported materials. The maximum distance covered by single flying of the
29 whitefly is 17 m (Maruthi, Jeremiah, Mohammed, & Legg, 2017), but in a dense plant population the insect
30 can move from plant to plant easily, and if the insect is viruliferous, massive virus spread is inevitable.

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31 The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in
32 blocking flying insect and had no effect on the insect behavior. This was different from barrier crop which not
33 only block the flying insects but also attract or repel the insect, depended on the crop species. Flying insect
34 such as *B. tabaci* could not easily differentiate host and nonhost plants which made barrier crop more effective
35 than net barrier (Udiarto, Setiawati, Muharam, & Dadi, 2023). *B. tabaci* actually has two type of flight behavior,
36 foraging flight and migratory flight. Foraging flight is close to earth surface or within flight boundary, while
37 migratory flight is above the boundary where the insect could be picked up and carried by air currents
38 (Reynolds, Chapman, & Drake, 2017), and the insect containing mature eggs has been trapped at 150 m
39 above ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier
40 could actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect with body
41 width wider than mesh size of the net.

CONCLUSIONS AND SUGGESTION

The first experiment of seed borne transmission of PYLCV concluded and verified that the virus infecting red chili was a seed borne virus. Hot water treatment and crude extract of red ginger could lengthen the average incubation period of PYLCV up to 6.61 days to 8.02 days respectively, and crude extract red ginger reduced the infection frequency and disease severity up to 66.67% and 73.13% respectively, compared to control. In the intercropping experiment, tomato was good enough as intercrops for red chili in term of reducing PYLCV infections which could reduce disease incidence up to 71.41%. Basil and marigold were functional as trap crops to protect chili from incoming *B. tabaci* and could reduce incidence and severity of disease transmitted by the insect up to 72.42% and 72.83% respectively. In the experiment of physical barrier, the use of 50 mesh cheese cloth as side barrier at the height of 125 cm could reduce PYLCV infection frequency up to 70.59%. In all cultural techniques applied in the field experiments, PYLCV infection on red chili caused yield reduction of 40 to 53%.

Infection of PYLCV on red chili is easily spread by *B. tabaci* and the disease is very damaging to the crop yield. Even though some cultural techniques are effective to reduce PYLCV transmission by each vector, regular disease monitoring is important and destroy infected plants is necessary to eliminate virus inoculum.

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1 **Tables**

2 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
3 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

4 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
5 Significant Difference Test.

6 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
7 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54
HSD 5%	12.18	-	-

8 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
9 Significant Difference Test.

10 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
11 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

12 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
13 Significant Difference Test.

1 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 2 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

3 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 4 Significant Difference Test.

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- Good
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The effects of some different cultural techniques on the transmission and infectious development of *Pepper yellow leaf curl Indonesia virus* on red chili

(Running title: Effects of cultural techniques on *Pepper yellow leaf curl Indonesia virus*)

ABSTRACT

An experiment was conducted to investigate the effects of cultural techniques on pepper yellow leaf curl disease caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV). The experiment was conducted in the area where the disease has been an endemic and *Bemisia tabaci* was abundant. Four cultural techniques were applied in separate lands and could not interfere each other. The techniques applied were seed treatment, intercropping, trap cropping, and physical barrier. Seeds harvested from infected plants were used for seed treatment experiment, and commercial seeds used by local farmers were used for the other experiments. The results confirmed that PepYLCIV was a seedborne virus affected by hot water treatment at 65°C for 30 minutes. Turmeric crude extract could reduce incidence and severity of the disease. Tomato was a better intercrop compared to eggplant, mung bean and soybean in reducing the disease incidence but their effects on disease severity and yield reduction were not significantly different. Basil and marigold were better barrier crops compare to cosmos and zinnia. A 125 cm high physical barrier using 50 mesh cheese cloth could reduce the disease incidence, but not the lower ones. Under different cultural techniques, PepYLCIV caused chili yield reduction of 40.00 – 52.32%.

KEYWORDS

Bemisia tabaci, cultural technique, *Pepper yellow leaf curl Indonesia virus*, seed treatment

INTRODUCTION

Red chilli, is an important horticultural crop in Indonesia and is cultivated almost everywhere in the country, with some provinces have become the production centres of this commodity from where the majority of nation demand is fulfilled. The eleven biggest red chili producing provinces in 2022 were West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra with total production of 1229262 tons. The chili production varied among different provinces and the three provinces in Java contributed to 58.3% to the national production (Siregar & Suroso, 2021).

Production of red chilli fluctuated due to several factors which inevitably caused significant fluctuation in chilli price which eventually made farmers and traders under inconvenience situations. Plant diseases has been the main factor causing yield reduction of red chilli in the country. The diseases include bacterial leaf spot which causes yield reduction up to 66% (Utami, Meale & Young, 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprpta, 2022), fusarium which had a record to cause 40% yield losses (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), a begomovirus, which under favourable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al, 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower abortion and fruit discoloration (Lavanya & Arun 2021).

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1 Disease caused by PepYLCIV often reached its high incidence and intensity during dry season when the
2 weather is favourable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci*
3 Genn (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek, Hariton-Shalev,
4 Sobol, Gorovits, & Ghanim, 2017). The disease is increasingly frightening because it has spread to all chilli
5 production centres in the country since its first appearance detected in 1999 (Gaswanto, Syukur, Hidayat, &
6 Gunaeni, 2016). The virus causing yellow leaf curl on pepper was previously known as *Pepper yellow leaf curl*
7 *virus* (PYLCV), however due to its own specification, the virus has received its own name as *Pepper yellow*
8 *leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila et al, 2020). Begomovirus is a member
9 of Family Geminiviridae, a big plant virus group with a lot of members known as damaging viruses. The virus
10 is characterised by 30 x 20 nm gemini (twin) particles with 2 components of single stranded DNA genome
11 inside. Geminiviridae comprises of 4 genera based on their vector and genome organization. Members of the
12 family transmitted persistently by white fly (*B. tabaci*) are grouped into Genus Begomovirus, a bipartite virus
13 having 2 components of single stranded DNA recognized as DNA A and DNA B, both have the same
14 measurement, 2.8 kb (Czosnek, Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). In Indonesia, *Pepper*
15 *yellow leaf curl Indonesia virus* (PepYLCIV) was recognized to have two strains i.e. PepYLCIV-Tomato which
16 also infects tomato and PepYLCIV-Ageratum which also infects ageratum. Instead of infecting chilli and
17 tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damages.
18 The other crops found to be infected by begomovirus included melon, water melon, pepper and eggplant
19 (Subiastuti, Hartono, Daryono, 2019).

20 As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently
21 transmit numbers of viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato*
22 *yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Suryapal, Singh, &
23 Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included
24 tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant,
25 chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is
26 known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm.
27 The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from
28 fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al., 2014).

29 *B. tabaci* has at least 43 species complex, (Shah & Liu, 2013) and is able to transmit more than 200 plant
30 viruses (Lu, Chen, Li, Shi, Gu, & Yan, 2019; MacLeod, Canty, & Polaszek, 2022), and 90% of plant viruses
31 transmitted by the vector belong to *begomovirus* (Kanakala & Ghanim, 2016). To transmit PepYLCIV
32 successfully, *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8
33 hours (Czosnek, Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). The vector remains viruliferous until 13
34 days after virus acquisition or until the vector dies (Roy, Chakraborty, & Ghosh, 2021).

35 Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However,
36 controlling whitefly with synthetic insecticide is not economical (Patra & Hath, 2022) because insecticide could
37 *enerate resistant genotypes of the vector and reduced natural enemies* (Wang et al., 2020). Under such
38 conditions, cultural control should be better alternative to solve the problems. Cultural control is to conserve
39 natural enemies and increase biological diversity through management of biotic and abiotic environment.
40 Abiotic environment of the crop can be manipulated by modifying soil tillage, fertilization, irrigation, and
41 mulching. Biotic environment might be manipulated by applying crop rotation, intercropping, trap cropping, and

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1 crop spacing (Zaefarian & Rezvani, 2016). Cultural control of insects has become better alternative since most
2 insect has strong ability to evolve pesticide resistance (Basit, 2019).

3 Cultural controls is also important in managing whitefly *B. tabaci* to avoid inappropriate crop management
4 which may lead to serious whitefly problems, and more seriously, virus problems. Some cultural techniques
5 by modifying various production practices such as mulching, intercropping, trap cropping and physical barrier
6 were reported to be effective to reduce whitefly infasion into the protected areas (Simmons & Shapiro-Ilan,
7 2021). The degree of whitefly exclusion might be not too significant, but it should be considered that the
8 techniques have enough contribution to the implementation of integrated pest management (Lapidot, Legg,
9 Wintermantel, & Polston, 2014).

10 Cultural practices have been implemented as parts of integrated pest management with various level of
11 success and failure depended on the pest and the crops in concern (Kenyon, Kumar, Tsai, & Hughes, 2014),
12 and there have been no report on the effects of cultural control on the appearance and development of
13 PepYLCIV on red chilli.

14 The objective of the research was to observe the effects of seed treatments, intercropping, trap cropping, and
15 physical barrier on the natural transmission and infection development of PepYLCIV by its vector, *B. tabaci*.
16 All of the experiments were conducted separately so all treatments were applied and analyzed independently
17 and not comparable each other.

18 MATERIALS AND METHODS

19 Study area

20 Research on the application of cultural techniques to control PepYLCIV infection on red chili was conducted
21 in 2022. The research was experimental research consisted of four different experiments. The first experiment
22 was a seed treatment effect on PepYLCIV experiment, conducted in Insectarium and insect-proof screenhouse
23 of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three
24 experiments were field experiments on the effects of intercropping, trap cropping and physical barrier on the
25 natural transmission and infection of PepYLCIV. The field experiments were conducted in experimental garden
26 of Universitas Sriwijaya located in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers'
27 fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

28 Procedures

29 Seed treatment effect on PepYLCIV experiment

30 Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were
31 sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then
32 underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated
33 seeds were not used because they were obviously not viable. No healthy seeds used as negative control in
34 this experiment because the experiment itself was carried out inside insect-proof house to guarantee that all
35 PepYLCIV infection were seed borne. The experiment was arranged in a completely randomized design with
36 five treatment and 5 replications. The treatments were hot water, crude extract of red ginger (*Zingiber
37 officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma
38 zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200
39 holes per unit. For hot water treatment, seeds were soaked in 65°C water for 30 minutes (Singh, Awasthi,

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Commented [A18]: So what is your novelty?

Commented [R19R18]: We used different crops in intercropping and trap cropping experiment and uses different barrier in physical barrier experiment.

Commented [A20]: There are many recent reports that begomovirus are seedborne, as well as PYLCV by Fadhila et al. This statement should also been supported in molecular detection. This statement is better to delete

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Commented [R23R22]: The explanation has been added.

Commented [A24]: Why there is no negative control of certified seeds as healthy indicated seeds?

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Commented [A26]: Please separate this as Seeds and Seedlings Preparation, because it will be used for all treatments, right?

Commented [R27R26]: Seeds from infected plants were only used in seed treatment experiment, while for other experiments were used certified healthy seed.

1 Jangre, & Nirmalkar, 2020). To make crude red ginger, crude turmeric, and crude Javanese ginger extracts,
2 50 gr of their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double
3 cheesecloth to obtain the crude extracts. The treated chilli seeds were dipped in the extracts accordingly for
4 30 minutes (Kabede, Ayalew, & Yeessuf, 2013); John, Ithum, Olusolape, & Janfa, 2018). The treated seeds
5 were sown accordingly in each double trays, and the trays were then placed in an insect proof screen box for
6 seed germination. The seedlings germinated in each treatment unit were used to calculated the viability of the
7 treated seeds. To observed the seed borned PepYLCIV infection, 50 four-leaf seedlings were then transferred
8 individually to a 20 cm diameter polybag, and all polybags were then placed in insect proof screenhouse and
9 were arranged accordingly to completely randomized design with 4 treatments and 5 blocks where 50 plants
10 were placed in each block as replication within each block.

11 Intercropping effect on PepYLCIV experiment

12 Red chili seedlings were planted experimentally under intercropping pattern with mung bean (*Vigna radiata*),
13 soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop
14 as the control. The experiment was arranged in a randomized block design with five treatments and five
15 blocks/replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots
16 resulted in 12 red chili plants and 12 intercropping plants. Red chili and intercropping plants were planted at
17 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All seedlings were prepared from certified healthy
18 seeds in insect-proof boxes to ensure that all PepYLCIV infection was initiated in the field and brought in
19 by its vector, *B. tabaci*. Sources of PepYLCIV were infected chili plants that spread in farmers' fields around
20 the research site. Additionally, some infected chili plants were deliberately planted around the experimental
21 plots. The vector of PepYLCIV was abundant in the area since the insect is polyphagous with hundreds number
22 of host species (Pym et al., 2019) and most of vegetation in the area were host of the vector. Data collected
23 from this experiment included PepYLCIV disease incidence, PepYLCIV disease severity and yield
24 reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect
25 when the vector of PepYLCIV arrived and inoculated the virus to the experimental plants in the plots.

26 Trap cropping effect on PepYLCIV experiment

27 An experiment to investigate the effects of trap crops on the PepYLCIV infection was conducted using basil
28 (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and
29 with no trap crop as control. The trap crops selected were those belonged to refugia which normally attract
30 and trap invader insect (Hardiansyah, Hartini, & Musa, 2021). The experiment was arranged in a randomized
31 block design with 5 treatments and 5 replications resulting in 25 experimental units. The experimental unit was
32 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing, resulted in 24 plants per plot. Two layers of
33 trap crops were planted surrounding the experimental plots at 25 cm spacing accordingly to each treatment,
34 three (3) weeks before transplanting red chilli seedling to the plots. The crop were positioned as border crop
35 so that they could intercept insect before they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020).
36 Chilli seedlings used in this experiment were prepared in insect proof boxes, together with those used in
37 other field experiments. The parameters observed and the method of observation were similar to those
38 observed and applied in intercropping experiment.

39 Effect of physical barrier on PepYLCIV experiment

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Commented [A30]: Seed prep?

Commented [R31R30]: Seeds and seedling preparation for field experiments is describe in line16 - 18.

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Commented [R33R32]: In RCBD, the blocks represent the replication. In this experiment there were 5 treatments, 5 blocks and 12 reps within block.

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Commented [R35R34]: There were 5 treatments, 5 blocks and 24 reps within block

Commented [A36]: How many layers?

Commented [R37R36]: Two layers

1 An experiment to observe the effect of physical barrier on the invasion of PepYLCIV vectors was conducted
2 using cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device
3 in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and
4 diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical
5 barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent
6 the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental
7 garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect
8 net was used only as side barrier and let the top opened for the access of pollinator and other beneficial
9 insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect
10 with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish,
11 Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design
12 with 5 treatments and 5 replications for which 25 plots were prepared. Red chili was planted at 60 x 40 cm
13 spacing, resulted in 24 plants per block. Four level of physical barrier height were applied i.e. 50, 75, 100 and
14 125 cm and no side barrier as control. The net barriers were put in place before seedling transplanting.
15 Seedlings and virus resources used and data collection in this experiment were similar to those applied in
16 intercropping and trap crop experiments.

17 Crop maintenance and observation

18 Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and
19 mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed
20 transmission of PepYLCIV, observations were made to collect data on incubation period, disease incidence,
21 and disease severity. Incubation period was described as the period from seed sowing to the appearance of
22 the first symptom of PepYLCIV. Data on chilli production was not collected since the experimental plants were
23 not grown from good seed and the plants were not under optimal cultivation conditions. For the other
24 experiments, where the PepYLCIV brought in by its vector, data on incubation period could not be measured
25 because the entrance of the vector into the experimental plots could not be controlled. However, since
26 PepYLCIV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease
27 severity of PepYLCIV was calculated according to disease scores described by Yadav, Reddy, Ashwathappa,
28 Kumar, Naresh, & Reddy, (2022) as follow: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins
29 on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling
30 and cupping; and 4 = very severe leaf yellowing, and pronounced leaf curling. The severity was calculated
31 using the following formula:

$$32 \quad DS = \frac{\sum nxv}{ZxN} \times 100\%$$

33 Note: DS = disease severity

34 v = disease score (0 to 4)

35 n = number of plants showing disease score v

36 Z = the highest disease score

37 N = total number of plants observed

38 Yield reduction was calculated using the following formula

$$39 \quad YR = \frac{w}{w'} \times 100\%$$

40 YR = yield reduction

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Commented [R39R38]: There were 5 treatments, 5 blocks and 24 reps within block

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1 w = weight of first three harvests of infected plant

2 W = average weight of first three harvests of healthy plants in the same plot

3 **Data analysis**

4 Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all
5 collected data, and significant differences between means were determined using Honestly Significant
6 Difference (HSD) test a 95% degree of significance.

8 **RESULTS AND DISCUSSION**

9 **Effect of seed transmission of PepYLCIV**

10 *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) is a member of begomovirus which was suspected to be
11 transmissible through seeds. The use of seeds harvested from infected plants has resulted in low viability of
12 the seeds, ranged from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated,
13 all of the seed treatments could not significantly increase the viability. The effects of PepYLCIV on the seed
14 viability might be directly since infection of virus could make seeds more sensitive to deterioration as reported
15 by Bueso, Serrano, Pallás, & Sánchez-Navarro, (2017) who found that infection of *Cucumber mosaic virus*
16 (CMV) could reduce the viability of Arabidopsis seeds up to 65%. Nallathambi, Umamaheswari, Lal,
17 Manjunatha, & Berliner (2020) also reported that virus infection could cause abnormal physical function of
18 seeds and establish itself in any part of the seed which eventually affected their viability and potentially initiates
19 seedborne disease.

20 Some of the seedling also produced PepYLCIV infection symptoms, confirming that PepYLCIV was a seed
21 borne virus. The seed transmission of begomovirus had previously been reported by Kothandaraman,
22 Devadason, & Ganesan (2016) who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV),
23 and concluded that the virus was seed-borne. A similar finding was also reported by Kil et al. (2020) who
24 worked with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed
25 transmissible. Another begomovirus showing ability to spread as seed borne virus was *Tomato yellow leaf curl*
26 *virus* (TYLCV) as reported by Pérez-Padilla et al., (2020), who worked with the virus on more than 3000 tomato
27 plants and concluded that TYLCV was seed borne, but seed transmission was not a general property of the
28 virus. In this experiment, the seedborne infection of PepYLCIV was relatively high because the seed was
29 harvested from infected plants which potentially brought the virus, as reported by Fadhila et al. (2020) who
30 worked with PYLCV and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested
31 from infected plants.

32 As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of
33 PepYLCIV but had not significant effect on disease incidence and severity when compared to control. Hot
34 water treatment at 65°C did not harm the seeds but the heat of the water could reach virus particles inside the
35 seeds and weakened parts of them resulted the longer incubation period. According to Paylan, Erkan,
36 Cetinkaya, Ergun, & Pazarlar (2014), heated water at 65°C, together with HCl and ozon, were very effective
37 treatment to reduce virus concentration in the seeds and had no negative effect on the seeds. Even though
38 the seed treatments affected the virus infection, seed borne infection of the virus still occurred indicated that
39 the effect could not reach the embryo where the virus normally present. Seed borne viruses present in seed

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1 coat, endosperm, nucleus or embryo, but only viruses present in the seed embryo can be transmitted to the
2 next generation. For hot water treatment, the effect could be correlated not only to the temperature but also
3 to the dipping time. Longer dipping time might have better effect to seed borne virus infection, but it might also
4 affect the seed germination. According to Farajollahi, Gholinejad, & Jafari (2014), hot water treatment could
5 induce seed germination but duration of drenching the seeds in hot water could reduce seed viability. Instead
6 of reducing seed borne virus infection, hot water treatment was also reported to be effective to eradicate
7 bacterial seed borne pathogens and was highly recommended for pepper, eggplant and tomato seeds (Kim,
8 Shim, Lee, & Wangchuk, 2022).

9 Seed borne virus infection with the level as presented in Table 1 could be categorized as dangerous and
10 threatening, because under the presence of the virus vectors, infected seed as low as 0.1% was enough to
11 start an endemic (Pagán, 2022). High seed borne tobamovirus infection has also been reported by
12 Dombrovsky & Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic*
13 *virus*. Sastry (2013), also reported high rate of seed borne transmission of *Cucumber mosaic virus*.

14 All treatments in the experiment resulted in PepYLCIV infection which confirmed that, as a member of
15 begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed borne
16 nature of begomovirus had also been reported by (Kothandaraman, Devadason, & Ganesan, 2016) who
17 studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato*
18 *yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato
19 plants and concluded that the virus was seed borne but seed transmission was not a general property of the
20 virus.

21 In the seed treatment experiment using plant crude extracts, only turmeric crude extract could significantly
22 affect the PepYLCIV seed borne infection. The antiviral effects of turmeric have previously been reported which
23 led to the use of turmeric as herbal medicine. Turmeric has been widely used as herbal medicine and showed
24 antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A virus and
25 zika virus (Al Hadhrami, Battashi, & Al Hashami, 2022); Jennings & Parks 2020), and plant virus such as
26 *Cucumber mosaic virus* (Hamidson, Damiri, & Angraini, 2018).

27 Intercropping effect on PepYLCIV

28 Intercropping affected the incidence of PepYLCIV on red chili, especially when the intercropping plants were
29 of different family from the main crop. The use of mung bean and soybean (Family Leguminosae), tomato and
30 eggplant (Family Solanaceae) did not affect disease severity and yield reduction, but affected disease
31 incidence in the range of 39.30 to 71.41%, with only tomato caused significant reduction of PepYLCIV
32 infections (Table 2). The different effect of intercropping on disease incidence and disease severity caused
33 by the different disease initiation. Newer infection always showed less severity than the older ones, therefore,
34 disease severity might change when disease incidence stagnant. The reduction of viral disease under
35 intercropping was a direct effect of the reduction of incoming vector, as reported by Mir et al. (2022) that
36 intercropping could effectively control insect pest. The effect of intercropping to insect vector might be by
37 reducing the vector invasion to the crop due to the presence of intercrop as alternative host in the plots, as
38 suggested by Boudreau (2013) that intercrops affected a plant disease by causing alteration of vector
39 dispersal. The significant effect of tomato in reducing disease incidence could be caused by its effect on

Commented [A43]: Please note that except turmeric the effect is not statistically significant. Therefore could not be concluded they reduce the disease. Please revise the sentence because it could be misleading

Commented [R44R43]: The sentence has been revised.

Commented [A45]: I think in the case of viral disease, the severity is important. If the disease severity is not significant compare to control, how do you explain that the method is effective? Why don't you explain that disease severity is only reducing incidence but not severity, therefore other method is needed?

Commented [R46R45]: It has been explained in the added sentences.

1 incoming vector, since tomato has insect repellent effect, especially against mosquito and aphid
2 (Setyaningrum, Unih, Pratami, & Kanedi, 2023).

3 Research on the use of leguminous crops as intercrops has been frequent but mostly for the intention of
4 increasing the yield of main crops due to more efficient water use and better uptake of nitrogen, but no effect
5 on plant disease was reported. Most of the research were combinations between leguminous and food crops
6 such as maize-mung bean (Syafuddin & Suwardi, 2020), cotton-mung bean (Liang, He, & Shi, 2020),
7 sorghum-mungbean (Temeche, Getachew, Hailu, & Abebe, 2022), sorghum-soybean (Saber 2018), maize-
8 soybean (Berdjour, Dugje, Dzomeku, & Rahman, 2020), and rice-soybean (Putra & Sas, 2023).

9 **Trap crop effect on PepYLCIV.**

10 Infections of PepYLCIV were lower in chilli plots surrounded by trap crops compared to those of control. Basil,
11 cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect
12 pests (Sarjan, Haryanto, Supeno, & Jihadi, 2023), but the effects of the four crops were different when being
13 used barrier crops. Barrier crop could affect the main crops by intercepting, arresting or retaining pest thereby
14 limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence
15 of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could
16 significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The
17 significant effect of basil and marigold on PepYLCIV infection might be due to insect repellence. According to
18 Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the insect
19 oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5%
20 effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman, Srinivasan, Suganthi,
21 Ranganathan, Gimbut, & Shanmugam (2018), the extract was also effective against *Culex tritaeniorhynchus*,
22 *Aedesal bopictus* and *Anopheles subpictuat*. Husna et al. (2020) also reported that the extract of basil leaves
23 was a strong larvicidal against mosquito with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$. Marigold also displayed strong
24 repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal
25 against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali*
26 (Dikr & Belete, 2021), and against thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap
27 crop has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour
28 (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but
29 Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured
30 flower and one species (basil) produced green flower but with strong odor.

31 **Physical barrier effect on PepYLCIV**

32 Physical barrier using cheese cloth 50 mesh could reduce the infestation of PepYLCIV vectors indicated by
33 the reduction of disease incidence and severity up to 64.00% and 70.59% respectively, but only physical
34 barriers at 125 cm high could significantly reduce incidence and severity of pepper yellow leaf curl disease
35 (Table 4). This result was in accordance to that reported by (Harish, Chellappan, Kumar, Ranjith, &
36 Ambavane, 2016) that a barrier of fine mesh could effectively prevent *B. tabaci* to the protected plot. It is shown
37 in the table that the higher the barrier the lower the disease incidence caused by *Pepper yellow leaf curl virus*
38 (PYLCV), an indication that *B. tabaci*, the only vector of PYLCV, could fly higher than 100 cm and transmitting
39 the virus.. According to Tillman (2014), height of barrier was very important to effectively blocked insect to
40 infest the protected crop. In this experiment, the height of 125 cm could significantly reduce the number of *B.*

Commented [A47]: How about the marigold?

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1 *tabaci* but not totally, indicated that the insect could fly above such altitude even though the whitefly is not a
2 good flyer even though it might spread to long distance carried by wind or transported materials. The maximum
3 distance covered by single flying of the whitefly is 17 m (Maruthi, Jeremiah, Mohammed, & Legg, 2017), but
4 in a dense plant population the insect can move from plant to plant easily, and if the insect is viruliferous,
5 massive virus spread is inevitable. The significant effects of physical barrier on disease incidence and disease
6 severity did not follow by its effect on yield reduction. This could be caused by the measurement of yield
7 reduction which only used yield of the first three harvests as an indicator. If the measurement used the whole
8 yield of each infected plants, the effect could be different because of different disease stage in each infected
9 plant.

10 The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in
11 blocking flying insect and had no effect on the insect behavior. This was different from barrier crop which not
12 only block the flying insects but also attract or repel the insect, depended on the crop species. Flying insect
13 such as *B. tabaci* could not easily differentiate host and nonhost plants which made barrier crop more effective
14 than net barrier (Udiarto, Setiawati, Muharam, & Dadi, 2023). *B. tabaci* actually has two type of flight behavior,
15 foraging flight and migratory flight. Foraging flight is close to earth surface or within flight boundary, while
16 migratory flight is above the boundary where the insect could be picked up and carried by air currents
17 (Reynolds, Chapman, & Drake, 2017), and the insect containing mature eggs has been trapped at 150 m
18 above ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier
19 could actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect with body
20 width wider than mesh size of the net.

21 CONCLUSIONS AND SUGGESTION

22 The first experiment of seed borne transmission of PepYLCIV concluded and verified that the virus infecting
23 red chili was a seed borne virus. Hot water treatment and crude extract of red ginger could lengthen the
24 average incubation period of PepYLCIV up to 6.61 days to 8.02 days respectively. The effect of turmeric crude
25 extract was not significant on incubation period of PepYLCIV but it was significant on disease incidence and
26 severity amounted to 66.67% and 73.13% respectively. In the intercropping experiment, tomato was good
27 enough as intercrops for red chili in term of reducing PepYLCIV infections which could reduce disease
28 incidence up to 68.34%. Basil and marigold were functional as trap crops to protect chili from incoming *B.*
29 *tabaci*. Basil could reduce incidence and severity of disease transmitted by the insect up to 50.87% and 55.47%
30 respectively, while marigold reduce the disease incidence and severity up to 72.42 and 72.83% respectively.
31 In the experiment of physical barrier, the use of 50 mesh cheese cloth as side barrier at the height of 125 cm
32 could reduce PepYLCIV infection frequency up to 64.00% and disease severity up to 70.59%. In all cultural
33 techniques applied in the field experiments, PepYLCIV infection on red chili caused yield reduction of 40 to
34 53%.

35 Infection of PepYLCIV on red chili is easily spread by *B. tabaci* and the disease is very damaging to the crop
36 yield. Even though some cultural techniques are effective to reduce PepYLCIV transmission by each vector,
37 regular disease monitoring is important and destroy infected plants is necessary to eliminate virus inoculum.

38 REFERENCES

Commented [A49]: Please elaborate also, why the reducing yield is not significant compare to control? Does the treatment effective then?

Commented [R50R49]: It has been elaborated in lines 5-9 of the next page

Commented [A51]: The numbers of results are misleading. Please re-count

Commented [A52]: Red?

Commented [R53R52]: We did use red ginger but we did not mentioned previously, and it has been corrected.

Commented [A54]: Should be result of control - result of tomato. Because the results have already been in percentage. Should be only subtraction of control - treatment.

Commented [R55R54]: It has been corrected

Commented [A56]: Idem. Please re-count: control - treatment

Commented [R57R56]: Correction has been made.

Commented [A58]: idem

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1 **Tables**

2 Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and
3 disease severity of pepper yellow leaf curl disease of red chili.

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Red ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

4 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
5 Significant Difference Test.

6 Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and
7 yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54
HSD 5%	12.18	-	-

8 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
9 Significant Difference Test.

10 Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and
11 yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

12 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
13 Significant Difference Test.

1 Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and
 2 yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier height 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier height 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier height 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier height 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

3 Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty
 4 Significant Difference Test.

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The Effects of Some Different Cultural Techniques on the Transmission and Infectious Development of *Pepper Yellow Leaf Curl Indonesia Virus* on Red Chili (Running title: Developing Virus on Red Chili)

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ABSTRACT

An experiment is conducted to investigate the effects of cultural techniques on pepper yellow leaf curl disease caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV). The investigation is conducted in the area where the disease has been endemic and *Bemisia tabaci* is abundant. Four cultural techniques are applied in separate lands and cannot interfere with each other. The methods applied are seed treatment, intercropping, trap cropping, and physical barrier. Seeds harvested from infected plants are used for seed treatment experiments, and local farmers use commercial sources for other experiments. The results confirmed that PepYLCIV was a seed-borne virus affected by hot water treatment at 65°C for 30 minutes. Turmeric crude extract could reduce the incidence and severity of the disease. The tomato is a better intercrop than eggplant, mung bean, and soybean in reducing disease incidence, but their effects on disease severity and yield reduction were not significantly different. Basil and marigolds were better barrier crops compared to cosmos and zinnia. A 125 cm high physical barrier using 50 mesh cheesecloth could reduce the disease incidence, but not the lower ones. Under different cultural techniques, PepYLCIV causes a 40.00–52.32% chili yield reduction.

Keywords: *Bemisia tabaci*; Cultural technique; *Pepper yellow leaf curl Indonesia virus*; Seed treatment

INTRODUCTION

Red chili is an essential horticultural crop in Indonesia. It is cultivated almost everywhere in the country, with some provinces having become the production centers of this commodity from where most of the nation's demand is fulfilled. The eleven biggest red chili-producing provinces in 2022 were West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung, and South Sumatra, with a total production of 1,229,262 tons. The chili production varied among different provinces, and the three provinces in Java contributed 58.3% to the national production (Siregar & Suroso, 2021).

Production of red chili fluctuated due to several factors, which inevitably caused significant fluctuation in chili prices, eventually making farmers and traders uncomfortable. Plant diseases have been the main factor driving the country's yield reduction of red chili. The diseases include bacterial leaf spot, which causes yield reduction of up to 66% (Utami et al., 2022), anthracnose, which, under severe infection, might cause yield losses of up to 80% (Suprpta, 2022), fusarium which had a record to generate 40% yield losses (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), a begomovirus, which under favorable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al, 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic that appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower abortion, and discoloration (Lavanya & Arun, 2021).

Disease caused by PepYLCIV often reaches its high incidence and intensity during the dry season when the weather is favorable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn (Hemiptera: Aleyrodidae)) with an incubation period ranging from 2 to 4 weeks (Czosnek et al., 2017). The disease is increasingly frightening because it has spread to all chili production centers in the country since its first appearance in 1999 (Gaswanto et al., 2016). The virus causing yellow leaf curl on pepper was previously known as *Pepper yellow leaf curl virus* (PYLCV). However, due to its

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specification, the virus has received its name as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), which belongs to begomovirus (Fadhila et al., 2020). Begomovirus is a member of the family Geminiviridae, a big plant virus group with many members known as damaging viruses. The virus is characterized by 30 x 20 nm Gemini (twin) particles with two components of a single-stranded DNA genome inside. Geminiviridae comprises four genera based on their vector and genome organization. Members of the family transmitted persistently by white fly (*B. tabaci*) are grouped into genus Begomovirus, a bipartite virus having two components of single-stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al., 2017). In Indonesia, *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) was recognized to have two strains, i.e., PepYLCIV-Tomato, which also infects tomato, and PepYLCIV-Ageratum which also infects ageratum. Instead of infecting chili and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damage. The other crops infected by begomovirus included melon, watermelon, pepper, and eggplant (Subiastuti et al., 2019).

As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector and also able to efficiently transmit viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Singh, Singh, et al., 2020). Furthermore, the insect was also reported to infest more than 600 plant species, including tomato, watermelon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li et al., 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10-0.25 mm. The eggs are laid on a lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appearing from fertilized eggs, while males are haploid from unfertilized eggs (Xie et al., 2014).

B. tabaci has at least 43 species complex (Shah & Liu, 2013) and can transmit more than 200 plant viruses (Lu et al., 2019; MacLeod et al., 2022), and 90% of plant viruses transmitted by the vector belong to begomovirus (Kanakala & Ghanim, 2016). To transmit PepYLCIV successfully, *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8 hours (Czosnek et al., 2017). The vector remains viruliferous until 13 days after virus acquisition or until the vector dies (Roy et al., 2021).

Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However, controlling whitefly with synthetic insecticide is not economical (Patra & Kumar Hath, 2022) because insecticide can generate resistant genotypes of the vector and reduce natural enemies (Wang et al., 2020). Under such conditions, cultural control should be a better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological diversity by managing biotic and abiotic environments. The abiotic environment of the crop can be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. The biotic environment might be applied by crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian & Rezvani, 2016). Cultural control of insects has become a better alternative since most insects can evolve pesticide resistance (Basit, 2019).

Cultural controls are also crucial in managing whitefly *B. tabaci* to avoid inappropriate crop management, which may lead to serious whitefly problems and virus problems. Some cultural techniques by modifying various production practices, such as mulching, intercropping, trap cropping, and physical barriers, are reported to effectively reduce whitefly invasion into the protected areas (Li et al., 2021). The degree of whitefly exclusion might not be too significant, but it should be considered that the techniques have enough contribution to implementing integrated pest management (Lapidot et al., 2014).

Cultural practices have been implemented as parts of integrated pest management with various levels of success and failure depending on the pest and the crops in concern (Kenyon et al., 2014), and there has been no report on the effects of cultural control on the appearance and development of PepYLCIV on red chili.

The research objective is to observe the effects of seed treatments, intercropping, trap cropping, and physical barriers on the natural transmission and infection development of PepYLCIV by its vector, *B. tabaci*. All of the experiments were conducted separately, so all treatments were applied and analyzed independently and not comparable to each other.

MATERIALS AND METHODS

Study Area

Research on cultural techniques to control PepYLCIV infection in red chili was conducted in 2022. The research was experimental and consisted of four different experiments. The first experiment was a seed treatment effect on the PepYLCIV experiment, completed in the Insectarium and insect-proof screen house of the Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three experiments were field experiments on the effects of intercropping, trap cropping, and physical barriers on the natural transmission and infection of PepYLCIV. The field experiments were conducted in the experimental garden of Universitas Sriwijaya in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers' fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

Procedures

Seed Treatment Effect on PepYLCIV Experiment

Chilli fruits were harvested from infected red chili plants in the farmers' fields for seed preparation. Seeds were sorted based on size and color, and the tiny, crinkled, black seeds were set aside. The selected seeds then underwent freshwater screening, and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were not viable. No healthy seeds were used as negative control in this experiment because the experiment was carried out inside an insect-proof house to guarantee that all PepYLCIV infections were seed-borne.

The experiment was arranged in a completely randomized design with five treatments and 5 replications. The treatments were hot water, crude extract of red ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*), and freshwater as the control. The experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, the seeds were soaked in 65°C water for 30 minutes (Singh, Awasthi, et al., 2020). To make crude red ginger, crude turmeric, and crude Javanese ginger extracts, 50 g of their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to obtain the crude extracts. The treated chili seeds were dipped in the sections for 30 minutes (Kabede, et al., 2013; John et al., 2018).

The treated seeds were sown accordingly in each double tray, and the trays were then placed in an insect-proof screen box for seed germination. The seedlings germinated in each treatment unit were used to calculate the treated seeds' viability. To observe the seed-borne PepYLCIV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and all polybags were then placed in the insect-proof screen house and were arranged accordingly to completely randomized design with 4 treatments and 5 blocks where 50 plants were placed in each block as replication within each block.

Intercropping Effect on PepYLCIV Experiment

Red chili seedlings were planted experimentally under an intercropping pattern with mung bean (*Vigna radiata*), soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop as the control. The experiment was arranged in a randomized block design with five treatments and five blocks/replications, resulting in 25 experimental plots measuring 4 x 2 m, with a 1 m distance among the plots resulting in 12 red chili plants and 12 intercropping plants. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Aini et al., 2020). All seedlings were prepared from certified healthy seeds in insect-proof boxes to ensure that all PepYLCIV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PepYLCIV were infected chili plants that spread in farmers' fields around the research site.

Additionally, some infected chili plants were deliberately planted around the experimental plots. The vector of PepYLCIV was abundant in the area since the insect is polyphagous with hundreds of host species (Pym et al., 2019), and most of the vegetation in the area was a host of the vector. Data collected from this experiment included PepYLCIV disease incidence, PepYLCIV disease severity, and yield reduction caused by the disease. Data on the incubation period was not collected since it was difficult to detect when the vector of PepYLCIV arrived and inoculated the virus to the experimental plants in the plots.

Trap Cropping Effect on PepYLCIV Experiment

An experiment to investigate the effects of trap crops on the PepYLCIV infection was conducted using basil (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and with no trap crop as control. The selected trap crops belonged to refugia, which generally attract and trap invader insects (Hardiansyah et al., 2021). The experiment was arranged in a randomized block design with 5 treatments and 5 replications, resulting in 25 experimental units. The experimental unit was a 4 x 2 m plot on which red chili was planted at 60 x 40 cm spacing, resulting in 24 plants per plot.

Two layers of trap crops were planted surrounding the experimental fields at 25 cm spacing for each treatment three (3) weeks before transplanting red chili seedlings to the plots. The crops were positioned as border crops to intercept insects before they attacked the main crops (Pribadi et al., 2020). Chili seedlings used in this experiment were prepared in insect-proof boxes and those used in other field experiments. The parameters observed, and the observation method was similar to those surveyed and applied in the intercropping experiment.

Effect of a Physical Barrier on the PepYLCIV Experiment

An experiment to observe the effect of a physical barrier on the invasion of PepYLCIV vectors was conducted using cheesecloth as a physical net barrier. Cheese net was selected because it was previously used as an anti-hail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, the net had been used as a nonaggressive pest and disease control device (Grasswitz, 2019). Physical barrier covering crop cultivation with an insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji et al., 2015). The experiment was conducted in the experimental garden, where 4 x 2 m plots were made as experiment units.

In this experiment, a physical barrier using an insect net was used only as a side barrier, leaving the top open to access pollinators and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insects with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish et al., 2016). The experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Red chili was planted at 60 x 40 cm spacing, producing 24 plants per block. Four levels of physical barrier height were applied, i.e., 50, 75, 100, and 125 cm, and no side barrier as control. The net barriers were put in place before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were similar to those in intercropping and trap crop experiments.

Crop Maintenance and Observation

Crop maintenance was conducted daily to ensure that all the chili plants could grow optimally, and mechanical technique was applied to control weeds, pests, and diseases. For the experiment of seed transmission of PepYLCIV, observations were made to collect data on the incubation period, disease incidence, and disease severity. The incubation period was described as the period from seed sowing to the appearance of the first symptom of PepYLCIV. Data on chili production was not collected since the experimental plants were not grown from good seed, and the plants were not under optimal cultivation conditions. For the other experiments, where the PepYLCIV was brought in by its vector, data on the incubation period could not be measured because the entrance of the vector into the experimental plots could not be controlled. However, since PepYLCIV did not stop the host from growing and fruiting, data was collected at harvest time.

Disease severity of PepYLCIV was calculated according to disease scores described by Yadav et al. (2022) as follows: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping; and 4 = very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following formula:

$$DS = \frac{\sum nxv}{ZxN} \times 100\% \dots\dots\dots 1)$$

Where: DS = disease severity, v = disease score (0 to 4), n = number of plants showing disease score v, Z = the highest disease score, N = total number of plants observed.

Yield reduction was calculated using the following formula:

$$YR = \frac{w}{W} \times 100\% \dots\dots\dots 2)$$

Where: YR = yield reduction; w = weight of first three harvests of infected plant; W = average weight of first three harvests of healthy plants in the same plot.

Data Analysis

Data of PYLCV infections was expressed as mean ± standard deviation. ANOVA was used to analyze all collected data, and significant differences between means were determined using the Honestly Significant Difference (HSD) test with a 95% degree of significance.

RESULTS AND DISCUSSION

Effect of Seed Transmission of PepYLCIV

Pepper yellow leaf curl Indonesia virus (PepYLCIV) is a member of begomovirus suspected to be transmissible through seeds. Using seeds harvested from infected plants has resulted in low viability, ranging from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all seed treatments could not significantly increase the viability. The effects of PepYLCIV on seed viability might be direct since infection of the virus could make seeds more sensitive to deterioration, as reported by Bueso et al., 2017, who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of *Arabidopsis* seeds up to 65%. Nallathambi et al. (2020) also reported that virus infection could cause the abnormal physical function of seeds and establish itself in any part of the seed, which eventually affects their viability and potentially initiates seed-borne disease.

Some of the seedlings also produced PepYLCIV infection symptoms, confirming that PepYLCIV was a seed-borne virus. The seed transmission of begomovirus had previously been reported by Kothandaraman et al. (2016), who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus was seed-borne. A similar finding was also reported by Kil et al. (2020), who worked with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus showing the ability to spread as seed-borne virus was *Tomato yellow leaf curl virus* (TYLCV), as reported by Pérez-Padilla et al. (2020), who worked with the virus on more than 3000 tomato plants and concluded that TYLCV was seed-borne, but seed transmission was not a general property of the virus. In this experiment, the seed-borne infection of PepYLCIV was relatively high because the seed was harvested from infected plants, which potentially brought the virus, as reported by Fadhila et al. (2020), who worked with PYLCV and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and disease severity of pepper yellow leaf curl disease of red chili

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Red ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

Remarks: Means in the same column followed by different letters are significantly different at p<0.05 according to Honestly Significant Difference Test

As shown in Table 1, hot water and ginger crude extract could significantly lengthen the incubation period of PepYLCIV but had no significant effect on disease incidence and severity compared to control. Hot water treatment at 65°C did not harm the seeds, but the water's heat could reach virus particles inside the seeds and weaken parts of them, resulting in a more extended incubation period. According to Paylan et al. (2014), heated water at 65°C, together with HCl and Ozon, was a very effective treatment for reducing virus concentration in the seeds and had no adverse effect on the seeds. Even though the seed treatments affected the virus infection, seed-borne infection of the virus still occurred, indicating that the outcome could not reach the embryo where the virus usually is present. Seed-borne viruses are present in the seed coat, endosperm, nucleus, or embryo, but only viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might better affect seed-borne virus infection, but it might also affect seed germination. According to Farajollahi et al. (2014), hot water treatment could induce seed germination, but the duration of drenching the seeds in hot water could reduce seed viability. Instead of lowering seed-borne virus infection, hot water treatment was also reported to eradicate bacterial seed-borne pathogens effectively and was highly recommended for pepper, eggplant, and tomato seeds (Kim et al., 2022).

Seed-borne virus infection with the level presented in Table 1 could be categorized as dangerous and threatening because, under the presence of the virus vectors, infected seed as low as 0.1% was enough to start an endemic (Pagán, 2022). High seed-borne tobamovirus infection has also been reported by Dombrovsky & Smith (2017), who recorded a 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) also explains the high rate of seed-borne transmission of *Cucumber mosaic virus*.

All treatments in the experiment resulted in PepYLCIV infection, which confirmed that, as a member of begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed-borne nature of begomovirus has also been reported by Kothandaraman et al. (2016) who studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato plants and concluded that the virus was seed-borne but seed transmission was not a general property of the virus.

Only turmeric crude extract could significantly affect the PepYLCIV seed-borne infection using plant crude extracts in the seed treatment experiment. The antiviral effects of turmeric have previously been reported, leading to using turmeric as herbal medicine. Turmeric has been widely used as an herbal medicine. It has shown antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A virus, and Zika virus (Al Hadhrami et al., 2022); Jennings & Parks, 2020), and plant viruses such as *Cucumber mosaic virus* (Hamidson et al., 2018).

Intercropping Effect on PepYLCIV

Intercropping affected the incidence of PepYLCIV on red chili, especially when the intercropping plants were of a different family from the main crop. Mung bean, soybean (family Leguminosae), tomato, and eggplant (family Solanaceae) did not affect disease severity or yield reduction. Still, it affected disease incidence in the range of 39.30 to 71.41%, with only tomatoes causing a substantial reduction in PepYLCIV infections (Table 2)—the different effects of intercropping on disease incidence and disease severity caused by the other disease initiation. Newer infections always showed less severity than older ones. Therefore, disease severity might change when disease incidence is stagnant. Reducing viral disease under intercropping affected the decline of incoming vectors, as Mir et al. (2022) reported that intercropping could effectively control insect pests. The effect of intercropping on insect vectors might be by reducing the vector invasion to the crop due to the presence of intercrop as an alternative host in the plots, as Boudreau (2013) suggested that intercrops affected a plant disease by causing alteration of vector dispersal. Its effect on incoming vector could significantly affect tomato in reducing disease incidence since tomato has a repellent effect, especially against mosquito and aphid (Setyaningrum et al., 2023).

Research on the use of leguminous crops as intercrops has been frequent but mainly to increase the yield of main crops due to more efficient water use and better nitrogen uptake. Still, no effect on plant disease was reported. Most of the research was combinations between leguminous and food crops such as maize-mung bean (Syafuruddin & Suwardi, 2020), cotton-mung bean (Liang et al., 2020), sorghum-

mungbean (Temeche et al., 2022), sorghum-soybean (Saber, 2018), maize-soybean (Berdjour et al., 2020), and rice-soybean (Putra & Sas, 2023).

Trap Crop Effect on PepYLCIV

Infections of PepYLCIV are lower in chili plots surrounded by trap crops compared to those in control. Basil, cosmos, tagetes, and zinnia are refugia that farmers frequently plant to attract natural enemies of insect pests (Sarjan et al., 2023). Still, the four crops' effects differ when used as barrier crops. Barrier crops can affect the main crops by intercepting, arresting, or retaining pests, thereby limiting the number of insect pests and insect vectors reaching the main crop, which eventually reduces the incidence of viral disease (Waweru et al., 2021). In this experiment, basil can significantly reduce the disease incidence and severity to 72.42% and 72.83%, respectively (Table 3).

The significant effect of basil and marigold on PepYLCIV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had the repellent trait against *B. tabaci* and prevented insect oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman et al. (2018), the extract was also effective against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus*. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against mosquitoes with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$.

Marigolds also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick et al., 2020), against eggplant fruit and shoot borer *Leucinodes orbonalis* (Dikr & Belete, 2021), and thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap crop has no insect-repellent trait, it works by intercepting, arresting or retaining insects through its color (Acharya et al., 2021) or odor (Shao et al., 2021) and all Homopteran insects, but family Aphididae, are attracted to color. Three trap crops used in this experiment produced distinct colored flowers, and one species (basil) made green flowers but with a strong odor.

Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54
HSD 5%	12.18	-	-

Remarks: Means in the same column followed by different letters are significantly different at $p<0.05$ according to Honesty Significant Difference Test

Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

Remarks: Means in the same column followed by different letters are significantly different at $p<0.05$ according to Honesty Significant Difference Test

Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier height 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier height 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier height 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier height 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

Remarks: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honest Significant Difference Test

Physical Barrier Effect on PepYLCIV

Physical barrier using cheesecloth 50 mesh could reduce the infestation of PepYLCIV vectors, indicated by the reduction of disease incidence and severity up to 64.00% and 70.59%, respectively. Still, only physical barriers at 125 cm high can significantly reduce the incidence and severity of pepper yellow leaf curl disease (Table 4). This result follows that reported by [Harish et al. \(2016\)](#) that a delicate mesh barrier it is shown in the table that the higher the barrier, the lower the disease incidence caused by *Pepper yellow leaf curl virus* (PYLCV), an indication that *B. tabaci*, the only vector of PYLCV, could fly higher than 100 cm and transmitting the virus. According to Tillman (2014), the height of the barrier is significant in effectively blocking insects to infest the protected crop. In this experiment, the size of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicating that the insect could fly above such altitude even though the whitefly is not a good flyer, even though it might spread to long distances carried by wind or transported materials. The maximum distance covered by a single flying whitefly is 17 m ([Maruthi et al., 2017](#)). Still, in a dense plant population, the insect can move from plant to plant quickly, and if the insect is viruliferous, massive virus spread is inevitable. Its effect on yield reduction did not follow the significant effects of physical barriers on disease incidence and severity. This could be caused by the measurement of yield reduction, which only used the yield of the first three harvests as an indicator. If the size used the whole yield of each infected plant, the effect could be different because of different disease stages in each infected plant.

The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in blocking flying insects and had no effect on the insect behavior. This differed from barrier crops that block the flying insects and attract or repel the insects, depending on the crop species. Flying insects such as *B. tabaci* could not easily differentiate host and nonhost plants, making barrier crops more effective than net barriers ([Udiarto et al., 2023](#)). *B. tabaci* has two types of flight behavior: foraging and migratory. Foraging flight is close to the earth's surface or within the flight boundary, while migratory flight is above the boundary where the insect can be picked up and carried by air currents ([Reynolds et al., 2017](#)). The insect containing mature eggs has been trapped at 150 m above ground, which could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier could block not only *B. tabaci*, for entering the protected plot, but also other flying insects with a body width more expansive than the net's mesh size.

CONCLUSION AND SUGGESTION

The first experiment of seed-borne transmission of PepYLCIV concluded and verified that the virus infecting red chili was a seed-borne virus. Hot water treatment and crude extract of red ginger could lengthen the average incubation period of PepYLCIV up to 6.61 days and 8.02 days, respectively. The effect of turmeric crude extract was not significant on the incubation period of PepYLCIV, but it was substantial on disease incidence and severity, amounting to 66.67% and 73.13%, respectively. In the intercropping experiment, the tomato was good enough as intercrops for red chili to reduce PepYLCIV infections, which could reduce disease incidence by up to 68.34%. Basil and marigolds were functional as trap crops to protect chili from incoming *B. tabaci*. Basil could reduce the incidence and severity of disease

transmitted by the insect up to 50.87% and 55.47%, respectively, while marigolds reduce the disease incidence and severity to 72.42 and 72.83%, respectively. In the experiment of physical barrier, using 50 mesh cheesecloth as a side barrier at a height of 125 cm could reduce PepYLCIV infection frequency by up to 64.00% and disease severity by up to 70.59%. In all cultural techniques applied in the field experiments, PepYLCIV infection on red chili caused a yield reduction of 40 to 53%.

B. tabaci quickly spreads infection of PepYLCIV on red chili, and the disease is very damaging to the crop yield. Even though some cultural techniques effectively reduce PepYLCIV transmission by each vector, regular disease monitoring is essential, and destroying infected plants is necessary to eliminate virus inoculum.

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The Effects of Some Different Cultural Techniques on the Transmission and Infectious Development of *Pepper Yellow Leaf Curl Indonesia Virus* on Red Chili (Running title: Developing Virus on Red Chili)

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ABSTRACT

An experiment is conducted to investigate the effects of cultural techniques on pepper yellow leaf curl disease caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV). The investigation is conducted in the area where the disease has been endemic and *Bemisia tabaci* is abundant. Four cultural techniques are applied in separate lands and cannot interfere with each other. The methods applied are seed treatment, intercropping, trap cropping, and physical barrier. Seeds harvested from infected plants are used for seed treatment experiments, and local farmers use commercial sources for other experiments. The results confirmed that PepYLCIV was a seed-borne virus affected by hot water treatment at 65°C for 30 minutes. Turmeric crude extract could reduce the incidence and severity of the disease. The tomato is a better intercrop than eggplant, mung bean, and soybean in reducing disease incidence, but their effects on disease severity and yield reduction were not significantly different. Basil and marigolds were better barrier crops compared to cosmos and zinnia. A 125 cm high physical barrier using 50 mesh cheesecloth could reduce the disease incidence, but not the lower ones. Under different cultural techniques, PepYLCIV causes a 40.00–52.32% chili yield reduction.

Keywords: *Bemisia tabaci*; Cultural technique; *Pepper yellow leaf curl Indonesia virus*; Seed treatment

INTRODUCTION

Red chili is an essential horticultural crop in Indonesia. It is cultivated almost everywhere in the country, with some provinces having become the production centers of this commodity from where most of the nation's demand is fulfilled. The eleven biggest red chili-producing provinces in 2022 were West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung, and South Sumatra, with a total production of 1,229,262 tons. The chili production varied among different provinces, and the three provinces in Java contributed 58.3% to the national production (Siregar & Suroso, 2021).

Production of red chili fluctuated due to several factors, which inevitably caused significant fluctuation in chili prices, eventually making farmers and traders uncomfortable. Plant diseases have been the main factor driving the country's yield reduction of red chili. The diseases include bacterial leaf spot, which causes yield reduction of up to 66% (Utami et al., 2022), anthracnose, which, under severe infection, might cause yield losses of up to 80% (Suprpta, 2022), fusarium which had a record to generate 40% yield losses (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), a begomovirus, which under favorable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al, 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic that appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower abortion, and discoloration (Lavanya & Arun, 2021).

Disease caused by PepYLCIV often reaches its high incidence and intensity during the dry season when the weather is favorable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn (Hemiptera: Aleyrodidae)) with an incubation period ranging from 2 to 4 weeks (Czosnek et al., 2017). The disease is increasingly frightening because it has spread to all chili production centers in the country since its first appearance in 1999 (Gaswanto et al., 2016). The virus causing yellow leaf curl on pepper was previously known as *Pepper yellow leaf curl virus* (PYLCV). However, due to its

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specification, the virus has received its name as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), which belongs to begomovirus (Fadhila et al., 2020). Begomovirus is a member of the family Geminiviridae, a big plant virus group with many members known as damaging viruses. The virus is characterized by 30 x 20 nm Gemini (twin) particles with two components of a single-stranded DNA genome inside. Geminiviridae comprises four genera based on their vector and genome organization. Members of the family transmitted persistently by white fly (*B. tabaci*) are grouped into genus Begomovirus, a bipartite virus having two components of single-stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al., 2017). In Indonesia, *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) was recognized to have two strains, i.e., PepYLCIV-Tomato, which also infects tomato, and PepYLCIV-Ageratum which also infects ageratum. Instead of infecting chili and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damage. The other crops infected by begomovirus included melon, watermelon, pepper, and eggplant (Subiastuti et al., 2019).

As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector and also able to efficiently transmit viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Singh, Singh, et al., 2020). Furthermore, the insect was also reported to infest more than 600 plant species, including tomato, watermelon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li et al., 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10-0.25 mm. The eggs are laid on a lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appearing from fertilized eggs, while males are haploid from unfertilized eggs (Xie et al., 2014).

B. tabaci has at least 43 species complex (Shah & Liu, 2013) and can transmit more than 200 plant viruses (Lu et al., 2019; MacLeod et al., 2022), and 90% of plant viruses transmitted by the vector belong to begomovirus (Kanakala & Ghanim, 2016). To transmit PepYLCIV successfully, *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8 hours (Czosnek et al., 2017). The vector remains viruliferous until 13 days after virus acquisition or until the vector dies (Roy et al., 2021).

Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However, controlling whitefly with synthetic insecticide is not economical (Patra & Kumar Hath, 2022) because insecticide can generate resistant genotypes of the vector and reduce natural enemies (Wang et al., 2020). Under such conditions, cultural control should be a better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological diversity by managing biotic and abiotic environments. The abiotic environment of the crop can be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. The biotic environment might be applied by crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian & Rezvani, 2016). Cultural control of insects has become a better alternative since most insects can evolve pesticide resistance (Basit, 2019).

Cultural controls are also crucial in managing whitefly *B. tabaci* to avoid inappropriate crop management, which may lead to serious whitefly problems and virus problems. Some cultural techniques by modifying various production practices, such as mulching, intercropping, trap cropping, and physical barriers, are reported to effectively reduce whitefly invasion into the protected areas (Li et al., 2021). The degree of whitefly exclusion might not be too significant, but it should be considered that the techniques have enough contribution to implementing integrated pest management (Lapidot et al., 2014).

Cultural practices have been implemented as parts of integrated pest management with various levels of success and failure depending on the pest and the crops in concern (Kenyon et al., 2014), and there has been no report on the effects of cultural control on the appearance and development of PepYLCIV on red chili.

The research objective is to observe the effects of seed treatments, intercropping, trap cropping, and physical barriers on the natural transmission and infection development of PepYLCIV by its vector, *B. tabaci*. All of the experiments were conducted separately, so all treatments were applied and analyzed independently and not comparable to each other.

MATERIALS AND METHODS

Study Area

Research on cultural techniques to control PepYLCIV infection in red chili was conducted in 2022. The research was experimental and consisted of four different experiments. The first experiment was a seed treatment effect on the PepYLCIV experiment, completed in the Insectarium and insect-proof screen house of the Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three experiments were field experiments on the effects of intercropping, trap cropping, and physical barriers on the natural transmission and infection of PepYLCIV. The field experiments were conducted in the experimental garden of Universitas Sriwijaya in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers' fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

Procedures

Seed Treatment Effect on PepYLCIV Experiment

Chilli fruits were harvested from infected red chili plants in the farmers' fields for seed preparation. Seeds were sorted based on size and color, and the tiny, crinkled, black seeds were set aside. The selected seeds then underwent freshwater screening, and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were not viable. No healthy seeds were used as negative control in this experiment because the experiment was carried out inside an insect-proof house to guarantee that all PepYLCIV infections were seed-borne.

The experiment was arranged in a completely randomized design with five treatments and 5 replications. The treatments were hot water, crude extract of red ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*), and freshwater as the control. The experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, the seeds were soaked in 65°C water for 30 minutes (Singh, Awasthi, et al., 2020). To make crude red ginger, crude turmeric, and crude Javanese ginger extracts, 50 g of their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to obtain the crude extracts. The treated chili seeds were dipped in the sections for 30 minutes (Kabede, et al., 2013; John et al., 2018).

The treated seeds were sown accordingly in each double tray, and the trays were then placed in an insect-proof screen box for seed germination. The seedlings germinated in each treatment unit were used to calculate the treated seeds' viability. To observe the seed-borne PepYLCIV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and all polybags were then placed in the insect-proof screen house and were arranged accordingly to completely randomized design with 4 treatments and 5 blocks where 50 plants were placed in each block as replication within each block.

Intercropping Effect on PepYLCIV Experiment

Red chili seedlings were planted experimentally under an intercropping pattern with mung bean (*Vigna radiata*), soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop as the control. The experiment was arranged in a randomized block design with five treatments and five blocks/replications, resulting in 25 experimental plots measuring 4 x 2 m, with a 1 m distance among the plots resulting in 12 red chili plants and 12 intercropping plants. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Aini et al., 2020). All seedlings were prepared from certified healthy seeds in insect-proof boxes to ensure that all PepYLCIV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PepYLCIV were infected chili plants that spread in farmers' fields around the research site.

Additionally, some infected chili plants were deliberately planted around the experimental plots. The vector of PepYLCIV was abundant in the area since the insect is polyphagous with hundreds of host species (Pym et al., 2019), and most of the vegetation in the area was a host of the vector. Data collected from this experiment included PepYLCIV disease incidence, PepYLCIV disease severity, and yield reduction caused by the disease. Data on the incubation period was not collected since it was difficult to detect when the vector of PepYLCIV arrived and inoculated the virus to the experimental plants in the plots.

Trap Cropping Effect on PepYLCIV Experiment

An experiment to investigate the effects of trap crops on the PepYLCIV infection was conducted using basil (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and with no trap crop as control. The selected trap crops belonged to refugia, which generally attract and trap invader insects (Hardiansyah et al., 2021). The experiment was arranged in a randomized block design with 5 treatments and 5 replications, resulting in 25 experimental units. The experimental unit was a 4 x 2 m plot on which red chili was planted at 60 x 40 cm spacing, resulting in 24 plants per plot.

Two layers of trap crops were planted surrounding the experimental fields at 25 cm spacing for each treatment three (3) weeks before transplanting red chili seedlings to the plots. The crops were positioned as border crops to intercept insects before they attacked the main crops (Pribadi et al., 2020). Chili seedlings used in this experiment were prepared in insect-proof boxes and those used in other field experiments. The parameters observed, and the observation method was similar to those surveyed and applied in the intercropping experiment.

Effect of a Physical Barrier on the PepYLCIV Experiment

An experiment to observe the effect of a physical barrier on the invasion of PepYLCIV vectors was conducted using cheesecloth as a physical net barrier. Cheese net was selected because it was previously used as an anti-hail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, the net had been used as a nonaggressive pest and disease control device (Grasswitz, 2019). Physical barrier covering crop cultivation with an insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji et al., 2015). The experiment was conducted in the experimental garden, where 4 x 2 m plots were made as experiment units.

In this experiment, a physical barrier using an insect net was used only as a side barrier, leaving the top open to access pollinators and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insects with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish et al., 2016). The experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Red chili was planted at 60 x 40 cm spacing, producing 24 plants per block. Four levels of physical barrier height were applied, i.e., 50, 75, 100, and 125 cm, and no side barrier as control. The net barriers were put in place before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were similar to those in intercropping and trap crop experiments.

Crop Maintenance and Observation

Crop maintenance was conducted daily to ensure that all the chili plants could grow optimally, and mechanical technique was applied to control weeds, pests, and diseases. For the experiment of seed transmission of PepYLCIV, observations were made to collect data on the incubation period, disease incidence, and disease severity. The incubation period was described as the period from seed sowing to the appearance of the first symptom of PepYLCIV. Data on chili production was not collected since the experimental plants were not grown from good seed, and the plants were not under optimal cultivation conditions. For the other experiments, where the PepYLCIV was brought in by its vector, data on the incubation period could not be measured because the entrance of the vector into the experimental plots could not be controlled. However, since PepYLCIV did not stop the host from growing and fruiting, data was collected at harvest time.

Disease severity of PepYLCIV was calculated according to disease scores described by Yadav et al. (2022) as follows: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping; and 4 = very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following formula:

$$DS = \frac{\sum nxv}{ZxN} \times 100\% \dots\dots\dots 1)$$

Where: DS = disease severity, v = disease score (0 to 4), n = number of plants showing disease score v, Z = the highest disease score, N = total number of plants observed.

Yield reduction was calculated using the following formula:

$$YR = \frac{w}{W} \times 100\% \dots\dots\dots 2)$$

Where: YR = yield reduction; w = weight of first three harvests of infected plant; W = average weight of first three harvests of healthy plants in the same plot.

Data Analysis

Data of PYLCV infections was expressed as mean ± standard deviation. ANOVA was used to analyze all collected data, and significant differences between means were determined using the Honestly Significant Difference (HSD) test with a 95% degree of significance.

RESULTS AND DISCUSSION

Effect of Seed Transmission of PepYLCIV

Pepper yellow leaf curl Indonesia virus (PepYLCIV) is a member of begomovirus suspected to be transmissible through seeds. Using seeds harvested from infected plants has resulted in low viability, ranging from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all seed treatments could not significantly increase the viability. The effects of PepYLCIV on seed viability might be direct since infection of the virus could make seeds more sensitive to deterioration, as reported by Bueso et al., 2017, who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of *Arabidopsis* seeds up to 65%. Nallathambi et al. (2020) also reported that virus infection could cause the abnormal physical function of seeds and establish itself in any part of the seed, which eventually affects their viability and potentially initiates seed-borne disease.

Some of the seedlings also produced PepYLCIV infection symptoms, confirming that PepYLCIV was a seed-borne virus. The seed transmission of begomovirus had previously been reported by Kothandaraman et al. (2016), who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus was seed-borne. A similar finding was also reported by Kil et al. (2020), who worked with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus showing the ability to spread as seed-borne virus was *Tomato yellow leaf curl virus* (TYLCV), as reported by Pérez-Padilla et al. (2020), who worked with the virus on more than 3000 tomato plants and concluded that TYLCV was seed-borne, but seed transmission was not a general property of the virus. In this experiment, the seed-borne infection of PepYLCIV was relatively high because the seed was harvested from infected plants, which potentially brought the virus, as reported by Fadhila et al. (2020), who worked with PYLCV and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and disease severity of pepper yellow leaf curl disease of red chili

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Red ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

Remarks: Means in the same column followed by different letters are significantly different at p<0.05 according to Honestly Significant Difference Test

As shown in Table 1, hot water and ginger crude extract could significantly lengthen the incubation period of PepYLCIV but had no significant effect on disease incidence and severity compared to control. Hot water treatment at 65°C did not harm the seeds, but the water's heat could reach virus particles inside the seeds and weaken parts of them, resulting in a more extended incubation period. According to Paylan et al. (2014), heated water at 65°C, together with HCl and Ozon, was a very effective treatment for reducing virus concentration in the seeds and had no adverse effect on the seeds. Even though the seed treatments affected the virus infection, seed-borne infection of the virus still occurred, indicating that the outcome could not reach the embryo where the virus usually is present. Seed-borne viruses are present in the seed coat, endosperm, nucleus, or embryo, but only viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might better affect seed-borne virus infection, but it might also affect seed germination. According to Farajollahi et al. (2014), hot water treatment could induce seed germination, but the duration of drenching the seeds in hot water could reduce seed viability. Instead of lowering seed-borne virus infection, hot water treatment was also reported to eradicate bacterial seed-borne pathogens effectively and was highly recommended for pepper, eggplant, and tomato seeds (Kim et al., 2022).

Seed-borne virus infection with the level presented in Table 1 could be categorized as dangerous and threatening because, under the presence of the virus vectors, infected seed as low as 0.1% was enough to start an endemic (Pagán, 2022). High seed-borne tobamovirus infection has also been reported by Dombrovsky & Smith (2017), who recorded a 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) also explains the high rate of seed-borne transmission of *Cucumber mosaic virus*.

All treatments in the experiment resulted in PepYLCIV infection, which confirmed that, as a member of begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed-borne nature of begomovirus has also been reported by Kothandaraman et al. (2016) who studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported that *Tomato yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato plants and concluded that the virus was seed-borne but seed transmission was not a general property of the virus.

Only turmeric crude extract could significantly affect the PepYLCIV seed-borne infection using plant crude extracts in the seed treatment experiment. The antiviral effects of turmeric have previously been reported, leading to using turmeric as herbal medicine. Turmeric has been widely used as an herbal medicine. It has shown antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A virus, and Zika virus (Al Hadhrami et al., 2022); Jennings & Parks, 2020), and plant viruses such as *Cucumber mosaic virus* (Hamidson et al., 2018).

Intercropping Effect on PepYLCIV

Intercropping affected the incidence of PepYLCIV on red chili, especially when the intercropping plants were of a different family from the main crop. Mung bean, soybean (family Leguminosae), tomato, and eggplant (family Solanaceae) did not affect disease severity or yield reduction. Still, it affected disease incidence in the range of 39.30 to 71.41%, with only tomatoes causing a substantial reduction in PepYLCIV infections (Table 2)—the different effects of intercropping on disease incidence and disease severity caused by the other disease initiation. Newer infections always showed less severity than older ones. Therefore, disease severity might change when disease incidence is stagnant. Reducing viral disease under intercropping affected the decline of incoming vectors, as Mir et al. (2022) reported that intercropping could effectively control insect pests. The effect of intercropping on insect vectors might be by reducing the vector invasion to the crop due to the presence of intercrop as an alternative host in the plots, as Boudreau (2013) suggested that intercrops affected a plant disease by causing alteration of vector dispersal. Its effect on incoming vector could significantly affect tomato in reducing disease incidence since tomato has a repellent effect, especially against mosquito and aphid (Setyaningrum et al., 2023).

Research on the use of leguminous crops as intercrops has been frequent but mainly to increase the yield of main crops due to more efficient water use and better nitrogen uptake. Still, no effect on plant disease was reported. Most of the research was combinations between leguminous and food crops such as maize-mung bean (Syafuruddin & Suwardi, 2020), cotton-mung bean (Liang et al., 2020), sorghum-

mungbean (Temeche et al., 2022), sorghum-soybean (Saber, 2018), maize-soybean (Berdjour et al., 2020), and rice-soybean (Putra & Sas, 2023).

Trap Crop Effect on PepYLCIV

Infections of PepYLCIV are lower in chili plots surrounded by trap crops compared to those in control. Basil, cosmos, tagetes, and zinnia are refugia that farmers frequently plant to attract natural enemies of insect pests (Sarjan et al., 2023). Still, the four crops' effects differ when used as barrier crops. Barrier crops can affect the main crops by intercepting, arresting, or retaining pests, thereby limiting the number of insect pests and insect vectors reaching the main crop, which eventually reduces the incidence of viral disease (Waweru et al., 2021). In this experiment, basil can significantly reduce the disease incidence and severity to 72.42% and 72.83%, respectively (Table 3).

The significant effect of basil and marigold on PepYLCIV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had the repellent trait against *B. tabaci* and prevented insect oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman et al. (2018), the extract was also effective against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus*. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against mosquitoes with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$.

Marigolds also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick et al., 2020), against eggplant fruit and shoot borer *Leucinodes orbonalis* (Dikr & Belete, 2021), and thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap crop has no insect-repellent trait, it works by intercepting, arresting or retaining insects through its color (Acharya et al., 2021) or odor (Shao et al., 2021) and all Homopteran insects, but family Aphididae, are attracted to color. Three trap crops used in this experiment produced distinct colored flowers, and one species (basil) made green flowers but with a strong odor.

Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54
HSD 5%	12.18	-	-

Remarks: Means in the same column followed by different letters are significantly different at $p<0.05$ according to Honesty Significant Difference Test

Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

Remarks: Means in the same column followed by different letters are significantly different at $p<0.05$ according to Honesty Significant Difference Test

Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier height 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier height 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier height 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier height 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

Remarks: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honest Significant Difference Test

Physical Barrier Effect on PepYLCIV

Physical barrier using cheesecloth 50 mesh could reduce the infestation of PepYLCIV vectors, indicated by the reduction of disease incidence and severity up to 64.00% and 70.59%, respectively. Still, only physical barriers at 125 cm high can significantly reduce the incidence and severity of pepper yellow leaf curl disease (Table 4). This result follows that reported by [Harish et al. \(2016\)](#) that a delicate mesh barrier it is shown in the table that the higher the barrier, the lower the disease incidence caused by *Pepper yellow leaf curl virus* (PYLCV), an indication that *B. tabaci*, the only vector of PYLCV, could fly higher than 100 cm and transmitting the virus. According to Tillman (2014), the height of the barrier is significant in effectively blocking insects to infest the protected crop. In this experiment, the size of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicating that the insect could fly above such altitude even though the whitefly is not a good flyer, even though it might spread to long distances carried by wind or transported materials. The maximum distance covered by a single flying whitefly is 17 m ([Maruthi et al., 2017](#)). Still, in a dense plant population, the insect can move from plant to plant quickly, and if the insect is viruliferous, massive virus spread is inevitable. Its effect on yield reduction did not follow the significant effects of physical barriers on disease incidence and severity. This could be caused by the measurement of yield reduction, which only used the yield of the first three harvests as an indicator. If the size used the whole yield of each infected plant, the effect could be different because of different disease stages in each infected plant.

The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in blocking flying insects and had no effect on the insect behavior. This differed from barrier crops that block the flying insects and attract or repel the insects, depending on the crop species. Flying insects such as *B. tabaci* could not easily differentiate host and nonhost plants, making barrier crops more effective than net barriers ([Udiarto et al., 2023](#)). *B. tabaci* has two types of flight behavior: foraging and migratory. Foraging flight is close to the earth's surface or within the flight boundary, while migratory flight is above the boundary where the insect can be picked up and carried by air currents ([Reynolds et al., 2017](#)). The insect containing mature eggs has been trapped at 150 m above ground, which could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier could block not only *B. tabaci*, for entering the protected plot, but also other flying insects with a body width more expansive than the net's mesh size.

CONCLUSION AND SUGGESTION

The first experiment of seed-borne transmission of PepYLCIV concluded and verified that the virus infecting red chili was a seed-borne virus. Hot water treatment and crude extract of red ginger could lengthen the average incubation period of PepYLCIV up to 6.61 days and 8.02 days, respectively. The effect of turmeric crude extract was not significant on the incubation period of PepYLCIV, but it was substantial on disease incidence and severity, amounting to 66.67% and 73.13%, respectively. In the intercropping experiment, the tomato was good enough as intercrops for red chili to reduce PepYLCIV infections, which could reduce disease incidence by up to 68.34%. Basil and marigolds were functional as trap crops to protect chili from incoming *B. tabaci*. Basil could reduce the incidence and severity of disease

transmitted by the insect up to 50.87% and 55.47%, respectively, while marigolds reduce the disease incidence and severity to 72.42 and 72.83%, respectively. In the experiment of physical barrier, using 50 mesh cheesecloth as a side barrier at a height of 125 cm could reduce PepYLCIV infection frequency by up to 64.00% and disease severity by up to 70.59%. In all cultural techniques applied in the field experiments, PepYLCIV infection on red chili caused a yield reduction of 40 to 53%.

B. tabaci quickly spreads infection of PepYLCIV on red chili, and the disease is very damaging to the crop yield. Even though some cultural techniques effectively reduce PepYLCIV transmission by each vector, regular disease monitoring is essential, and destroying infected plants is necessary to eliminate virus inoculum.

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The Effects of Some Different Cultural Techniques on the Transmission and Infectious Development of *Pepper Yellow Leaf Curl Indonesia Virus* on Red Chili

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ABSTRACT

An experiment is conducted to investigate the effects of cultural techniques on pepper yellow leaf curl disease caused by Pepper yellow leaf curl Indonesia virus (PepYLCIV). The investigation is conducted in the area where the disease has been endemic and *Bemisia tabaci* is abundant. Four cultural techniques are applied in separate lands and cannot interfere with each other. The methods applied are seed treatment, intercropping, trap cropping, and physical barrier. Seeds harvested from infected plants are used for seed treatment experiments, and local farmers use commercial sources for other experiments. The results confirmed that PepYLCIV was a seed-borne virus affected by hot water treatment at 65°C for 30 minutes. Turmeric crude extract could reduce the incidence and severity of the disease. The tomato is a better intercrop than eggplant, mung bean, and soybean in reducing disease incidence, but their effects on disease severity and yield reduction were not significantly different. Basil and marigolds were better barrier crops compared to cosmos and zinnia. A 125 cm high physical barrier using 50 mesh cheesecloth could reduce the disease incidence, but not the lower ones. Under different cultural techniques, PepYLCIV causes a 40.00–52.32% chili yield reduction.

INTRODUCTION

Red chili is an essential horticultural crop in Indonesia. It is cultivated almost everywhere in the country, with some provinces having become the production centers of this commodity from where most of the nation's demand is fulfilled. The eleven biggest red chili-producing provinces in 2022 were West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung, and South Sumatra, with a total production of 1,229,262 tons. The chili production varied among different provinces, and the three provinces in Java contributed 58.3% to the national production (Siregar & Suroso, 2021).

Production of red chili fluctuated due to several factors, which inevitably caused significant fluctuation in chili prices, eventually making farmers and traders uncomfortable. Plant diseases have been the main

factor driving the country's yield reduction of red chili. The diseases include bacterial leaf spot, which causes yield reduction of up to 66% (Utami et al., 2022), anthracnose, which, under severe infection, might cause yield losses of up to 80% (Suprpta, 2022), fusarium which had a record to generate 40% yield losses (Parihar et al., 2022), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), a begomovirus, which under favorable condition caused yield losses 20 to 100% in Indonesia (Fadhila et al, 2020). Symptoms of diseases caused by begomovirus are obvious and dominated by yellowing or yellow mosaic that appears on infected leaves. Other symptoms include internode shortening, stunting, leaf curling, smaller fruit, flower abortion, and discoloration (Lavanya & Arun, 2021).

Disease caused by PepYLCIV often reaches its high incidence and intensity during the dry season

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when the weather is favorable to the virus and its vector. The virus is transmitted persistently by whitefly (*Bemisia tabaci* Genn (Hemiptera: Aleyrodidae)) with an incubation period ranging from 2 to 4 weeks (Czosnek et al., 2017). The disease is increasingly frightening because it has spread to all chili production centers in the country since its first appearance in 1999 (Gaswanto et al., 2016). The virus causing yellow leaf curl on pepper was previously known as *Pepper yellow leaf curl virus* (PYLCV). However, due to its specification, the virus has received its name as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), which belongs to begomovirus (Fadhila et al., 2020). Begomovirus is a member of the family Geminiviridae, a big plant virus group with many members known as damaging viruses. The virus is characterized by 30 x 20 nm Gemini (twin) particles with two components of a single-stranded DNA genome inside. Geminiviridae comprises four genera based on their vector and genome organization. Members of the family transmitted persistently by white fly (*B. tabaci*) are grouped into genus Begomovirus, a bipartite virus having two components of single-stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek et al., 2017). In Indonesia, *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) was recognized to have two strains, i.e., PepYLCIV-Tomato, which also infects tomato, and PepYLCIV-Ageratum which also infects ageratum. Instead of infecting chili and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damage. The other crops infected by begomovirus included melon, watermelon, pepper, and eggplant (Subiastuti et al., 2019).

As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector and also able to efficiently transmit viruses such as *Pepper yellow leaf curl virus*, *Lettuce infectious yellows virus*, *Tomato yellow leaf curl virus*, *African cassava mosaic virus*, and *Cassava brown streak virus* (Singh, Singh, et al., 2020). Furthermore, the insect was also reported to infest more than 600 plant species, including tomato, watermelon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li et al., 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10-0.25 mm. The eggs are laid on a lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appearing from fertilized eggs, while males are haploid from unfertilized eggs (Xie et al., 2014).

B. tabaci has at least 43 species complex (Shah & Liu, 2013) and can transmit more than 200 plant viruses (Lu et al., 2019; MacLeod et al., 2022), and 90% of plant viruses transmitted by the vector belong to *begomovirus* (Kanakala & Ghanim, 2016). To transmit PepYLCIV successfully, *B. tabaci* requires an acquisition period of at least 90 minutes and a latent period of at least 8 hours (Czosnek et al., 2017). The vector remains viruliferous until 13 days after virus acquisition or until the vector dies (Roy et al., 2021).

Controlling plant viruses is mostly only possible by controlling their vectors using insecticide. However, controlling whitefly with synthetic insecticide is not economical (Patra & Kumar Hath, 2022) because insecticide can generate resistant genotypes of the vector and reduce natural enemies (Wang et al., 2020). Under such conditions, cultural control should be a better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological diversity by managing biotic and abiotic environments. The abiotic environment of the crop can be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. The biotic environment might be applied by crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian & Rezvani, 2016). Cultural control of insects has become a better alternative since most insects can evolve pesticide resistance (Basit, 2019).

Cultural controls are also crucial in managing whitefly *B. tabaci* to avoid inappropriate crop management, which may lead to serious whitefly problems and virus problems. Some cultural techniques by modifying various production practices, such as mulching, intercropping, trap cropping, and physical barriers, are reported to effectively reduce whitefly invasion into the protected areas (Li et al., 2021). The degree of whitefly exclusion might not be too significant, but it should be considered that the techniques have enough contribution to implementing integrated pest management (Lapidot et al., 2014).

Cultural practices have been implemented as parts of integrated pest management with various levels of success and failure depending on the pest and the crops in concern (Kenyon et al., 2014), and there has been no report on the effects of cultural control on the appearance and development of PepYLCIV on red chili.

The research objective is to observe the effects of seed treatments, intercropping, trap cropping, and physical barriers on the natural transmission and

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infection development of PepYLCIV by its vector, *B. tabaci*. All of the experiments were conducted separately, so all treatments were applied and analyzed independently and not comparable to each other.

MATERIALS AND METHODS

Study Area

Research on cultural techniques to control PepYLCIV infection in red chili was conducted in 2022. The research was experimental and consisted of four different experiments. The first experiment was a seed treatment effect on the PepYLCIV experiment, completed in the Insectarium and insect-proof screen house of the Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three experiments were field experiments on the effects of intercropping, trap cropping, and physical barriers on the natural transmission and infection of PepYLCIV. The field experiments were conducted in the experimental garden of Universitas Sriwijaya in District Indralaya, Ogan Ilir, South Sumatra, surrounded by local farmers' fields where pepper yellow leaf curl disease has been an endemic and *B. tabaci* was abundant.

Procedures

Seed Treatment Effect on PepYLCIV

Experiment

Chilli fruits were harvested from infected red chili plants in the farmers' fields for seed preparation. Seeds were sorted based on size and color, and the tiny, crinkled, black seeds were set aside. The selected seeds then underwent freshwater screening, and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were not viable. No healthy seeds were used as negative control in this experiment because the experiment was carried out inside an insect-proof house to guarantee that all PepYLCIV infections were seed-borne.

The experiment was arranged in a completely randomized design with five treatments and 5 replications. The treatments were hot water, crude extract of red ginger (*Zingiber officinale*), crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*), and freshwater as the control. The experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, the seeds were soaked in 65°C water for 30 minutes (Singh, Awasthi, et al.,

2020). To make crude red ginger, crude turmeric, and crude Javanese ginger extracts, 50 g of their rhizomes were separately blended in 250 ml water for 3 minutes and filtered through double cheesecloth to obtain the crude extracts. The treated chili seeds were dipped in the sections for 30 minutes (Kabede, et al., 2013); John et al., 2018).

The treated seeds were sown accordingly in each double tray, and the trays were then placed in an insect-proof screen box for seed germination. The seedlings germinated in each treatment unit were used to calculate the treated seeds' viability. To observe the seed-borne PepYLCIV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and all polybags were then placed in the insect-proof screen house and were arranged accordingly to completely randomized design with 4 treatments and 5 blocks where 50 plants were placed in each block as replication within each block.

Intercropping Effect on PepYLCIV Experiment

Red chili seedlings were planted experimentally under an intercropping pattern with mung bean (*Vigna radiata*), soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop as the control. The experiment was arranged in a randomized block design with five treatments and five blocks/replications, resulting in 25 experimental plots measuring 4 x 2 m, with a 1 m distance among the plots resulting in 12 red chili plants and 12 intercropping plants. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Aini et al., 2020) Brondong Sub-District of Lamongan Regency, Province of East Java. The research was performed with the aim of examining and obtaining appropriate combinations of plant spacing and planting model for red chili (*Capsicum annum* L.). All seedlings were prepared from certified healthy seeds in insect-proof boxes to ensure that all PepYLCIV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PepYLCIV were infected chili plants that spread in farmers' fields around the research site.

Additionally, some infected chili plants were deliberately planted around the experimental plots. The vector of PepYLCIV was abundant in the area since the insect is polyphagous with hundreds of host species (Pym et al., 2019), and most of the vegetation in the area was a host of the vector. Data collected from this experiment included PepYLCIV

disease incidence, PepYLCIV disease severity, and yield reduction caused by the disease. Data on the incubation period was not collected since it was difficult to detect when the vector of PepYLCIV arrived and inoculated the virus to the experimental plants in the plots.

Trap Cropping Effect on PepYLCIV Experiment

An experiment to investigate the effects of trap crops on the PepYLCIV infection was conducted using basil (*Ocimum basilicum*), cosmos (*Cosmos caudatus*), marigold (*Tagetes erecta*), and zinnia (*Zinnia elegans*), and with no trap crop as control. The selected trap crops belonged to refugia, which generally attract and trap invader insects (Hardiansyah et al., 2021). The experiment was arranged in a randomized block design with 5 treatments and 5 replications, resulting in 25 experimental units. The experimental unit was a 4 x 2 m plot on which red chili was planted at 60 x 40 cm spacing, resulting in 24 plants per plot.

Two layers of trap crops were planted surrounding the experimental fields at 25 cm spacing for each treatment three (3) weeks before transplanting red chili seedlings to the plots. The crops were positioned as border crops to intercept insects before they attacked the main crops (Pribadi et al., 2020). Chili seedlings used in this experiment were prepared in insect-proof boxes and those used in other field experiments. The parameters observed, and the observation method was similar to those surveyed and applied in the intercropping experiment.

Effect of a Physical Barrier on the PepYLCIV Experiment

An experiment to observe the effect of a physical barrier on the invasion of PepYLCIV vectors was conducted using cheesecloth as a physical net barrier. Cheese net was selected because it was previously used as an anti-hail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, the net had been used as a nonaggressive pest and disease control device (Grasswitz, 2019) and have an acknowledged role in providing other biological and societal benefits, including the conservation of agricultural biodiversity and enhancement of local food security. Despite this, the small-farm sector is currently underserved in relation to the development and implementation of scale-appropriate Integrated Pest Management

(IPM). Physical barrier covering crop cultivation with an insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji et al., 2015). The experiment was conducted in the experimental garden, where 4 x 2 m plots were made as experiment units.

In this experiment, a physical barrier using an insect net was used only as a side barrier, leaving the top open to access pollinators and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insects with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish et. al., 2016). The experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Red chili was planted at 60 x 40 cm spacing, producing 24 plants per block. Four levels of physical barrier height were applied, i.e., 50, 75, 100, and 125 cm, and no side barrier as control. The net barriers were put in place before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were similar to those in intercropping and trap crop experiments.

Crop Maintenance and Observation

Crop maintenance was conducted daily to ensure that all the chili plants could grow optimally, and mechanical technique was applied to control weeds, pests, and diseases. For the experiment of seed transmission of PepYLCIV, observations were made to collect data on the incubation period, disease incidence, and disease severity. The incubation period was described as the period from seed sowing to the appearance of the first symptom of PepYLCIV. Data on chili production was not collected since the experimental plants were not grown from good seed, and the plants were not under optimal cultivation conditions. For the other experiments, where the PepYLCIV was brought in by its vector, data on the incubation period could not be measured because the entrance of the vector into the experimental plots could not be controlled. However, since PepYLCIV did not stop the host from growing and fruiting, data was collected at harvest time.

Disease severity of PepYLCIV was calculated according to disease scores described by Yadav et al. (2022) as follows: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling

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and cupping; and 4 = very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following formula:

$$DS = \frac{\sum nxv}{ZxN} \times 100\% \quad \dots\dots\dots 1)$$

Where: DS = disease severity, v = disease score (0 to 4), n = number of plants showing disease score v, Z = the highest disease score, N = total number of plants observed.

Yield reduction was calculated using the following formula:

$$YR = \frac{w}{W} \times 100\% \quad \dots\dots\dots 2)$$

Where: YR = yield reduction; w = weight of first three harvests of infected plant; W = average weight of first three harvests of healthy plants in the same plot.

Data Analysis

Data of PYLCV infections was expressed as mean \pm standard deviation. ANOVA was used to analyze all collected data, and significant differences between means were determined using the Honestly Significant Difference (HSD) test with a 95% degree of significance.

RESULTS AND DISCUSSION

Effect of Seed Transmission of PepYLCIV

Pepper yellow leaf curl Indonesia virus (PepYLCIV) is a member of begomovirus suspected to be transmissible through seeds. Using seeds harvested from infected plants has resulted in low viability, ranging from 43% to 59% (Table 1). Even though the seeds have been sorted before being treated, all seed treatments could not significantly increase the viability. The effects of PepYLCIV on seed viability might be direct since infection of the virus could make seeds more sensitive to deterioration, as reported by Bueso *et al.*, 2017, who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of *Arabidopsis* seeds up to 65%. Nallathambi *et al.* (2020) also reported that virus infection could cause the abnormal physical function of seeds and establish itself in any part of the seed, which eventually affects their viability and potentially initiates seed-borne disease.

Some of the seedlings also produced PepYLCIV infection symptoms, confirming that PepYLCIV was a seed-borne virus. The seed transmission of begomovirus had previously been reported by Kothandaraman *et al.* (2016), who studied the seed transmission of *Mungbean yellow mosaic virus* (MYMV), and concluded that the virus

was seed-borne. A similar finding was also reported by Kil *et al.* (2020), who worked with *Tomato yellow leaf curl New Delhi virus* (ToLCNDV) and found that the virus was also seed transmissible. Another begomovirus showing the ability to spread as seed-borne virus was *Tomato yellow leaf curl virus* (TYLCV), as reported by Pérez-Padilla *et al.* (2020), who worked with the virus on more than 3000 tomato plants and concluded that TYLCV was seed-borne, but seed transmission was not a general property of the virus. In this experiment, the seed-borne infection of PepYLCIV was relatively high because the seed was harvested from infected plants, which potentially brought the virus, as reported by Fadhila *et al.* (2020), chili pepper (*Capsicum annum*) who worked with PYLCV and could detect DNA of the virus in 67-100% of seedlings grown from seeds harvested from infected plants.

As shown in Table 1, hot water and ginger crude extract could significantly lengthen the incubation period of PepYLCIV but had no significant effect on disease incidence and severity compared to control. Hot water treatment at 65°C did not harm the seeds, but the water's heat could reach virus particles inside the seeds and weaken parts of them, resulting in a more extended incubation period. According to Paylan *et al.* (2014), heated water at 65°C, together with HCl and Ozon, was a very effective treatment for reducing virus concentration in the seeds and had no adverse effect on the seeds. Even though the seed treatments affected the virus infection, seed-borne infection of the virus still occurred, indicating that the outcome could not reach the embryo where the virus usually is present. Seed-borne viruses are present in the seed coat, endosperm, nucleus, or embryo, but only viruses present in the seed embryo can be transmitted to the next generation. For hot water treatment, the effect could be correlated not only to the temperature but also to the dipping time. Longer dipping time might better affect seed-borne virus infection, but it might also affect seed germination. According to Farajollahi *et al.* (2014), hot water treatment could induce seed germination, but the duration of drenching the seeds in hot water could reduce seed viability. Instead of lowering seed-borne virus infection, hot water treatment was also reported to eradicate bacterial seed-borne pathogens effectively and was highly recommended for pepper, eggplant, and tomato seeds (Kim *et al.*, 2022).

Seed-borne virus infection with the level presented in Table 1 could be categorized as

dangerous and threatening because, under the presence of the virus vectors, infected seed as low as 0.1% was enough to start an endemic (Pagán, 2022). High seed-borne tobamovirus infection has also been reported by Dombrovsky & Smith (2017), who recorded a 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) also explains the high rate of seed-borne transmission of *Cucumber mosaic virus*.

All treatments in the experiment resulted in PepYLCIV infection, which confirmed that, as a member of begomovirus, the virus is transmitted vertically from generation to generation of the host plant. The seed-borne nature of begomovirus has also been reported by Kothandaraman et al. (2016) who studied the seed transmission of *Mungbean yellow mosaic virus*, by Kil et al. (2020) who reported

that *Tomato yellow leaf curl New Delhi virus*, and by Pérez-Padilla et al. (2020) who worked with more than 3000 tomato plants and concluded that the virus was seed-borne but seed transmission was not a general property of the virus.

Only turmeric crude extract could significantly affect the PepYLCIV seed-borne infection using plant crude extracts in the seed treatment experiment. The antiviral effects of turmeric have previously been reported, leading to using turmeric as herbal medicine. Turmeric has been widely used as an herbal medicine. It has shown antiviral activities against human viruses such as herpes simplex virus, dengue virus, influenza A virus, and Zika virus (Al Hadhrami et al., 2022); Jennings & Parks, 2020), and plant viruses such as *Cucumber mosaic virus* (Hamidson et al., 2018).

Table 1. Effects of seed treatments on red chili seed viability and on incubation period, disease incidence and disease severity of pepper yellow leaf curl disease of red chili

Seed treatment	Seed viability (%)	Pepper Yellow Leaf Curl Disease		
		Incubation period (day)	Disease incidence (%)	Disease severity (%)
Hot water	58.90±1.68	29.45±0.54 b	6.80±0.77 ab	4.10±0.60 ab
Red ginger crude extract	54.00±1.87	30.86±1.09 b	8.40±1.30 ab	4.90±0.82 ab
Turmeric crude extract	51.17±2.29	21.40±1.02 a	3.20±0.37 a	1.80±0.26 a
Javanese ginger crude extract	51.74±3.03	23.84±1.61 a	5.60±0.74 ab	4.00±0.49 ab
Control	43.66±3.11	22.84±1.06 a	9.60±0.57b	6.70±0.41 b
F Calculated	2.76 ^{ns}	12.12*	1.94*	2.14*
P Value	0.08	0.001	0.04	0.02
HSD 5%		5.15	5.61	3.56

Remarks: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty Significant Difference Test

Table 2. Effects of intercropping incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Intercrop	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Eggplant	16.18±2.35 ab	10.95±1.04	47.19±2.29
Mung bean	15.23±2.39 ab	9.52±1.58	49.22±2.20
Soybean	14.58±1.97 ab	8.57±1.25	46.41±3.27
Tomato	7.62±0.88 a	4.52±0.66	40.00±1.47
Control	26.66±0.88 b	14.28±1.10	46.26±2.04
F Calculated	5.74*	3.44 ^{ns}	0.82 ^{ns}
P Value	0.01	0.05	0.54
HSD 5%	12.18	-	-

Remarks: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty Significant Difference Test

Intercropping Effect on PepYLCIV

Intercropping affected the incidence of PepYLCIV on red chili, especially when the intercropping plants were of a different family from the main crop. Mung bean, soybean (family Leguminosae), tomato, and eggplant (family Solanaceae) did not affect disease severity or yield reduction. Still, it affected disease incidence in the range of 39.30 to 71.41%, with only tomatoes causing a substantial reduction in PepYLCIV infections (Table 2)—the different effects of intercropping on disease incidence and disease severity caused by the other disease initiation. Newer infections always showed less severity than older ones. Therefore, disease severity might change when disease incidence is stagnant. Reducing viral disease under intercropping affected the decline of incoming vectors, as Mir et al. (2022) reported that intercropping could effectively control insect pests. The effect of intercropping on insect vectors might be by reducing the vector invasion to the crop due to the presence of intercrop as an alternative host in the plots, as Boudreau (2013) suggested that intercrops affected a plant disease by causing alteration of vector dispersal. Its effect on incoming vector could significantly affect tomato in reducing disease incidence since tomato has a repellent effect, especially against mosquito and aphid (Setyaningrum et al., 2023).

Research on the use of leguminous crops as intercrops has been frequent but mainly to increase the yield of main crops due to more efficient water use and better nitrogen uptake. Still, no effect on plant disease was reported. Most of the research was combinations between leguminous and food crops such as maize-

mung bean (Syafuruddin & Suwardi, 2020), cotton-mung bean (Liang et al., 2020), sorghum-mungbean (Temeche et al., 2022), sorghum-soybean (Saber, 2018), maize-soybean (Berdjour et al., 2020), and rice-soybean (Putra & Sas, 2023).

Trap Crop Effect on PepYLCIV

Infections of PepYLCIV are lower in chili plots surrounded by trap crops compared to those in control. Basil, cosmos, tagetes, and zinnia are refugia that farmers frequently plant to attract natural enemies of insect pests (Sarjan et al., 2023). Still, the four crops' effects differ when used as barrier crops. Barrier crops can affect the main crops by intercepting, arresting, or retaining pests, thereby limiting the number of insect pests and insect vectors reaching the main crop, which eventually reduces the incidence of viral disease (Waweru et al., 2021). In this experiment, basil can significantly reduce the disease incidence and severity to 72.42% and 72.83%, respectively (Table 3).

The significant effect of basil and marigold on PepYLCIV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had the repellent trait against *B. tabaci* and prevented insect oviposition. Husna et al. (2020) reported that crude extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes aegypti* larvae, and according to Purushothaman et al. (2018), the extract was also effective against *Culex tritaeniorhynchus*, *Aedesal bopictus* and *Anopheles subpictuat*. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against mosquitoes with $LC_{50}=0.97\%$ and $LC_{90}=1.42\%$.

Table 3. Effects of trap cropping on incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Trap crop	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Basil	8.14±1.36 a	5.85±0.86 a	50.21±2.41
Cosmos	10.28±1.29 ab	7.12±0.57 ab	48.41±1.54
Marigold	4.57±0.53 a	3.57±0.38 a	42.80±1.22
Zinnia	13.71±1.59 ab	11.28±1.31 ab	47.32±1.35
control	16.57±0.81 b	13.14±1.00 b	52.01±1.62
F Calculated	5.51*	7.70*	1.50 ^{ns}
P Value	0.01	0.003	0.27
HSD 5%	8.41	6.06	-

Remarks: Means in the same column followed by different letters are significantly different at $p<0.05$ according to Honesty Significant Difference Test

Marigolds also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick et al., 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Dikr & Belete, 2021), and thrips *Megalurothrips sjostedti* Trybom (Diabate et al., 2019). When a trap crop has no insect-repellent trait, it works by intercepting, arresting or retaining insects through its color (Acharya et al., 2021) or odor (Shao et al., 2021) and all Homopteran insects, but family Aphididae, are attracted to color. Three trap crops used in this experiment produced distinct colored flowers, and one species (basil) made green flowers but with a strong odor.

Physical Barrier Effect on PepYLCIV

Physical barrier using cheesecloth 50 mesh could reduce the infestation of PepYLCIV vectors, indicated by the reduction of disease incidence and severity up to 64.00% and 70.59%, respectively. Still, only physical barriers at 125 cm high can significantly reduce the incidence and severity of pepper yellow leaf curl disease (Table 4). This result follows that reported by Harish et al. (2016) that a delicate mesh barrier It is shown in the table that the higher the barrier, the lower the disease incidence caused by *Pepper yellow leaf curl virus* (PYLCV), an indication that *B. tabaci*, the only vector of PYLCV, could fly higher than 100 cm and transmitting the virus. According to Tillman (2014), the height of the barrier is significant in effectively blocking insects to infest the protected crop. In this experiment, the size of 125 cm could significantly reduce the number of *B. tabaci* but not totally, indicating that the insect could fly above such altitude even though the whitefly is

not a good flyer, even though it might spread to long distances carried by wind or transported materials. The maximum distance covered by a single flying whitefly is 17 m (Maruthi et al., 2017). Still, in a dense plant population, the insect can move from plant to plant quickly, and if the insect is viruliferous, massive virus spread is inevitable. Its effect on yield reduction did not follow the significant effects of physical barriers on disease incidence and severity. This could be caused by the measurement of yield reduction, which only used the yield of the first three harvests as an indicator. If the size used the whole yield of each infected plant, the effect could be different because of different disease stages in each infected plant.

The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in blocking flying insects and had no effect on the insect behavior. This differed from barrier crops that block the flying insects and attract or repel the insects, depending on the crop species. Flying insects such as *B. tabaci* could not easily differentiate host and nonhost plants, making barrier crops more effective than net barriers (Udiarto et al., 2023). *B. tabaci* has two types of flight behavior: foraging and migratory. Foraging flight is close to the earth's surface or within the flight boundary, while migratory flight is above the boundary where the insect can be picked up and carried by air currents (Reynolds et al., 2017). The insect containing mature eggs has been trapped at 150 m above ground, which could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier could block not only *B. tabaci*, for entering the protected plot, but also other flying insects with a body width more expansive than the net's mesh size.

Table 4. Effects of side barrier on incidence and severity of pepper yellow leaf curl disease of red chili and yield reduction caused by the disease

Treatment	Pepper yellow leaf curl disease		
	Disease incidence (%)	Disease severity (%)	Yield reduction (%)
Side barrier height 50 cm	11.42±1.53 ab	8.71±0.86 bc	49.87±0.85
Side barrier height 75 cm	9.71±0.52 ab	8.00±0.47 abc	47.42±1.41
Side barrier height 100 cm	8.00±1.26 ab	6.57±0.71 ab	49.00±1.36
Side barrier height 125 cm	5.14±0.81 a	3.57±0.74 a	43.40±1.08
Control	14.28±0.68 b	12.14±0.92 c	52.32±2.11
F Calculated	5.28*	8.456*	1.73 ^{ns}
P Value	0.01	0.002	0.21
HSD 5%	6.40	4.58	

Remarks: Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Honesty Significant Difference Test

CONCLUSION AND SUGGESTION

The first experiment of seed-borne transmission of PepYLCIV concluded and verified that the virus infecting red chili was a seed-borne virus. Hot water treatment and crude extract of red ginger could lengthen the average incubation period of PepYLCIV up to 6.61 days and 8.02 days, respectively. The effect of turmeric crude extract was not significant on the incubation period of PepYLCIV, but it was substantial on disease incidence and severity, amounting to 66.67% and 73.13%, respectively. In the intercropping experiment, the tomato was good enough as intercrops for red chili to reduce PepYLCIV infections, which could reduce disease incidence by up to 68.34%. Basil and marigolds were functional as trap crops to protect chili from incoming *B. tabaci*. Basil could reduce the incidence and severity of disease transmitted by the insect up to 50.87% and 55.47%, respectively, while marigolds reduce the disease incidence and severity to 72.42 and 72.83%, respectively. In the experiment of physical barrier, using 50 mesh cheesecloth as a side barrier at a height of 125 cm could reduce PepYLCIV infection frequency by up to 64.00% and disease severity by up to 70.59%. In all cultural techniques applied in the field experiments, PepYLCIV infection on red chili caused a yield reduction of 40 to 53%.

B. tabaci quickly spreads infection of PepYLCIV on red chili, and the disease is very damaging to the crop yield. Even though some cultural techniques effectively reduce PepYLCIV transmission by each vector, regular disease monitoring is essential, and destroying infected plants is necessary to eliminate virus inoculum.

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