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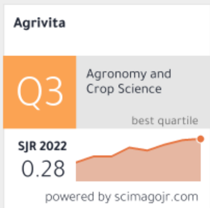

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COVER PAGE

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

Suparman [1*], Arsi[2] a Yulia Pujiastuti[1] and Rahmat Pratama[1] (Arial 10)

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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

Pepper Yellow Leaf Curl Virus (PYLCV) has been increasingly threatening to most red chili cultivation and many efforts to control the disease has been applied but the results is less satisfying except when involving insecticides. Non insecticide control measures could be better alternative to minimize the unwanted side impact of the chemical. Experiments of using cultural methods to suppress the disease initiation in the field has been conducted in the area where the disease has been an endemic and 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 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, mungbean and soybean in reducing disease incidence but their effects on disease severity and yield reduction were not significantly different. Basil and marigold were better trap crops compare to cosmos and zinnia, even though the effect on yield reduction was not significant. Side net barrier using 50 mesh cheese cloth with 125 cm high could reduce the initiation of the disease because *Bemisia tabaci* as the main vector of the disease tended to fly close to the ground when they came from short distance. Even though all of the experiments showed the potential of cultural technique in reducing incoming vector to bring in and transmit PYLCV, roguing is still required to prevent secondary and subsequent transmissions in the field

KEYWORDS

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

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 provinces as the red chili production centres are West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra with total production of 1229262 tons in 2022 (Statistik 2022) which contribute approximately 75% of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered all over the country.

Production of red chilli has been fluctuating due to several factors eventually causing significant fluctuation in chilli price which finally made farmers and traders under inconvenience situations. Plant diseases has been the main factor causing production reduction of chilli in the country. The diseases include bacterial leaf spot which causes yield reduction up to 66% (Utami et al. 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprapta 2022), fusarium which had a record to cause 50% yield losses (Sutarini et al. 2015), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a begomovirus, which under favourable condition might cause yield losses up to 70% (Novrianty et al. 2013). Symptoms of viral 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 and Arun 2021)

PYLCV often reach 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 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks) (Czosnek et al. 2017). The disease is increasingly frightening because it has spread to all chilli production centres in the country since its first appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) belongs to begomovirus (Sakata et al. 2008). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4 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 2 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, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum. Both strains have bipartite genome (Sharma et al. 2010). Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon, 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 numbers of 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* (Suryapal Singh et al. 2020). Furthermore, the insect also reported to infest more than 500 plant species include tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Schuster et al. 2009). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Brown et al. 1995)

B. tabaci has at least 39 biotypes, feeds on more than 361 species from 89 families (Li et al. 2011) and is able to 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 and Ghanim 2016). To transmit PYLCV 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 (Chen et al. 2011; Horowitz et al. 2011)

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

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

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

MATERIALS AND METHODS

Study area

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

Procedures

Seed transmission of PYLCV

Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water test and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. The experiment was arranged in a completely randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of ginger (*Zingiber officinale),* crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65ºC water for 30 minutes (Singh et al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, 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 calculated the viability of the treated seeds. To observed the seed born PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and all polybags were then placed in insect proof house and were arranged accordingly to completely randomized design.

Intercropping effect on PYLCV

Red chili plants were planted experimentally under intercropping pattern with mungbean (*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 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous and good flyer especially from 06.00 to 13.00 hours (Blackmer and Byrne 1993). Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

Trap crop effect on PYLCV

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

Effect of physical barrier on PYLCV infection

An experiment to verify the effect of physical barrier on the invasion of PYLCV vectors was conducted using cheesecloth as physical net barrier treatment. Nets were previously used as an antihail device in fruit production. However, having a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had been considered to be used as a nonaggressive pest and diseases control device (Grasswitz 2019). The experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units. Physical barrier covering crop cultivation with insect net has been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji et al. 2015). In this experiment, physical barrier using insect net was used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width 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 experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no barrier as control. The barriers were put in place before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were similar to those applied in intercropping and trap crop experiments.

Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control unwanted weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Lapidot et al. (2006) as follow: 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:

$$DS = \frac{\sum nxv}{ZxN} x100\%$$

Note: 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

$$YR = \frac{w}{W} x100\%$$

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

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

RESULTS AND DISCUSSION

Seed transmission of PYLCV

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 direct since infection of 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%. Ali and Kobayashi (2010) also reported that virus infection could cause premature aging of the seeds which eventually affected their viability.

Some of the seedling also produced PYLCV infection symptoms indicating that PYLC 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 had also been 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**.** Fadhila et al. (2020) who worked with PepYLCIV, 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^{o}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 et al. (2014), heated water at 65^{o}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. According to Ojuederie et al. (2010), 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 Nega et al. (2003), hot water treatment at temperature 53^{o} for 30 minutes did not affect seed germination and provided a good phytosanitary. 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 (Miller and Ivey 2005).

Seed borne virus infection in this research were relatively high because the seeds used were harvested from infected plants. 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 start an endemic (Pagán 2022). Such high seed borne virus infection has also been reported by Bashir et al. (2002) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus.*

All treatments in the experiment resulted in PYLCV infection which confirmed that the virus is transmitted vertically from generation to generation of the host plant. The seed borne nature of begomovirus had 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.

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

Intercropping effect on PYLCV

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

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

Trap crop effect on PYLCV.

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

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

Physical barrier effect on PYLCV Infection

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

CONCLUSIONS AND SUGGESTION (Arial 10)

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

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Tables

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

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

Suparman [1*], Arsi[2] a Yulia Pujiastuti[1] and Rahmat Pratama[1] (Arial 10)

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

Pepper Yellow Leaf Curl Virus (PYLCV) has been increasingly threatening to most red chili cultivation and many efforts to control the disease has been applied but the results is less satisfying except when involving insecticides. Non insecticide control measures could be better alternative to minimize the unwanted side impact of the chemical. Experiments of using cultural methods to suppress the disease initiation in the field has been conducted in the area where the disease has been an endemic and 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 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, mungbean and soybean in reducing disease incidence but their effects on disease severity and yield reduction were not significantly different. Basil and marigold were better trap crops compare to cosmos and zinnia, even though the effect on yield reduction was not significant. Side net barrier using 50 mesh cheese cloth with 125 cm high could reduce the initiation of the disease because *Bemisia tabaci* as the main vector of the disease tended to fly close to the ground when they came from short distance. Even though all of the experiments showed the potential of cultural technique in reducing incoming vector to bring in and transmit PYLCV, roguing is still required to prevent secondary and subsequent transmissions in the field

KEYWORDS

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

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

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 provinces as the red chili production centres are West Java, North Sumatra, Central Java, East Java, West Sumatra, Jambi, Aceh, Bengkulu, Yogyakarta, Lampung and South Sumatra with total production of 1229262 tons in 2022 (Statistik 2022) which contribute approximately 75% of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered all over the country.

Production of red chilli has been fluctuating due to several factors eventually causing significant fluctuation in chilli price which finally made farmers and traders under inconvenience situations. Plant diseases has been the main factor causing production reduction of chilli in the country. The diseases include bacterial leaf spot which causes yield reduction up to 66% (Utami et al. 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprapta 2022), fusarium which had a record to cause 50% yield losses (Sutarini et al. 2015), and pepper yellow leaf curl disease caused by *Pepper yellow leaf curl virus* (PYLCV), a begomovirus, which under favourable condition might cause yield losses up to 70% (Novrianty et al. 2013). Symptoms of viral 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 and Arun 2021)

PYLCV often reach 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 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks) (Czosnek et al. 2017). The disease is increasingly frightening because it has spread to all chilli production centres in the country since its first appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) belongs to begomovirus (Sakata et al. 2008). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4 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 2 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, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum. Both strains have bipartite genome (Sharma et al. 2010). Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon, 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 numbers of 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* (Suryapal Singh et al. 2020). Furthermore, the insect also reported to infest more than 500 plant species include tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Schuster et al. 2009). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Brown et al. 1995)

B. tabaci has at least 39 biotypes, feeds on more than 361 species from 89 families (Li et al. 2011) and is able to 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 and Ghanim 2016). To transmit PYLCV 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 (Chen et al. 2011; Horowitz et al. 2011)

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

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

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

The objective of the research is …..

MATERIALS AND METHODS (when and where?)

Study area

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

Procedures

Seed transmission of PYLCV

Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water test and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. The experiment was arranged in a completely randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of ginger (*Zingiber officinale),* crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65ºC water for 30 minutes (Singh et al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, 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 calculated the viability of the treated seeds. To observed the seed born PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and all polybags were then placed in insect proof house and were arranged accordingly to completely randomized design.

Intercropping effect on PYLCV

Red chili plants were planted experimentally under intercropping pattern with mungbean (*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 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous and good flyer especially from 06.00 to 13.00 hours (Blackmer and Byrne 1993). Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

Trap crop effect on PYLCV

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

Effect of physical barrier on PYLCV infection

An experiment to verify the effect of physical barrier on the invasion of PYLCV vectors was conducted using cheesecloth as physical net barrier treatment. Nets were previously used as an antihail device in fruit production. However, having a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had been considered to be used as a nonaggressive pest and diseases control device (Grasswitz 2019). The experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units. Physical barrier covering crop cultivation with insect net has been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji et al. 2015). In this experiment, physical barrier using insect net was used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width 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 experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no barrier as control. The barriers were put in place before seedling transplanting. Seedlings and virus resources used and data collection in this experiment were similar to those applied in intercropping and trap crop experiments.

Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control unwanted weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Lapidot et al. (2006) as follow: 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:

$$DS = \frac{\sum n x v}{Z x N} x 100\%$$

Note: 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

$$YR = \frac{w}{W} x 100\%$$

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

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

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

Seed transmission of PYLCV

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 direct since infection of 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%. Ali and Kobayashi (2010) also reported that virus infection could cause premature aging of the seeds which eventually affected their viability.

Some of the seedling also produced PYLCV infection symptoms indicating that PYLC 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 had also been 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**.** Fadhila et al. (2020) who worked with PepYLCIV, 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^oC 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 et al. (2014), heated water at 65^oC, 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. According to Ojuederie et al. (2010), 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 Nega et al. (2003), hot water treatment at temperature 53^o for 30 minutes did not affect seed germination and provided a good phytosanitary. 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 (Miller and Ivey 2005).

Seed borne virus infection in this research were relatively high because the seeds used were harvested from infected plants. 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 start an endemic (Pagán 2022). Such high seed borne virus infection has also been reported by Bashir et al. (2002) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus.*

All treatments in the experiment resulted in PYLCV infection which confirmed that the virus is transmitted vertically from generation to generation of the host plant. The seed borne nature of begomovirus had 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.

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

Intercropping effect on PYLCV

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

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

Trap crop effect on PYLCV.

Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However, only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests (Sarjan et al. 2023). Trap crop could also affect the main crops by intercepting, arresting or retaining pest thereby limiting the number of insect pest reaching the main crop (Shelton and Badenes 2006). Furthermore, the significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Maia and Moore (2011), basil and tagetes had trait of repellent against thrips and variety of flies. Husna et al. (2020) that crude extract of basil leaves at a concentration of 1.5% effectively caused mortality of *Aedes aegypti* larvae, and according to Farindira (2015), at higher concentration the crude extract could be used as repellent against the mosquito. Chokechaijaroenporn et al. (1994) also reported that the extract of basil leaves was a strong larvicidal against mosquito with EC_{50} = 81 ppm and EC_{90} = 113 ppm. Marigold also displayed strong repellent against mosquito (Ponkiya et al. 2018), and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick et al. 2020) and against eggplant fruit and shoot borer *Leucinodes orbonali* (Calumpang and Ohsawa 2015). When a trap crop has no insect repellent trait, it works by intercepting, arresting or retaining insects through its colour or odor. According to Junker et al. (2010), insects are attracted to colour and odour and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odour.

Physical barrier effect on PYLCV Infection

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

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

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

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

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Tables

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

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

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

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^{o}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, hot water treatment, intercropping, physical barrier, trap cropping.

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

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 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 in 2022 (Statistics Indonesia 2022) which contribute approximately 75% of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered all over the country.

Production of red chilli fluctuated due to several factors which eventually caused significant fluctuation in chilli price which finally 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 et al. 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprapta 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 up to 70% (Novrianty et al. 2013). 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 and 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 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek et al. 2017). The disease is increasingly frightening because it has spread to all chilli production centres in the country since its first appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila et al. 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4 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 2 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, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum. Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon, 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 numbers of 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* (Suryapal et al. 2020). Furthermore, the insect also reported to infest more than 600 plant species include tomato, water melon, 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 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al. 2014).

B. tabaci has at least 43 species complex, (Shah and Liu 2013) and is able to 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 and Ghanim 2016). To transmit PYLCV 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 (Hema et al. 2014) and generated resistant genotypes and reduced natural enemies (Wang et al. 2020). Under such conditions, cultural control should be better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological diversity through management of biotic and abiotic environment. Abiotic environment of the crop can be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian and Rezvani 2016). Cultural control of insects has become better alternative sincemost insect has strong ability to evolve pesticide resistance (Basit 2019).

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

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

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

MATERIALS AND METHODS (when and where?)

Study area

Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment of PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of Department of Plant Pest and Disease, Faculty of Agriculture, Sriwijaya University. The other three experiments were filed experiments on the effects of intercropping, trap cropping and physical barrier on the natural transmission and infection of PLCV. The field experiments were conducted in experimental garden of Sriwijaya University located 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 PYLCV experiment

Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. The experiment was arranged in a completely randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of ginger (*Zingiber officinale),* crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65^{o}C water for 30 minutes (Singh et al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, 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 calculated the viability of the treated seeds. To observed the seed born

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

Intercropping effect on PYLCV experiment

Red chili plants were planted experimentally under intercropping pattern with mungbean (*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 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous (Kumar et al. 2023) and most of vegetation in the area were host of the vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

Trap cropping effect on PYLCV experiment

An experiment to verify the effects of trap crops on the PYLCV 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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (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 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing.Trap crops were planted surrounding experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi et al. 2020).. Chilli seedlings used in this experiment were prepared in insect proof boxes together with those used in other field experiments. The parameters observed and the method of observation were also similar to those observed and applied in intercropping experiment.

Effect of physical barrier on PYLCV experiment

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

prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no 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 applied in intercropping and trap crop experiments.

Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Sharma et al. (2018) as follow: 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 = $\frac{\sum nxv}{ZxN} x100\%$

Note: 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 = $\frac{w}{W} x100\%$

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 Honestly Significant Difference (HSD) test a 95% degree of significance.

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

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 et al. (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis seeds up to 65%. Nallathambi at al. (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 indicating that PYLC 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 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^{o}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 et al. (2014), heated water at 65^{o}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 et al. (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 et al. 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 start an endemic (Pagán 2022). High seed borne tobamovirus infection has also been reported by Dombrovsky and Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus.*

All treatments in the experiment resulted in PYLCV 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 had 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.

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

Intercropping effect on PYLCV

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

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

Trap crop effect on PYLCV.

Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However, only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests (Sarjan et al. 2023). Barrier crop could also affect the main crops by intercepting, arresting or retaining pest thereby limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence of viral disease (Waweru et al. 2021). Furthermore, the significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the 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 Farindira (2015), at higher concentration the crude extract could be used as repellent against the mosquito. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito (Ponkiya et al. 2018), 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* (Calumpang and Ohsawa 2015), and against 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 colour (Acharya et al. 2021) or odor (Shao et al. 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

Physical barrier effect on PYLCV

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

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

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

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Tables

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty 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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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 (**maximum 200 words, it must : the objective, method, research products**)

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 (just primer supporting references, less than 10 years)

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

As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently transmit numbers of 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* (Suryapal, Singh, & Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie at al., 2014).

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

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

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

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

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

MATERIALS AND METHODS (when and where?)

Study area

Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three experiments were filed experiments on the effects of intercropping, trap cropping and physical barrier on the natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden of Universitas Sriwijaya located 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 PYLCV experiment

Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. The experiment was arranged in a completely randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of ginger (*Zingiber officinale),* crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65^{o}C water for 30 minutes (Singh, Awasthi, Jangre, & Nirmalkar, 2020). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes (Kabede, Ayalew, & Yeesuf, 2013); John, Ihum, Olusolape, & Janfa, 2018). The treated seeds were sown accordingly in each double trays, 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 calculated the viability of the treated seeds. To observed the seed borned PYLCV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and all polybags were then placed in insect proof screenhouse and were arranged accordingly to completely randomized design.

Intercropping effect on PYLCV experiment

Red chili plants were planted experimentally under 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 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous with hundreds number of host species (Pym et al., 2019) and most of vegetation in the area were host of the vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

Trap cropping effect on PYLCV experiment

An experiment to investigate the effects of trap crops on the PYLCV 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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (Hardiansyah, Hartini, & Musa, 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 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing.Trap crops were planted surrounding the experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chilli seedlings used in this experiment were prepared in insect proof boxes, together with those used in other field experiments. The parameters observed and the method of observation were similar to those observed and applied in intercropping experiment.

Effect of physical barrier on PYLCV experiment

An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect net was used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Four level 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 applied in intercropping and trap crop experiments.

Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy, (2022) as follow: 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:

$$DS = \frac{\sum nxv}{ZxN} x100\%$$

Note: 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

$$YR = \frac{w}{W} x100\%$$

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 Honestly Significant Difference (HSD) test a 95% degree of significance.

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

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 PYLC 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^{o}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^{o}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

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

All treatments in the experiment resulted in PYLCV 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 had also been reported by (Kothandaraman, Devadason, & Ganesan, 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.

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

Intercropping effect on PYLCV

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

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

Trap crop effect on PYLCV.

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

of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the 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, Srinivasan, Suganthi, Ranganathan, Gimbun, & Shanmugam (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 mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Dikr & Belete, 2021), and against 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 colour (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

Physical barrier effect on PYLCV

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

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

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

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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Tables

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

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

Suparman [1*], Arsi[2] a Yulia Pujiastuti[1] and Rahmat Pratama[1]

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VII. Reviewer Candidates

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

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 $65^{o}C$ 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, hot water treatment, intercropping, physical barrier, trap cropping.

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

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 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 in 2022 (Statistics Indonesia 2022) which contribute approximately 75% of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered all over the country.

Production of red chilli fluctuated due to several factors which eventually caused significant fluctuation in chilli price which finally 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 et al. 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprapta 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 up to 70% (Novrianty et al. 2013). 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 and 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 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek et al. 2017). The disease is increasingly frightening because it has spread to all chilli production centres in the country since its first appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila et al. 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4 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 2 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, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum. Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon, 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 numbers of 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* (Suryapal et al. 2020). Furthermore, the insect also reported to infest more than 600 plant species include tomato, water melon, 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 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al. 2014).

B. tabaci has at least 43 species complex, (Shah and Liu 2013) and is able to 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 and Ghanim 2016). To transmit PYLCV 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 (Hema et al. 2014) and generated resistant genotypes and reduced natural enemies (Wang et al. 2020). Under such conditions, cultural control should be better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological diversity through management of biotic and abiotic environment. Abiotic environment of the crop can be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian and Rezvani 2016). Cultural control of insects has become better alternative sincemost insect has strong ability to evolve pesticide resistance (Basit 2019).

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

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

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

MATERIALS AND METHODS (when and where?)

Study area

Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment of PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of Department of Plant Pest and Disease, Faculty of Agriculture, Sriwijaya University. The other three experiments were filed experiments on the effects of intercropping, trap cropping and physical barrier on the natural transmission and infection of PLCV. The field experiments were conducted in experimental garden of Sriwijaya University located 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 PYLCV experiment

Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. The experiment was arranged in a completely randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of ginger (*Zingiber officinale),* crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65^{o}C water for 30 minutes (Singh et al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, 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 calculated the viability of the treated seeds. To observed the seed born

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

Intercropping effect on PYLCV experiment

Red chili plants were planted experimentally under intercropping pattern with mungbean (*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 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous (Kumar et al. 2023) and most of vegetation in the area were host of the vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

Trap cropping effect on PYLCV experiment

An experiment to verify the effects of trap crops on the PYLCV 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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (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 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing.Trap crops were planted surrounding experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi et al. 2020).. Chilli seedlings used in this experiment were prepared in insect proof boxes together with those used in other field experiments. The parameters observed and the method of observation were also similar to those observed and applied in intercropping experiment.

Effect of physical barrier on PYLCV experiment

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

prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no 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 applied in intercropping and trap crop experiments.

Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Sharma et al. (2018) as follow: 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:

$$DS = \frac{\sum n x v}{Z x N} x 100\%$$

Note: 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

$$YR = \frac{w}{W} x 100\%$$

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 Honestly Significant Difference (HSD) test a 95% degree of significance.

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

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 et al. (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis seeds up to 65%. Nallathambi at al. (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 indicating that PYLC 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 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^{o}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 et al. (2014), heated water at 65^{o}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 et al. (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 et al. 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 start an endemic (Pagán 2022). High seed borne tobamovirus infection has also been reported by Dombrovsky and Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus.*

All treatments in the experiment resulted in PYLCV 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 had 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.

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

Intercropping effect on PYLCV

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

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

Trap crop effect on PYLCV.

Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However, only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests (Sarjan et al. 2023). Barrier crop could also affect the main crops by intercepting, arresting or retaining pest thereby limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence of viral disease (Waweru et al. 2021). Furthermore, the significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the 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 Farindira (2015), at higher concentration the crude extract could be used as repellent against the mosquito. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito (Ponkiya et al. 2018), 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* (Calumpang and Ohsawa 2015), and against 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 colour (Acharya et al. 2021) or odor (Shao et al. 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

Physical barrier effect on PYLCV

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

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

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

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Tables

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

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

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

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 65ºC 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, hot water treatment, intercropping, physical barrier, trap cropping.

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

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 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 in 2022 (Statistics Indonesia 2022) which contribute approximately 75% of national production (Yanuarti and Afsari 2016) and the rest are produced by small producing areas scattered all over the country.

Production of red chilli fluctuated due to several factors which eventually caused significant fluctuation in chilli price which finally 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 et al. 2022), anthracnose which under severe infection might cause yield losses up to 80% (Suprapta 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 up to 70% (Novrianty et al. 2013). 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 and 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 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek et al. 2017). The disease is increasingly frightening because it has spread to all chilli production centres in the country since its first appearance detected in 1999 (Gaswanto et al. 2016). More intensive research revealed that PYLCV in Indonesia has its own specification and was diagnosed as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila et al. 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4 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 2 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, the virus is known as *Pepper yellow leaf curl Indonesia virus* (PeYLCIV) which has two strains i.e. PeYLCIV-Tomatowhich also infects tomato and PeYLCIV-Ageratum which also infects ageratum. Instead of infecting chilli and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon, 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 numbers of 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* (Suryapal et al. 2020). Furthermore, the insect also reported to infest more than 600 plant species include tomato, water melon, 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 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie et al. 2014).

B. tabaci has at least 43 species complex, (Shah and Liu 2013) and is able to 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 and Ghanim 2016). To transmit PYLCV 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 (Hema et al. 2014) and generated resistant genotypes and reduced natural enemies (Wang et al. 2020). Under such conditions, cultural control should be better alternative to solve the problems. Cultural control is to conserve natural enemies and increase biological diversity through management of biotic and abiotic environment. Abiotic environment of the crop can be manipulated by modifying soil tillage, fertilization, irrigation, and mulching. Biotic environment might be manipulated by applying crop rotation, intercropping, trap cropping, and crop spacing (Zaefarian and Rezvani 2016). Cultural control of insects has become better alternative sincemost insect has strong ability to evolve pesticide resistance (Basit 2019).

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

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

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

MATERIALS AND METHODS (when and where?)

Study area

Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment of PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of Department of Plant Pest and Disease, Faculty of Agriculture, Sriwijaya University. The other three experiments were filed experiments on the effects of intercropping, trap cropping and physical barrier on the natural transmission and infection of PLCV. The field experiments were conducted in experimental garden of Sriwijaya University located 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 PYLCV experiment

Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. The experiment was arranged in a completely randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of ginger (*Zingiber officinale),* crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65^{o}C water for 30 minutes (Singh et al. 2020; Toporek et al. 2017). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes (Kabede et al. 2013; John et al. 2018). The treated seeds were sown accordingly in each double trays, 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 calculated the viability of the treated seeds. To observed the seed born

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

Intercropping effect on PYLCV experiment

Red chili plants were planted experimentally under intercropping pattern with mungbean (*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 replications, resulting in 25 experimental plots measuring 4 x 2 m. Red chilli and intercropping plants were planted at 60 x 40 cm spacing (Ain et al. 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous (Kumar et al. 2023) and most of vegetation in the area were host of the vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

Trap cropping effect on PYLCV experiment

An experiment to verify the effects of trap crops on the PYLCV 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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (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 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing.Trap crops were planted surrounding experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi et al. 2020).. Chilli seedlings used in this experiment were prepared in insect proof boxes together with those used in other field experiments. The parameters observed and the method of observation were also similar to those observed and applied in intercropping experiment.

Effect of physical barrier on PYLCV experiment

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

prepared. Four level of physical barrier height were applied i.e. 50, 75, 100 and 125 cm and no 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 applied in intercropping and trap crop experiments.

Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector in the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Sharma et al. (2018) as follow: 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:

$$DS = \frac{\sum nxv}{ZxN} x100\%$$

Note: 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

$$YR = \frac{w}{W} x100\%$$

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 Honestly Significant Difference (HSD) test a 95% degree of significance.

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

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 et al. (2017) who found that infection of *Cucumber mosaic virus* (CMV) could reduce the viability of Arabidopsis seeds up to 65%. Nallathambi at al. (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 indicating that PYLC 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 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^{o}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 et al. (2014), heated water at 65^{o}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 et al. (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 et al. 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 start an endemic (Pagán 2022). High seed borne tobamovirus infection has also been reported by Dombrovsky and Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) , also reported high rate of seed borne transmission of *Cucumber mosaic virus.*

All treatments in the experiment resulted in PYLCV 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 had 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.

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

Intercropping effect on PYLCV

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

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

Trap crop effect on PYLCV.

Infections of PYLCV were lower in chilli plots surrounded by trap crops compared to those of control. However, only basil and marigold could significantly reduce the disease incidence and severity (Table 3). Basil, cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests (Sarjan et al. 2023). Barrier crop could also affect the main crops by intercepting, arresting or retaining pest thereby limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence of viral disease (Waweru et al. 2021). Furthermore, the significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the 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 Farindira (2015), at higher concentration the crude extract could be used as repellent against the mosquito. Husna et al. (2020) also reported that the extract of basil leaves was a strong larvicidal against mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito (Ponkiya et al. 2018), 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* (Calumpang and Ohsawa 2015), and against 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 colour (Acharya et al. 2021) or odor (Shao et al. 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

Physical barrier effect on PYLCV

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

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

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

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Tables

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty 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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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 65ºC 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 (just primer supporting references, less than 10 years)

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

As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently transmit numbers of 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* (Suryapal, Singh, & Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie at al., 2014).

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

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

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

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

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

MATERIALS AND METHODS (when and where?)

Study area

Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three experiments were filed experiments on the effects of intercropping, trap cropping and physical barrier on the natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden of Universitas Sriwijaya located 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 PYLCV experiment

Chilli fruits were harvested from infected red chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. The experiment was arranged in a completely randomized design with five treatment and 5 replications. The treatments were hot water, crude extract of ginger (*Zingiber officinale),* crude extract of turmeric (*Curcuma longa*), crude extract of Javanese ginger (*Curcuma zanthorrhiza*) and fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65^{o}C water for 30 minutes (Singh, Awasthi, Jangre, & Nirmalkar, 2020). To make crude ginger, turmeric, and Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes

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

Intercropping effect on PYLCV experiment

Red chili plants were planted experimentally under 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 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous with hundreds number of host species (Pym et al., 2019) and most of vegetation in the area were host of the vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

Trap cropping effect on PYLCV experiment

An experiment to investigate the effects of trap crops on the PYLCV 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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (Hardiansyah, Hartini, & Musa, 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 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing.Trap crops were planted surrounding the experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chilli seedlings used in this experiment were prepared in insect proof boxes, together with those used in other field experiments. The parameters observed and the method of observation were similar to those observed and applied in intercropping experiment.

Effect of physical barrier on PYLCV experiment

An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect net was used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Four level 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 applied in intercropping and trap crop experiments.

Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy, (2022) as follow: 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:

$$DS = \frac{\sum nxv}{ZxN} x100\%$$

Note: 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

$$YR = \frac{w}{W} x100\%$$

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 Honestly Significant Difference (HSD) test a 95% degree of significance.

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 PYLC 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^{o}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^{o}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

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

All treatments in the experiment resulted in PYLCV 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 had also been reported by (Kothandaraman, Devadason, & Ganesan, 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.

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

Intercropping effect on PYLCV

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

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

Trap crop effect on PYLCV.

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

of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the 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, Srinivasan, Suganthi, Ranganathan, Gimbun, & Shanmugam (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 mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Dikr & Belete, 2021), and against 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 colour (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

Physical barrier effect on PYLCV

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

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

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

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

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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 65ºC 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%.~~

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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% (Suprapta, 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).

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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

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

As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently transmit numbers of 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* (Suryapal, Singh, & Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie at al., 2014).

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

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

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

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

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

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This statement should also been supported in molecular detection.
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MATERIALS AND METHODS

Study area

Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three experiments were filed experiments on the effects of intercropping, trap cropping and physical barrier on the natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden of Universitas Sriwijaya located 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 PYLCV experiment

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

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

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Intercropping effect on PYLCV experiment

Red chili plants were planted experimentally under 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 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous with hundreds number of host species (Pym et al., 2019) and most of vegetation in the area were host of the vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

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Trap cropping effect on PYLCV experiment

An experiment to investigate the effects of trap crops on the PYLCV 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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (Hardiansyah, Hartini, & Musa, 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 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing.Trap crops were planted surrounding the experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chilli seedlings used in this experiment were prepared in insect proof boxes, together with those used in other field experiments. The parameters observed and the method of observation were similar to those observed and applied in intercropping experiment.

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Effect of physical barrier on PYLCV experiment

An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect net was used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Four level 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 applied in intercropping and trap crop experiments.

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Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy, (2022) as follow: 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:

$$DS = \frac{\sum nxv}{ZxN} x100\%$$

Note: 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

Commented [A21]: Literature?

$$YR = \frac{w}{W} x100\%$$

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 Honestly Significant Difference (HSD) test a 95% degree of significance.

RESULTS AND DISCUSSION

~~Seed transmission of PYLCV~~

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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 PYLC 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^{o}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^{o}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

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

All treatments in the experiment resulted in PYLCV 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 had also been reported by (Kothandaraman, Devadason, & Ganesan, 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.

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

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Please revise the sentence because it could be misleading

Intercropping effect on PYLCV

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

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Why don't you explain that disease severity is only reducing incidence but not severity, therefore other method is needed?

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

Trap crop effect on PYLCV.

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

of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the 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, Srinivasan, Suganthi, Ranganathan, Gimbun, & Shanmugam (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 mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Dikr & Belete, 2021), and against 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 colour (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

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Physical barrier effect on PYLCV

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

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The ineffective low cheesecloth net barrier indicated that the net barrier could only work mechanically in blocking flying insect and had no effect on the insect behavior. This was different from barrier crop which not only block the flying insects but also attract or repel the insect, depended on the crop species. Flying insect such as *B. tabaci* could not easily differentiate host and nonhost plants which made barrier crop more effective than net barrier (Udiarto, Setiawati, Muharam, & Dadi, 2023). *B. tabaci* actually has two type of flight behavior, foraging flight and migratory flight. Foraging flight is close to earth surface or within flight boundary, while migratory flight is above the boundary where the insect could be picked up and carried by air currents (Reynolds, Chapman, & Drake, 2017), and the insect containing mature eggs has been trapped at 150 m above ground and this could be among the 30% of vertically distributed *B. tabaci* in the air. The net barrier could actually block not only *B. tabaci*, for entering the protected plot, but also other flying insect with body 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 [A28]: Red?

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

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Because the results have already been in percentage.
Should be only substraction of control - treatment.

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Tables

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

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

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

ABSTRACT

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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 65ºC 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%.~~

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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% (Suprapta, 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).

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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

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

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As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently transmit numbers of 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* (Suryapal, Singh, & Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie at al., 2014).

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

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

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

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

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

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MATERIALS AND METHODS

Study area

Research on the application of cultural techniques to control PYLCV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment effect on PYLCV experiment, conducted in Insectarium and insect-proof screenhouse of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Sriwijaya. The other three experiments were filed experiments on the effects of intercropping, trap cropping and physical barrier on the natural transmission and infection of PYLCV. The field experiments were conducted in experimental garden of Universitas Sriwijaya located 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 PYLCV experiment

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

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

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Intercropping effect on PYLCV experiment

Red chili plants were planted experimentally under 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 replications, resulting in 25 experimental plots measuring 4 x 2 m, with 1 m distance among the plots. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 2020). All seedlings were prepared in insect proof boxes to ensure that all PYLCV infection was initiated in the field and brought in by its vector, *B. tabaci*. Sources of PYLCV 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 PYLCV was abundant in the area since the insect is polyphagous with hundreds number of host species (Pym et al., 2019) and most of vegetation in the area were host of the vector. Data collected from this experiment included PYLCV disease incidence, PYLCV disease severity and yield reduction caused by the disease. Data on incubation period was not collected since it was difficult to detect when the vector of PYLVC arrived and inoculated the virus to the experimental plants in the plots.

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Trap cropping effect on PYLCV experiment

An experiment to investigate the effects of trap crops on the PYLCV 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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (Hardiansyah, Hartini, & Musa, 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 4 x 2 m plot on which red chilli was planted at 60 x 40 cm spacing.Trap crops were planted surrounding the experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chilli seedlings used in this experiment were prepared in insect proof boxes, together with those used in other field experiments. The parameters observed and the method of observation were similar to those observed and applied in intercropping experiment.

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Effect of physical barrier on PYLCV experiment

An experiment to observe the effect of physical barrier on the invasion of PYLCV vectors was conducted using cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect net was used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016). The experiment was arranged in a randomized block design with 5 treatments and 5 replications for which 25 plots were prepared. Four level 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 applied in intercropping and trap crop experiments.

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Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli plants could grow optimally and mechanical technique was applied to control weeds, pests and diseases. For the experiment of seed transmission of PYLCV, observations were made to collect data on incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PYLCV. Data on chilli 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 PYLCV brought in by its vector, data on incubation period could not be measured because the entrance of the vector into the experimental plots could not be controlled. However, since PYLCV did not stop the host growing and fruiting, data collection was made at the harvest time. Disease severity of PYLCV was calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy, (2022) as follow: 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:

$$DS = \frac{\sum nxv}{ZxN} x100\%$$

Note: 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

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$$YR = \frac{w}{W} x100\%$$

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 <u>+</u> standard deviation. ANOVA was used to analyze all collected data, and significant differences between means were determined using Honestly Significant Difference (HSD) test a 95% degree of significance.

RESULTS AND DISCUSSION

Seed transmission of PYLCV

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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 PYLC 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^{o}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^{o}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

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

All treatments in the experiment resulted in PYLCV 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 had also been reported by (Kothandaraman, Devadason, & Ganesan, 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.

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

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Intercropping effect on PYLCV

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

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Why don't you explain that disease severity is only reducing incidence but not severity, therefore other method is needed?

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

Trap crop effect on PYLCV.

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

of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could significantly reduce the disease incidence and severity up to 72.42% and 72.83% respectively (Table 3). The significant effect of basil and marigold on PYLCV infection might be due to insect repellence. According to Gonzales-Valdivia et al. (2017), basil had trait of repellent against *B. tabaci* and prevented the 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, Srinivasan, Suganthi, Ranganathan, Gimbun, & Shanmugam (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 mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Dikr & Belete, 2021), and against 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 colour (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

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Physical barrier effect on PYLCV

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

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

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
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

Note: 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	-	-

Note: 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	-

Note: 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 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	

Note: Means in the same column followed by different letters are significantly different at $p<0.05$ according to Honesty 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: 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 65ºC 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% (Suprapta, 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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Disease caused by PepYLCIV 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 (Hemiptera: Aleyrodidae) with incubation period ranges from 2 to 4 weeks (Czosnek, Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). The disease is increasingly frightening because it has spread to all chilli production centres in the country since its first appearance detected in 1999 (Gaswanto, Syukur, Hidayat, & Gunaeni, 2016). The virus causing yellow leaf curl on pepper was previously known as *Pepper yellow leaf curl virus* (PYLCV), however due to its own specification, the virus has received its own name as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), belongs to begomovirus (Fadhila et al, 2020). Begomovirus is a member of Family Geminiviridae, a big plant virus group with a lot of members known as damaging viruses. The virus is characterised by 30 x 20 nm gemini (twin) particles with 2 components of single stranded DNA genome inside. Geminiviridae comprises of 4 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 2 components of single stranded DNA recognized as DNA A and DNA B, both have the same measurement, 2.8 kb (Czosnek, Hariton-Shalev, Sobol, Gorovits, & Ghanim, 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 chilli and tomato, begomovirus was reported to infect other crop species and cause similar symptoms and damages. The other crops found to be infected by begomovirus included melon, water melon, pepper and eggplant (Subiastuti, Hartono, Daryono, 2019).

As the only vector of PYLCV, *B. tabaci* has been identified as a very efficient vector, and also able to efficiently transmit numbers of 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* (Suryapal, Singh, & Bharat, 2020). Furthermore, the insect was also reported to infest more than 600 plant species included tomato, water melon, pumpkin, cauliflower, cabbage, melon, cotton, carrot, sweet potato, cucumber, eggplant, chili, rose, poinsettia, lantana, and lily (Li, Mbata, Punnuri, Simmons, & Shapiro-Ilan, 2021). The insect is known to reproduce with high fecundity. A female can produce 50 to 400 eggs measuring 0.10 mm - 0.25 mm. The eggs are laid on lower surface of host plant leaves. Females *B. tabaci* are diploid individuals appear from fertilized eggs, while the males are haploid appear from unfertilized eggs (Xie at al., 2014).

B. tabaci has at least 43 species complex, (Shah & Liu, 2013) and is able to transmit more than 200 plant viruses (Lu, Chen, Li, Shi, Gu, & Yan, 2019; MacLeod, Canty, & Polaszek, 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, Hariton-Shalev, Sobol, Gorovits, & Ghanim, 2017). The vector remains viruliferous until 13 days after virus acquisition or until the vector dies (Roy, Chakraborty, & Ghosh, 2021).

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

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Commented [R15R14]: The sentence has been revised crop spacing (Zaefarian & Rezvani, 2016). Cultural control of insects has become better alternative since most insect has strong ability to evolve pesticide resistance (Basit, 2019).

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

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

The objective of the research was to observe the effects of seed treatments, intercropping, trap cropping, and physical barrier 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 each other.

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MATERIALS AND METHODS

Study area

Research on the application of cultural techniques to control PepYLCIV infection on red chili was conducted in 2022. The research was experimental research consisted of four different experiments. The first experiment was a seed treatment effect on PepYLCIV experiment, conducted in Insectarium and insect-proof screenhouse of 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 barrier on the natural transmission and infection of PepYLCIV. The field experiments were conducted in experimental garden of Universitas Sriwijaya located 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 chilli plants in the farmers' fields for seed preparation. Seeds were sorted based on size and colour and set aside the small, crinkle and black seeds. The selected seeds then underwent fresh water screening and only the sunk seeds were used for the experiment, while the floated seeds were not used because they were obviously not viable. No healthy seeds used as negative control in this experiment because the experiment itself was carried out inside insect-proof house to guarantee that all PepYLCIV infection were seed borne. The experiment was arranged in a completely randomized design with five treatment 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 fresh water as the control. Experiment unit was two seed trays of 100 holes to make 200 holes per unit. For hot water treatment, seeds were soaked in 65^{o}C water for 30 minutes (Singh, Awasthi,

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Jangre, & Nirmalkar, 2020). To make crude red ginger, crude turmeric, and crude Javanese ginger extracts, 50 gr 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 chilli seeds were dipped in the extracts accordingly for 30 minutes (Kabede, Ayalew, & Yeesuf, 2013); John, Ihum, Olusolape, & Janfa, 2018). The treated seeds were sown accordingly in each double trays, 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 calculated the viability of the treated seeds. To observed the seed borned PepYLCIV infection, 50 four-leaf seedlings were then transferred individually to a 20 cm diameter polybag, and all polybags were then placed in insect proof screenhouse 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.

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Intercropping effect on PepYLCIV experiment

Red chili seedlings were planted experimentally under 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 1 m distance among the plots resulted in 12 red chili plants and 12 intercropping plants. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Ain, Yamika, Aini, & Firdaus, 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 number of host species (Pym et al., 2019) and most of vegetation in the area were 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 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.

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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 trap crops selected were those belonged to refugia which normally attract and trap invader insect (Hardiansyah, Hartini, & Musa, 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 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 trap crops were planted surrounding the experimental plots at 25 cm spacing accordingly to each treatment, three (3) weeks before transplanting red chilli seedling to the plots . The crop were positioned as border crop so that they could intercept insect before they attack the main crops (Pribadi, Purnawati, & Rahmadhini, 2020). Chilli seedlings used in this experiment were prepared in insect proof boxes, together with those used in other field experiments. The parameters observed and the method of observation were similar to those observed and applied in intercropping experiment.

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Effect of physical barrier on PepYLCIV experiment

An experiment to observe the effect of physical barrier on the invasion of PepYLCIV vectors was conducted using cheesecloth as physical net barrier. Cheese net was selected it was previously used as an antihail device in fruit production, and due to a lot of beneficial impacts on agriculture, especially in controlling pests and diseases, net had been used as a nonaggressive pest and diseases control device (Grasswitz, 2019). Physical barrier covering crop cultivation with insect net has also previously been used in tobacco cultivation to prevent the invasion of *B. tabaci* (Aji, Hartono, & Sulandari, 2015). The experiment was conducted in experimental garden where 4 x 2 m plots were made as experiment units. In this experiment, physical barrier using insect net was used only as side barrier and let the top opened for the access of pollinator and other beneficial insects. The 50 mesh cheesecloth was used in this experiment because it could prevent the entry of insect with body width more than 0.25 mm, while the body width of *B. tabaci* ranged from 0.253 to 0.288 mm (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 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, resulted in 24 plants per block. Four level 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 applied in intercropping and trap crop experiments.

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Crop maintenance and observation

Crop maintenance was conducted daily to ensure that all of the chilli 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 incubation period, disease incidence, and disease severity. Incubation period was described as the period from seed sowing to the appearance of the first symptom of PepYLCIV. Data on chilli 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 brought in by its vector, data on 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 growing and fruiting, data collection was made at the harvest time. Disease severity of PepYLCIV was calculated according to disease scores described by Yadav, Reddy, Ashwathappa, Kumar, Naresh, & Reddy, (2022) as follow: 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 = $\frac{\sum nxv}{ZxN} x100\%$

Note: 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 = $\frac{w}{W} x100\%$

YR = yield reduction

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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 Honestly Significant Difference (HSD) test a 95% degree of significance.

RESULTS AND DISCUSSION

Effect of seed transmission of PepYLCIV

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Pepper yellow leaf curl Indonesia virus (PepYLCIV) 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 PepYLCIV 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 PepYLCIV infection symptoms, confirming that PepYLCIV 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 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.

As shown in Table 1, hot water and ginger crude extract could significantly lengthen incubation period of PepYLCIV but had not significant effect on disease incidence and severity when compared to control. Hot water treatment at 65ºC 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 65ºC, 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 start an endemic (Pagán, 2022). High seed borne tobamovirus infection has also been reported by Dombrovsky & Smith (2017) who recorded 6.9% seed transmission incidence of *Blackeye cowpea mosaic virus*. Sastry (2013) , also reported 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 had also been reported by (Kothandaraman, Devadason, & Ganesan, 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.

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

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Please revise the sentence because it could be misleading

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Intercropping effect on PepYLCIV

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

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Why don't you explain that disease severity is only reducing incidence but not severity, therefore other method is needed?

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

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

Trap crop effect on PepYLCIV.

Infections of PepYLCIV were lower in chilli plots surrounded by trap crops compared to those of control. Basil, cosmos, tagetes and zinnia are refugia which frequently planted by farmers to attract natural enemies of insect pests (Sarjan, Haryanto, Supeno, & Jihadi, 2023), but the effects of the four crops were different when being used barrier crops. Barrier crop could affect the main crops by intercepting, arresting or retaining pest thereby limiting the number of insect pest and insect vector reaching the main crop which eventually reduce incidence of viral disease (Waweru, Rukundo, Kilalo, Miano, & Kimenju, 2021). In this experiment, basil and could significantly reduce the disease incidence and severity up 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 trait of repellent against *B. tabaci* and prevented the 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, Srinivasan, Suganthi, Ranganathan, Gimbun, & Shanmugam (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 mosquito with LC_{50}=0.97% and LC_{90}=1.42%. Marigold also displayed strong repellent against mosquito and contained extractable toxicants which effective as repellent and larvicidal against *B. tabaci* (Fabrick, Yool, & Spurgeon, 2020), against eggplant fruit and shoot borer *Leucinodes orbonali* (Dikr & Belete, 2021), and against 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 colour (Acharya et al., 2021) or odor (Shao, Cheng, Wang, Zhang, & Yang, 2021), and all Homopteran insect, but Family Aphididae, are attracted to colour. Three trap crops used in this experiment produced distinct coloured flower and one species (basil) produced green flower but with strong odor.

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Physical barrier effect on PepYLCIV

Physical barrier using cheese cloth 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, but only physical barriers at 125 cm high could significantly reduce incidence and severity of pepper yellow leaf curl disease (Table 4). This result was in accordance to that reported by (Harish, Chellappan, Kumar, Ranjith, & Ambavane, 2016) that a barrier of fine mesh could effectively prevent *B. tabaci* to the protected plot. 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), height of barrier was very important to effectively blocked insect to infest the protected crop. In this experiment, the height of 125 cm could significantly reduce the number of *B.*

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

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

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

CONCLUSIONS AND SUGGESTION

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

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 to 8.02 days respectively. The effect of turmeric crude extract was not significant on incubation period of PepYLCIV but it was significant on disease incidence and severity amounted to 66.67% and 73.13% respectively. In the intercropping experiment, tomato was good enough as intercrops for red chili in term of reducing PepYLCIV infections which could reduce disease incidence up to 68.34%. Basil and marigold were functional as trap crops to protect chili from incoming *B. tabaci.* Basil could reduce incidence and severity of disease transmitted by the insect up to 50.87% and 55.47% respectively, while marigold reduce the disease incidence and severity 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 PepYLCIV infection frequency up to 64.00% and disease severity up to 70.59%. In all cultural techniques applied in the field experiments, PepYLCIV infection on red chili caused yield reduction of 40 to 53%.

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 substraction 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

Commented [R59R58]: Correction has been made

Infection of PepYLCIV 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 PepYLCIV transmission by each vector, regular disease monitoring is important and destroy infected plants is necessary to eliminate virus inoculum.

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Tables

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

Note: 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	-	-

Note: 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	-

Note: 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	

Note: Means in the same column followed by different letters are significantly different at p<0.05 according to Honesty 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 65ºC 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% (Suprapta, 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).

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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 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 65ºC 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). Chilli 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:

$$DS = \frac{\sum nxv}{ZxN} x100\% \quad .... 1)$$

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:

$$YR = \frac{w}{W} x100\% \text{ .... 2)}$$

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 Honesty 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^{o}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^{o}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 (Syafruddin & Suwardi, 2020), cotton-mung bean (Liang et al., 2020), sorghum-mungbean (Temeche et al., 2022), sorghum-soybean (Saberi, 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%.

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.

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 Honesty 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 65ºC 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% (Suprapta, 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 65ºC 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). Chilli 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:

$$DS = \frac{\sum nxv}{ZxN} x100\% \text{ .... 1)}$$

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:

$$YR = \frac{w}{W} x 100\% \text{ .... 2)}$$

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 Honesty 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 65ºC 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 65ºC, 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 (Syafruddin & Suwardi, 2020), cotton-mung bean (Liang et al., 2020), sorghum-mungbean (Temeche et al., 2022), sorghum-soybean (Saberi, 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%.

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.

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 Honesty 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 65oC 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% (Suprapta, 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 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^{o}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 annuum 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). Chilli 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 and cupping; and 4 = very severe leaf yellowing, and pronounced leaf curling. The severity was calculated using the following formula:

$$DS = \frac{\sum nxv}{ZxN} x100\% \quad \text{1)}$$

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:

$$YR = \frac{w}{W} x100\% \quad \text{2)}$$

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), chili pepper (Capsicum annuumwho 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 (Syafruddin & Suwardi, 2020), cotton-mung bean (Liang et al., 2020), sorghum-mungbean (Temeche et al., 2022), sorghum-soybean (Saberi, 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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