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Protection of chili pepper from mosaic virus disease and *Aphis gossypii* by a fermented water extract of compost

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Abstract. Mosaic and leaf curl diseases caused by multiple infections of viruses are the most important diseases of chili pepper. Bioactive compounds that are able to improve plant tolerance toward virus infections is promisingly developed for plant protection in organic chili cultivation. The potential of fermentation liquids from enrichment of SWCE (water extract of shrimp waste compost) with shrimp paste (TS, TSN, and TSNJK) that contains amino acids to control mosaic and leaf curl diseases and its aphid vector was demonstrated in a naturally infested curly red chili (*Capsicum annuum*). Fermentation liquids was sprayed weekly at 2 concentrations on potted *C. annuum* growing in an aphid-infested field. Treatment with all 3 fermentation products significantly reduced disease progression. Disease suppression was demonstrated following spraying at concentration as low as 0.2% and treatment with a higher concentration (2.0%) resulted in a higher suppression. Colonization of *Aphis gossypii* was significantly reduced on plants treated with fermentation liquids. The compost extract containing amino acids showed a remarkable potential to develop into an effective biostimulant for protection from virus disease and its insect vector, *Aphis gossypii*.

1. Introduction

Virus diseases are epidemic and responsible for substantial yield losses on chili cultivation. Mosaic are the most common symptoms of multiple viral infection on chili pepper. In Indonesia, mosaic disease was reported to be associated with *Cucumber mosaic virus* (CMV), *Pepper yellow leaf curl virus* (PYLCV), *Tobacco mosaic virus* (TMV), *Chili vein mottle virus* (ChiVMV), and *Pepper vein yellowing virus* (PeVYV) [1]. Infected plant produces malformed leaves and stunting with reduced internode extension and smaller leaves. Infection during early growth stage can cause total losses due to flower dropping and fruit setting. Diseased plant produces unmarketable fruit (small and hard fruit) when infected during generative stage. Sukada *et al.* [2] reported that yield losses due to mosaic disease on chili pepper was 54%-80%.

Plant viral diseases have generally been considered practically difficult to control as thus far, no agrochemical been developed for directly targeting viral life cycles [3]. The use of resistant plants is one of the most effective and widely employed strategies to control virus infections in fields [4]. Numerous studies have demonstrated that resistance inducers of natural origin such as microbial fermentation products can improve plant resistance against virus infection [5-7]. A water-based



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compost preparation, referred to as compost tea and compost-water extract are widely applied for improving plant growth and enhancing plant resistance against pathogen in organic farming systems [8–11].

Despite its potential, field application of compost extract alone had been reported to have no or a minor control efficacy against viral diseases [12]. Fortification of compost extract with microorganism or bioactive substances may increase its control efficacy against viral infection. A mixture of compost extract from vegetable wastes and siderophores-producing *Pseudomonas aeruginosa* Ch1 suppressed infection of CMV on tobacco as studied in a greenhouse test [13]. Fortification of compost with shrimp waste products resulted in an increase the contains of amino acids in its water extract [14]. The water extract from the shrimp fortified compost is then called shrimp-waste compost extract (SWCE). SWCE exhibited biostimulant activities when applied to seeds, roots or plant leaves. We demonstrated that seed treatment with the fortified compost extract improved tolerance of rice seedling from salt stress [15]. Our field trial also suggested that the bark treatment with the compost extract exhibited protective effects against tapping panel dryness, a physiological disorder of rubber tree [16]. This study demonstrated the protection effects of a fermented liquid made from mixture of SWCE and shrimp paste (TS, TSN, and TSNJK) against natural infestation of mosaic disease and its vector *Aphis gossypii*.

2. Methods

2.1. Plant material

Seedlings of CMV free-certified chili cultivar F1 Lado were grown on insect-free room for 3 w. Seedlings were transferred to a field soil amended with a fortified compost (N: 1.0 P: 6.1 K: 5.2 Mg: 8.6) as planting media in a-15L-polyethylene bag (polybag). Polybags were placed in 70-cm spacing in the experiment field, where surrounding chili pepper plants were severely infected with mosaic disease. Plants were fertilized weekly with 250 mL/plant using a fortified liquid compost extract (10% (v/v) cow manure compost, 0.5% (v/v) microbial starter and 1% (w/v) NPK 16-16-16 fertilizer). Weeds were removed manually and pesticides were not used throughout experiment.

2.2. Fermentation liquids and treatment

Fermentation liquids were prepared through fermentation of SWCE [14,16] in mixture with 5% (w/v) shrimp paste and 5% (w/v) sucrose. SWCE contained naturally occurring fermentative microorganisms such as *Lactobacillus* spp. and *Saccharomyces* spp. as starter for fermentation. The mixture was then allowed to be fermented for 7 d before used. Three types of fermentation liquids, TS, TSN and TSNJK were applied in the study. TS and TSN used shrimp paste produced from Selapan and Sungang area in South Sumatra, respectively. TSNJK was formulated by mixing juice of *Citrus amblycarpa* (Hassk.) Ochse (jeruk limau) at 5% (v/v) into TSN preparation. Content of amino acid in the TS and TSN preparation as analysed using the UPLC Amino Acid Analysis System was listed in Table 1. Treatment of fermentation liquids were performed weekly by spraying at concentration 0.2 or 2.0% on 15 plants as replications. Experiment was arranged in a completely randomized block factorial design with 1 control treatment.

2.3. Assessment of disease and insect vector

Both incidence and severity of naturally infected mosaic disease were recorded weekly for disease assessment. Incidence of diseased leaves was counted as percentage of diseased leaves and measured at 7 to 10 w after transplanting. Severity of naturally occurring mosaic disease was evaluated weekly at 3 to 10 w after transplanting on a 0-to-5 scale [17]. Severity was calculated as percentage of total ratings out of the number of plants multiplied by 5. Area under disease progress curve (AUDPC) was calculated to analyse effect of treatment on development of mosaic severity. The AUDPC of mosaic severity was computed following the method defined by Simko and Piepho [18]. Blackman and

Eastop [19] was used as reference for identification of aphid species. Number of aphids and % plant infested were counted weekly

Table 1. Content of amino acids in compost-water extract preparations (TS and TSN).

Amino acid	Amino acid content in TS (mg/L)	Amino acid content in TSN (mg/L)
Alanine	1159	1419
Arginine	493	283
Aspartic acid	1791	815
Glutamic acid	2128	922
Glycine	1207	842
Histidine	250	4678
Isoleucine	863	388
Leucine	1462	815
Lysine	1417	13605
Phenylalanine	860	683
Proline	874	391
Serine	830	830
Threonine	818	4848
Tyrosine	554	2127
Valine	937	603

2.4. Data analysis

Data were analyzed using Proc Glimmix based on mixed model for factorial design with 1 control treatment and performed under SAS University Edition 2.8.9.4 M6 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

The effect of fermentation liquids on development of naturally occurring mosaic disease was evaluated based on the progress curve of disease incidence and severity. Plant sprayed with fermentation liquids showed a delaying disease progress curve both of disease incidence (Fig. 1) and severity (Fig. 1) compared to that of control (water treated) plants. Suppression of disease progress curve was recorded as early as 1 w after spraying and continued to a similar pattern as increasing of plant ages. Disease suppression was prominently observed after 6 w of transplanting when mosaic disease developed well in all plots. When treated at the same concentration, all preparations resulted in a similar manner on disease progress curve. Treatment with a higher concentration (2.0%) resulted in a slower progress of both disease incidence and severity compared to treatment with lower concentration (0.2%). Development of disease severity was less progressive compared to that of diseased leaves (Figs. 1).

Protection from mosaic disease by treatment with fermentation liquids was evaluated based on AUDPC analysis. AUDPC of treated plants had significantly ($p < 0.001$) lower than water treated plant. AUDPC amongst 3 preparation of compost extract was not significantly different (p main effect of preparation types = 0.709). Concentration significantly affected AUDPC value (p main effect of concentration <0.001) whereas concentration at 2.0% resulted in significantly lower in AUDPC. Effect of concentration on AUDPC was not affected by preparation type (p interaction between concentration and preparation types = 0.268). Based on AUDPC, reduction in disease development on treated plots was ranged from 30.7 to 49.1% and 63.8 to 71.6% for concentration 0.2% and 2.0%, respectively. Significant lower AUDPC or highest suppression of mosaic disease was achieved following spraying with 2.0% TS mixture (Table 2).

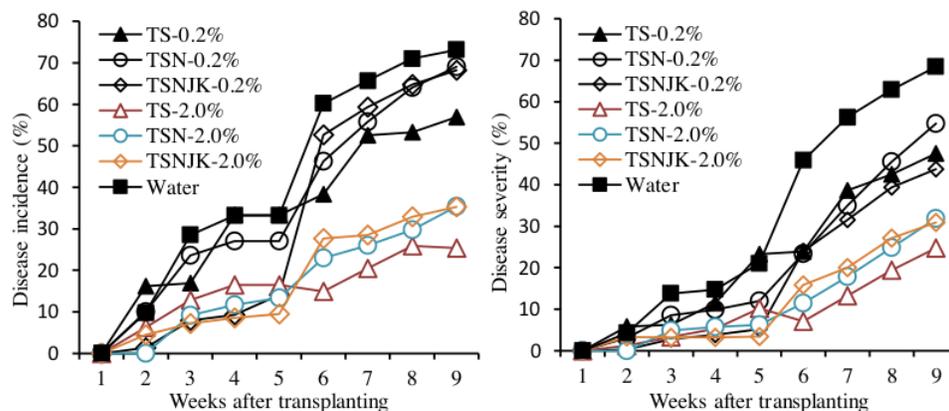


Figure 1. Mosaic incidence (left) and severity (right) progress curves of *Capsicum annum* sprayed weekly with different fermentation liquids (TS, TSN, TSNJK).

Abundant aphid colonization was observed at 8 and 9 w after transplanting. Aphid was found colonizing on 80 and 99% fermentation liquids treated plants at 8 and 9 w after transplanting, respectively, but all control plants was colonized by the insect vector. Colonization of aphid on plants treated with compost extract was significantly lower ($p < 0.001$) compared to those of on water treated plants. Aphid colonization was significantly affected by preparation types (p main effect of preparation types < 0.001) with lower aphid number on TSN and TS compared to TSNJK treated plants. Plants sprayed with higher concentration of preparation (2.0%) significantly colonized by a lower number of aphid than those treated at concentration 0.2% (p main effect of concentration < 0.001). Interaction between types of liquids and concentration was significant ($p = 0.028$). Treatment with 2% TS and TSN preparation resulted in lowest aphid colonization (Fig. 2).

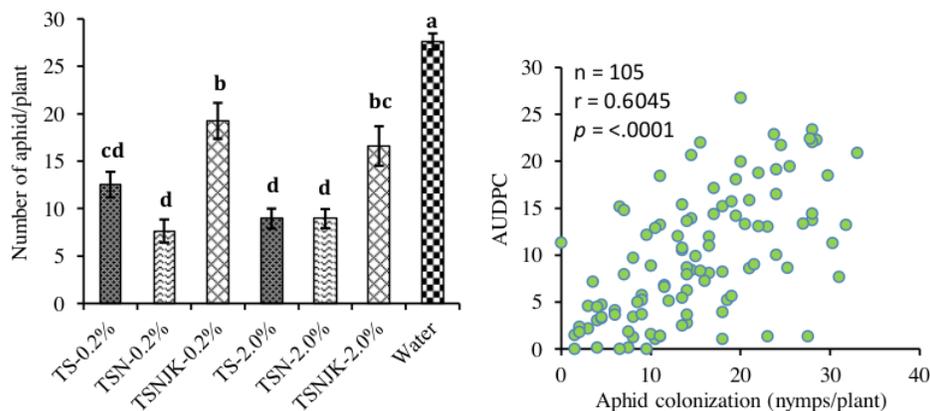


Figure 2. Colonization of aphid at 8 and 9 w after transplanting (left) and correlation plot between aphid colonization of individual plant and AUDPC of *Capsicum annum* sprayed weekly with different fermentation liquids (TS, TSN, TSNJK).

To assess the role of aphid colonization on disease progress, a correlation analysis was performed in individual and overall treatment. A highly correlation (Pearson correlation coefficient = 0.6045, $p =$

< 0.0001) was observed between aphid colonization and AUDPC in overall treatment (Fig. 2). An increased in aphid colonization has resulted in more progressive disease. Plant treated with TSNJK showed an inconsistency association between aphid colonization and AUDPC as disease progress of some individual plants was not developed well as increasing of aphid population.

Table 2. Area under disease progress curve (AUDPC) of mosaic severity after treatment with different fermentation liquids.

Fermentation liquids	AUDPC ^a	Disease suppression relative to control (%) ^b
TS-0.2%	12.29 ± 1.75 ab	30.7
TSN-0.2%	11.53 ± 1.37 bc	34.9
TSNJK-0.2%	9.02 ± 1.66 bcd	49.1
TS-2.0%	5.04 ± 0.98 d	71.6
TSN-2.0%	6.08 ± 1.26 cd	65.7
TSNJK-2.0%	6.41 ± 1.47 bcd	63.8
Water (control)	17.72 ± 1.41 a	-

^a Mean ± S.E.M = Mean values ± Standard error of means of fifteen replications. Means followed by different letters are significantly different according to the Tukey's HSD-test at $p < 0.05$.

^b % disease suppression = [(AUDPC of treatment - AUDPC of control) / AUDPC of control] * 100

In this study, we revealed the protective effects of fermentation liquids made from enrichment of SWCE with shrimp paste on naturally occurring virus disease of curly red chili and its aphid vector, *Aphis gossypii*. The compost extract containing amino acids showed a remarkable potential to develop into an effective biostimulant for protection from virus disease and insect vector. Plants treated with all 3 preparations showed both significantly less progressive development of disease and less colonization of *A. gossypii* compared to control plant. Suppression of both disease and aphid following drenching with TSN preparation was also confirmed in a growth-room experiment (unpublished data). Control of mosaic disease as demonstrated here could be caused by bioactive compounds present in the fermentation liquids. Fermentation liquids used in the study contains at least 15 types of amino acid. Amino acids and their metabolites have a critical role during signaling activities as well as in improving plant immunity [20]. Numerous reports have revealed that exogenous application of the amino acids enhanced plant resistance against biotic and abiotic injuries. Accumulation of homoserine in *Arabidopsis* floral tissue had been determined to be associated with enhanced resistance to *Fusarium graminearum* and *F. culmorum* and exogenous treatment of the amino acid could improve resistance against the pathogen [21]. Induced systemic resistance against blast pathogen had been revealed to occur on rice roots exposed to low dose glutamine [22]. Improved plant tolerance against virus following application with a low concentration of a biostimulant containing amino acid has been demonstrated by Betti *et al.* [23]. Mosaic disease severity of chili pepper inoculated with *PepMV* had been suppressed following foliar spray with the amino acid preparation at concentration 0.2-0.3%. The protection from viral infection had been suggested to be linked with correction of amino acid ratio impairment of diseased plant through complementation with amino acids from the biostimulant.

Preparation of compost extracts showed a slight variation in their efficacy against viral disease. When treated at 2% concentration, TS preparation showed the highest protection efficacy, though it has no suppressive effect at 0.2%. Protection of plant by exogenous amino acid treatment is known to be affected by the protein source, method and degree of hydrolysis, as well as the amino acid and peptide composition [24]. TS contains a higher concentration of glutamic acid. Glutamine has been suggested to act as regulators of the plant defense pathway [25]. TSN has a lower concentration of glutamic acid, but contains a higher composition of lysine. L-lysine metabolic pathway has recently discovered to play a major role in plant systemic acquired resistance (SAR) to pathogen infection

through its catabolism product, N-hydroxypipicolinic acid. N-hydroxypipicolinic acid induces the expression of a set of major plant immune genes to enhance a variety of defense mechanisms [26].

There was a significant positive correlation between aphid colonization and disease progress in overall treatment. It was likely that disease protection by the compost extract could be explained through the indirect suppression against *A. gossypii*. Amino acids found in phloem including both protein and nonprotein amino acids are known to be a limiting factor for aphid growth [27]. However, interaction between individual amino acid and aphid host plant resistance is host-specific [27], and therefore need to be further studied.

4. Conclusion

Regularly spraying of curly red chili with amino acid containing compost extracts could suppress both natural infestation of mosaic diseases and its aphid vector, *A. gossypii*. Plant protection was demonstrated at concentration as low as 0.2% and treatment with a higher concentration (2.0%) resulted in a higher suppression.

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