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

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ABSTRACT

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

INTRODUCTION

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

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

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

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

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

Study Area

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

Procedures

Seed Treatment Effect on PepYLCIV

Experiment

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

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

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

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

Intercropping Effect on PepYLCIV Experiment

Red chili seedlings were planted experimentally under an intercropping pattern with mung bean (*Vigna radiata*), soybean (*Glycine max*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) and without intercrop as the control. The experiment was arranged in a randomized block design with five treatments and five blocks/replications, resulting in 25 experimental plots measuring 4 x 2 m, with a 1 m distance among the plots resulting in 12 red chili plants and 12 intercropping plants. Red chili and intercropping plants were planted at 60 x 40 cm spacing (Aini et al., 2020) Brondong Sub-District of Lamongan Regency, Province of East Java. The research was performed with the aim of examining and obtaining appropriate combinations of plant spacing and planting model for red chili (*Capsicum 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). Chili seedlings used in this experiment were prepared in insect-proof boxes and those used in other field experiments. The parameters observed, and the observation method was similar to those surveyed and applied in the intercropping experiment.

Effect of a Physical Barrier on the PepYLCIV Experiment

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

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

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

Crop Maintenance and Observation

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

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

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

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

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

Yield reduction was calculated using the following formula:

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

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

Data Analysis

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

RESULTS AND DISCUSSION

Effect of Seed Transmission of PepYLCIV

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Intercropping Effect on PepYLCIV

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

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

mung bean (Syafuruddin & Suwardi, 2020), cotton-mung bean (Liang *et al.*, 2020), sorghum-mungbean (Temeche *et al.*, 2022), sorghum-soybean (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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