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Effects of Selected Low-Risk Insecticides and Rainfall Rate on *Bemisia tabaci* Population and Pepper Yellow Leaf Curl Indonesia Virus Incidence on Chili

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Abstract: Pepper Yellow Leaf Curl Indonesia Virus (PepYLCIV) is a damaging chili disease in Indonesia. Chili growers depend on synthetic insecticides to control the disease by suppressing its vector population, *Bemisia tabaci* Genn. This study assessed the efficacy of several low-risk insecticides in suppressing the *B. tabaci* population and PepYLCIV incidence during the dry and rainy seasons. Treatments consisted of different insecticide active ingredients, namely: Abamectin, azadirachtin, imidacloprid, deltamethrin, and spinosad. Each chemical was applied with three application frequencies: Once weekly, twice weekly, and unsprayed control. Each insecticide was applied through foliar spray at the rate recommended by the respective manufacturer. The treatments were arranged in a split-plot design, application frequency, and active ingredient served as the main plots and sub-plots, respectively. Each treatment combination had four replications of a plot each. Data were analyzed using ANOVA and Duncan's multiple range test (p = 0.5). In the rainy season, the vector population decreased as the season progressed with increasing rainfall. However, the PepYLCIV incidence continuously increased throughout the season. This seemed to be due to the initial disease infection taking place when the rainfall rate was low and the *B. tabaci* population was high early in the season. In contrast, during the dry season, the vector population and PepYLCIV incidence went up as the season progressed due to the decrease in the rainfall rate. All insecticides tested were effective in controlling the disease and its vector. One and two weekly applications provided the same efficacy of the pest control, thus, the current local farmers' practice of 2-3 applications per week is unnecessary.

Keywords: Low-Risk Insecticide, *Bemisia tabaci*, Pepper Yellow Leaf Curl Indonesia Virus (PepYLCIV), Rainfall Rate, Chili

Introduction

Chilies (*Capsicum annum* L. and *Capsicum frutescens* L.) belonging to the family *Solanaceae* are important crops worldwide. In Indonesia, chili is used mostly as food spices containing high nutrition, such as proteins, lipids, carbohydrates, calcium, and vitamins A, B1, and C (Harpenas and Dermawan, 2010). Besides that, chili is more economically valuable than other main crops in the country, including rice and corn (Anggriani, 2015; Rismawanto *et al.*, 2016). However, chili growers continuously face the problems of a number of pests and diseases attacking their crops. *Bemisia tabaci* Genn. (*Hemiptera*: *Aleyrodidae*), the Sweet Potato Whitefly (SPW), is one of the most important pests of chili in Indonesia. The pest can cause direct and indirect damage to plants by feeding on the plant sap and spreading Pepper Yellow Leaf Curl Indonesia Virus (PepYLCIV), respectively. Direct damage causes distortions on young leaves and silver chlorotic spots on the leaves and reduces yield by up to 80% (Sulandari *et al*., 2001), while indirect damage can cause total yield loss (Setiawati *et al*., 2007). Pepper yellow leaf curl Indonesia virus is a *Begomovirus* belonging to the family *Geminiviridae* with bisegmented particles, persistently spread by *B. tabaci* (Hidayat *et al*., 2009).

The virus was recorded for the first time infecting chili plants in 1999 in West Java (Rusli *et al*., 1999). Since then, the disease spread very quickly and by 2003, it had been found throughout Java Island (Sulandari *et al.*, 2006). In 2005 the disease was detected in Lampung and West Sumatera provinces (Trisno *et al*., 2010). Furthermore, PepYLCIV was reported in the provinces of North Sulawesi (Tsai *et al*., 2009) and Bali (Selangga and Listihani, 2021).

Plants infected by PepYLCIV express typical symptoms which are leaf yellowing and curling, plant stunting, and flowers and fruits prematurely dropping (Sulandari, 2006; Sumardiyono *et al*., 2003). Early symptoms are yellow spots around the leaf veins then they develop into vein clearing. Young leaves change in color to yellow and then bright yellow, veins become thicker and the leaves become smaller and curled up or down (cupping) (Sulandari *et al*., 2006).

Chili growers in the area rely mainly on synthetic insecticides to directly control *B. tabaci* and at the same time, indirectly control PepYLCIV. Researchers have reported that the virus incidence is positively correlated with the vector population in the field (Saeed and Samad, 2017; Singarimbun *et al*., 2017; Temaja *et al*., 2022). Growers usually apply insecticides based on schedule and up to three applications per week to reduce the vector population (Nasruddin *et al*., 2020). The control practice raises concerns about its potentially damaging effects on non-target organisms and the environment.

Low-risk insecticides provide effective control of the SPW and are relatively safer for beneficial organisms and the environment. Low-risk insecticides are insecticides that meet at least one of the following characteristics: Low risks to human health, non-target organisms, and the environment and can be incorporated into integrated pest management strategies (EPA, 1993). Several low-risk insecticides, including pyrethroid (Price and Schuster, 1991), imidacloprid (Oetting and Anderson, 1990), spinosad, abamectin, and azadiractin (Setiawati *et al.,* 2007) are effective against *B. tabaci*. However, no reports are available on how effective the insecticides are in suppressing PepYLCIV incidence and its vector, *B. tabaci* population in the region. Therefore, the current study was conducted to evaluate the impact of selected low-risk insecticides and their application frequency and the rainfall rate on the *B. tabaci* population and PepYLCIV incidence.

Materials and Methods

Study Site

Two field experiments were carried out at the Experiment Station, Faculty of Agriculture, Hasanuddin University (5°07’42” S, 119°28’47” E), Makassar, Indonesia, from August 2022-December 2022 (rainy season) and from March-August 2023 (dry season). The station’s altitude is around 7 m from the sea level with a mean temperature of 27.5°C. Both experiments were identical in all aspects, except one was conducted in the dry season and the other was in the rainy season. In the station, chili had been continuously planted for more than eight years. During that time, the Pepper yellow leaf curl Indonesia virus (PepYLCIV) and its vector, *Bemisia tabaci* were present in the station. Climatic data were obtained from the Station of Meteorology, Climatology, and Geophysics, Maros, South Sulawesi.

Seeding and Plot Preparation

The seedbeds were prepared using a hand tractor passed two times throughout the experimental plot. The seedbeds were elevated approximately 20 cm and composted chicken manure was added into the topsoil. Certified chili seeds cv Kastilo were evenly spread on the seedbed and then covered with a 1 cm layer of top soil-manure mixture (1:1). The seedbed size was 1.5×2.0 m confined in an insect-proof cage and treated once a week with imidacloprid (Confidor 200SL) to protect the seedlings from PepYLCIV infection and whitefly infestation. The seedbed was manually weeded and irrigated as needed during the seeding period. Three weeks after emergence, the seedlings were ready for transplanting.

Before the transplanting, the field was prepared using a hand tractor passed twice throughout the field. One week before the transplantation, chicken dung compost, equivalent to two tons per ha, was evenly scattered on all plots and then mixed with the topsoil using a hand tractor. To control weeds, reflective silver plastic mulch 2 m and 0.08 mm in width and thickness, respectively (CV. Sumber Tani, Jember), was used to cover the plots.

Local recommendations for chili cultivation were followed during the experiment. Twenty-one day-old seedlings were transplanted at the beginning of the planting seasons, 10 August 2022 for the rainy season and 15 April 2023 for the dry season. Plant bed size was 1.5×6 m and each bed contained two plant rows with a planting spacing of 60×50 cm. Two weeks after transplanting, plants were fertilized with 50 kg/ha of a compound fertilizer of Phonska (NPKS = 15:15:15:10) (Petrokimia Gresik). When the plants began to blossom, they were fertilized every 10 days or after each harvesting at a rate of 10 kg/ha with NPKS and SP36 (1:1) mixture to induce the formation of new flowers. An overhead sprinkler system was used to irrigate the plants as needed.

Experimental Layout

Insecticides with the following active ingredients were tested in this experiment: Abamectin (Demolish 10EC, PT. Dharma Guna Wibawa, Jakarta), azadirachtin (neem oil spray, urban farming, Malang), imidacloprid (Confidor 200SL, PT. Bayer Indonesia, Jakarta), deltamethrin (Revitrin 25EC, Bayer, Jakarta) and spinosad (Endure 120SC, PT. Dow agro sciences Indonesia, Jakarta). Each chemical was applied with three insecticide application frequencies: Once weekly, twice weekly, and unsprayed control (plot sprayed with water). The insecticides were used at the rates suggested by the respective manufacturers: Abamectin (0.5 mL/L), azadirachtin (5.00 mL/L), imidacloprid (0.75 mL/L), deltamethrin (2.00 mL/L) and spinosad (1.00 mL/L). The insecticides were applied using a backpack sprayer. The field experiments were laid out in a split-plot design. The main and sub-plots were the application frequency and the active ingredient, respectively. Each combination of treatments had four replicates of a plot each with a size of two rows wide by 6 m long as described before. Row and plant spaces used were 60 and 50 cm, thus, there were 24 plants in each plot.

Data Collection

Every seven days, one day before insecticide application, the biweekly population and weekly populations of whiteflies were determined during the dry and rainy seasons, respectively. From each replication plot, four plants were chosen at random as plant samples. Three leaves were observed from each plant sample and the number of adults on the abaxial leaf surface was recorded. The same leaves were collected and placed in separate Ziploc bags before being transported to our laboratory for nymph identification and counting using a dissecting microscope (Zeiss Stemi DV-4). The nymph number on the abaxial leaf surface was assessed by placing a card with a 1×1 cm hole next to the midrib in the center of the leaf. The number of nymphs present per cm^2 was then recorded. The incidence of PepYLCIV disease was expressed in the percentage of diseased plants in each replicate plot.

Data Analysis

The data of the whitefly count were transformed using the square root transformation and PepYLCIV incidence was transformed using the arcsine-square root transformation before ANOVA was performed. Duncan's multiple range test (p = 0.05) was used to compare the treatment means if significant differences were found among the treatments. Correlation between rainfall rate and the SPW population and between the SPW population and PepYLCIV incidence were calculated using Pearson's linear regression analysis (SPSS Statistics 27.0.1.0) (SPSS, 2021).

Results

Rainy Season

For the rainy season, weekly observations of the experiment were conducted from 20 September-29 November 2022 (Fig. 1). The population of *B. tabaci* adults per plant and the associated rainfall data are presented in Fig. 1. During the rainy season experiment, the rainfall rate was 385 mm per month with a range from 20-880 mm. One day before insecticide application, there were no significant differences among the treatments in the numbers of whiteflies, but the numbers were the highest during the season. On the same date, the rainfall rate was the lowest during the experiment. One week after the first insecticide application, the number of whiteflies for all treatments went down, except the control. The results of the second observation showed that the number of SPW in the control, azadirachtin, and deltamethrin were not significantly different from each other. However, they were significantly higher than those in the abamectin, imidacloprid, and spinosad treatments. The numbers of SPW in the abamectin, imidacloprid, and spinosad were not significantly different from each other. In the third observation and on, SPW populations were low across all the treatments and there were no significant variations among them. The season's average numbers of SPW adults and nymphs across all the treatments was low and no significant differences were found between the treatments (Table 1). The low populations were coincident with the high rainfall rates towards the end of the season.

Person's correlation analysis showed a strong negative relationship between rainfall rate and whitefly number ($y = -0.30x + 20.65$, $R^2 = 0.76$, $p<0.001$) (Fig. 2). This confirms that as the rainfall increased the number of adult whiteflies decreased.

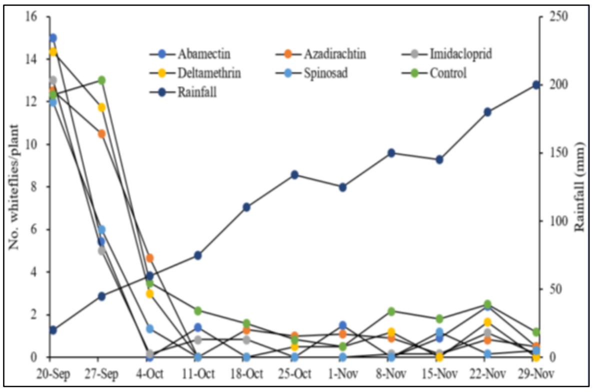

Fig. 1: Whitefly population per plant for every insecticide treatment and weekly rainfall rate on each observation date during the rainy season

Table 1: Populations of adult and nymph whiteflies for every combination of insecticides and their spraying frequency during the rainy season

Spray frequency	Insecticide	No. adults	No. nymphs
Once a week	Abamectin	0.6	0.0
	Azadirachtin	0.5	0.8
	Imidacloprid	0.3	0.1
	Deltamethrin	0.7	0.1
	Spinosad	0.4	0.7
Twice a week	Abamectin	0.5	0.0
	Azadirachtin	0.2	0.1
	Imidacloprid	0.4	0.1
	Deltamethrin	0.4	0.0
	Spinosad	0.9	0.3
	Control	0.3	0.0

No significant discrepancies among the treatments in the populations of nymph and adult whitefly per plant (Duncan's multiple range test, p>0.05)

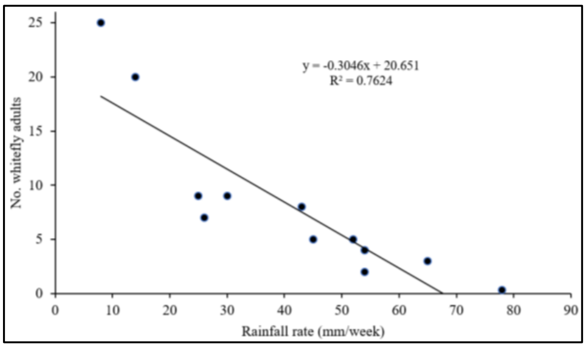

Fig. 2: Relationship between the average weekly rainfall (mm) and the population of whiteflies during the rainy season

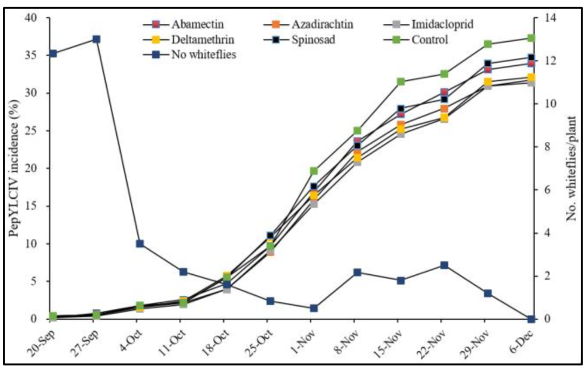

Fig. 3: Average incidence of PepYLCIV and SPW number on each observation date during the rainy season

The relationship between the whitefly population and PepYLCIV incidence is shown in Fig. 3. The whitefly populations in control plants for the first and the second observations were high, about 35 and 37 individuals per plant, respectively, then it dropped sharply to about 10 in the third observation. From there, the vector population remained low until the end of the experiment. On the other hand, PepYLCIV steadily increased for all treatments throughout the experiment. For every observation, there were no statistically significant variations in the disease incidence between the treatments.

The results of the Person's correlation analysis showed a strongly negative and statistically significant relationship between whitefly number and PYLCIV incidence ($y = -1.25x + 26.92$, $R^2 = 0.63$, $p<0.01$) (Fig. 4). This indicates that PepYLCIV was low when the whitefly population was high early in the season. However, the disease incidence kept increasing towards the end of the season, even though, the population of *B. tabaci* was consistently low till the end of the season.

Dry Season

For the dry season, biweekly observations were conducted from 14 May-15 August 2023. The population of *B. tabaci* adults, disease incidence, and the associated rainfall data are presented in Fig. 5. The biweekly rainfall during the dry season experiment ranged from 0-151 mm, with an average of 51.1 mm. Early in the season, the rainfall rate increased and reached its peak on 15 May (about 170 mm). During that time, the average number of whiteflies and PepYLCIV incidence were low, 3 individuals per plant and 4%, respectively. After that, the rainfall dropped sharply to around 58 mm and then it continuously declined and reached 0 mm in the middle of August. At the end of August, the rainfall rate started to increase and kept increasing till the end of the season.

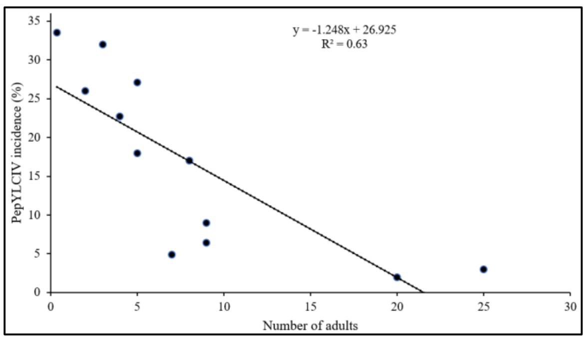

Fig. 4: Relationship between the whitefly population and PepYLCIV incidence during the rainy season

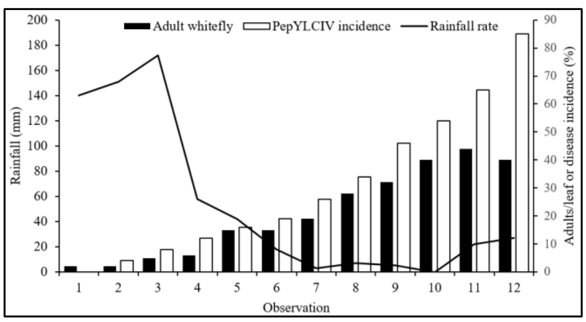

Fig. 5: Average number of SPW per plant and PepYLCIV prevalence in the control (unsprayed plants) and the associated biweekly rainfall data during the dry season

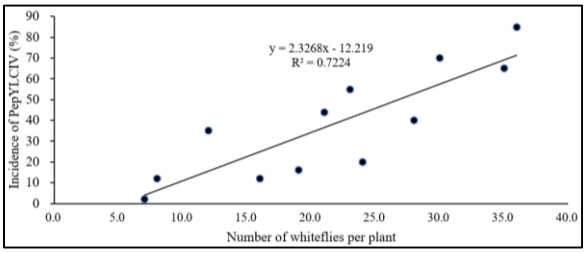

Fig. 6: Relationship between the whitefly population and the PepYLCIV incidence in control plants during the dry season

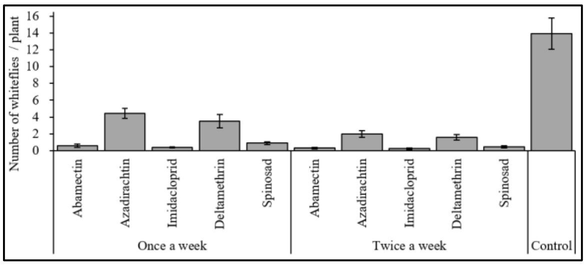

Fig. 7: Average number of *B. tabaci* per plant for different treatment combinations of insecticide and application frequency during the dry season

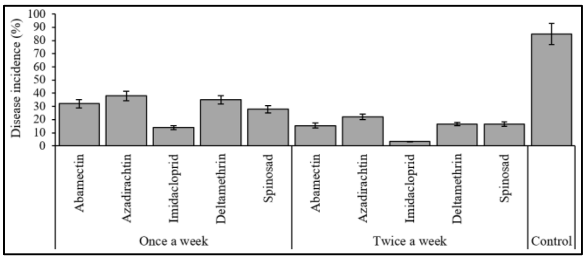

Fig. 8: Average incidence of PepYLCIV on different treatment combinations of insecticide and application frequency in the dry season

As the rainfall rate decreased, the number of whiteflies continuously increased and reached its peak (45 individuals per plant in the control plants) in early September, which was concomitant with the increase in the rainfall rate. In parallel with the rise of the SPW population, the incidence of PepYLCIV grew gradually throughout the season and peaked at the end of the season (85%).

The rainfall rate and the adult SPW population had a substantial negative correlation (y = -3.13x +120.20; R^2 = 0.61; p<0.01). On the other hand, there was a strong positive correlation (y = 2,33x -12,22, R^2 = 0.72, p<0.01) between the incidence of PepYLCIV and the number of SPW adults (Fig. 6).

The population of SPW was significantly affected by the insecticide and their application frequency (Fig. 7). For all insecticides tested, the whitefly populations were substantially lower than those in the control. For abamectin, imidacloprid, and spinosad, the populations of *B. tabaci* were not significantly different from each other. For these insecticides, application frequency did not significantly affect the number of SPW. On the contrary, *B. tabaci* populations were considerably lower when azadirachtin and deltamethrin were applied twice a week as opposed to once a week. Within the same application frequency, the numbers of whiteflies in azadirachtin and deltamethrin were not significantly different from each other.

The average incidence of PepYLCIV was significantly affected by the insecticide and their application frequency in comparison to the control (Fig. 8). The PepYLCIV incidences for all insecticides tested were significantly lower than was for the control. Within each application frequency, imidacloprid had the lowest disease incidence and substantially lower than the other treatments but there were no statistical differences among the other treatments. The average disease incidence for each insecticide studied was considerably lower on plants sprayed twice a week than on plants sprayed once a week.

Discussion

During the rainy planting season, the population of *B. tabaci* decreased as the season progressed; however, PepYLCIV incidence continuously went up throughout the season (Fig. 1). The SPW population was negatively correlated with the disease incidence, which contradicted previous reports that population vector is positively correlated with the disease incidence (Singarimbun *et al*., 2017; Temaja *et al*., 2022). The reason for this appears to be that the disease spread early in the season during a period of high insect population, but it took several weeks for symptoms to manifest in the middle to the end of the season (Ganefianti *et al*., 2011). That is why the disease incidence kept going up, though the vector population declined during the rainy season experiment. Our results suggested that to effectively control the PepYLCIV incidence and its vector population in the rainy season, the spray should be administered at the beginning of the season when high vector populations occur in the field, but most likely sprays become unnecessary later in the season when the population very low due to the high rainfall rate. Thus, scouting is needed to monitor the vector population early in the season to assess whether insecticide applications are warranted.

In contrast to the rainy season, during the dry season, the SPW population increased as the season progressed, while the rainfall rates continuously declined until the experiment ended. Consequently, the number of whiteflies and the rate of rainfall had a substantial negative relationship, which is parallel to the reports of (Leite *et al*., 2005; Nasruddin *et al*., 2021). Similarly, the PepYLCIV incidence increased as the season progressed and in tandem with the increase of the vector population (Fig. 5). Pearson's regression analysis showed a substantial positive association between the number of SPW per plant and the PepYLCIV incidence (Fig. 6). This is in agreement with previous reports demonstrating that the incidence of PepYLCIV and some other *Geminiviruses* is higher when its vector population is bigger and vice versa (Bonato *et al*., 2007; Singarimbun *et al*., 2017; Temaja *et al*., 2022). In addition, Naveed *et al*. (2015) reported that epidemics of tomato leaf curl virus, another *Geminivirus*, occur when the SPW population is high during the dry season. Thus, this finding suggested that to effectively control the *B. tabaci* population and the virus infection in the dry season, the vector population should be monitored regularly during the season and spray should be applied when the population reaches the action threshold of the pest.

In the dry season, all insecticides tested were effective in suppressing the vector population in comparison to the control (Fig. 7). A single application had the same impact as two applications every week in suppressing the insect population, except deltamethrin and azadirachtin had significantly lower number of SPW in the plants sprayed twice per week than in the plants sprayed once in a week. Similarly, all insecticides tried successfully suppressed the viral disease incidence to levels substantially lower than the control (Fig. 8). Our results suggested that the current farmers' practice of spraying their plants 2-3 times a week to control the SPW and PepYLCIV (Nasruddin *et al*., 2020) is unneeded, resource wasting and potentially harmful to beneficial organisms and the environment. However, insecticide application of once a week throughout the season as tested in the current experiment is still considered very high. Therefore, lower application frequency and proper timing for insecticide application, based on an action threshold, should be studied further in order to control the disease and its vector more effectively, and efficiently and at the same time reduce its potential detrimental effects on non-target organisms and the environment.

Conclusion

All insecticides tried in the current study provided effective control of the *B. tabaci* population and PepYLCIV incidence in the dry season. One application per week was sufficient to curb the vector population and the disease incidence. Thus, local farmers' practice of spraying insecticides 2-3 times a week is unnecessary. Since one spray per week for the whole season as studied in this experiment is still considered very high, more research needs to be done to elucidate the appropriate timing and frequency of insecticide application based on the pest's economic thresholds as a pest and a vector. During the rainy season, on the other hand, pesticide applications may be necessary to avoid PepYLCIV inoculation only early in the season, when the rainfall rate is low and the SPW population is large. However, for the rest of the season, no sprays are needed. It is interesting to note that, in contrast to earlier publications suggesting a positive association between the vector population and the PepYLCIV incidence, we discovered a negative correlation between the two variables, during the rainy season. This finding suggests that different insecticide application schemes should be used to manage the PepYLCIV and its vector in the dry and rainy seasons.

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Author's Contributions

Andi Nasruddin: Designed the experiments, supervised the whole work, validated the data, prepared the original manuscript and critically revised it, approved the manuscript, and acted as corresponding author.

Iftitah Kartika Amaliah, Nurul Arfiani, Ernawati Djaya, Firdaus Firdaus, Erwin Erwin and Rahmat Mahadir: Conducted the field experiments, collected the data, analyzed the data, critically revised the manuscript, and approved the manuscript.

Melina Melina and Muhammad Junaid: Supervised field work, validated and analyzed the data, and critically revised and approved the manuscript.

Ethics

This is an original article containing unpublished data. The work has no ethical concerns and each author has reviewed and given their approval for the manuscript.

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