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Seedling protection and barrier crops in chili pepper to reduce whitefly denseness and prevalence of pepper yellow leaf curl virus

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Abstract. Yellow leaf curl disease caused by pepper yellow leaf curl virus (PepYLCV) and transmitted by the whitefly (*Bemisia tabaci*), becomes the main problem in major horticultural crops, including chili pepper. The control of the insect vector is crucial to overwhelm the diseases. The experiment was designed to verify the efficacy of integrated vector and virus control focused on barrier crops and healthy seedling techniques on chili pepper to reduce whitefly crowdedness, the prevalence of the virus, and crop yield. This experiment used a randomized block design. The treatments were two kinds of seedlings, with and without seedling cover, combined with four kinds of borders i.e., maize, Sunn–hemp, screen (chiffon), and control (non-border). The result indicated that the use of protected seedlings was able to defend against the attack of *B. tabaci* and delay virus infection for about 2 weeks. The use of protected seedlings together with barrier crops (maize and sunn-hemp) reduced the number of pests, and PepYLCV damage up to 49.94 – 50.80%, and gave the highest yields. The positive correlation (r) between the population of *B. tabaci* and disease incidence was 0.925, whereas the correlation between disease incidence and yield crop was negative (r = -0.8886). These results will provide an advance of more valuable and practical methods for safeguarding chili pepper production.

Keywords: *Bemisia tabaci*, chili pepper, PepYLCV, seedling protection, border crop

1. Introduction

The whitefly, called *Bemisia tabaci* (Aleyrodidae, Hemiptera) is an important pest of major horticultural crops in subtropical and tropical regions of the world [1 – 3]. Two main types of damage are caused by *B. tabaci* on chili pepper plants. The first one is produced by the direct feeding of both immature and adult stages, while the second is triggered by vectoring and the spread of pepper yellow leaf curl virus (PepYLCV). It includes the genus *Begomovirus* of *the Geminiviridae* which mainly leads to significant yield loss in chili crops [4 – 7]. Transmission of PepYLCV in the field only happened by the insect vector *B. tabaci* persistently, but not propagative [8]. These pathogens bring about tremendously destructive maladies in a broad range of crops, i.e., bean golden mosaic (BGM) in the Americas; beet curly top in North America and the Middle East; african cassava mosaic in Africa; cotton leaf curl in Asia; maize streak in Africa; and tomato yellow leaf curl in Asia, Europe, Africa, and Americas, [9]. Plants infested with PepYLCV exhibit varied symptoms depending on varieties, such as leaf crumpling, curling, veinal and interveinal yellowing, yellow spots in leaves, stunting and leaf distortion, and some combination of golden-light, and green-yellow mosaic or mottle [10]. Further infection of PepYLCV could conduct to bright yellow and smaller leaves, and stunted plants as well. Chili infecting PepYLCV occurs in Africa, Central America India, Indonesia, Mexico, and the southern United States.

The occurrence of PepYLCV in Indonesia was first reported in West Java in 1999. The virus spread into Central Java in 2003, South Sumatera, South Borneo, and Lampung [11-12]. Its infection caused severe yield losses, particularly in the Cucurbitaceae and Solanaceae [13], and the yield loss of chili reached 100% [12 – 17].

Control of *B. tabaci* depends upon frequent pesticide usage because of high population masses fortified by the overlapping availability of multiple crop hosts throughout the year. The use of abundant insecticides also contributed to the increase of PepYLCV diversity [13]. Therefore, viable management of the pest in these cropping systems hinges on a large coverage of the readiness of various insecticidal chemicals and their continuous efficacy. In such cropping systems, repetitive spraying is necessary and often leads to the overuse of these chemicals. Despite the heavy use of pesticides, the average yield loss caused by insects remains high [18]. *B. tabaci* has increased resistance to many common insecticides around the world. *B. tabaci* are highly resistant to distinctive groups of insecticides i.e., carbamates, pyrethroids, and organophosphates [19-22]. Mota-Sanchez and Wise [23] stated that approximately 650 occurrences of insecticidal resistance of the genus Bemisia have been reported, and such resistance of more than 60 active substances has been detected**.** It should also be understood that the management of whiteflies and PepYLCV cannot be undertaken by any single control method, especially with dependency on insecticides. This is the reason why an IPM approach is needed.

The integrated healthy seedling technique that is composed of the protection of nurseries with insect nets and barrier crops has been suggested as another cultural control option for reducing the incidence of PepYLCV. Non-host plants, such as grasses (Gramineae), consisting of sorghum, *Sorghum bicolor* (L.) Moench [4], pearl millet, *Pennisetum typhoides* (Burm. f.) Stapf & Hubbard [24], and maize, *Zea mays* L. [25] have been effectively applied to reduce virus transmission and whitefly colonization. Divanzo et al. [26] and Fereres [27] reported that barrier plants have been utilized as a control strategy since the early 1950s to reduce the distribution of non-persistent aphid-borne viruses. Potato plots surrounded with maize as the barrier reduces aphid density and PVY virus transmission. Manandhar & Hooks [28] reported that sunn-hemp (*Crotalaria juncea*), decreased the prevalence of Papaya ring spot virus-watermelon strains by acting as a virus on zucchini.

The experiment was designed to determine the efficacy of IPM (Integrated Pest Management) focused on barrier crops and healthy seedling techniques on chili pepper to reduce whitefly masses, the prevalence of PepYLCV, and crop yield. It is expected that the incorporation of these methods will provide appropriate management of the pest, and guarantee sustainable yields in the future.

2. Materials and Methods

The research was conducted at the Indonesian Vegetable Research Institute (IVEGRI) at Cikole-Lembang, West Java, elevation 1,200 m sea above level (sal), from June 2017 until January 2018. The experiment was arrayed in a completely randomized block design with eight treatments and three replications. Two kinds of seedlings, with and without protected seedlings, combine with four kinds of barriers i.e., sunn-hemp, maize, screen (chiffon), and non-border. Eight treatments were established :(A) Protected Seedling + Sunn - hemp; (B) Protected Seedling + Maize; (C) Protected Seedling + Screened (chiffon); (D) Protected Seedling + No border; (E) Non-Protected Seedling + Sunn - hemp; (F) Non-Protected Seedling + Maize; (G) Non-Protected Seedling + Screened (chiffon), and (H) Non-Protected Seedling + No border (Control).

The chili pepper variety "TM 999" used as the main crop was surrounded by three rows of different border crops as treatments. Each plot was 14 m x 12.5 m and was separated by min 2 m. Six beds were located in each plot, with two rows of plants 50 cm apart. Plant spacing within rows was 70 cm. The number of plants per bed: 30 plants. Each treatment consisted of 180 plants per plot. Border crops (maize and sunn-hemp) were planted two times during the planting season (4-5 weeks before planting and 6 weeks after planting of chili pepper). The inter-row spacing of border crops was 25 cm, but intra-row spacing depended upon the plants, for maize was 25 cm, and sunn–hemp was 30 cm.

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The protected seedling technique was applied by sowing the seeds under a 60-mesh plastic covering net, and then seedlings were treated with thiamethoxam 25% WG through soil drenching three days before transplanting at four weeks after sowing. Silver black cover mulches and yellow sticky traps were used for repelling and attracting insects, respectively. Chili pepper seeds were sown in banana leaves, and were placed at nurseries depending on the treatment. Nurseries were covered with insect netting (4.0 m x 1.5 m x 1.5 m) with double layers of the entrance (Covered seedlings). Seedlings (40 days old) were transplanted into the field. The soil was prepared before transplanting, according to IVEGRI recommendations. Manure (400 kg ha^{-1}) and TSP (200 kg ha^{-1}) were applied a week before transplanting. Silvery plastic mulch was used to cover the beds. Urea (200 kg ha^{-1}), ZA (500 kg ha^{-1} and KCl (200 kg ha^{-1}) were added three times at 3, 6, and 9 weeks after planting.

2.1. Sampling Methods
Adult whiteflies activity was observed by using yellow sticky traps of 6.0 cm x 15.0 cm, and three traps were located in each plot. The traps were put above the canopy of the crop because whiteflies are commonly attracted to the young growth of the plants. The traps were changed daily at the nursery and weekly in the field and were collected and brought to the laboratory for inspection, observation, and counting the number of trapped whitefly adults. Some undeveloped phases of *B. tabaci* on chili were observed at weekly intervals from 2 WAP until 12 WAP. Sampling was carried out at three different positions of plant height namely upper, middle, and lower levels. Leaf samples were preserved in plastic bags labeled with plot number and position within the canopy. The leaves were then brought to the laboratory where the nymphs and adults of *B. tabaci* were counted using a dissecting microscope.

2.2. Disease Intensity
The disease intensity (IP) was analyzed to verify the severity of the virus infection on the tested chili genotypes utilizing the formula:

$$IP = [\Sigma (n_i \times z_i) / (N \times Z) \times 100\%$$

where: IP = Intensity of the disease; i: 0 to 5, n_i = number of symptomatic plants showing the value of a particular score; z_i = value of symptom score; N = the total number of plants observed, and Z = the value of the highest symptom scores.

Symptom severity scales were classified as follows : 0 = no symptom; 1 = light yellow and mosaic symptoms; 2 = moderate yellow and mosaic symptoms; 3= severe yellow and mosaic symptoms; 4= yellow, malformation, dwarf symptoms.

AUDPC (Area Under the Diseases Progress Curve) was computed with the AP (All Points) method (AUDPCAP) as recommended by Shaner and Finney (29):

$$AUDPC = \sum_{i=1}^{n-1} \frac{(x_i + x_i + 1)}{2} (t_i + 1 - t_i)$$

where: AUDPC = Area Under Diseases Progress Curve; x_i = the part of host tissue damaged on the i^{th} day; t_i = the time period (days) after the emergence of the disease on the i^{th} day; n = the total number of inspections.

$$IP = \left(1 - \frac{AUDPC\ treatment}{AUDPC\ control}\right) X\ 100\%$$

Where: IP = Inhibition percentage

2.3. Yield Assessment
Fruits per plot of chili pepper were pondered for each treatment to notice the total yield. Data are performed in weight t ha^{-1}.

2.4. Statistical Analysis
The data were treated to one-way ANOVA (SAS Program). Significantly different means ($P < 0.05$) were detached using Duncan Multiple Range Test (DMRT) at 5% probability. Count data were converted by the square root of $(x + 0.5)$, and transformation of percentage data by arcsine-square root. The original data and their standard errors are presented in graphs and figures.

3. Results and Discussion

3.1. Adult Population of Whiteflies in Nurseries and the Field
The number of trapped adult whiteflies in yellow sticky traps in protected seedling technique treatment was significantly lower (no adult trapped) and without covered protected seedlings ranged between 5 – 18 adults/days (Fig. 1). From these data it was indicated that whiteflies have occurred at the beginning of planting time. Karungi *et al.* [30] reported that protected seedlings reduced the population of whiteflies by up to 28%. This study demonstrated that covered seedlings reduced the number of adult whiteflies on chili pepper and the incidence of PepYLCV. The timing of PepYLCV infection is the main factor that affects the severity of the virus disease. The perilous feeding period for whiteflies on chili plants was identified in the 4th week after germination, resulting in yield losses of up to 100% [12]. The virus infection on tomato plants 20 days after planting resulted in a yield loss of 92%, but when the infection occurred in older plants 35 days after planting, the yield loss dropped to 74%. It was indicated that the earlier the virus infection, the greater the damage [31].

Trapped adult whiteflies were first noticed on all barrier treatments 2 weeks after transplanting (WAT). Significant differences were detected in the average weekly trap catch between treatments (Fig. 2). The flying ability of whiteflies enabled them to quickly hunt their food. Whitefly numbers in the treated plots were significantly lower than those of the untreated plots (without protected seedlings and barrier treatment). The mean numbers of whiteflies weekly in barrier treatments with and without protected seedlings were significantly lower compared to another treatment. The whitefly numbers on the treatment ranged from 18.33 to 110.67 and 36.67 to 131.33 on the control. It indicated that barrier treatments reduced whiteflies adults from 15 to 50%.

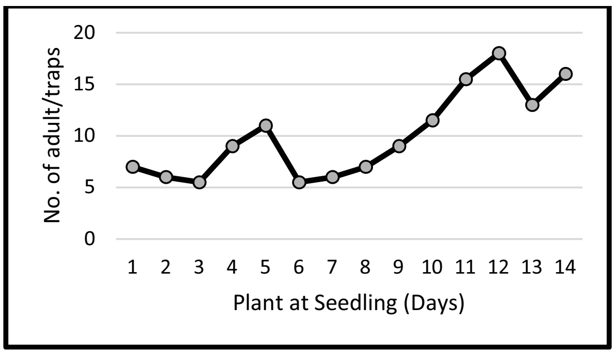

Figure 1. The average adult of whiteflies trapped at the nursery for 14 days by using a yellow sticky trap

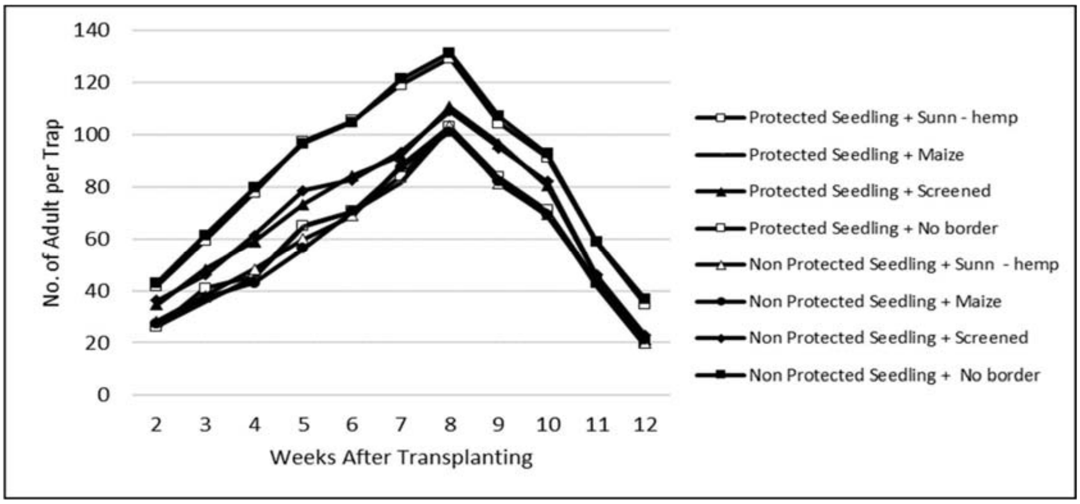

Figure 2. The average number of trapped adult whiteflies

A similar trend of trapped adult whiteflies among treatments was observed. Adult densities on chili were lower at the first observation and increased in line with plant age. The number of trapped adult whiteflies varied between 18.33 – 131.33 adults per trap during the plant growth period. The peak incidence in all the samples of trapped whiteflies occurred in 6 - 8 WAT and declined at the end of the cropping period. This might be due to the decrease in plant quality as the plant matured. The results also indicated that the number of adult whiteflies reduced very rapidly after 9 WAT until the end of the cropping period (12 WAT). Therefore regular monitoring of the whiteflies should be established from the early phase of the crop. The utilization of the traps inside the nursery and in fields is an advantageous tool for a suitable whitefly management program on chili. The traps can be applied for whiteflies monitoring and can reduce oviposition and subsequent nymph population on plants [32 - 33].

The average number of adult whitefly catches was higher on the trap located on the control plot than that on the border plot, and this indicated that maize, sunn-hemp, and screen consistently reduced the population of whiteflies. Nevertheless, maize and sunn-hemp the most effective barrier crop delayed entering the adult of whiteflies to chili pepper. This suggested that screen (chiffon) only acts as a physical barrier, meanwhile maize and sunn–hemp not only act as a physical barrier but also whiteflies cannot distinguish hosts from non-hosts. Manandhar & Hooks [28] reported that sunn-hemp (*Crotalaria juncea*) decreased the occurrence of Papaya ring spot virus-watermelon strain on zucchini. According to Friarini *et al*. [34], maize was effective to reduce the spread of whiteflies. This suggests that the crops could serve as mechanical barriers and also as a source of natural enemies of the vector as well [35].

The distribution average of whiteflies varied among the treatments and plant age. Statistical analysis revealed that protected seedlings and barrier crops had the significantly highest effect in reducing the population density of whiteflies on chili pepper (Fig. 3). The population of whitefly nymphs was intensely dependent on treatments during cropping periods. This was similar to that of adults described previously. The number of immature whiteflies was notably correlated with the number of adult whiteflies. The present study also showed that the population of adult whiteflies was extensively influenced by the existence of immature whiteflies.

Nymph populations on the plant were slightly lower from the beginning until the end of the cultivation period (Fig. 3). It was indicated that adult whiteflies trapped during the planting season were migrating adult not colonizing adult on the chili pepper. The present study indicated that the traps can be used both for monitoring insects and controlling whiteflies. Abdel-Megeed et al. (1998) also showed that the traps can decrease whitefly density in the field. Although whiteflies have a wide host range, however, it more prefers to colonize host that has trichome on the underside of the leaves such as cotton and eggplant [36 – 37].

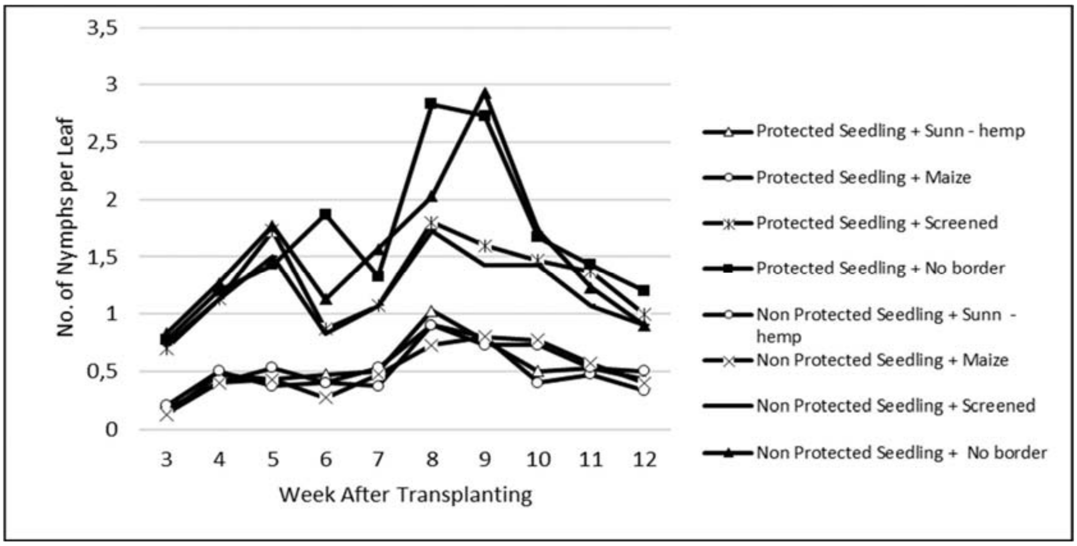

Figure 3. Number of nymphs whiteflies on each chili plant (average of nymphs in three leaf strata)

A higher number of whitefly nymphs per leaf was observed at weeks 8 and 9. However, the number of immature whiteflies declined at the end of the cropping period. This might be due to the reduction of plant quality as the plant matured. Barrier crops considerably suppressed the increase of whiteflies population. Regular chemical control of whiteflies is very complicated because the immature stages of the pest commonly appear on the lower surface of leaves and in the below part of the plant canopy [38]. The copiousness of both immature and adult whiteflies on spring collard was certainly correlated with plant height and maturity. The numbers of adult and immature whiteflies decrease at the end of the cropping period [6].

3.2. Incidence of PepYLCV

The incidence of disease varied based on the treatments and time observed. The incidence of the virus was delayed by approximately 2 weeks on the protected seedling treatment. The time of infection is a factor that influences the harshness of the disease caused by PepYLCV. It was indicated that the younger plants infected with the virus, the greater the damage (Fig. 4). The study demonstrated that all the treatments reduced the disease incidence significantly in comparison to the control (unprotected) chili pepper plot. Two treatment-protected seedlings with maize and sun-hemp as a barrier crop can significantly reduce the subsequent transmission of PepYLCV on chili pepper by whitefly. Disease incidence increased with the increase in the growth stage. The highest damage occurred at 12 WAT, the maximum disease incidence found at the treatment without protected seedling combined with screen barrier treatments was 81.67% and without protected seedling and barrier was 80.56%. The lowest PepYLCV incidence was found at covered seedling treatment combined with barrier plant (maize and sun–hemp) up to 50.56% and 51.11%, respectively. The experiment indicated that leaf curl virus prevalence under field conditions corresponds with the whitefly vector population on plants.

The value of AUDPC obtained from this experiment indicates the disease incidence performance. The lowest AUDPC value, the highest suppressed incidence of PepYLCV. The lowest AUDPC (1543.92 – 1571.14) was found at the protected seedling treatment combined with barrier crops (maize and sunn-hemp) with the suppressed incidence of the virus being 49.94 – 50.80%. The contribution exhibited by barriers to reducing whiteflies may depend on the crowdedness of the whiteflies population. Barriers such as maize and sunn-hemp may be more applicable when implemented with other control measures.

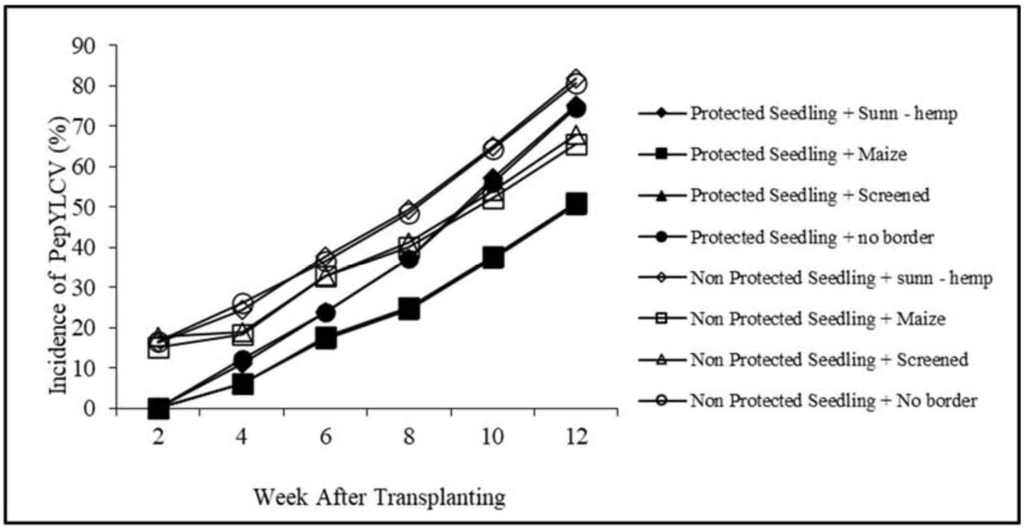

Figure 4. Incidence (%) of PepYLCV on chili pepper

This data suggested that the use of protected seedlings combined with the barrier (maize and sunn-hemp) reduced the incidence of PepYLCV up to 49.94 – 50.80%. Barrier crops were able to decrease the spread of PepYLCV.

Regression analysis correlation between the incidence of PepYLCV and the size of the whitefly's population was y = 6.165 x – 93.466 with R^2 = 0.8559 (F = 35.62; P, 0.001) dan r = 0.925 (t = 5.91; P, 0.001 (Fig.5). This data suggested that there was a high correlation between the incidence of PepYLCV and the number of adults caught on the traps. PepYLCV incidence was certainly correlated with whitefly population density. However, single whitefly can transfer the virus to chili plants [11, 39].

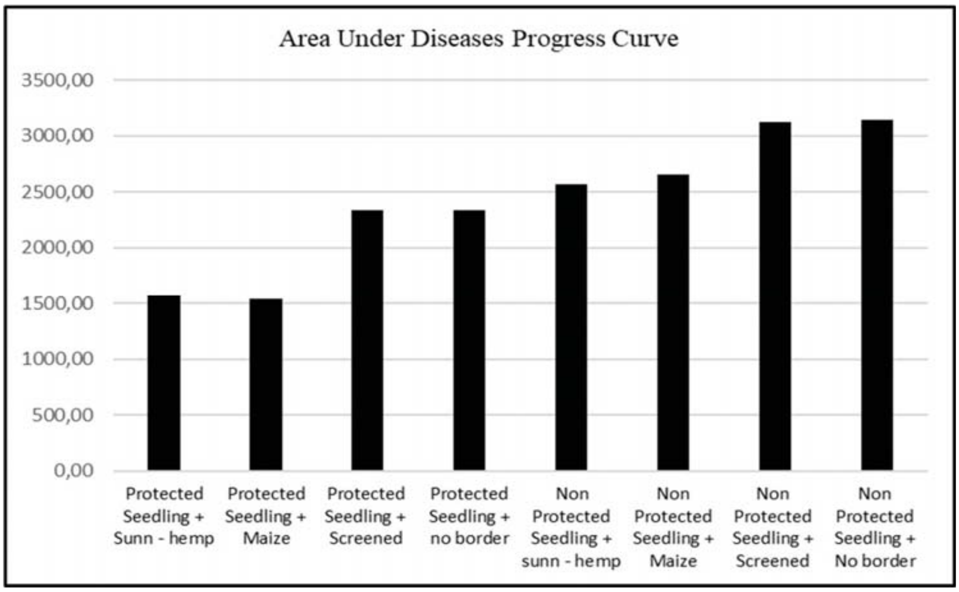

Figure 5. AUDPC using disease incidence on chili pepper

Chili pepper yields gained are shown in Figure 6. Results proved that yields were influenced by the treatments. Both adults and nymphs of the pest suck the cell liquid from different plant parts causing losses of plant vigor and reducing crop yield, and the virus infection caused shorter fruit length. Among all the treatments protected seedlings combine with barrier crops (maize and sunn-hemp) gave the highest yields followed by a physical barrier (Screen), whereas the lowest yield was recorded in the plot without protected seedlings with barrier crops. This result revealed that chili pepper bordered by maize and sunn- hemp contributed significantly in reducing the population of whiteflies and incidence of PepYLCV and contributed significantly in blocking the whiteflies coming from the neighboring field. It can be concluded that chili can be cultivated with less incidence of PepYLCV with protected seedlings and maize as a barrier crop. Intercropping was more efficient to decrease whitefly density confirming Zhang's results [40].

Regression analysis correlation on the incidence of PepYLCV with a yield of chili pepper have P = 54.122 – 0.501 K, with R2 = 0.7897 (F = 82.60; P < 0.001) and r = - 0.8886 (t = 9.09; P, 0.001). This data suggested that there was a negative correlation between the incidence of PepYLCV with the yield of chili pepper. Increasing the virus incidence reduced chili pepper yield.

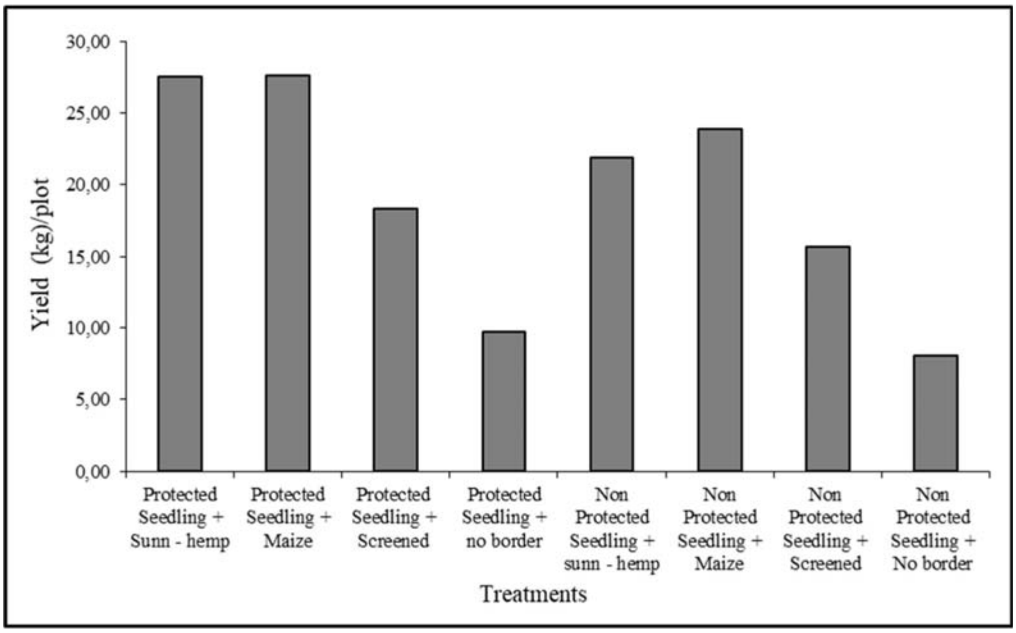

Figure 6. Average yield [kg/plot] of chili pepper under different treatments

4. Conclusions

The use of protected seedlings combined with barrier crops is the potential to suppress the incidence of PepYLCV and reduce the population of B. tabaci, the vector of the virus. Delayed viral infection on the protected seedling decreased the severity of the virus on the chili pepper. The combination of the treatments significantly promoted the highest yields of chili pepper.

The results will contribute to the development of more effective and practical approaches for protecting chili pepper not only from *B. tabaci* and PepYLCV but also from other major virus diseases with their insect vectors. Further similar research should be carried out for any other important vegetable crops to decrease the incidence of viral diseases together with their insect vectors. Furthermore, the combination treatments will decrease the use of chemical insecticides which are commonly used by farmers to control insect pests.

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