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Management of tomato leaf curl virus disease transmission by whiteflies vector

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Abstract

In this paper, Tomato leaf curl disease which caused by Tomato leaf curl (ToLCV) and tomato leaf curl virus and damaged 80-90% yield losses was found in Kompot and Siem Reap provinces, Cambodia. This study was conducted to evaluated the management of tomato leaf curl virus disease and it vector whiteflies in Cambodia. Specifically, this study determined the differences application treatments to controlling insect vector and reduced leaf curl disease severity. There are two types of data collection: crop components and disease assessment. The data were collection after selection four normal size plant from each plot for measuring some parameter like TAS – ELIS. The data disease severity scale, disease incidence and percent disease reduction. Based on the result analysis of symptom severity score of treated plants regardless of induction time showed that those treated with SA has lower symptom severity score than the untreated control within the treatments with Neem extract resulted in symptom severity score at 2 wpi was significant lower mean value of (ss = 0.59, 1.31 and 2.46) and 3wpi showed result (ss = 2.44, 2.85 and 4.29) as compared to control. There was a significant reduction of ToLCV disease incidence in all the treatments compared with the untreated control which trial A, disease incidence at 2 wpi was lower in plants treated with treatment of T1 at 10, 15 and 20dbi having means value (12% to 18%) as compared with the control and tria Management, l B, incidence at 3 wpi was high in treated plant that the untreated control (Fig B). In case of treatments plants, the three treatments at 10dbi incidence had the lowest (less than 12%). The efficacy was affected by difference concentration, and by the induction time depending on the treatment.

Keywords: disease, management, resistance, tomato leaf curl (ToLCV), whiteflies vector.

Introduction

One of the most popular grown vegetables, the tomato is a nourishing vegetable that contains a diversity of vitamins and minerals (Bankole,1996) and also plays an essential role as a cash crop for worldwide trade. There are approximately 4.5 million hectares of cultivated land and produce 122.6 million metric tons of yield (Chittaranjan and Timothy, 2008). To cultivate this crop, there is a considerable number of abiotic and biotic constraints to reduce the yield and the quality of tomatoes (Arya and Perello,2010). The Leaf curl virus disease which caused by Tomato leaf curl (ToLCV) and tomato leaf curl virus and damaged 80-90% yield losses was found in Kompot province, Cambodia (Basavaraj and Rayapati, 2016). This disease is located in the Begomovirus genus and Geminiviridae Family. ToLCV can impact the early stage of tomato growth illustrating the symptom of stunting, curling leaf upward, reducing the margin of the leaf, stunting new shoot, and turning to yellow (Salati et al., 2002). Commonly, whitefly (*Bemisia tabac*) is an agent of spreading the begomovirus to tomato plants by starting to oviposit the egg underside of tomato leaves. The nymph of a whitefly can transmit begomovirus to tomatoes (Cohen and Nitzany, 1966). The ability of virus translocation is related to both genders of whitefly. The 3week-old female can infect 60% of the plant while there is no infection by a male at the same age (Czosnek et al., 2002). To control ToLCV, agrochemical pesticides are commonly used in the field. To manage the disease control of its vector is very important which is done by using different approaches. Management of ToLCV and Bemisia tabac through plants extracts, nutrients and insecticides are useful. However, chemical substances can have detrimental impacts on both human health and the environment. As a consequence of this, alternative methods should be considered, especially the natural product plays a vital role instead of the chemical substances (Siebert et al.,2012). Similarly, Plant extract is one of eco-friendly products which uses to control pests and pathogens (Jayaraj et al., 2008; Kapooria, 2007) and does not impact on plant growth (Ning et al., 2003). In addition, there are a wide variety of plants that contain the potential compound against the pathogens and safe for the environment (Elsharkawy et al., 2015). Therefore, the objective of this study was to assess the appropriate methods to prevent ToLCV and to identify the symptom of ToLCV on tomato crops.

Methods

The experiment was conducted at the greenhouse of the faculty of Agronomy, Royal University of Agriculture started from October 2018 till December 2019. This experiment was designed in Randomized Completely Block Design (RCBD) consisting five treatments untreated control, Salicylic acid, Carrageenan, Neem extract, *Trichoderma harzianum* and three induction time 10 ,15 and 20day before inoculation and four replications which account four plants. The seeds of susceptible tomato varieties were chosen to sown in sterile soil on plastic trays. After germination for 20 days, the seedlings were transplanted into the pots and kept in whitefly-proof net cages to avoid pathogens before inoculating the virus. The leaf curl infected tomato plants were used as a source of virus inoculation and given whiteflies to feed and increase its population on these plants. The 35-day-old of healthy tomatoes were inoculated with the viruliferous whiteflies by putting 10 whiteflies per plant for an acquisition feeding period for 24 hours. After the inoculation, the plants were observed to identify the development of the disease. Whiteflies were directly put-on tomato leaves between 6:00am – 7:00am. After inoculation whiteflies were killed with treatments and symptoms development was recorder for 1,2 and 3 weeks.

TAS – ELISA

To identify the Tomato leaf curl virus, Triple Antibody Sandwich Enzyme Linked Immunosorbent Assay (TAS-ELISA, alkaline phosphatase conjugate) was used. Testing with TAS – ELISA, the second, third, fourth and fifth leaf of tomatoes were selected randomly from four different plants for each replication in all plots both treated and control treatments. After selecting the leaves, all leaf samples were ground (1g sample/10ml buffer) in extracting buffer: Coating buffer 100μl, Antibody coating buffer 100μl, PBST 14.4g, Tween-20, for PBST and sample buffer 4.0g, SB1 buffer 5.8g, SB4 grape sample buffer 24.7g, SB5 blueberry sample buffer 8.6g. pH 7.3 and incubated at 40C overnight. Regarding the positive of TLCV infected tomato leaf tissue sap, the positive samples were diluted in ELISA extraction buffer and further steps were carried as normal TAS – ELISA. The protein concentration was adjusted 250mg/ml in all samples before loading into the wells and the plats were washed four time with antibody coating 100μl and incubated for 4h at 40C. When the plates were already washed, phosphatase buffer saline pH 7.3 containing 0.05% Tween-20(PBST) was added to wells and incubated at room temperature for 21- 240C. Following to the previous steps, the wells loaded with 100 μl of the enzyme alkaline phosphatase conjugate at 1:10 ratio and were incubated at 240C for 2.5h. The absorbance value of each sample was measured at 650 nm using a microplate reader 60 min after the addition the negative control at least a factor of two.

Disease severity: The symptom severity was determined at 3wpi, following the rating scale.

Table 1: Rating scale use in evaluating the symptom severity of Tomato leaf curl.

Rating scale	*Symptom description*
0	No leaf curl disease symptom
1	Leaf curl disease symptom on the shoot apex
2	Leaf curl disease symptom on the shoot apex, and on the first and second leaf petioles
3	Leaf curl disease symptom on upper half portion of the plant
4	Leaf curl disease symptom on the whole plant
5	Leaf curl disease symptom on the whole plant with severe stunting

Disease incidence (%): The disease incidence was determined by using the following formula.

$$\text{Disease incidence} = \frac{No.of.infected\ plant}{No.of\ plants\ examined} \times 100$$

Percent Disease reduction (%): The reduction in disease incidence was calculated by following formula

$$\text{Percent Disease reduction} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}} \times 100$$

Where, C is percent disease incidence in untreated,
T is percent disease incidence in treated plants.

Evaluation of plant growth and Disease Assessment

Crop components and disease assessment were identified by choosing four normal tomato plant from each plot to measure TAS – ELIS, Chlorophyll contents and disease severity scale and disease incidence (%) and percent disease reduction (%).

Statistical Analysis

All data in the present study were subjected to analysis of Variance (ANOVA) and means were compared using the Least Significant Different Test (LSD, $P \leq 0.05$) using the Statistics8 Too for Agriculture research (Statistics8 version 8.0).

Results

The data describe in the following of ToLCV virus was identified by TAS-ELISA by randomly selecting 96 samples from all treatments. According to the result of TAS-ELISA, there are significant difference at P≤0.05 in the first year between control treatment and treated treatments. The control treatment illustrated the highest concentration agent, consisting of 0.92. The following year there are significant different between Salicylic acid treatment and control treatment at P≤0.05, with 0.29 and 0.35, respectively. (Table.2).

Table 2: The mean TAS-ELISA absorbances of the tomato sample after infection tomato leaf curl virus at 2018-2019.

Treatments	Detection of ToLCV in test plants by TAS-ELISA	
	2018	2019
Control	0.92 a	0.35 a
Salicylic acid	0.68 b	0.29 c
Carrageenan	0.64 b	0.31bc
Neem extract	0.69 b	0.32 ab
T. harzianum	0.66 b	0.32 ab
LSD ($P \leq 0.05$)	8.11	1.76
CV (%)	11.97	2.45

The value in table followed by the different letter indicates significant difference among treatment according to the last significant difference test $P \leq 0.05$.

According to the chlorophyll measurement by used sped 502 plus and the 2 seasonal experiments between 2018-2019 of TLCV impacting on leaf chlorophyll, shown that during season in 2018, the control treatment got the lower number in 31.33 μg/cm2 as comparing to all other three treatments. But there is also gotten the lower number of plant chlorophyll in 39.19μg/cm^2 as comparing to other application treatments in significant different 95% ($p<0.05$). So, this result could be express based all those applications have efficiently to control plant chlorophyll of season 1 (2018) get better result than season 2 (2019) (Table 2).

Table 3: The effect of treatments to chlorophyll.

Treatments	Chlorophyll index reading (μg/cm^2)	
	2018	2019
Control	31.33 b	39.19 d
Salicylic acid	37.81 a	49.45 a
Carrageenan	36.90 a	46.70 ab
Neem extract	36.77 a	42.78 cd
T. harzianum	36.16 a	45.18 bc
LSD ($P \leq 0.05$)	2.08	3.70
CV (%)	5.09	5.38

The values in table followed by the different letters indicate significant difference among treatment using the least significant difference test $P \leq 0.05$.

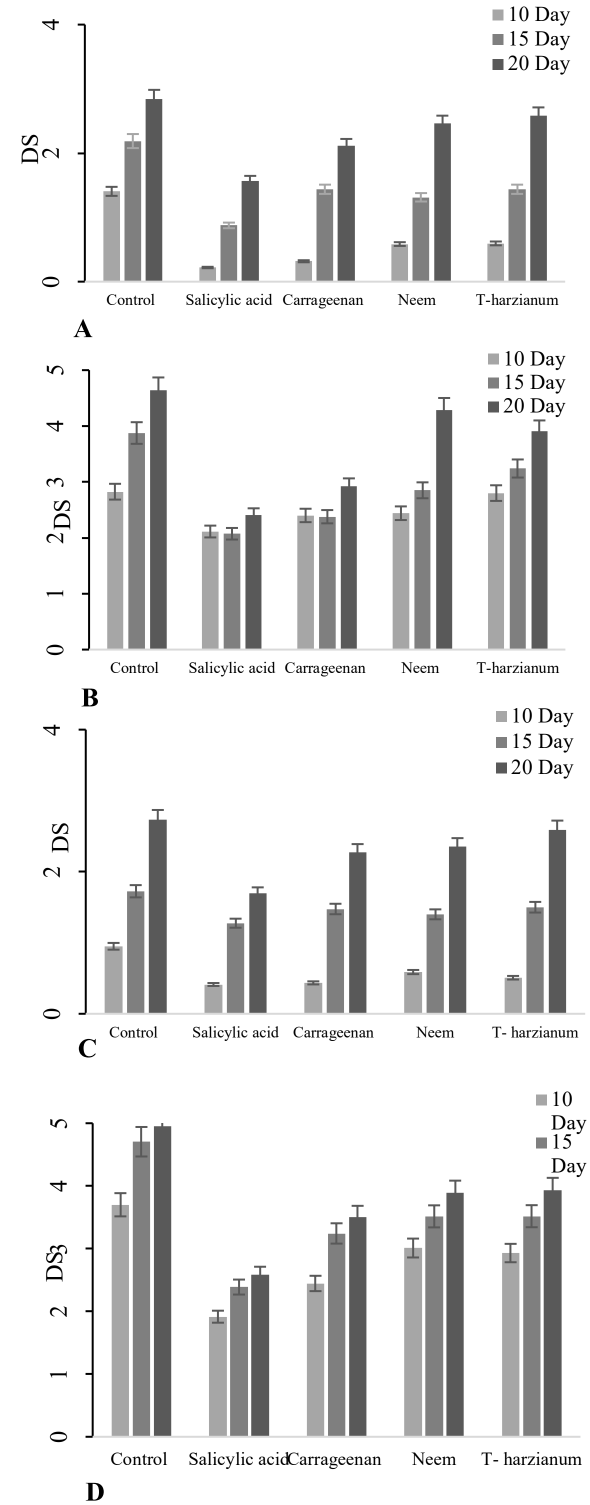

Fig 1: Symptom severity score for tomato leaf curl at 2,3 weeks after inoculation of plant treatment in Trial1 (A-B) at each time point season 2018 with 2 and 3 week and Trial (C-D) at each time point season 2019 with 2and 3week, significant difference among treatment according to the last significant difference test $P \leq 0.05$.

Symptom severity score

Analysis of symptom severity score of treated plants regardless of induction time showed that those treated with SA has lower symptom severity score than the untreated control in both trial (A-B). Fig1. In trial (A), symptom severity score at 2 wpi was lower in plant treated with varying concentrations of SA at 10, 15 or 20 dbi mean value of 0.22, 0.88 and 1.57 as compared to control. At 3 wpi was significant lower mean value of 2.11, 2.07 and 2.41that the control (Fig B). Likewise, symptom severity of application of carrageenan means value of (ss = 0.32, 1.44 and 2.12) was significantly lower than the control (Fig A) more 3wpi showed significant has means value of (ss = 2.40, 2.38 and 2.92) as compared with control (Fig 3). Treatments with Neem extract resulted in symptom severity score at 2 wpi was significant lower mean value of (ss = 0.59,1.31 and 2.46) and 3wpi showed result (ss = 2.44, 2.85 and 4.29) as compared to control (Fig A-B). Plant treated with *T. harzianum* has symptom severity score of (ss= 0.60,1.44 and 2.58) (Fig A) which was significantly lower than the control, However, at 3wpi symptom severity scores of plants treated with *T. harzianum* (ss= 2.88, 3.34 and 3.91) as compared with control (Fig B).

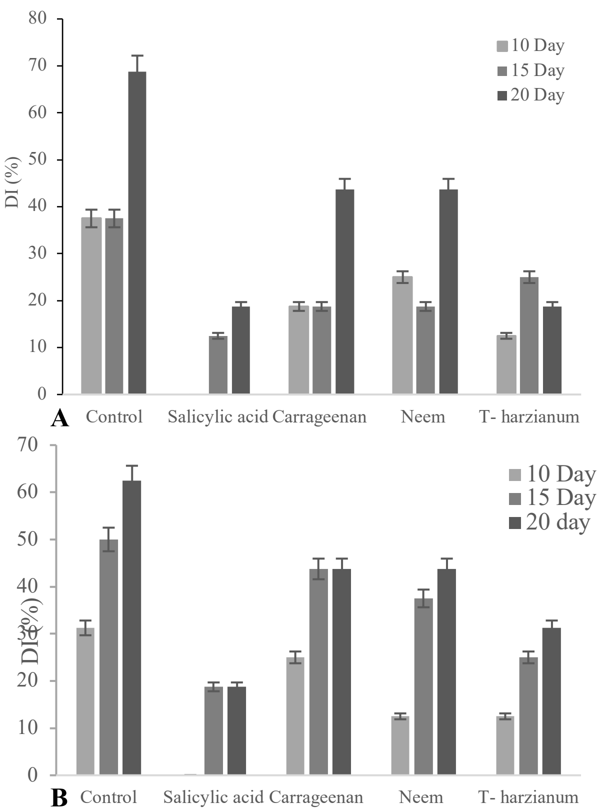

Fig. 2: Disease incidence for tomato leaf curl in treated at difference induction time with treatments in Trial (A) and Trial (B) at weeks post inoculation significant difference among treatment according to the last significant difference test $P \leq 0.05$.

Disease incidence (%)

There was a significant reduction of ToLCV disease incidence in all the treatments compared with the untreated control. The efficacy was affected by difference concentration, and by the induction time depending on the treatment of Salicylic acid (Fig A). In trial A, disease incidence at 2 wpi was lower in plants treated with treatment of Salicylic acid at 10, 15 and 20dbi having means value (12% to 18%) as compared with the control (Fig A). At induction time of 10 or 15dbi, treatment with Carrageenan resulted to lower incidence (both 19%) compared to 20dbi (44%). However, incidence at 10 or 15dbi the treatment Neem extract had the lower with (25% or 19%) compare to 20dbi (44%). At 2wpi treatment with *T. harzianum* at 10, 15dbi was slightly lower (12% to 25%) than with 20dbi (18%), while the treated untreated control had comparable incidence (68%) (Fig A). In, trial B, incidence at 3 wpi was high in treated plant that the untreated control (Fig B). In case of treatments plants, the three treatments at 10dbi incidence had the lowest (less than 12% or 25%).

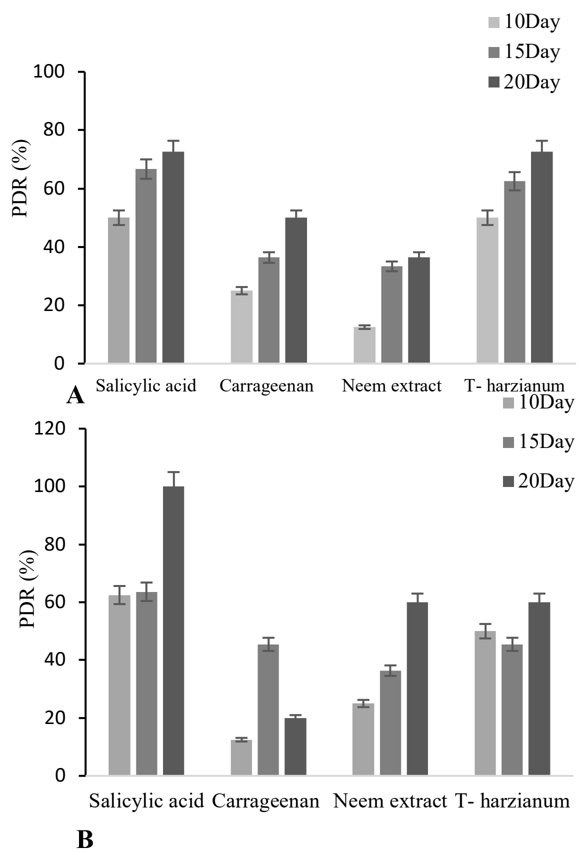

Fig 3: Precent Disease reduction for tomato leaf curl in plant treated at difference induction time with varying concentration of treatments in Trial (A) and Trial(B) at each time point (weeks post inoculation), value followed by the different letters indicate significant difference among treatment according to the last significant difference test $P \leq 0.05$.

Precent disease reduction (%)

The percentage of disease incidence of ToLCV was measured by the number of infected plants divide by the number of plants examined and multiple with 100. The highest percentage of disease infected plants in both years was observed in untreated treatment, while the lowest percentage of infected plants was found in the plots which treated with Salicylic acid and *T. harzianum*. Other plots which applied Carrageenan and Neem extract demonstrated more or less similar influence on the disease incidence caused by ToLCV in both years. Regarding the percentage of disease reduction, plots under Salicylic acid treatment showed the highest percentage of reducing diseases compared to other treatments in both years. Another treatment which proved the second highest percentage of reducing disease in the first year was the *T. harzianum* treatment, while *T. harzianum*, Carrageenan and Neem extract proved more or less similar influence in the second year.

Discussion

According to the result of the end of experimented and analysed in statistix 8 found that the determinate of ELISA DNA extractions of Salicylic acid got the highest positive value and compare to control treatment. Salicylic acid has effective to inhibit of the plan viral against DNA on crop. Salicylic acid is the primary molecule that easily to absorbance into the plant cell and also has the influence in blocking or delaying on ToLCV symptom of tomato leaf curl (Mohammad and Alzohairy, 2016). The activity of Salicylic acid reduces the disease severity of ToLCV regarding to the experiment of (Amin et al., 2016; Tong, Li et al., 2019). Salicylic acid is produced (PR-protein) that synthesis of the energy and sugar contents during the productivity of photosynthesis so this compound is to preventative or developing the plant tolerance of the disease viral against in Actually ToLCV. During the experiment (Amin et al., 2016) also reported that Salicylic acid would be complex of the decrease the symptom severity of ToLCV and tomato yields thought out curling, stunting, yellowing symptom lose their chlorophyll component on leaf. Moreover, Salicylic acid also reduce the yield loss of tomato production in average number between 28 to 92% or in higher amount until 100% that was compared to the control treatment (Nakhla and Maxwell,1998). Carrageenan, Neem extract, *T. harzianum*, Salicylic acid use to control the disease incidence and the percentage of reduction of ToLCV showing the efficiency compared to the control treatment. Because of Carrageenan is involved with chitine is the effectiveness to interact on plasmodium cell on plant into small size and delaying the dropped of viral DNA impaction in cell-wall (Vera et al., 2012). Carrageenan is also playing role in producing the sulphate for protecting the genes on plant leaves and making the resistance systemic related to adoption with plant abiotic and biotic stress condition (Bi et al., 2011; Shukla et al., 2016). Neem extract is the natural synthesis that have an importantly role in to inhibiting oxidation interaction of the growth of some bacterial and change the genes and convert to the plant cell molecules to treat ToLCV and the injure of whiteflies [Mohammad and Alzohariy, 2016; Canakci, 2011). Neem extract also release out the nitrogen and phosphate as the plant nutrient contents of developing the plant growth and yields (Oladimeji and Kannike, 2009). T- harzinum is the bio-fungicide not only use to control plant diseases such bacterial and fungus. In this component also produced the phosphate (P) and Zins (Zn) to promote plant growth and yield (Cuevas, 2006). According to the study (Mastouri et al.,2011) also showing that. Use *T. harzinum* has the special role in water stocking on soil that easily for soil moisture as plant nutrient converter Sometime Trichoderma spp. is the complex against to prevent plant from the demonstrated of plant disease. The importantly of use *T. harzinum* is the best ways to lead the plant photosynthesis to absorbance the oxygen to interact in innovated enzyme of developing plant chlorophylls and release out the blocking of symptom severity of some plant pathogen. Salicylic acid is the synthesis component chemical substance from phenylalanine. Phenylalanine is the naturel enzyme or antiviral substance or has benefit to prevent plant. Cell-wall from the attacking of viral against.

Conclusion

In conclusion, two treatments, SA and *T. harzinum*, show the effective in controlling ToLCV with strongest component and whiteflies vector. The interaction of those chemical substances is the bio-control against that have benefit of reducing disease severity, disease incidence and percent disease reduction of ToLCV. Therefore, the chemical substances of SA and T- harzinum can prevent the main vector and ToLCV in safe agriculture innovation in integrated pest management practices.

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