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Full Length Article

Potential of Neonicotinoids, Botanicals and Plant Defense Activators against Whitefly Infestation and Tomato Leaf Curl Virus Disease (TLCVD)

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Abstract

Tomato leaf curl virus disease (TLCVD) is a major constraint in tomato production that is caused by tomato leaf curl virus (TLCV). TLCV is transmitted in tomato crop by whitefly in a circulative and persistent manner. As there is no viricide available, TLCV disease management is relied upon to control the whitefly vector. Whitefly management by using common insecticides results in environmental deterioration and development of resistance. The current study was undertaken to evaluate the efficacy of relatively safer and durable strategies for the management of whitefly and TLCVD. Five varieties Carmen, Roker, Uovo Roseo, Po-02, and Lyp#1 were sown in a randomized complete block design (RCBD) with three replications. New chemistry insecticides (neonicotinoids i.e. Imidacloprid and Acetamiprid), botanicals (*Azadirachta indica* and *Eucalyptus globulus*), micronutrient solution (Classic™ of Ali Akbar group comprising 6% Zn (Zinc) and4% B (Boron) solution) and 0.35% salicylic acid were evaluated against whiteflies and TLCVD. All the treatments significantly reduced the whitefly infestation in five varieties. Imidacloprid was the most effective in reducing the whitefly population and gave 68.21% inhibition followed by acetamiprid (68.19%), *A. indica* (56.21%), salicylic acid (55.63%), Zn & B solution (28.43%) and *E. globulus* (15.01%), respectively. The maximum efficacy of imidacloprid in reducing TLCVD incidence was 74.13% followed by acetamiprid (63.00%), neem extract (54.34%), Zn & B solution (39.50%), salicylic acid and eucalyptus extract (36.17%). The study suggests that neonicotinoids, plant extracts, and micronutrients proved as effective eco-friendly approaches for whitefly and TLCVD management.

Keywords: Plant extracts; Evaluation; Salicylic acid; Insecticides; *Bemisia tabaci*

Introduction

Tomato leaf curl virus disease (TLCVD) is a notable biotic stress for the production of tomato worldwide (Chakraborty 2008). This disease is of economic importance (Valizadeh *et al*. 2011) as the yield of infected plants is reduced both in qualitative and quantitative (Fang *et al*. 2013). TLCVD is differentiated by stunting, chlorosis, upward curling of leaves, crinkling, puckering, and yellowing with reduced flower and fruit set. Infected plants have a bushy appearance due to the shortening of internodal length with more lateral branches (Kumar *et al*. 2012). TLCV is a species of the genus Begomovirus in the family Geminiviridae and is exclusively transmitted by the whitefly *Bemisia tabaci* in a persistent and circulative manner (Ghanim *et al*. 2001; Haq *et al*. 2018). The whitefly is described as 'superbug' because of its effect on agricultural production (Dalton 2006; Liu *et al*. 2007; Barro 2008).

Whitefly *B. tabaci* (Gennadious) belongs to the order *Hemiptera* and family *Aleyrodidae* (Boykin *et al*. 2007). Whitefly *B. tabaci* (Genn.) is the most damaging pest of tomato crop in the tropical and subtropical areas which cause heavy losses by direct feeding and transmitting the geminiviruses (Inbar and Gerling 2008; Haider *et al*. 2017). It has become a global threat for many greenhouse crops and could be able to infect plants at any stage of growth (Martin *et al*. 2000). *B. tabaci* induces phytotoxic disorders to crops by phloem-feeding, excretion of honeydew, and transmission of plant viruses. It infests more than 600 plant species and transmits Begomoviruses (Oliveira *et al*. 2001).

Chemical control methods remained a major approach for the management of insect infestations, but this approach has become less effective because the insect populations develop resistance against insecticides (Siebert *et al*. 2012). Due to the increasing trend of resistance development in insects against commonly used insecticides and environmental hazards; insect control programs have relied upon the use of new chemistry insecticides (Jeschke and Nauen 2008). New chemistry insecticides are environmentally safer and specific (Cloyd and Bethke 2011). The chloronicotinyls or neonicotinoids (imidacloprid, acetamiprid, nitenpyram, and thiamethoxam) have shown good efficacy in controlling insects (Bacci *et al*. 2007; Ishaaya *et al*. 2007). Imidacloprid and acetamiprid have a systemic mode of action and these have negligible impacts on the environment (Tomizawa and Casida 2005). The botanicals obtained from plant extracts also act as effective insecticides in reducing the problems such as insecticide resistance and environmental hazards caused by synthetic compounds (Abou-Yousef *et al*. 2010). The aqueous extracts of plants are efficient in repelling the whiteflies because of the elevated amount of hydrocarbons that they contain (Patel 2011). The plant health plays an important role in pest management (Altieri and Nicholls 2003). Nutrient management improves plant health which enables the plant to tolerate the incidence and herbivory of sucking as well as of chewing insect-pests. In a study, the nutrients (Zn and B) significantly reduced the population of whitefly in treated plots as compared to control (Gogi *et al*. 2012). Zinc has an essential role in the plant defense against insects and pathogenic attacks (Machado *et al*. 2018). It expresses the defense-related genes and enhances the function of the concerned proteins (Li *et al*. 2016). Zn affects the plant-microbe interaction by the activation of metalloenzymes which helps to overcome the stress (Deepak *et al*. 2006). Boron plays a vital role in the activation of dehydrogenase enzymes, sugar translocation, strengthening cell wall structure, and fruit setting (El-Sheikh *et al*. 2007). It regulates the carbohydrate and sugar contents in phloem which have been impaired by the insect and pathogen attack (Jonathan 2012). Plant defense activators provide effective control against sucking insects (Boughton *et al*. 2006). Salicylic acid (SA) is one of the prominent defense activators that activate resistance in plants (Kamel *et al*. 2016). The use of SA reduces the infestation of sucking insects in tomato plantations (Goggin 2005). SA reduces the harmful effects on plants caused by extensive insect attack (Catinot *et al*. 2008). Tomato plants treated with SA produce more quantity of terpenes that results in the repulsion of whiteflies (War *et al*. 2011).

Extensive studies have been conducted on the management of whitefly and TLCVD by using conventional insecticides that revealed less control in horticultural production systems due to repeated use (Gravalos *et al*. 2015). Whitefly develops resistance against synthetic insecticides (Palumbo *et al*. 2001). The studies about the effects of neonicotinoids, micronutrients, and defense activators against whitefly and TLCVD in tomato crops are lacking. Moreover, the effect of the above said chemicals on rice growth and yield was also evaluated. It is hypothesized that the application of treatments with varied modes of action may decrease the whitefly infestation in tomato crops and subsequently TLCV disease incidence. The present study was planned to evaluate the relative effectiveness of neonicotinoids (imidacloprid and acetamiprid), plant extracts (*Azadirachta indica, Eucalyptus globulus*), micronutrients (Zn & B solution), and salicylic acid against whitefly infestation and TLCV disease incidence in different tomato genotypes.

Materials and Methods

Experimental layout

The experiments were conducted at the research area of the Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan during two consecutive crop growing seasons of the years 2014 and 2015. In both the seasons, five tomato varieties (Carmen, Roker, Uovo Roseo, Po-02, and Lyp#1) were sown in the rows of 3m length with 70cm row to row and 30 cm plant to plant distance. These genotypes were obtained from Vegetables Research Institute, Faisalabad, Pakistan. The recommended agronomic practices (irrigation, fertilizers, weeding) were opted to keep the crop in good condition. The field was plowed and leveled thoroughly. Farmyard manure (FYM) and NPK (1:2:2) was added as basal. Seedlings were transplanted on 25 cm high ridges by maintaining a 40cm plant to plant distance. Irrigation was applied weekly basis that was reduced after flowering. Weeding was done routinely just after transplanting with a garden hoe. The experiments were laid down in a randomized complete block design (RCBD) with three replications. The plot size was 1.3 Kanal with dimensions (60×100 ft^2). All the treatments were randomly applied in the sub-plots with fifteen rows each.

Preparation of plant extracts

For the preparation of aqueous extracts, fresh leaves from the healthy plants of *A. indica*, and *E. globules* were collected and macerated with sterilized water at the dose of 1 kg/L and then thoroughly homogenized. Neem and Eucalyptus were selected for extract preparation because of their active ingredients (*A. indica* and Eucalyptol) which act as insect growth regulators. These plant extracts have been reported effective in the management of plant virus diseases (Kumar and Singh 2012). Many studies revealed that these two plant extracts are more effective than other plant extracts in terms of insect repellency, plant growth, and disease management (Ali *et al*. 2011). The composition neem extract (*A. indica*, tannins, alkaloids, oxalate, hydrogen cyanide, phenols, flavonoids, saponins, and steroids (Shah *et al*. 2017). Eucalyptus extract contains Eucalyptol (1-8, cineol), globulol, transpinocarveol, terpineol. The macerated extracts were passed through two folds of muslin cloth and diluted up to ten times and stored at 4°C until use. To prepare the required concentration, 5 mL of each plant extract was measured and dissolved in 100 mL of water. A knapsack sprayer was used to apply these solutions. The spray was done until drip off occurred and control was not sprayed with any insecticide/chemical (Ashfaq *et al*. 2006).

Application of treatments

For the management of *B. tabaci* and TLCVD, plant extracts (*A. indica* and *E. globulus*), insecticides (Imidacloprid and Acetamiprid), micronutrients consisting of 6% Zn and 4% B solution and salicylic acid (0.35%) were applied randomly to each row of experimental plot. Micronutrients were applied by following the direction of use as provided with the product. Salicylic acid was used at a very low dose to avoid toxicity. The above-mentioned treatments were applied as Neem (*A. indica*) extract (5 mL/L); Eucalyptus (*E. globulus*) extract (5 mL/L); Acetamiprid (2 mL/L); Imidacloprid (3mL/L); Classic™ comprising 6% Zn (Zinc) and 4% B (Boron) solution (6 mL/L); Salicylic acid (3.5 g/L) and Control (Water)

Whitefly (*B. tabaci*) identification and data recording

The effect of treatments on whitefly infestation was calculated by selecting three plants randomly from each row and recording the whitefly population data from upper, middle, and lower leaves and the average was calculated. For the identification of *B. tabaci*, pseudo pupae were observed under a microscope, and pairs of setae and transverse molting suture were examined (Bellows *et al*. 1994).

TLCV disease incidence recording

Disease incidence of TLCV infected plants on each variety was recorded weekly basis from the ratio of infected plants to the total number of plants and was expressed in percentage.

Data recording for growth and yield parameters

Fresh weight was calculated by selecting ten plants randomly from each variety applied with the same treatment at the time of harvesting. The harvested plants were separated into leaves, stems, and roots, all the parts were weighed and the average of ten plants was calculated. The plant parts were dried in an open-air draught oven at 80°C for 72 h, and then their dry weights were estimated. Plant height was taken with measuring tape.

Fruit weight, fruit yield/plant, and number of fruits/plant was recorded by selecting ten plants randomly from all varieties having the same treatment and average was calculated.

Statistical analysis

Data for the evaluation of the above-mentioned treatments on *B. tabaci* population and TLCV disease incidence was recorded before and after the application of treatments and analyzed through Statistix 8.1 software. All possible interactions and comparisons of treatments were determined through analysis of variance. All the treatments were compared with one another and with control by the least significant difference (LSD) test at $P= 0.05$ (Steel *et al*. 1997).

Results

The individual effect of year, spray, variety, and treatment was significant against *B. tabaci* population (Table 1). The two-way interactions of spray with year, variety with year, treatment with year, and variety with spray were also significant, whereas the interaction of variety with treatment and spray with treatment were non-significant. The three-way interaction between variety, spray and the year was significant, whereas the interaction of variety with spray and treatment, variety with treatment and year, spray with treatment and year were non-significant. The four-way interaction of variety with spray, treatment, and year was also not significant.

All the treatments were significantly effective in reducing *B. tabaci* population compared to untreated control during 2014 and 2015 (Table 2). Imidacloprid was the most effective in reducing the *B. tabaci* population (68.21%) as compared to control followed by Acetamiprid (68.19%), neem extract (56.21%), salicylic acid (55.63%), classic™ (Zn and B solution, 28.39%) and eucalyptus extract (15.01%), respectively.

All the treatments were effective in reducing *B. tabaci* population compared to untreated control during the years 2014 and 2015 (Table 2). In 2014, all the treatments showed significantly different results in reducing *B. tabaci* population, while in 2015 the salicylic acid and neem extract were not significantly different from each other in reducing the *B. tabaci* population as compared to control. In 2014, three treatments *i.e*., imidacloprid, classic (Zn and B solution), and eucalyptus extract showed significantly different results as compared to their respective treatments in 2015. In 2014, three treatments *i.e*., acetamiprid, salicylic acid, and neem extract were not significantly different from their respective treatments in the year 2015. During both years (2014 and 2015), imidacloprid was the most effective in reducing *B. tabaci* populations compared to other treatments and control.

Table 1: Analysis of variance for *B. tabaci* population and TLCVD during two seasons

Source of variation	DF	MS for *B. tabaci*	MS for TLCVD
Year	1	0.14*	230.91*
Spray	2	124.47*	170.46*
Variety	4	21.12*	16489.73*
Treatment	6	18.03*	1370.32*
Spray × Year	2	0.62*	1.37*
Variety × Year	4	0.69*	107.83*
Treatment × Year	6	0.44*	9.54*
Variety × Spray	8	0.05*	3.28*
Variety × Treatment	24	0.09 NS	11.93 NS
Spray × Treatment	12	2.71 NS	1.12 NS
Variety × Spray × Year	8	0.86*	0.53*
Variety × Spray × Treatment	48	0.001 NS	0.54 NS
Variety × Treatment × Year	24	0.35 NS	4.82 NS
Spray × Treatment × Year	12	0.32 NS	0.63 NS
Variety × Spray × Treatment × Year	48	0.04 NS	0.24 NS
Error	418	0.002	0.37
Total	629		

SOV source of variation, DF degree of freedom, MS mean sum of square
*Significant at $P < 0.05$ NS=Non-significant

Table 2: Comparisons of different treatments against whitefly population during two seasons

Treatment	Mean *B. tabaci* Population		Mean *B. tabaci* Population		% Inhibition
	2014	2015	Before Spray	After Spray	
Imidacloprid	1.28 i	1.07 j	3.27 g	1.04 g	68.21
Acetamiprid	1.78 h	1.79 h	3.68f	1.17f	68.19
Classic (Zn and Boron)	4.87 d	4.14 e	4.72 c	3.38 c	28.39
Salicylic acid	3.18 f	3.16 f	5.95 d	2.64 d	55.63
Neem extract	3.06 g	3.13 g	4.59 e	2.01 e	56.21
Eucalyptus Extract	6.91 b	5.89 c	5.86 b	4.98 b	15.01
Control	10.71 a	10.69 a	9.75 a*	9.69 a*	

*Means with similar letters in a column are not significantly different at $P = 0.05$, LSD = 0.018

Table 3: Whitefly *B. tabaci* population on all the varieties after different sprays during two seasons

	Mean *B. tabaci* Population		Varieties				
Sprays	2014	2015	Carmen	PO-02	Roker	Uovo Roseo	Lyp#1
1st Spray	3.78 a	3.75 b	2.94 e	3.95 a	2.97 e	3.48 b	3.22 d
2nd Spray	2.73 d	2.84 c	2.32 m	3.39 c	2.52 l	2.86 f	2.51 l
3rd Spray	1.25 f	1.41e	1.62 q	2.72 k	1.77 p	2.18 n	1.87 o

*Means with similar letters in a row and column are not significantly different at $P = 0.05$, LSD = 0.013

Three sprays were applied for the management of *B. tabaci* during two years (2014 and 2015). There was a significant difference in *B. tabaci* population after each spray during 2014 and 2015 (Table 3).

The mean *B. tabaci* population was significantly reduced in all the tested genotypes *i.e*., Carmen, Po-02, Roker, Uovo Roseo, and Lyp#1in first, second and third sprays (Table 3). In the first spray, three genotypes i.e., Po-02, Uovo Roseo, and Lyp#1 had a significant difference in *B. tabaci* population while Carmen and Roker showed non-significant difference. All the genotypes showed a significant difference in *B. tabaci* population after the second spray except Roker and Lyp#1 which showed a non-significant difference with each other. In the third spray, all genotypes (Carmen, PO-02, Roker, Uovo Roseo, and Lyp#1) genotype showed a significant difference in reducing *B. tabaci* population.

The mean *B. tabaci* population significantly reduced in all genotypes *i.e*., Carmen, Po-02, Roker, Uovo Roseo, and Lyp#1in first, second and third sprays during two years i.e. 2014 and 2015 (Fig. 1). All genotypes had a significant difference in mean *B. tabaci* population after the third spray with respect to the first and second sprays during 2014 and 2015. In the first spray all genotypes showed a significant difference in mean *B. tabaci* population during 2014 and 2015. In second spray all the genotypes *i.e*., Carmen, Po-02, Roker, Uovo Roseo, and Lyp#1 showed a significant difference in *B. tabaci* population during the years 2014 and 2015. All the genotypes showed a significant difference in mean *B. tabaci* population third spray during the year 2015 but Carmen and Lyp#1 showed a non-significant difference in mean *B. tabaci* population during 2014.

Comparisons of different treatments against TLCVD incidence

The individual effect of year, spray, variety and treatment was significant for disease incidence (Table 1). The two-

Table 4: Comparisons of different treatments against TLCVD incidence during two seasons

Treatments	Disease incidence (%)		Disease incidence (%)		% Efficacy
	2014	2015	Before Spray	After Spray	
Imidacloprid	13.83 h	11.85 i	34.95 g	11.34 g	67.56
Acetamiprid	16.34 g	16.02 g	36.25 f	16.47 f	54.57
Classic (Zn and Boron)	21.24 d	20.97 d	47.42	26.71 c	43.67
Salicylic acid	19.42 e	18.26 f	44.91	28.18 d	37.25
Neem extract	18.12 f	17.94 f	42.15 e	20.16 e	52.17
Eucalyptus Extract	24.23c	23.71 c	37.68 b	23.52 b	37.25
Control	49.09 b	54.21 a	56.15 a*	57.04 a*	

*Means with similar letters in a column are not significantly different at $P = 0.05$, LSD = 0.16

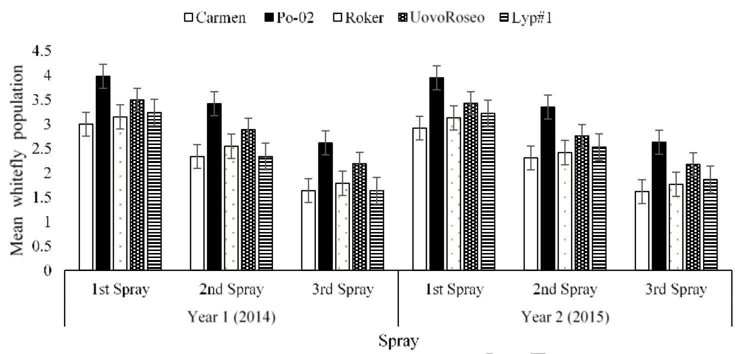

Fig. 1: Comparisons of whitefly population with variety, spray and year

way interactions of spray with year, variety with year, treatment with year and variety with spray were significant; whereas the two way interactions of variety with treatment and spray with treatment were not significant. The three-way interaction between variety, spray and year was significant. Three-way interactions between variety, spray and treatment; variety, treatment and year; spray, treatment and year were not significant. The four-way interaction of variety with spray, treatment and year was also non-significant.

All the treatments were significantly effective in reducing TLCVD incidence compared to untreated control. The comparative efficacy of all treatments was significantly different from each other. Imidacloprid was the most effective in reducing TLCVD incidence as compared to control followed by acetamiprid, neem extract, salicylic acid, classic (Zn and B solution), and eucalyptus extract (Table 4).

All the treatments were effective in reducing TLCVD incidence compared to untreated control during the years 2014 and 2015 (Table 4). In 2014 all the treatments showed significantly different results in reducing TLCVD incidence while in 2015 salicylic acid and neem extract were not significantly different from each other in reducing the TLCVD incidence. In 2014, the efficacy of imidacloprid and salicylic acid against TLCVD incidence was significantly different from their respective treatments in 2015. In 2014, three treatments *i.e*., acetamiprid, classic (Zn and B solution) and neem extract were not significantly different from their respective treatments in the year 2015. During both years (2014 and 2015) imidacloprid was the most effective in reducing TLCVD incidence as compared to other treatments and control.

Three sprays were applied for the management of TLCVD during two years (2014 and 2015). There was a significant difference in TLCVD incidence after each spray during 2014 and 2015 (Table 5). After the first spray, 38.65% disease incidence was recorded which reduced to 17.25% after the third spray during 2014, while disease incidence reduced from 36.03–17.41% after first and third spray, respectively during 2015.

The mean TLCVD incidence significantly reduced in all genotypes *i.e*., Carmen, Po-02, Roker, Uovo Roseo, and Lyp#1in first, second and third sprays (Table 5). In the first spray, three genotypes *i.e*., Po-02, Uovo Roseo, and Lyp#1 had significant differences with respect to disease incidence while Carmen and Roker showed a non-significant difference. All the genotypes showed a significant difference in TLCVD incidence in second spray. In the third spray, only Carmen showed significant difference as compared to all other varieties/lines, while the disease incidence was non-significant in Po-02 and Uovo Roseo; Roker and Lyp#1.

The TLCVD incidence significantly reduced in all genotypes *i.e*., Carmen, Po-02, Roker, Uovo Roseo, and Lyp#1in first, second and third sprays during two years 2014 and 2015 (Fig. 2). All genotypes had a significant difference in disease incidence in the third spray with respect to first and second sprays during 2014 and 2015. In the first spray all genotypes showed a significant difference in disease incidence during 2014 and 2015. In second and third sprays, three genotypes *i.e*., Po-02, Uovo Roseo, and Lyp#1 showed significant difference with respect to disease incidence while two genotypes Carmen and Roker showed non-significant

Table 5: TLCVD incidence on all the varieties after different sprays during two seasons

	Disease incidence (%)		Varieties				
Sprays	2014	2015	Carmen	PO-02	Roker	Uovo Roseo	Lyp#1
1st Spray	38.65 a	36.03 a	22.13 e	53.67 a	22.19 e	47.55 b	27.94 de
2nd Spray	24.73 b	27.21 b	13.72 hij	28.15 de	15.36 h	31.83 c	18.19 g
3rd Spray	17.25 c	17.41 c	5.46 lm	14.79 hi	9.33 kl	18.19 g	9.46 k

*Means with similar letters in a row and column are not significantly different at $P = 0.05$, LSD = 0.26

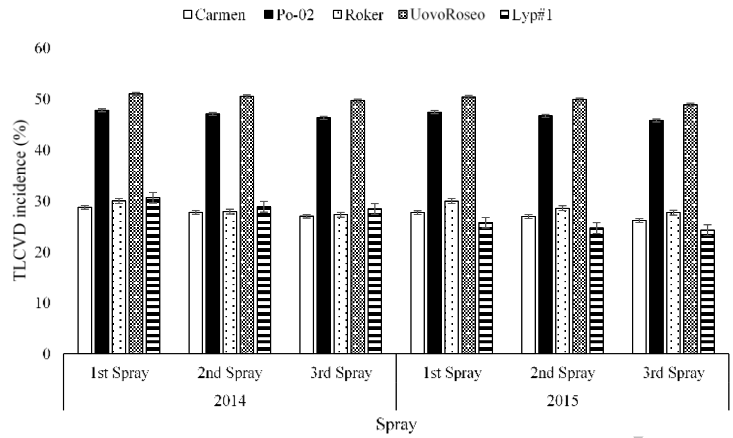

Fig. 2: Comparisons of TLCVD incidence with variety, spray and year

difference with each other during the year 2014. All the genotypes showed a significant difference in disease incidence in second and third sprays during the year 2015.

The growth and yield of treated plants were significantly higher than the untreated tomato plants in all genotypes during both years (Table 6). The plants treated with imidacloprid showed significantly higher values of growth and yield parameters as compared to other treatments and control. The maximum plant height (39.01 cm) was recorded in imidacloprid treated plants in 2015 which was significantly higher than control. There was a non-significant difference between plant height in imidacloprid treated plants during both years. Among treated plants, the minimum plant height (31.19 cm) and (30.95 cm) was recorded in 2014 and 2015, respectively. A similar trend for other growth and yield parameters (fresh weight, dry weight, no. of fruits/plant, fruit weight and fruit yield/plant) was recorded in case of all the treatment during two seasons. The maximum growth and yield were recorded in imidacloprid treated plants followed by acetamiprid, neem extract, salicylic acid, Zn & B solution, and Eucalyptus extract.

Discussion

Tomato leaf curl virus disease (TLCVD) causes severe damage to tomato crops worldwide every year (Kumar *et al*. 2012). TLCV transmission is accomplished by the phloem-feeding of whitefly (Boykin *et al*. 2007). Different insecticides are used against whitefly to minimize the virus transmission (Aktar *et al.* 2008). The repeated use of conventional insecticides results in the development of resistance (Nauen *et al*. 2015).

The present study describes that there are diversified ways to minimize the losses caused by whitefly such as transmission of TLCV. Genetic resistance of the host plant can play a significant role to avoid yield losses. The cultivation of resistant varieties is the most economical method to manage the plant diseases (Bosch *et al*. 2006) but when the disease appears suddenly and at a very rapid rate in the field, the farmers have no option except to spray the crop with some effective chemicals (Pal and Gardener 2006). Whitefly infestation was recorded in all the varieties and none was found resistant or immune. As none of the tested varieties showed resistance, different insecticides, plant extracts, and nutrients were applied for the management of insect vector of TLCV; the whitefly *B. tabaci*. All the treatments reduced *B. tabaci* population significantly compared to untreated control. Among the insecticides, imidacloprid was the most effective to manage the *B. tabaci* population followed by acetamiprid in that order. The imidacloprid and acetamiprid being the member of neonicotinoids, bind to the acetylcholine receptors (AChRs) in the Central Nervous System (CNS) of insects (Zhang *et al*. 2000). Neonicotinoids mimic acetylcholine and induce abnormal excitement in the insect by disturbing the systematic synaptic transmission. Subsequently, the insect undergoes excitation and paralysis, followed by death. The neonicotinoids are effective on contact and through stomach action (Lind *et al*. 1999). New chemistry insecticides caused maximum reduction in whitefly infestation resulting in minimum TLCV transmission (Abbas *et al*. 2012).

In the present study, the plants treated with neonicotinoids (imidacloprid and acetamiprid) exhibited a minimum disease incidence than other treatments. TLCV

Table 6: Effect of different treatments on growth and yield parameters of tomato during two seasons

Treatments	Parameters						
	Years	Plant height (cm)	Fresh weight (kg)	Dry weight (kg)	No. of fruits/plant	Fruit weight (g)	Fruit yield/plant (kg)
Imidacloprid	2014	38.34 g	3.06 g	1.03 g	84.6 g	177.2 g	8.92 g
	2015	39.01 g	3.12 g	1.05 g	84.3 g	177.3 g	8.77 g
Acetamiprid	2014	36.23 f	3.42 f	0.97 f	73.2 f	147.5 f	8.52 f
	2015	36.65 f	3.45 f	0.98 f	73.5 f	146.9 f	8.33 f
Neem extract	2014	35.67 e	2.74 e	0.84 e	65.8 e	144.7 e	7.42 e
	2015	35.99 e	2.66 e	0.81 e	65.7 e	143.4 e	7.53 e
SA	2014	34.82 d	2.46 d	0.77 d	62.4 d	142.6 d	6.72 d
	2015	34.78 d	2.49 d	0.75 d	62.9 d	142.3 d	6.57 d
Zn & B	2014	33.75 c	2.35 c	0.68 c	58.3 c	124.5 c	5.26 c
	2015	33.54 c	2.34 c	0.63 c	58.5 c	125.6 c	5.41 c
Eucalyptus extract	2014	31.19 b	2.14 b	0.59 b	55.5 b	116.3 b	4.74 b
	2015	30.95 b	2.18 b	0.55 b	55.4 b	116.8 b	4.83 b
Control	2014	23.25 a	1.82 a	0.47 a	41.9 a	103.7 a	3.07 a
	2015	23.17 a	1.85 a	0.43 a	41.7 a	103.2 a	3.07 a

*Means with similar letters in a row and column are not significantly different at $P = 0.05$, LSD = 1.2

infection is delayed in early growth stages of tomato plants if treated with imidacloprid because it protects the plant by following a systemic pathway (Karim *et al*. 2008). Neonicotinoids stimulate plant defense by expressing the salicylic acid (SA) pathway (Ford *et al*. 2010). These insecticides stimulate the SA pathway by expressing pathogenesis-related (PR) proteins (Karthikeyan *et al*. 2009). Imidacloprid is absorbed by the plants systemically and translocated thus controlling the sucking insects (Kagabu 2003). The use of imidacloprid increases resistance against pathogens and is regarded as induction of stress shield (Thielert 2006) because the resultant PR proteins suppresses the viral replication and movement (Ahmed *et al*. 2001). Due to slow virus movement, minimum TLCD incidence was recorded in neonicotinoids treated tomato seedlings (Dempsey *et al*. 2017). After absorption into plants, imidacloprid is converted into metabolites like 2-chlorothiazolyl-5-carboxylic acid (CTA) that enhances the plant growth and vigor apart from insect control (Gonias *et al*. 2008). Reduced diseased severity and improvement in plant growth and yield parameters are attributed to the imidacloprid driven SA pathway that helped in resuming NAC transcription factors of tomato from the replication enhancer protein of TLCV (Riley and Srinavasan 2019). Neonicotinoids trigger soluble protein content in plants that increases their ability to fix more CO_2 and photosynthesis resulting in enhanced yield (Li *et al*. 2020).

Although chemical control is easy, direct and rapid action to solve pest and disease problems but continuous dependence on pesticides has contributed to environmental pollution and degradation (Palumbo *et al*. 2001) and has become less effective due to the development of resistance against insecticide in insects (Siebert *et al*. 2012). Bio-pesticides can solve the problems of insecticidal resistance and environmental hazards (Abou-Yousef *et al*. 2010). In the current experiment, the extract of *A. indica* (neem) was very effective against the *B. tabaci* population and TLCVD incidence after the synthetic insecticides (imidacloprid and acetamiprid) followed by the extract of *E. globulus* (Eucalyptus). The insecticidal activity of neem extract is due to the components that are capable of influencing the physiology and behavior of a wide range of insects (Schaaf *et al*. 2000). *A. indica* interacts with the corpus cardiacum, thus blocking the activity of the molting hormone and acts as an insect growth regulator, suppresses fecundity, molting, pupation and adult emergence (Ascher 1993). *A. indica* produces antifeedant effects by stimulating specific deterrent chemoreceptors and blocking the sugar receptors in the mouthparts of whitefly (Butler *et al*. 1991). The anti-feeding and deterrent effects of neem had forced the insects to leave the locality or chronic effect of the neem compounds (Khattak *et al*. 2006). Eucalyptol present in the aqueous extract of *E. globules* causes toxicity and repellent effects against the insects (Lee *et al*. 2002). It has serious neurotoxic, cytotoxic, and phytotoxic effects on the sucking insects (Bakkali *et al*. 2008). The neurotoxic effect is attributed to the inhibition of acetylcholine esterase (AChE) activities after the exposure of whitefly to the eucalyptus extract-treated plants (Lionetto *et al*. 2013). Volatile secondary metabolites present in eucalyptus extract are released into the air that disrupts the olfactory orientation of whitefly (Deletre *et al*. 2015).

The efficient control of whitefly led to a considerable reduction in TLCVD incidence. In another study, the phyto-pesticides significantly reduced the TLCVD incidence and severity (Bhyan *et al*. 2007). Eucalyptus extract manages the disturbed balance of production and scavenging of active oxygen species under stress situations (Wan *et al*. 2012) by producing catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Apel and Hirt 2004). Eucalyptus extract contains eucalyptol (1-8, cineol), and many types of terpenes that initiate the systemic defense in plants by following the JA pathway (Hong *et al*. 2012). TLCV infection results in a decrease of enzymes and photosynthetic pigments (Montasser *et al*. 2012) the deficiency of which is compensated by the application of *A. indica* (Sujanya *et al*. 2008). Apart from *A. indica*, neem extract also contains tannins, nimbin, nimbidine, and terpenoids (Mondali *et al*. 2009), all of these stimulates plant defense mechanisms, hormones and proteins production that is disturbed due to virus infection (Kumar 2019). *A. indica* increases the phenylalanine ammonia-lyase (PAL) activity which is suppressed by the viral attack. The suppressed PAL activity results in reduced plant growth, curling of leaves, and thinner cell walls of phloem in virus-infected plants (Paul and Sharma 2002). It also boosts the production of tyrosine ammonia-lyase (TAL) which helps in resuming the halted metabolic activities by the viral infection (Maeda 2016).

Pathogenic attack destroys the physiology of the plants such as nutrient uptake, assimilation, translocation from the root to shoot and utilization (Marschner 1995). In the present study, Classic (Zn and Boron) solution significantly reduced the whitefly population as compared to control. Nutrients improve the plant health by regulating metabolic and cellular functions which enable the plant to tolerate the attack of sucking and chewing insects. The nutrients such as N, P, K, Zn, and B significantly reduced the whitefly population in cotton (Gogi *et al*. 2012). The nutrients status of the plant determines its ability to defend against pests and pathogens (Walters and Binghum 2007). Several nutrient elements act as catalytically active cofactors in enzymes while others stabilize the proteins structurally (Hansch and Mendel 2009). Zn affects the plant defense by the activation of metalloenzymes after insect attack (Fones and Preston 2012). Zn application helps in the production of secondary metabolites that are reduced due to the whitefly attack in tomato plants (Lehman *et al*. 2015). High leaf concentration of Zn contributes to increased structural defense of the plant and defense-related signals (Martos *et al.* 2016). Boron may affect the physiology and biochemistry of the plants by strengthening the cell wall and membrane through binding of apoplastic proteins to cis-hydroxyl groups and by interfering with enzymatic reactions (Blevins and Leukaszewski 1998). Viruses alter the physiology of plants by affecting the growth and development and interrupting with defense mechanism. The concentration of reactive oxygen species (ROS) and free radicals increase up to two-fold due to the viral attack in Zn deficient cells causing significant damage to the plants. Zinc improves the defense system of plant cells against ROS by interfering with membrane-bound NADPH oxidase that produces ROS and protects membrane lipids, proteins, chlorophyll, enzymes, and DNA of the cell from oxidation (Cakmak 2000). Boron reduces the severity of many diseases as well as the susceptibility of plants because it affects the structure of the cell wall, plant membrane, and metabolism of phenolics or lignin (Brown *et al*. 2002).

In current experiments, salicylic acid (SA) was found the most effective after neonicotinoids and neem extract for whitefly management. Plant defense responses are regulated by a complex network of signal molecules and growth regulators. Resistance genes identify the pathogen and start defense responses. Salicylic acid (SA), jasmonic acid (JA), naphthalene acetic acid (NAA), and ethylene (ET) mediates both specific as well as basal defense responses (Jalali *et al*. 2006). SA at 3% concentration was found superior in reducing the egg hatchability, adult emergence, adult whitefly population, and CLCuVD severity both in soil drenching and foliar sprays (Khan *et al*. 2003). According to Doorn *et al*. (2015) SA stimulates the plant defense responses to fight against the whitefly attack. Thaler *et al.* (2010) found a minimum infestation of sucking insects on the SA treated plants. SA activates defense cascades in plants to repel the phloem-feeding insects (Walling 2009). The exogenously applied SA reduces the fecundity and longevity of whiteflies (Shi *et al*. 2013). The disease incidence was also reduced in SA treated plants because it inhibits the systemic movement of the virus from cell to cell and induces a signal transduction pathway (Mayers *et al*. 2005).

Conclusion

New chemistry insecticide (Imidacloprid) effectively controlled the whitefly infestation in all tomato genotypes. The active ingredients of plant extracts (*Azadiracht indica* and Eucalyptol) resulted in significant whitefly mortality by disturbing the hormonal activities of whitefly. Micronutrients and salicylic acid stimulated the defense signals of the plants thus decreasing the whitefly infestation.

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Author Contributions

Muhammad Ahmad Zeshan conceived the idea and conducted research. Muhammad Aslam Khan supervised the experiment and reviewed the manuscript. Safdar Ali helped in planning of experiment, analytical work and data collection. Muhammad Arshad helped in identification of Bemisia tabaci. Ghulam Mustafa Sahi proof read for technical details in the manuscript. Muhammad Sagheer provided technical assistance in whitefly data recording. Nadeem Ahmed did the statistical analysis. Rana Binyamin assisted in data recording on all the aspects, sample collection and yield estimations. Muhammad Usman Ghani assisted in making formulations, plant extract formation, spray applications and other field activities.

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