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Full Length Article

Differential Response of Micronutrients and Novel Insecticides to Reduce Cotton Leaf Curl Virus Disease and its Vector in *Gossypium hirsutum* Varieties

Humaira Kalsoom[1], Safdar Ali[1*], Ghulam Mustafa Sahi[1], Amer Habib[1], Muhammad Ahmad Zeshan[2], Romana Anjum[1], Maryam Yousaf[2] and Ahsan Abdullah[1]
[1]*Department of Plant Pathology, University of Agriculture, Faisalabad, 38000, Pakistan*
[2]*Department of Plant Pathology, University of Sargodha, Sargodha, Pakistan*
[*]For correspondence: safdar147m@uaf.edu.pk

Abstract

The present research evaluated insecticides flonicamid (ulala, 1 g/L) and pyriproxyfen (flyban, 5 mL/L) applied to control the adults and nymphs of *Bemisia tabaci* under field conditions. Micronutrients (33% $ZnSO_4$ (zinc sulphate)) 3 g/L of water and 13% H_3BO_3 (boric acid) 2 g/L of water were applied to young cotton plants. Minimum disease severity (26.77%) was recorded in flonicamid treated plants and maximum (27.76%) was in pyriproxyfen as compared to the control (77.25%). Whitefly mortality was higher due to flonicamid application than other treatments. The boric acid application showed good control of whitefly and subsequent reduction in disease severity followed by zinc sulphate. The results revealed that treated plants showed significant response to decrease the disease severity (65.34%) and whitefly infestation (58.62%) in all the varieties. The application of micronutrients proved useful for the management of cotton leaf curl virus disease.

Keywords: CLCuV; Viruliferous; Infestation; Flonicamid; Pyriproxyfen; Insecticides

Introduction

Cotton leaf curl disease caused by cotton leaf curl virus (CLCuV) is the most damaging to cotton. CLCuV has single stranded DNA genome that belongs to genus Begomovirus of family Geminiviridae (Idris and Brown, 2004). In Pakistan, the disease gained importance in last few decades when it became a severe problem after 1991–1992 and continued till the development of cotton leaf curl resistant variety CIM-1100 in 1996 (Sharma and Bombawale, 2008). This problem appeared again during 2001 in Burewala, breaking the resistance of all the available cotton cultivars/lines (Mansoor *et al.*, 2003; Hussain *et al.*, 2017). CLCuV is transmitted by whitefly (*Bemisia tabaci*) that belongs to order Hemiptera and family Aleyrodidae. Virus is transmitted in a persistent and circulative manner (Oliveira *et al.*, 2001).

The symptoms of leaf curl disease comprises of upward curling of leaves with thickened veins which are most distinct on the underside (Mansoor *et al.*, 2003). Infected plants show development of leaf enations (oval or cuplike foliar growth) on the underside of leaf. Leaves become brittle and thicker than normal and produce a rattling sound when plant is shaken (Harrison *et al.*, 1997). Susceptibility of cotton plants to viral attack is based upon the stage of plant growth. Plants in early stage are more vulnerable to disease attack as compared to later stages (Alegbejo, 2001).

As the virus is not transmitted through soil and seed, the whitefly being the most efficient vector is focused upon in order to reduce the CLCuV transmission (Ali *et al.*, 1995, 2014). Whitefly is mainly controlled by using insecticides with different mode of actions under various agricultural systems (Jadhav *et al.*, 2018; Schlaeger *et al.*, 2018). Conventional insecticides became less effective in controlling whitefly due to development of resistance by repeated and excessive exposure. The problem of insecticidal resistance has put a pressure on the novel insecticides (Nauen *et al.*, 2015). Flonicamid is a novel insecticide used against sucking insects of order Hemiptera. It produces antifeeding behavior and convulsions in hemipterous insects exposed to it (Morita *et al.*, 2007). Flonicamid through its selective activities increase mortality of whitefly and cause delay in nymphal development (Roditakis *et al.*, 2014). Pyriproxyfen controls all growth stages of whitefly with considerable efficiency. It is one of the novel insecticides to which *B. tabaci* has not developed resistance (Caballero *et al.*, 2013). The other way to reduce whitefly infestation and virus transmission is to use nutrient solutions. Soil conditions have promising effect on improving plant health that is helpful in controlling the insect infestation (Altieri and Nicholls, 1999).

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Nutrient application affects the relationship between plant and insect (Abro *et al.*, 2004). Insect pests and pathogenic attack is more prevalent on week plants that have nutritional deficiency (Huber and Thompson, 2007).

Novel insecticides may gave the effective control of whitefly and reduce the CLCuV transmission while nutrients may assist the plant to defend against viral attacks. The present research was conducted to assess the efficiency of insecticides and micronutrients application for the management of CLCuV and its vector.

Materials and Methods

The experiment was conducted at research area Department of Plant Pathology, University of Agriculture, Faisalabad. Land was prepared by properly pulverizing the soil and ploughing. Delinted cotton seed of four varieties (BS-70, CIM-616, MM-58 and S-32) were sown by using randomized complete block design (RCBD) with three replications (10 plants/treatment/variety in each replication). There was 75 cm row to row and 30 cm plant to plant distance. Management of CLCuV and its insect vector was done by using two insecticides (pyriproxyfen and flonicamid (ulala) and two micronutrients solutions (H_3BO_3 and $ZnSO_4$).

Insecticides were sprayed on cotton plants when whitefly population reached economic threshold level (ETL) *i.e.*, 4–5/leaf. Nutrient solutions *i.e.*, 33% $ZnSO_4$ and 13% H_3BO_3 was sprayed 15 days after sowing, thereafter, insecticides and micronutrients were sprayed to plants at weekly intervals. Applications were done early in the morning to minimize the chances of drift. The treatments of insecticides pyriproxyfen (flyban) and flonicamid (ulala) and the micronutrients solutions of H_3BO_3 and $ZnSO_4$ were applied at the dose of 5 ml/L, 1 g/L, 2 gL and 3 gL, respectively (Table 1). The control plants were treated only with distilled water.

Disease severity was recorded by counting number of infected leaves and total number of leaves in one plant. Three plants were randomly selected in each row and average was taken and disease severity was determined by following the formula:

Disease severity = Number of infected leaves/Total number of healthy leaves x100

Whiteflies were counted from upper, middle and lower leaves of randomly selected three plants in each row. After counting, their mean was taken and recorded against each variety (Razaq *et al*., 2003).

Efficacy of treatments was determined by using following formula:

Efficacy = (Disease severity in control – Disease severity in treated plants/Disease severity in control) x100

Efficacy = Whitefly infestation in control - Whitefly infestation in treated plants/Whitefly infestation in control x100

Analysis of data was performed statistically and comparison of means was performed using Least Significance Test (LSD-Test) with 5% significance level. Statastix 8.1 software was used for analysis of the data (Steel *et al.*, 1997).

Results

Response of Cotton Germplasm against Whitefly Infestation after Treatment with Novel Insecticides

The insecticides (Pyriproxyfen and Flonicamid) were applied to check the whitefly mortality and reduction in CLCuV transmission. Flonicamid at its recommended dose showed maximum mortality of whiteflies and thus found to be the most effective in reducing the whitefly infestation. Pyriproxyfen also gave significant decrease in whitefly infestation as compared to untreated plants (Table 4).

The efficacy of insecticides was also recorded in different cotton cultivars. In case of Flonicamid, S-32 gave maximum mortality and minimum whitefly infestation (1.67/leaf) followed by CIM-616 (1.79/leaf). Minimum mortality and maximum infestation was recorded in MM-58 (2.64/leaf) followed by MM-58 (2.33/leaf). Pyriproxyfen proved most effective in BS-70 with minimum whitefly infestation (2.05/leaf) while BS-70 (2.38/leaf), S-32 (2.91/leaf) and CIM-616 (3.03/leaf) as compared to control (5.10/leaf).

Response of Cotton Germplasm against Whitefly Infestation after Treatment with Nutrients

Application of micronutrients affected the whitefly population. Boric acid at 13% lowered the whitefly population to 37.01%. Zinc sulphate effectively reduced the infestation (29.65) at 33% when compared to non-treated plants (Table 5).

Such effect of micronutrients also varied in cotton varieties individually. Minimum infestation of whitefly was shown by S-32 as a result of boric acid application followed by CIM-616, S-32 and MM-58. Whitefly population decreased to a minimum level in BS-70 when treated with zinc sulphate whereas BS-70, CIM-616 and MM-58 demonstrated promising control of whitefly respectively than untreated check (Table 5).

Efficacy of Insecticides against CLCuV Disease Severity in Different Varieties

The control of whitefly through insecticides significantly reduced CLCuV transmission and disease severity. Flonicamid was the most effective in reducing disease severity (65.34%) while pyriproxyfen also gave considerable (64.06%) disease control as compared to control (Table 2).

Flonicamid and pyriproxyfen affected the disease severity in different varieties. The lowest disease severity was recorded in S-32 treated with flonicamid. Maximum disease severity was shown by MM-58 in case of flonicamid application followed by CIM-616 and BS-70.

Table 1: Treatments applied for the management of CLCuV and its vector

Active ingredient	Trade name	Category	Dose/ha
Pyriproxyfen	FlyBan	Insecticide	1235 mL
Flonicamid	Ulala	Insecticide	247 g
Zinc sulphae	Kiran	Micronutrient	741 g
Boric acid	Boric acid	Micronutrient	494 g

Table 2: Effect of insecticides on CLCuV disease severity in different cotton varieties

Insecticides	Varieties				Mean
	BS-70	CIM-616	MM-58	S-32	
	Disease severity (%)				
Flonicamid	26.17	27.67	29.23	25.00	26.77
Pyriproxyfen	28.14	27.53	29.35	26.02	27.76
Control	74.26	72.03	79.06	83.62	77.25

LSD = 2.075 at $P < 0.05$

Table 3: Effect of nutrients on CLCuV disease severity in different cotton varieties

Nutrients	Varieties				Mean
	BS-70	CIM-616	MM-58	S-32	
	Disease severity (%)				
Boric acid	37.17	36.35	39.11	35.07	36.93
Zinc sulphate	37.23	36.85	38.62	34.98	36.92
Control	79.68	73.25	82.14	80.05	78.78

LSD = 2.027 at $P < 0.05$

Table 4: Effect of insecticides on whitefly infestation in different cotton varieties

Insecticides	Varieties				Mean
	BS-70	CIM-616	MM-58	S-32	
	Whitefly population				
Flonicamid	2.33	1.79	2.64	1.67	2.11
Pyriproxyfen	2.38	3.03	2.05	2.91	2.59
Control	5.43	5.01	4.99	4.97	5.10

LSD = 0.012 at $P < 0.05$

Pyriproxyfen performed best on S-32 and exhibited minimum disease severity. CIM-616 responded effectively to pyriproxyfen treatment and showed significantly low disease severity as compared to control. Slightly higher disease severity was recorded in BS-70 and MM-58 respectively).

Efficacy of Nutrients against CLCuV Disease Severity in Different Varieties

Proper plant nutrition strengthens its vigour and stimulates the defence mechanisms. Zinc sulphate gave maximum control (53.13%) of the disease as compared to control. The efficacy of boric acid was also best in reducing the disease severity (53.12%). Zinc sulphate and boric acid have very slight difference in their efficacies.

Zinc sulphate provided minimum disease severity on variety S-32 as compared to non-treated plants of the same variety. Maximum disease severity was found in MM-58 followed by BS-70 and CIM-616. The efficacy of boric acid against CLCuV disease severity was more in S-32 than BS-70, CIM-616 and MM-58 in that order (Table 3).

Table 5: Effect of nutrients on whitefly infestation in different cotton varieties

Nutrients	Varieties				Mean
	BS-70	CIM-616	MM-58	S-32	
	Whitefly population				
Boric acid	3.47	3.31	4.09	2.12	3.25
Zinc sulphate	3.29	3.64	4.44	3.13	3.63
Control	4.95	5.23	5.48	4.98	5.16

LSD = 0.033 at $P < 0.05$

Table 6: Evaluation of different treatments against whitefly population

Treatments	Whitefly population	Efficacy (%)
Flonicamid	1.53	81.18a*
Pyriproxyfen	2.06	74.66b
Boric acid	2.79	65.68c
Zinc sulphate	3.62	55.47d
Control	8.13	

*Different letters indicating insignificant means at $P < 0.05$

Table 7: Evaluation of different treatments against CLCuV

Treatments	Disease severity (%)	Efficacy (%)
Flonicamid	9.82	74.01b
Pyriproxyfen	11.14	70.61c
Boric acid	8.05	78.75a
Zinc sulphate	7.81	79.38a*
Control	37.79	

*Different letters indicating insignificant means at $P < 0.05$

Evaluation of Insecticides and Nutrients against Whitefly Infestation and Severity

Whitefly population was significantly reduced by the application of flonicamid (81.18%) followed by pyriproxyfen (74.66%) was the second most efficient insecticide in controlling whitefly followed by boric acid and zinc sulphate (Table 6). Zinc sulphate treatment reduced CLCuV disease severity with 79.38% efficiency. The efficacy of boric acid was high (78.75%) as compared to pyriproxyfen (70.61%) and flonicamid (74.01%, Table 7).

Discussion

Cotton leaf curl virus causes huge losses worldwide and 30% yield reduction is recorded in Pakistan (Hassan *et al.*, 2015). Different insecticides are used against whitefly to minimize the virus transmission and disease severity (Aktar *et al.*, 2008). Commonly used insecticides from organophosphate (OP), organochlorine (OC) and carbamates groups become less effective against whitefly after repeated use (Nauen *et al.*, 2015). Resistance to insecticides is pretended as the failure of the chemical. That's why OPs and OCs have been replaced with neonicotenoids and novel insecticides (Jeschke *et al.*, 2010). One of the unique novel insecticide is flonicamid that blocks the feeding of sucking insects like whitefly (Roditakis *et al.*, 2014).

In present study, the mean whitefly population was 2.11 from all the varieties treated with flonicamid.

Flonicamid was 81.18% efficient in controlling the whitefly infestation. Previously 95% whitefly mortality have been recorded after exposure to flonicamid (Roditakis *et al.*, 2014). Devine and Denholm (1998) studied the effect of flonicamid application on nymphs of whitefly and found delay in its development. The results of the following experiment revealed that whitefly management by flonicamid affected the disease severity and 74% efficacy was calculated. Flonicamid inhibits the feeding of sucking insects and it has no side effect on natural enemies (Morita *et al.*, 2007).

The whitefly population in pyriproxyfen treated plants was 2.59 and its efficiency was 74.66%. Wen *et al.* (2014) recorded 77% mortality of whitefly eggs through the use of pyriproxyfen. It was further concluded that pyriproxyfen is more toxic against whitefly eggs than neonicotenoids. It binds to the ryanodine receptors and kills the eggs and larvae of the *B. tabaci* (Jeanguenat, 2013). Li *et al.* (2012) reported pyriproxyfen is an anthranilic diamide to which whitefly has no resistance. Reduction in egg hatchability and mortality of larvae was described by Lee *et al.* (2002). Vijay *et al.* (2014) used pyriproxyfen to manage whitefly infestation in tomato plantations 200 g/acre. Pooled analysis of treatments applied showed that maximum whitefly mortality was calculated from pyriproxyfen with 76% efficiency. Pyriproxyfen act as insect growth regulator that suppresses the process of embryo formation and development of adult by badly interrupting with hormones of insects (Ishaaya and Horowitz, 1998). Bi *et al.* (2002) sprayed esteem (Pyriproxyfen) on the leaves of strawberry and found it effective against whitefly egg hatching. These findings are further strengthened by (Mckee and Zalom, 2007) who described that foliar spray of pyriproxyfen at early growth stages minimize the whitefly population. In this study, CLCuV disease severity was reduced upto 70% in the plants treated with flonicamid due to maximum mortality of the insect vector. Detailed study was made on novel insecticides pyriproxyfen, buprofezin, imidacloprid, pymetrozine and diafenthiuron in 1990. It was observed that the insecticides do not directly kill or affect the *Bemisia tabaci* as conventional pesticides (immediately knocking down the pests). Buprofezin adversely affect the female's viability, eggs fertility and larvae molting of insects is highly affected. Pyriproxyfen directly or indirectly affects all stages of life cycle (Dittrich *et al.*, 1990). Three successful applications of novel pesticides pyriproxyfen and flonicamid control the whitefly infestation and subsequently CLCuV transmission successfully. Pyriproxyfen (juvenile hormone mimic) suppresses embryogenesis and adult formation of whitefly (Imtiaz *et al.*, 2010).

Pyriproxyfen mode of action revealed that it acts like analog hormone of juvenile stage and mimics the hormone action (playing major role in control of whiteflies). Pyriproxyfen played significant role in control of *B. tabaci* especially in Israel and North America (Palumbo *et al.*, 2001).

Micronutrients enriched plants are less vulnerable to insect attack as compared to under nourished cotton plants (Rashdi, 1998). Khan *et al.* (2003) used salicylic acid at 3% concentration that effectively controlled adult whitefly, emergence and egg hatchability. In present study, whitefly infestation was controlled with 65% efficiency in different cotton cultivars by using boric acid. The insecticidal efficacy of boric acid was studied against the sucking insect pests and 81% mortality was recorded (Javaid *et al.*, 2018). Gore and Schal (2004) observed significant mortality of sucking insects in laboratory when treated with aqueous solution of boric acid. Boric acid has been used as a successful chemical to control a wide range of sucking insects in different plantations (Bicho *et al.*, 2015). Deterrence of insects was also observed during the contact with boric acid treated sites (Tsunoda, 2001). Boric acid destroys the inner portion of foregut and produces toxin in the neuron of insects (Habes *et al.*, 2006). Murray (1998) described that boric acid is less toxic to environment and highly water soluble. In present experiment, CLCuV disease severity minimized up to 78% due to boric acid application. Boric acid increases the plant integrity and thus helps to manage the physiological disturbance caused by viral infection. Boric acid caused significant increase in total chlorophyll content and proline of the plants (Kucukakyuz*et al.*, 2018). Evaluation of zinc sulphate against whitefly population indicated 55% mortality. This finding is supported by the results obtained from experiments of (Sharaby *et al.*, 2013) where plant yield is increased due to zinc sulphate application. Actually, zinc sulphate acts as protective chemical that disrupt the endocrine system of insect. Padhee and Mishara (1993) recorded that zinc application induce resistance in plants against insect pests. Sarwar (2011) found that zinc sulphate affects the growth, development and reproduction of insects.

Plant nutrition should be improved to provide an active defence system against attacking virus (Pervez *et al.*, 2007). Nutrients can decrease disease occurrence to an acceptable level by improving plant growth and development. Application of adequate nutrients improves plant resistance by enhancing defence system. In current study, boric acid and zinc sulphate effectively reduced CLCuV disease severity and their efficacy was 79 and 75, respectively. Provision of zinc sulphate and boric acid controlled disease incidence to an appropriate level (Abro *et al.*, 2004). Zinc is involved in many physiological functions and limited availability of this vital nutrient limits plant yield by stunting the growth, increasing crop maturity time, chlorosis, shorter leaves and inferior production quality (Butler *et al.*, 1991). Virus infections alters the synthesis and functions of plant hormones especially indole acetic acid. Zinc takes part in the synthesis of tryptophan that is used for the indole acetic acid formation. Indole acetic acid triggers the plant defense mechanism (Spiegel-Roy and Goldschmidt, 2008). Puzina (2004) described that zinc sulphate and boric acid application enhances the hormonal status of plants which contributes towards increased growth and development. Geminiviruses interrupt with plant cell cycle by disturbing proteins that are integral part of cell cycle (Kong *et al.*, 2000). Boron increases the vegetative growth by boosting carbohydrate and protein metabolism (Asad *et al.*, 2003) and provides integrity to the cell wall in plants (Altieri and Nicholls, 1999).

Conclusion

The insecticides (Flonicamid and Pyriproxyfen) effectively controlled the whitefly infestation that resulted in significant reduction in disease severity. Nutrients application not only enhanced the plant defence against CLCuV but also created harsh conditions for the whitefly vector.

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