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EFFECT OF SALICYLIC ACID, KH_2PO_4 and K_2HPO_4 ON THE EGG HATCHABILITY, ADULT EMERGENCE AND POPULATION OF *BEMISIA TABACI* AND COTTON LEAF CURL VIRUS SEVERITY

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Abstract

Salicylic acid, potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) at 2 and 3 % concentrations were drenched in pots in wooden cages covered with muslin cloth and as foliar sprays under field conditions on cotton cvs. S-14 and NIAB-Krishma. *Bemisia tabaci* collected from virus infected plants were released on these plants placed in wooden cages. Data recorded one day before spray and then 24, 48 and 72 hrs after spray on egg hatchability, adult emergence, adult whitefly population and CLCuV severity were recorded at the end of the experiment and statistically analysed. Seven samples from each of the treated and control plants of S-14 and NIAB-Krishma were collected and their protein contents were estimated by Kjeldahl method. Salicylic acid at 3 % concentration proved to be the best in reducing egg hatchability, adult emergence, adult whitefly population and severity of CLCuV and increasing different levels of proteins *viz.* thaumatin, β 1, 4-gluconasis and chitinasis in S-14 and NIAB Krishma. KH_2PO_4 and K_2HPO_4 applied at 3 % concentration also significantly reduced egg hatchability, adult emergence and whitefly population and CLCuV severity compared to control.

Introduction

Plants do not possess an immune system like humans and animals as they do not produce antibodies. Since the early 1990's, however, transgenic plants have been produced that have been genetically engineered to incorporate in their genome and to express foreign genes, such as mouse genes that produce antibodies against certain plant pathogens. Such antibodies encoded by animal genes but produced in and by the plant are called plantibodies (Agrios, 1997). It is expected that this type of plant immunization will yield great dividends by expressing in plants, animal antibody genes that would produce antibodies directed against specific essential proteins of the pathogens, such as viral replicases or movement proteins and fungal and bacterial enzymes.

Most of the biochemical defenses in plants are triggered by pathogen induced signal or to a treatment with certain natural or synthetic chemical compounds. Resistance induced by infection or through chemicals may be local, acquired resistance (LAR) or systemic acquired resistance (SAR). Salicylic acid, arachidonic acid and 2, 6-dichloro isonicotinic acid may induce localized and systemic resistance in plants at levels not causing tissue necrosis. Such chemicals may be effective in inducing resistance in plants when they are applied through the roots, as foliar spray or by stem injection.

Leaf curl virus has induced significant crop losses in the past and it continues to be a major threat to future cotton production in Pakistan (Anon., 1992; Mirza *et al.*, 1994; Ali *et al.*, 1995). Extensive use of pesticides to control *Bemisia tabaci* is neither economical

nor beneficial for the environment. The induced resistance offers a potential alternative to traditional disease control methods. The objective of these studies was to investigate the effect of application of certain chemicals (as foliar spray and drenching) for developing resistance in cotton plants against leaf curl virus.

Materials and Methods

Salicylic acid, dipotassium hydrogen phosphate and potassium dihydrogen phosphate at 2 and 3 % concentrations were used as drench in pots and foliar spray in the field on two cotton varieties *viz.* S-14 and NIAB-Krishma grown in a randomized complete block design. Whitefly population collected from virus infected plants were released on the plants of the above mentioned varieties arranged in completely randomized design under controlled conditions in the wooden cages covered with muslin cloth.

The data on the egg hatchability, adult emergence and adult population of *B. tabaci* one were recorded day before spray and then 24, 48 and 72 hrs after the spray. The observations on these parameters were taken on nine randomly selected plants of each variety from upper, middle and lower parts of the crop canopy. Egg hatchability was recorded in the laboratory after marking eggs and nymphs on 10 leaves collected randomly from each treatment of the two varieties from the field. Cotton leaf curl virus disease severity was recorded at the end of the experiment according to a CLCuV disease rating scale (Anon., 1992).

For the estimation of protein, 7 samples from each treatment of S-14 and NIAB-Krishma using Kjeldhal method was used (Hiller *et al.*, 1948). One to two gram of oven dried sample was placed in 500 mL long neck pyrex glass Kjeldhal flask mixed with 25-30 mL of concentrated Sulphuric acid and 5 g of digestion mixture containing K_2SO_4 100 mL, $CuSO_4$ 10 mL, $FeSO_4$ 5 mL and boiled until it became light green or almost colourless. On complete oxidation of the sample, the flask was allowed to cool and the contents diluted with ammonia free water in 250-mL flask. Ten ml of this diluted solution was put in micro Kjeldhal distillation apparatus and a concentrated solution of NaOH was added in excess. A receiving flask containing 10 mL N/10 standard H_2SO_4 solution and 2 % boric acid solution containing few drops methyl red indicator was used in such a way that the delivery tube coming through condenser was dipped into it. The steam generator plug was opened and the contents of the distillation tube allowed to boil until all the ammonia was liberated. The partly neutralized N/10 H_2SO_4 solution was titrated in the receiving flask by means of N/10 NaOH solution and the amount of H_2SO_4 neutralized by NH_3 was calculated. Percent nitrogen was calculated by the following formula:

$$\text{Nitrogen \%} = \frac{\text{mL N/10} H_2SO_4 \text{ neutralized by } NH_3 \times 0.0014 \times 100}{\text{Weight of the sample} \times \text{mL of dil. digested material distilled}}$$

Crude Protein = Nitrogen % x 6.25

Table 1. Effect of different chemicals on the egg hatchability, adult emergence and mean whitefly population on S-14 and Niab Krishma.

Treatments		Egg hatchability		Adult emergence		Whitefly population	
		S-14	Niab-Krishma	S-14	Niab-Krishma	S-14	Niab-Krishma
Salicylic acid	(2%)	51.25 c	48.52 b	51.50 c	47.05 c	1.73 c	1.25 c
	(3%)	45.27 c	42.72 b	45.87 b	49.75 b	1.43 c	1.38 c
K_2HPO_4	(2%)	45.27 c	42.72 b	45.87 b	49.75 b	1.43 c	1.38 c
	(3%)	53.97 bc	49.60 b	54.10 c	48.90 bc	2.71 b	1.99 b
KH_2PO_4	(2%)	46.57 bc	44.05 b	46.27 b	46.92 b	2.06 b	1.98 b
	(3%)	56.50 b	49.97 b	57.97 b	49.15 b	3.01 b	2.15 b
Control		48.60 b	44.67 b	47.20 b	47.12 b	2.36 b	1.94 b
		96.95 a	96.37 a	95.25 a	97.42 a	3.87 a	3.02 a
		97.15 a	98.50 a	96.52 a	96.50 a	4.15 a	3.64 a
LSD		3.98	2.41	3.55	1.94	0.57	0.44
		2.27	4.05	3.05	3.46	0.54	0.45

Results and Discussion

Salicylic acid proved to be the most effective in reducing the egg hatchability and adult emergence as compared to potassium di-hydrogen phosphate (Table 1). Whitefly population was also significantly less in case of salicylic acid treatment in both the varieties compared to untreated control. When potassium dihydrogen phosphate and dipotassium hydrogen phosphate was applied at 2 and 3 % concentrations mean whitefly population did not differ significantly, which means that these treatments were equally effective compared to untreated control.

Applications of salicylic acid resulted in reducing disease severity when applied either as foliar spray or as drench (Table 2). There was a significant reduction in the CLCuV incidence on S-14 and NIAB-Krishma plants grown in field or greenhouse when sprayed with 3 % salicylic acid. Other chemicals were also equally effective in reducing CLCuV compared to untreated control. In general, the incidence of CLCuV was higher in greenhouse grown plants compared to field grown plant. It shows that the treatments applied as drench were less effective in reducing disease severity compared to their application as spray. Salicylic acid seems to be involved in both the hypersensitive response and the systemic acquired resistance. It is present in the phloem of plants after the primary inoculation but before the onset of acquired resistance its concentration levels correlate with the induction of PR protein, and external application of salicylic acid activates the same sets of SAR genes that are expressed after SAR induction by the pathogens (Agrios, 1997). Proteins like chitinase, cellulase and thaumatin have been reported to induce artificial or abiotic resistance in host plants against viral, fungal and bacterial diseases (Mills & Wood, 1984; Malamy *et al.*, 1990; Yalpani *et al.*, 1991; Walters *et al.*, 1993; Vernoij *et al.*, 1994).

Salicylic acid when applied as foliar spray or as drench resulted in an increased cellulase and chitinase activity is responsible for inducing artificial resistance in host plants against different plant pathogens (Gottstein & Kuc, 1989; Reuveni *et al.*, 1994; Hashmi *et al.*, 1995). In the present study Chitinases, thaumatin and gluconases were higher in leaf samples of cotton plants when sprayed with different chemicals especially with salicylic acid at 3 % concentration indicating that application of this chemical was helpful in inducing artificial resistance in cotton plants against leaf curl virus. Induction of systemic acquired resistance through external application of salicylic acid has raised

very important questions of whether salicylic acid or other chemical compounds could be used to artificially induce systemic acquired resistance in plants against pathogens. Unfortunately, externally applied salicylic acid is not translocated efficiently in the plant and in addition, salicylic acid is strongly phytotoxic when applied even at slightly higher levels above those required for efficacy. In addition to salicylic acid, derivatives of isonicotinic acid and benzothiazoles have been shown to induce systemic acquired resistance in plants against several pathogens (Agrios, 1997).

Table 2. Mean severity of CLCuV in field and green house grown cotton varieties before and after spray of different chemicals.

Treatments		Under field conditions				Under greenhouse conditions			
		S-14		NIAB-Krishma		S-14		NIAB-Krishma	
		before	after	before	after	before	after	before	after
Salicylic acid	(2 %)	2.53 c	1.20 c	2.53 c	1.20 c	3.07 b	2.03 a	2.86 b	2.20 b
	(3 %)	2.10 c	1.10 c	2.10 c	1.10 c	3.10 b	1.76 c	3.10 b	2.10 b
K_2HPO_4	(2 %)	3.06 b	2.16 b	3.06 b	2.16 b	3.06 b	2.17 a	3.06 b	2.00 b
	(3 %)	3.10 b	2.36 b	3.10 b	2.36 b	3.20 b	2.26 bc	3.10 b	2.36 b
KH_2PO_4	(2 %)	3.03 b	2.16 b	2.80 bc	2.17 b	3.00 b	2.16 a	2.80 b	2.17 b
	(3 %)	2.33 b	3.23 b	2.33 b	3.23 b	2.33 b	3.23 b	2.33 b	3.23 b
Control		3.76 a	3.16 a	3.70 a	3.16 a	3.73 a	2.50 a	3.70 a	3.16 a
		4.06 a	3.46 a	4.06 a	3.46 a	4.07 a	3.46 a	4.06 a	3.46 a
LSD1	(2 %)	0.31	0.42	0.38	0.42	0.25	0.51	0.46	0.42
LSD2	(3 %)	0.18	0.32	0.18	0.33	0.24	0.55	0.18	0.32

Table 3. Protein contents after chemical spray to induce artificial resistance in cotton against leaf curl virus

		S-14
Salicylic acid	(2 %)	Chitinasis (3 %), Thaumatin (5 %), Gluconasis (3 %), ß 1,4 Gluconasis (3 %)
	(3 %)	Chitinasis (3.5 %),Thaumatin (6 %), Gluconasis (5 %), ß 1,4 Gluconasis (6 %)
K_2HPO_4	(2 %)	Chitinasis (2 %), Thaumatin (2 %), Gluconasis (1 %)
	(3 %)	Chitinasis (2 %), Thaumatin (3 %), Gluconasis (1.5 %)
KH_2PO_4	(2 %)	Chitinasis (1.5 %), Thaumatin (1.7 %), Gluconasis (1 %)
	(3 %)	Chitinasis (1.8 %), Thaumatin (3.5 %), Gluconais (1.7 %)
Control	(2 %)	Osmatin (4.5 %), Thaumatin (2 %), Glucan (1 %)
		NIAB-Krishma
Salicylic acid	(2 %)	Chitinasis (4 %), Thaumatin (11 %), Gluconasis (7 %), ß 1,4 Gluconasis (4 %)
	(3 %)	Chitinasis (6 %), Thaumatin (12 %), Gluconasis (8 %), ß 1,4 Gluconasis (5 %)
K_2HPO_4	(2 %)	Chitinasis (5%), Thaumatin (12%), Gluconasis (7%)
	(3 %)	(3 %) Chitinasis (5 %), Thaumatin (12 %), Gluconasis (8 %)
KH_2PO_4	(2 %)	Chitinasis (4 %), Thaumatin (11 %), Gluconasis (6.5 %)
	(3 %)	Chitinasis (5 %), Thaumatin (12 %), Gluconais (6.6 %)
Control		Osmatin (6 %), Thaumatin (10 %), Glucan (6 %)

Salicylic acid and isonicotinic acid are true SAR activators because not only do they induce resistance to the same spectrum of pathogens and induce expression of the same genes as do pathogens, but these chemicals have no antimicrobial activity. A large number of other compounds have been tested for their ability to induce systemic acquired resistance in plants but so far none have proved effective. The research for SAR-inducing compounds, however, has a tremendous commercial potential.

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