DUAL EFFICACY OF SAFE CHEMICALS AGAINST *MYZUS PERSICAE* AND CUCUMBER MOSAIC VIRUS IN TOMATO (*SOLANUM LYCOPERSICUM*)

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ABSTRACT: *Role of chemical elicitors have been widely documented as safe chemicals against various insect pests and diseases of crops. Two chemical elicitors Benzothiadiazole (BTH) and salicylic acid (SA) were used alone and in combination to determine their effect on the aphid infestation in tomato plants. Aphid population hence recorded, was further correlated to the relative incidence of cucumber mosaic virus (CMV). Results have indicated that synergistic application of BTH and SA on weekly basis has significantly reduced (95.63%) the aphid population on tomato. Regulation of aphid has also minimized (84%) the severity of CMV. Aphid fecundity can also be used as biological tool to forecast the occurrence of Shoe string disease in tomato.*

Keywords: Aphid, Elicitors, CMV.

INTRODUCTION:

Tomato (*Solanum lycopersicum*) belongs to family *Solanaceae*. It is the second most important vegetable crop grown throughout the world [1]. It contains vitamin C, potassium, carotenoids and folic acid; the important dietary sources. Carotenoids are formed during fruit ripening and they confer the red color to the tomato. Latest research on tomato nutrition emphasize over its disease suppression abilities in human like obesity, hyperglycemic and hypercholesterolemics, cardiovascular disorders, and cancer [2]. Shoestring disease caused by Cucumber Mosaic Virus is most destructive in tomato growing countries with a temperate climate [3]. It is transmitted principally with *Myzus persicae*; the green peach aphid. Aphid have various species that can transmit different Strains of CMV. Control of plant viruses is not practicable by common fungicide applications. Although considerable research has been conducted for inhibitors of virus infection and multiplication through Systemic acquired resistance (SAR) [4]. Elicitors of SAR could potentially revolutionize the crop protection. Benzothiadiazoles (BTH) is an important commercial elicitor that has been shown to reduce infestation of various diseases throughout a wide range of plant taxa and crops. Regarding viruses, BTH was shown to activate SAR response against CMV in tomato [5], *Tobacco mosaic virus* (TMV) in tobacco [6], *Turnip crinkle virus* (TCV) in arabidopsis [6], *Tomato spotted wilt virus* (TSWV) in tobacco [7], CMV in tomato [8] and in cantaloupe [9]. BTH has been reported as successful inducer of defense response in tomato against Myzus persicae [10]. Impact of BTH on against potato aphid *Macrosiphum euphorbiae* have indicated that SAR can be successfully triggered against arthropods and it can reduce the aphid fecundity[11].Similar response has been explored on the Arabidopsis plants artificially fed with *Myzus persicae*. Plants treated with Benzothiadiazolev(BTH) were comparatively more resistant to aphid infestation [12].

Salicylic Acid has a role of wide significance in the plant kingdom as being of the important components of defense signal transduction. In the case of viruses, SA target virus replication or trans-plasmodesmatal movement or transport through sculpture in the infectivity phase by minimizing the expression of plant genes that encode the factors safeguarding the replication and systemic spread of virus in the plant. Alternatively, SA induces the buildup of certain biological molecules that inhibit the infection cycles of pathogens. Overall conclusion drawn from the efficacy of SA is the interference with replication and systemic spread of plant viruses [13]. SA has also triggered the mechanisms in the crop plants that can disrupt the aphid population by inducing various defense related pathways [14].

MATERIALS AND METHODS:

Plant Material:

Tomato variety Nagina without special resistance against *Myzus persicae* and CMV [1] (Akhtar et al., 2010) was transplanted in high tunnel at Nuclear Institute for Agriculture and Biology, Faisalabad. One month old tomato nursery was transplanted following the one hour root dip into the freshly prepared solutions of salicylic acid @ 0.014g/L and BTH @ 0.5g/L. Control plants were dipped into the distilled water for the same time. After the transplantation, aphid (*Myzus persicae*) was naturally allowed to feed over without applying any protection measure to get high inoculum pressure throughout the experiment. Elicitors were applied by foliar mode of application after one week post transplantation. Single treatment was applied only once. While, weekly treatments were applied at 7 days intervals until the fruit set. Seven treatment combinations including a positive control were maintained as, DC= control, SA weekly= SA_W, SA one foliar spray(SA_1), BTH weekly=B_W, BTH single foliar spray=B_1, BTH+ SA weekly= $(B+SA)_W$, BTH+ SA single spray= $(B+SA)_1$.

Data for CMV symptoms was recorded following a five point (0-4) disease Rating scale (Table-I) [1].

TABLES & FIGURES

Table: I: Disease rating scale for tomato shoestring disease caused by CMV.

Symptoms	Disease severity	Disease reaction
No visible disease symptoms.	0	Highly resistant
Mild mosaic or mottling and leaf deformity	1	Resistant
Moderate mosaic or mottling, leaf deformity and	2	Tolerant

filiformity		
Severe mosaic or mottling or leaf deformity, filiformity, shoestringing, minor to medium stunting with minor flower shedding and minor reduction in fruit setting.	3	Susceptible
Severe mosaic or mottling, leaf deformity, filiformity, shoestringing, stunting with no or few fruit setting	4	Highly susceptible

Virus detection using ELISA:

Samples from tomato leaves were collected after the appearance of symptoms on the leaves. The presence or absence of CMV in the test plants was assayed by double antibody sandwich procedure (DAS-ELISA) [15] with commercial polyclonal antibodies to CMV (BIOREBA AG Switzerland) as recommended by the Manufacturers.

Wells of ELISA plate were coated with CMV antibodies diluted in coating buffer at 1:200. The coated plate was incubated at 4^0C for overnight. After incubation the plate was washed with PBS-Tween 3-4 times at 5-minute intervals. Then wells were filled with the sap of CMV- infected tissue extracted in extraction buffer and two wells were filled with each of buffer, CMV negative and positive sample. The plate was incubated for overnight at 4^oC and washed 3 times with PBST. Enzyme conjugate (@ 200 µl) diluted at 1:200 added and plates were incubated for overnight at 4^0C followed by washing with PBS-Tween 3-4 times. Freshly prepared substrate buffer (200µl) containing p- nitrophenyl phosphate (75ug/ml) was added to each well.

Incubation was done at room temperature for 30 minutes and reaction was observed visually for the development of yellow colour and read in reader at 405 nm. The reaction was stopped by adding 50µl 3M NaOH to each well.

Aphid Population:

Aphid population was recorded on fortnightly basis from three leaves (upper, middle and lower parts) of five plants from each treatment [1].

Data analysis:

Data was analyzed with XL-STAT software and means were compared using Tukey's HSD test.

RESULTS

ELISA:

Presence or absence of virus was confirmed by typical viral symptoms and ELISA. ELISA plate was assessed visually. Positive reaction (yellow colour) was observed with CMV-infected (symptomatic) plants but negative reaction was rare due to unchecked aphid feedings throughout the cropping season. Colour development was due to conversion of p-Nitro phenyl phosphate into p-Nitro phenol phosphate. Positive and negative control samples were also loaded as standards available in the ELISA kit.

Aphid Population:

As discussed earlier, aphid acts as insect vector of the CMV in various species of plants including tomato. Under the experimental conditions the aphid population was considerably disrupted by the foliar application of nontoxic SAR chemicals viz. BTH and SA (Table. II). Highest aphid population Plant^{-1} (151.8) was recorded in the control where no treatment was applied at all. While, weekly application of BTH combined with SA has reduced the aphid population (6.6) in the tunnel significantly. This phenomenon indicated the promising role of safe chemicals against insect vectors of economic importance. Aphid population in $(B+SA)_1$ (48.1) and B-w (64.4) was also minimized to much lower extent. However, B1 (106.8) and SA1(116.1) could be regarded as lower moderate treatments against *Myzus persicae*. While, SA-w (132.5) remained statistically non-significant for aphid control (Table:II).

TABLE II: Efficacy of elicitors in tomato var. Nagina against *Myzus persicae* during, 2015.

Treatments	Mean			
	2nd week of February	4th week of February	2nd week of March	Grand Mean
B1	48	189.5	83	106.8 ^{B}
BW	36.5	85	60	64.4 ^{C}
SA1	71.5	171	106	116.1 ^{B}
SAW	94	158	145.5	132.5 ^{AB}
(B+SA)1	54.5	62.5	27.5	48.1 ^{C}
(B+SA)W	4.5	14	1.5	6.6 ^{D}
Control (DC)	93	225	137.5	151.8 ^{A}

CMV infection:

Symptoms of cucumber mosaic virus (CMV) were initiated as minor mosaic on the emerging leaves. Later it was enhanced towards severe mosaic, leaf deformation and shoestringing with stunting growth. Fruit formation was little or none. Previouslt formed fruit was abnormal in shape. About 4-5 weeks after infection during filiformity an advance symptom was appeared in the form of a numbers of lateral leaflets. Early stage infection was much severe, while while mid-season infections was comparatively mild. Infection percentage varied from 16-100% (Table: III) indicating a

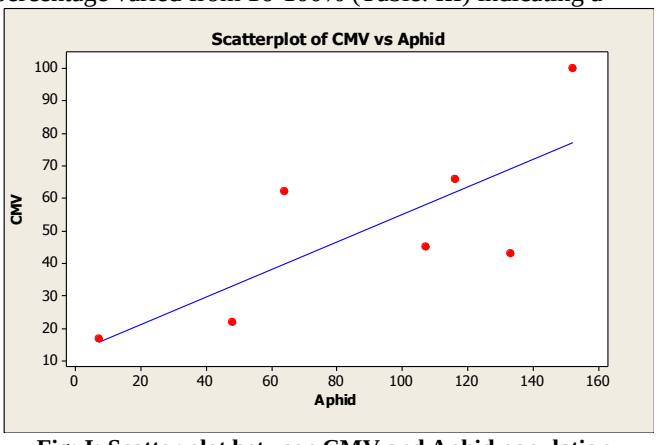

Fig: I: Scatter plot between CMV and Aphid population

varying response of the elicitors against CMV. Aphid has successfully transmitted the CMV and untreated plants (DC) received maximum level (100%) of infection level. First symptom of shoe string was appeared after 13-15 days of aphid colonization. All treatments contributed towards the protection of tomatoes against CMV however, $(B+SA)_W$ and $(B+SA)_1$ were least infected at 16.67% and 22.0% infection percentages, respectively. While, B1 (44.70%) and SA-w (43.33%) elucidated an intermediate response. But SA_1 (65.67%) and Bw (62%) expressed least efficacy against infection at 65.67% and 62% respectively.

Table III: Efficacy of elicitors in tomato var. Nagina against CMV Subgroup-I during, 2015.

Treatment	Infection percentage (%)	Disease response
B1	44.70	Tolerant
BW	62.00	Susceptible
SA1	65.67	Susceptible
SAW	43.33	Tolerant
(B+SA)1	22.00*	Tolerant
(B+SA)W	16.67*	Resistant
Control	100	Highly Susceptible

(* = Significant values)

B_1 = BTH single foliar spray, **B_W** = BTH weekly, **SA_1** = Salicylic acid single foliar spray, **SA_W** = Salicylic acid weekly, **(B+SA)1**= BTH+Salicylic acid single spray, **(B+SA)W** = BTH+ Salicylic acid weekly.

Correlation between Aphid population and CMV:

According to the results, the aphid infestation on tomato was positively correlated ($R^{2=}$ 0.745) to the incidence of CMV (Fig: I). High population pressure significantly transmitted the virus among all the treatments with varying response. Hence it was determined that aphid plays a key role for the orientation of CMV epidemics. Controlling the aphid with safe chemicals can reduce the risk of non-persistent viral transfer among various crop plants.

DISCUSSION:

Our early emphasis in the experiment was to investigate the role of chemical elicitors against Aphid infestation on the tomato (var. Nagina). According to the results, aphid population per plant was significantly reduced in the (B+SA)w (10) as compared to control (158). Earlier studies for the impact of elicitors on aphid populations have been documented in wheat crop where salicylic acid induced the systemic resistance against aphid and minimized the infestation on host plants [16][14]. SA has vital roles in establishing systemic acquired resistance (SAR), a form of long-term and broad-spectrum resistance throughout the entire plant [17] after it is challenged by a biotic or abiotic elicitor [18]. SA induces the defense pathways which are triggered to immunize the host against wide range of pathogens. Proteomic studies explored the molecular mechanisms in tomato in response to the aphid specie indicated the activation of defense related pathways similar to that of jasmonic acid (JA), salicylic acid (SA) playing a key role for the resistance of tomato to the aphid [19]. BTH has also been documented to control the phloem feeding aphid population on tomato [11]. However, individual application of BTH over the tomato is not encouraged due to its adverse impact on fruit quality and vigor a phenomenon termed as "allocation fitness cost" or "trade-off". The reduction in growth results from the competing metabolic demands of plant-related compound synthesis and the requirement of a substantial amount of energy for the induction of SAR [20]. But the role of BTH to combat CMV can never be overlooked [5]. Hence, the combined application of SA and BTH has led to the most effective and safe exploration of SAR chemicals in crop protection.

Our results indicated a significant correlation between aphid population and incidence of CMV in tomato. Aphid population in crop plants has been widely correlated to the occurrence of non-persistent viral diseases. It is now generally assumed that higher aphid fecundity means the higher incidence of CMV. Earlier studies have been carried out in snap beans, where a positive correlation between cucumber mosaic virus and aphid population has been documented [21]. Some results support the luring role of plant defense volatiles to aphid colonies, where SAR has already been triggered by CMV [22][23]. Most recent findings indicated that infection of host plants by CMV increases the predation of aphid by parasitoid *Aphidius colemani*. Such mode of parasitism may aid the rapid dispersal of aphid on large field areas, urging the epidemics of CMV [24]. After all, higher infection supports the aphid population or vice versa.

According to our experimental concerns, minimum severity of CMV (16%) was recorded in the combined application of BTH and salicylic acid applied on weekly basis. Previous studies about BTH against CMV have been documented for tomato, resulted in the significant disease suppression by this elicitor [5]. Salicylic acid has also been reported to play significant role for the control of cucumber mosaic virus in *Arabidopsis thaliana* triggered with PGPR as biotic elicitor [25][26]. BTH application as a drench, seven days before transmission of CMV-Y, protected plants against the necrosis caused by CMV-Y. The resistance was evident as a decreased disease incidence and severity. The disease incidence in BTH-treated plants did not exceed 12.5% whereas 91.7% of control plants were severely infected. The induced resistance in tomato plants markedly reduced the replication of viral RNA [8]. However, our experimental evidences also indicate that disease severity due to CMV was least affected by application of salicylic acid alone. The ability of CMV to escape from SA-induced inhibition of replication and/or local movement might have appeared due to the CMV 2b protein, that functions as an inhibitor of RNA interference (RNAi) induction [27]. Despite this, SA treatment can inhibit the systemic spread of CMV through the plant, by inhibiting the long-distance movement of this virus [28].

CONCLUSION: Use of eco-friendly and safe chemicals in plant protection is crucial, so as to handle the food toxicity issues. Application of BTH and Salicylic acid on weekly basis can protect the tomato from the colonization of aphid. Relieving the aphid population can further prevent the non-

persistent transmission of cucumber mosaic virus hence reducing the threat of epidemics and crop failures.

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