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Response of Tomato Plants to Salicylic Acid and Chitosan under Infection with *Tomato mosaic virus*

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Abstract: Tomato (*Solanum lycopersicum* L.) plants are considered sensitive to *tomato mosaic virus* especially during the reproductive growth phase. Hence, the present study aimed to investigate the effect of foliar application with salicylic acid (2 mM/l) alone or combined with chitosan (0.1%) with or without TMV inoculation on improving resistance, growth, productivity and quality of tomato Hybrid Super Jackal F_1. The study was conducted in The Experimental Farm, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt, during the two growing summer seasons of 2012 and 2013. All inoculated plants with TMV after 14 and 30 days from foliar application with combination of salicylic acid and chitosan, exhibited symptoms later and less severe than the plants inoculated with TMV in the other treatments. The SA plus CH foliar application without TMV inoculation gave the highest significant values of vegetative growth in both seasons. Combination treatment of SA plus CH increased significantly N, P, K, Fe and Zn concentration. This treatment was also effective in increasing tomato yield compared with treatment of infection alone. Our results showed that SA or CH alone or combined significantly increased ascorbic acid concentration compared with control treatment.

Key words: Chitosan · Growth · Salicylic acid · TMV · Yield

INTRODUCTION

The tomato belongs to the *Solanaceae* family along with other economically important crops such as pepper, eggplant and potato. The tomato was classified by Miller as *Lycopersicon esculentum* and renamed by Child and Peralta and Spooner as *Solanum lycopersicum* [1]. Tomato plants are one of the most important vegetable crops overall the world and was exposed to many pathogens and infections. The introduction of policies on reducing pesticide inputs in many countries is aimed at designing more environmentally friendly fungicides and strategies for disease control sustainable production involving the international policy on reduction of pesticide applications [2].

Chitosan is a natural carbohydrate polymer modified from chitin, which is derived from crustaceous shells such as crabs and shrimps. It has been reported as a high potential bio-molecule that increases plant growth and yield [3]. Chitosan is considered an environmental friendly product that has been widely used in agricultural applications mainly for stimulation of plant defense [4]. Chitosan triggers a defense response within the plant, leading to the formation of physical and chemical barriers against invading pathogens, chitosan has been used in seed, leaf, fruit and vegetable coating, as well as a fertilizer and in controlled agrochemical release [5]. The increase in phenolic substances following chitosan application has been reviewed [6]. Chitosan application improved vegetative growth, leaf content of N and K and yield components (number and weight) of strawberry plants [7]. Moreover, El-Nagar *et al.* [8] on pea green yield found that spraying the plants with chitosan at a rate of 1% increased vegetable growth and yield and its component. In addition, strawberry plants foliar application with chitosan at 1% significantly increased such assayed yield, leaf macronutrients and T.S.S. of fruit compared with the control treatment [9]. On the other hand, Hofgaard *et al.* [10] mentioned that preparations of chitin or chitosan have been reported to trigger defence reactions in plants

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and thereby potentially enhance resistance against pathogens on wheat plants. Chitosan is a safe material has antifungal activity against many plant pathogens. Moreover, Chitosan also was reported to induce resistance against soil borne fungi [11]. Notably, antibacterial action of chitosan has been widely reported for food and clinically important bacteria however, this fact has not been equally matched with phytopathogenic bacteria, also chitosan is reported to possess antifungal activity and is capable also of eliciting resistance in susceptible plants through the induction of the host's defence mechanisms [2, 12,13]. Also, chitosan improved the growth and yield of various crops such as tomato, sweet pepper and radish [14-16]. Oligochitosan was shown to induce resistance of plants against viral disease and fungal disease [17,18].

Salicylic acid (SA) is a phenolic derivative, distributed in a wide range of plant species. It is a natural product of phenylpropanoid metabolism. Decarboxylation of transcinnamic acid to benzoic acid and its subsequent 2- hydroxylation results to SA. It undergoes metabolism by conjugating with glucose to SA glucoside and an ester. SA has direct involvement in plant growth, flower induction and uptake of ions. Enhancement of the level of chlorophyll pigment, photosynthetic rate and modifying the activity of some of the important enzymes are other roles assigned to SA [19]. Salicylic acid which is a secondary plant product performs important actions in the growth and development processes of plants. It can stimulate plant growth, yield of vegetables [20]. Pacheco *et al.* [21] mentioned that SA is a phenolic phytohormone that acts as a key regulator of the signaling network in plants under abiotic and biotic stresses. Also, SA exerts stimulatory effects on various physiological processes related to plant growth and development. Many researches monitored the role of SA in defence and pathogen resistance, SA could contribute to maintaining cellular redox homeostasis through the regulation of antioxidant enzyme activities and induction of the alternative respiratory pathway [22]. The SA has been shown to regulate cell growth, stomatal aperture, seed germination, seedling development, thermotolerance, fruit yield, nodulation in legumes and the expression of senescence-related genes. It is mostly known for its central role in defense responses [23].

Considering the above facts, the present study was undertaken for further understanding of the response of tomato, in terms of induction systemic acquired resistance, vegetative growth, chemical constituents and productivity, to chitosan and salicylic acid foliar spray alone and in combination with TMV inoculation under controlled field conditions.

MATERIALS AND METHODS

The field experiment was carried out during the two growing summer seasons of 2012 and 2013, at The Experimental Farm, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt, in order to investigate the effect of foliar application of chitosan and salicylic acid under infection with TMV on improving resistance, growth, productivity and quality of tomato plants (*Solanum lycopersicum*, Super Jackal hybrid produced by TAKII & CO., Ltd, Japan). The experimental soil was clay loam. Cultural management, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture.

Five-week old tomato transplants were sown in the shading screen into the field on 1st of April in 2012 and 2013 summer seasons. The area of the experimental plot was 18 m^2 consisted of three ridges, each ridge was 6 m length and 1 m width. The plant distance was 40 cm apart on one ridge.

The experimental treatments and design
This experiment included eight treatments as follows:

- Foliar application with distilled water as a control treatment.
- Inoculation with TMV.
- Foliar application with 2 mM/l salicylic acid (SA).
- Foliar application with 2 mM/l SA and TMV inoculation.
- Foliar application with 0.1% chitosan (CH).
- Foliar application with 0.1% CH and TMV inoculation.
- Foliar application with 2 mM/l SA and 0.1% CH.
- Foliar application with 2 mM/l SA, 0.1% CH and TMV inoculation.

Chitosan is a commercial product by Oxford Laboratory, India. It includes chitosan 90 % (2-Amino-2-deoxy-beta-D-glucosamine) at 1%, i.e. 11.11 g from chitosan was dissolved in 0.1 NaOH and completed to one liter of distilled water. Salicylic acid (MW 132) of Sigma

Aldrich was used dissolved in distilled water. Foliar applications were applied after 30 and 50 days from transplanting. The Inoculation was done after 4 days from the first foliar application. *Tomato mosaic virus* (TMV) was kindly provided by Plant Virus Lab., Plant Pathology Department, Faculty of Agriculture, Ain Shams University, was used in the present study. Tomato plants were mechanically inoculated using phosphate buffer solution [24]. Leaves of control plants were dusted with carborandum and inoculated with buffer solution. The experiment was laid out in a complete randomized block design with three replicates.

Studied Characteristics: The TMV symptoms on tomato plants were recorded after 14 and 30 days from inoculation with TMV on all treatments. Relative concentration of TMV in infected plants was determined serologically after 14 and 30 days post inoculation (dpi), in the two seasons, by double antibody enzyme-linked immunosorbent assay (DAS-ELISA) was used to determine TMV levels in sap of young leaves [25]. Absorbance values were determined using ELISA reader (BIE & BERNTSEN A.S) at 405 nm [1 hr. after addition of the substrate].

After 65 days from transplanting, five plants per plot were randomly chosen to measure plant length, number of branches/plant, number of leaves/plant and plant fresh weight. Leaf chlorophyll reading (SPAD) was determined using the recently full expanded mature upper leaf of 10 plants in the middle row per plot. A digital chlorophyll meter, Minolta SPAD-502, (Minolta Company, Japan) was used. Total nitrogen, phosphorus and potassium were determined, in the fourth leaf, at 75 days after transplanting according the methods described by Horneck and Miller [26], Sandell [27] and Horneck and Hanson [28], respectively. Also, Zn and iron concentration in leaves were determined according to Black *et al.* [29] and Cottenie [30], respectively. Early yield (as the first two pickings), total yield of five plants (from the inner ridges) were recorded from each plot, the average weight of yield/plant was calculated and yield per feddan was calculated by multiplying the average yield per plant by number of plants per feddan. Fruit juice of randomly selected ripe-fruits was used to measure total soluble solids by using a hand refractometer, while vitamin C content was determined according to A.O.A.C. [31].

Data of the two seasons were arranged and statistically analyzed using Mstatic (M.S.) software. The comparison among means of the different treatments was determined as illustrated by Snedecor and Cochran [32].

RESULTS AND DISCUSSION

Symptomatology: Inoculated plants with TMV and treated with foliar application of combination of salicylic acid and chitosan exhibited symptoms later and less severe than the plants inoculated with TMV in the other treatments. In this respect, Huijsduijnen *et al.* [33] reported that the inhibition of the systemic replication in tobacco shows that the induced resistance does not necessarily involve the formation of local lesions. The results with the cowpea protoplasts demonstrate that inhibition does not occur at the level of cell-to-cell spread of the virus, but that salicylic acid is acting on primarily infected cells. Apparently, salicylic acid treatment blocks the synthesis of minus-strand and plus-strand RNAs by this replicase activity. Possibly, the inhibition occurs at the level of the synthesis of double-stranded replicative intermediates. The lack of coat protein synthesis may be a consequence of the block of viral RNA synthesis.

Concerning, reaction of tomato plants after 14 dpi, tomato plants treated with distilled water or chitosan showed moderate mosaic, on the other hand, tomato plants treated with salicylic acid or combination of salicylic acid and chitosan showed mild mosaic (Table 1). After 30 dpi, tomato plants treated with distilled water showed severe mosaic, stunting, leaf malformation and yellowing. Tomato treated with salicylic acid showed moderate mosaic and leaf malformation. Tomato treated with chitosan showed severs mosaic and leaf malformation. On the contrary, the combination of salicylic acid and chitosan showed less severe symptoms and only moderate mosaic. According to the results obtained and listed in Table 1, it can be concluded that tomato plants inoculated with TMV and treated with a combination of salicylic acid and chitosan exhibited mild symptoms. In this respect, Sudhakar *et al*. [34] stated that the rapid accumulation of salicylic acid after cucumber mosaic virus inoculation leads to increase in activity of enzymes known to be involved with systemic acquired resistance such as phenylalanine ammonialyase and peroxidase. Salicylic acid activates resistance mechanisms such as phytoalexin production, proteinase inhibitors, cell wall strengthening and lignification. Also, SA application on tobacco enhanced the resistance to CMV and the resistance was shown to be due to inhibition of systemic virus movement [35]. Raskin [36] found that it is possible that SA is an endogenous messengers that activities important element of host resistance pathogens. This hypothesis is supported by spraying tobacco plants with salicylic acid or acetylsalicylic acid that induces both the

Table 1: Effect of foliar application of salicylic acid and chitosan after 14 and 30 days from TMV inoculation on external symptoms of tomato plants in the two seasons (2012 and 2013)

	14 days		30 days				
Treatments	M.M	Mo. M	Mo.M	S.M	Stunting	L.Mal.	Y.
	1st season						
Control	-	-	-	-	-	-	-
TMV	-	+	-	+	+	+	+
2mM/l Salicylic acid (SA)	-	-	-	-	-	-	-
SA + TMV	+	-	+	-	-	+	-
0.1% Chitosan (CH)	-	-	-	-	-	-	-
CH + TMV	-	+	-	+	-	+	-
SA + CH	-	-	-	-	-	-	-
SA + CH + TMV	+	-	+	-	-	-	-
	2nd season						
Control	-	+	-	-	-	-	-
TMV	-	-	-	+	+	+	+
2mM/l Salicylic acid (SA)	-	-	-	-	-	-	-
SA + TMV	+	-	+	-	-	+	-
0.1% Chitosan (CH)	-	-	-	-	-	-	-
CH + TMV	-	+	-	+	-	+	-
SA + CH	-	-	-	-	-	-	-
SA + CH + TMV	+	-	+	-	-	-	-

M.M= Mild mosaic, Mo.M = moderate mosaic S.M= sever mosaic, L.Mal. = Leaf malformation, Y= yellowing, + = symptoms, - = no symptoms.

Table 2: Effect of foliar application of salicylic acid (SA) and chitosan (CH) after 14 and 30 days from TMV inoculation on relative concentration of TMV with ELISA (405 nm OD) of tomato plants in the two seasons (2012 and 2013)

	Treatments							
Days after inoculation	Control	TMV	2mM/l SA	SA + TMV	0.1% CH	CH + TMV	SA + CH	SA + CH + TMV
	1st season							
14 days	0.113	0.495	0.114	0.292	0.113	0.345	0.110	0.235
30 days	0.114	0.545	0.121	0.313	0.114	0.371	0.115	0.286
	2nd season							
14 days	0.121	0.511	0.119	0.327	0.116	0.381	0.115	0.248
30 days	0.120	0.583	0.125	0.371	0.118	0.401	0.116	0.320

Table 3: Effect of foliar application with salicylic acid, chitosan and inoculation with TMV on some vegetative growth characters of tomato plants at 65 days after transplanting in the two seasons (2012 and 2013)

	1st season				2nd season			
Treatments	Plant length (cm)	Number of branches	Number of leaves	Plant fresh weight(g)	Plant length (cm)	Number of branches	Number of leaves	Plant fresh weight(g)
Control	53.7 bc	9.9 de	42.2 cd	483.6 ef	52.9 cd	10.9 e	42.0 c-e	429.4 de
TMV	50.4 c	8.5 e	35.0 e	466.6 f	50.4 d	8.0 f	39.2 e	393.2 e
2mM/l Salicylic acid (SA)	58.3 a	12.9 bc	43.6 cd	530.2 cd	60.9 ab	12.7 b-d	44.4 bc	475.0 c
SA + TMV	52.2 bc	10.7 c-e	37.3 e	514.0 de	55.3 c	11.2 de	40.8 de	442.5 cd
0.1% Chitosan (CH)	60.5 a	16.4 a	47.5 ab	565.7 bc	62.7 ab	14.4 ab	47.2 b	554.6 ab
CH + TMV	53.9 b	12.6 b-d	41.1 d	544.1 cd	59.4 b	12.2 c-e	42.9 cd	524.1 b
SA + CH	58.9 a	16.7 a	50.3 a	602.2 a	63.9 a	15.5 a	50.8 a	594.1 a
SA + CH + TMV	54.2 b	14.2 ab	44.6 bc	586.3 ab	59.8 b	13.4 bc	46.8 b	550.9 b

Means followed by different letters are significantly different at P $\leq$ 0.5 level; Duncan's multiple range test synthesis of pathogenesis-related (PR) proteins and resistance to infection with tobacco mosaic virus (TMV). Moreover, a tobacco hybrid that produces PR proteins constitutively is highly resistant to TMV infection [33]. In this respect, Zhao *et al*. [37] suggested that, induction of TMV resistance may be to several reasons such as: expression of gene related with resistance in tobacco, transcripts of phenyl alanine ammonia layase (PAL) and chitinase.

DAS-ELISA was used to determine the relative concentration of TMV in tomato plants after 14 and 30 days post inoculation. Data in Table 2 showed that the absorbance values (at 405 nm) of the infectious sap containing TMV were generally increased after 30 days from inoculations with TMV and the lowest value of TMV was detected in combination of foliar application treatment than the other treatments.

Zhao *et al*. [37] reported that oligochitosan has the ability to inhibit local viral infection and development of lesion spots caused by TMV in tobacco plants in greenhouse. Also, consistent with the results of field experiments of oligochitosan controlling plant virus disease [17]. In the present study showed that salicylic acid approved low expression of symptoms and low relative concentration than chitosan alone these results may be due to results of Zhao *et al*. [37] they showed that the accumulation of PAL and chitinase mRNA in response to oligochitosan was markedly increased within 10 h after treating by oligochitosan, but the accumulation of PAL and chitinase mRNA was declined after the treating 12 h. efficiency of oligochitosan in induction of tobacco resistance to TMV was gradually declined after 48 h. So the amount of antiviral substance may be decreased after 48 h, and the efficiency of oligochitosan in induction of tobacco resistance to TMV was declined. Another reason, it may be related with the inactivation of oligochitosan on plant virus in vitro, after spraying oligochitosan 48 h, the amount of oligochitosan on leaf surface of tobacco may be reduced because of plant absorbing, so the efficiency of oligochitosan was declined. On the other hand the combination of the two inducer may be enhance each other the antiviral materials in tomato plants so it's showed the best results than each inducer alone.

Vegetative Growth: Data in Table 3 revealed, generally, that foliar application of SA plus CH without TMV inoculation gave the highest significant values of vegetative growth in the both seasons. Foliar application of CH or SA each alone or in combination gave the highest significant plant length in both seasons compared to the other treatments. In case of infection, SA plus CH or CH alone gave moderate values followed by SA, while infection alone induced significantly the lowest plant length in the two seasons. As for branch number and leaf number, plants treated with CH with or without SA as foliar application produced the highest number of branches and leaves in the two seasons. In case of infection, plants sprayed with CH with or without SA increased both branch and leaf number compared to those treated with TMV alone. Also, the combination treatment of SA plus CH gave significantly the highest fresh weight of tomato plant even with or without infection compared to other treatments. The CH was better than SA in increasing fresh weight. In case of inoculation, the combination of CH and SA improved the plant growth compared to other tested treatments.

The positive effect of treatments may be related to that salicylic acid application promotes cell division and cell enlargement [38]. Foliar application of salicylic acid increased the leaf area of sugarcane [39, 40]. Pacheco *et al*. [21] suggested that the observed increase in photosynthesis rate in plants sprayed with SA can be assigned to metabolic changes at the chloroplasts level (efficiency of photosystem II, Rubisco enzyme activity and supply of ATP and NADPH for the carbon reduction cycle). However, the stimulatory effect of SA on gas exchange parameters and plant development is dependent on several factors such as application mode, exposure time and ontogenetic stage of the plant [41]. Besides, the effective concentrations of SA differ among species and their domestication stage. This can explain the little effect of the treatment of SA alone. These results corroborate the findings of Khan *et al.* [42] who reported increases in shoot dry weight of soybean and corn plants treated with SA. Vicente and Plasencia [43] suggested that the growth-promoting effects of SA could be related to changes in the hormonal status. Also, Javaheri *et al*. [40] reported that the dry matter accumulation was significantly increased in tomato, when lower concentrations of salicylic acid were sprayed. In this respect, Fathy *et al*. [44] mentioned that salicylic acid increased eggplant plant height, number of branches and leaves per plant and dry weight.

Concerning the chitosan application, Nawar [45] found that the highest plant length and fresh and dry weights were obtained in tomato plants grown from transplants treated with chitosan. This result is in accordance with that of El-Tanahy *et al*. [46] who found that application of chitosan at 5% had the superior effect on all measured vegetative parameters of cowpea plants

Table 4: Effect of foliar application with salicylic acid, chitosan and inoculation with TMV on some chemical constituents of tomato leaves in the two seasons (2012 and 2013)

Treatments	Chlorophyll reading (SPAD)	N %	P %	K %	Fe (ppm)	Zn (ppm)
	1st season					
Control	42.0 b-d	3.28 bc	0.48 e	2.89 c	118.63 c	58.43 de
TMV	38.3 e	2.74 d	0.46 e	2.81 c	108.26 d	54.22 e
2mM/ l Salicylic acid (SA)	42.8 bc	3.41 bc	0.58 a	3.04 bc	119.97 c	62.60 b-d
SA + TMV	39.8 de	3.01 cd	0.55 bc	2.89 c	119.19 c	60.53 c-e
0.1% Chitosan (CH)	44.0 b	3.68 b	0.52 cd	3.05 bc	136.05 b	65.07 bc
CH + TMV	41.4 cd	3.24 b-d	0.52 d	2.87 c	124.03 c	62.06 cd
SA + CH	47.1 a	4.28 a	0.55 b	3.44 a	150.79 a	72.42 a
SA + CH + TMV	44.2 b	3.53 bc	0.52 d	3.32 ab	135.36 b	68.92 ab
	2nd season					
Control	39.9 cd	3.13 cd	0.43 d	2.61 cd	125.16 cd	52.09 e
TMV	36.2 e	2.78 d	0.36 e	2.59 d	117.14 d	48.76 e
2mM/ l Salicylic acid (SA)	41.9 bc	3.54 bc	0.52 e	2.91 b	131.33 b-d	64.14 c
SA + TMV	38.4 de	2.93 d	0.46 cd	2.85 bc	120.94 d	63.94 cd
0.1% Chitosan (CH)	43.6 ab	3.64 a-c	0.56 ab	3.22 a	141.32 bc	65.61 bc
CH + TMV	39.9 cd	3.26 cd	0.51 bc	2.90 b	129.72 b-d	60.58 d
SA + CH	46.2 a	4.21 a	0.59 a	3.27 a	153.54 a	71.78 a
SA + CH + TMV	42.1 bc	3.89 ab	0.52 b	3.24 a	144.24 ab	67.85 b

Means followed by different letters are significantly different at P $\leq$ 0.5 level; Duncan's multiple range test

Table 5: Effect of foliar application with salicylic acid, chitosan and inoculation with TMV on yield and its components of tomato leaves in the two seasons (2012 and 2013)

Treatments	Yield plant^{-1} (kg)	Early yield (ton / fed.)	Total yield (ton/fed.)	TSS %	Ascorbic acid (mg/100 g FW.)
	1st season				
Control	1.25 c	1.77 c	13.17 c	4.45 de	110.34 de
TMV	1.13 d	1.85 bc	11.88 d	4.38 e	93.58 f
2mM/ l Salicylic acid (SA)	1.58 a	1.99 b	16.71 a	4.46 de	121.29 bc
SA + TMV	1.36 bc	1.97 b	14.32 bc	4.70 c-e	102.00 ef
0.1% Chitosan (CH)	1.46 b	2.02 b	15.40 b	4.89 b-d	126.14 ab
CH + TMV	1.44 b	2.00 b	15.20 b	4.98 a-c	108.85 de
SA + CH	1.68 a	2.42 a	17.75 a	5.23 ab	134.99 a
SA + CH + TMV	1.44 b	2.39 a	15.15 b	5.35 a	114.09 cd
	2nd season				
Control	1.29 ef	1.94 e	13.64 ef	4.36 bc	105.10 cd
TMV	1.18 f	2.05 de	12.50 f	4.18 c	98.28 d
2mM/ l Salicylic acid (SA)	1.60 ab	2.16 c-e	16.85 ab	4.59 b	113.13 bc
SA + TMV	1.34 de	2.16 c	14.14 de	4.66 b	99.69 d
0.1% Chitosan (CH)	1.49 bc	2.31 bc	15.73 bc	5.19 a	119.89 ab
CH + TMV	1.42 cd	2.29 c	14.99 cd	5.21 a	105.78 cd
SA + CH	1.65 a	2.69 a	17.46 a	5.51 a	123.75 a
SA + CH + TMV	1.43 cd	2.43 b	15.10 cd	5.23 a	112.48 bc

Means followed by different letters are significantly different at P $\leq$ 0.5 level; Duncan's multiple range test

(plant height, number of leaves and shoots, fresh and dry weights of leaves and shoots) followed in decreasing order by chitosan at 3 then at 1%. Abu-Muriefah [47] found that foliar-applied chitosan increased common bean plant growth as compared to chitosan untreated plants. Besides, chitosan also promoted the growth and yield of various crops such as tomato [3].

Leaf Chemical Constituents: Data in Table 4 showed that SA plus CH treatment significantly increased chlorophyll reading without significant difference with CH in the second season, followed by SA plus CH with TMV infection. Combination treatment of SA plus CH significantly increased N, P and K followed by CH alone in infected and non-infected plant. SA induced the least values of the macro elements but it didn't differ significantly from CH in most cases. The SA plus CH as referred previously induced high values of Fe and Zn in both infected and non-infected plants.

This can be attributed to the role of salicylic acid to improve membrane permeability, absorption and utilization of mineral nutrients. Some researches indicated that salicylic acid increased membrane permeability that would facilitate absorption and utilization of mineral nutrients and transport of assimilates [40]. Also, El-Tanahy *et al.* [46] reported that the best results of N, P and K values were reported by chitosan in cowpea plants. The increment in total N content in the leaves may be brought about by the amino components in chitosan and or higher ability of the plant to absorb N from the soil when chitosan was degraded. Also, the higher content of K explains the higher quality of the fruits due to the presence of K which acts on photosynthate translocation from the leaves to the storage organs. On the contrary, Pacheco *et al*. [21] who observed that the chlorophyll content in SA treated plants did not differ significantly from the control plants. On the other hand, many investigators reported that application of chitosan increased key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improved the transportation of nitrogen in the functional leaves which enhanced plant growth and development [3].

Yield and its Components: Data in Table 5 elucidated that combination treatment with SA plus CH was effective in producing the highest tomato yield when compared with any other treatment followed by SA. Infected plants treated with SA, CH or with both produced higher yield compared to TMV infected plants only. Our results showed that SA and CH alone or and combined increased significantly ascorbic acid content compared with the control. The SA plus CH gave the highest TSS values in infected and non-infected plants compared to other treatments.

The increase in tomato yield in the present study might be due to the reduction in disease incidence, promotion of plant growth (Table 3) and increasing chlorophyll, N, P, K, Fe and Zn (Table 4) as influenced by foliar spray with SA and CH. The effect of SA on productivity was discussed by Boatwright and Mukhtar [23] who reported that some virus-encoded proteins have demonstrated the ability to inhibit or enhance SA-dependent signaling. In this scenario, the cucumber mosaic virus (CMV) encoding the 2b counter-defence protein (CMV 2b) exhibits a complex regulatory effect by interfering with JA-dependent signaling, RNA silencing, SA biosynthesis and SA-mediated gene expression. Our results suggested that application of SA alone was effective for increasing yield of tomato plant. Meanwhile, Javaheri *et al*. [40] showed that the application of salicylic acid increased tomato yield and vitamin C of fruits. According to Shakirova *et al.* [48], reported that the positive effect of salicylic acid on yield can be due to its influence on other plant hormones. Increasing of yield under foliar application of salicylic acid could be ascribed to the well-known roles of salicylic acid on photosynthetic parameters and plant water relations. In this respect, Fariduddin *et al.* [49] reported that exogenous application of salicylic acid enhanced the net photosynthetic rate in *Brassica juncea*. These results agree with those reported by El- Mougy *et al*. [11] who noted that chitosan treatment increased tomato yield more than 66.7%, whereas the moderate increase was obtained with individual treatments of chitosan recorded more than 40.0% increase as compared with untreated plants. Additionally, El-Tanahy *et al*. [46] pointed out that the best yield of cowpea plants were obtained by using chitosan.

Resistance property of SA and CH was studied by many researchers. In this regard, Pacheco *et al.* [21] stated that SA exogenous application induced the expression of many defense genes which encode particular enzymes of secondary metabolic pathway to form bioactive compounds such as phenolics. Hackmann *et al.* [50] reported that the phytohormone SA participates in regulating defence predominantly against biotrophic and hemibiotrophic pathogens.

Chitosan induced resistance, through increasing the chitinase activity. This finding are in accordance Hofgaard *et al.* [10] observed enhanced expression of chitinase genes after treatment with chitosan tested treatment, although no significant enhancement of snow mould resistance was found in this experiment. Enhanced expression of chitinase genes or increased chitinase enzyme activity is trigged in plants by pathogen infection. Mansilla *et al*. [13] mentioned that chitosan could exert its bactericide effect by cell membrane permeabilization unlike of other types of polycationic molecules which impacts on cell permeability but lacks of direct bactericidal activity. Also, to reinforce the idea that the antibacterial mechanism of chitosan is due to the electrostatic interaction, assays with a derivative of chitosan called N-propyl-N-methylene phosphonic chitosan were performed.

CONCLUSION

Combination of SA plus CH treatment had the best effect on vegetative growth, mineral concentration, yield and TSS in both infected and non infected tomato plants, followed by CH or SA which didn't differ significantly in most cases. This insures the defense property of CH or SA in induced resistance and promoting vegetative growth and yield. This potential value appears to be attributable to the combination of the antivirus and eliciting properties of chitosan in general with SA. Thus, in this study we suggest use combination of CH and SA to reduce the effect of viral infection of TMV and improve the production of tomato plants under field conditions.

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