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Control of Root Knot Nematode *(M. incognita*) via Improve Defense Ability by Using Salicylic Acid in Eggplant (*Solanum melongena*)

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ABSTRACT

Effect of salicylic acid (SA) as chemical resistance inducer against root knot nematode *M. incognita* (RKN) on eggplant was investigated during 2022-2023 growing season. Five salicylic acid concentrations 100, 150, 200, 250 and 300 uM as seedling roots dipping solutions. Studied treatments significantly (P≤0.05) reduced root -knot nematode parameters compare to control treatment. Highest significant effects were achieved under using of 300 uM treatment which caused reduction percentages of 68.83 %and 69.09 % for final population (Pf) and reproductive factor (RF) lower than control treatment, respectively. Antioxidant enzymes and antioxidant compounds showed significant effects in eggplant infected with (RKN). Activity of peroxidase, polyphenoloxidase and catalase antioxidant enzymes were increased as a result of SA treatments with the highest significantly effects of 300 uM treatments. Total and free phenols and total chlorophyll (a+b) contents in SA treating plants were significantly increased compare to control treatment. Carotenoids and free amino acids contents were increased with salicylic acid application while proline contents were decreased. All studied SA treatments significantly increased eggplant growth attributes *i.e.* shoot and root fresh weights, dry shoot weight, shoot and root lengths with the highest effects at 300 uM treatment, compare to control plants. It seems that SA at 300 uM concentration seedling roots dipping solutions as an abiotic resistance inducer treatment could be used to control root-knot *M. incognita* on eggplant by decreasing both cost and hazard effects of chemical nematicides.

Keywords: Salicylic acid, *M. incognita*, Antioxidant enzymes.

INTRODUCTION

Plant parasitic nematodes (PPN) are one of the most damaging pests of many vegetables, while *Meloidogyne* spp (RKN). are the most destructive species under tropical and subtropical conditions. Root knot nematodes (RKN) can result in yield decreasing of 33 % in melons 38% with tomato , 25% in potato and 20 % on eggplant yield decreasing Sikora and Fernandez (2005) and (Perry *et al.*, 2009). Although chemical nematicides have been used as one of the most effective nematode control with fast and efficient results, their use have negative hazard effects on both human and environment (Hemmati and Saeedizadeh, 2020) and (Enyiukwu *et al*., 2021)

Plant activate protective mechanisms with different pathogens along with constitutive resistance in the tissues around the infection site, pathogen can induced. Localized acquired resistance (LAR) while in distant, there is systemic acquired resistance, (SAR) which found in uninfected tissues. Resistance can be chemically induced via using salicylic acid (SA) .This inducer resistance can be protects plants which acts systemically while it is environmentally benign. Salicylic acid used as long-distance messenger signaling molecules which involved under different stress conditions like pathogenic infection (Klessig and Malamy, 1994); Oostendrop *et al*. (2001) and Hammerschmidt (2009).

Salicylic acid considers a key hormone required for resistance for different pathogens as signal molecules having activator defense role responses which inducing cells responses against pathogenic infection. Due to it is non-phytotoxic at the appropriate dosage application, the SA usage for inducer resistance to (RKN) is important. SA increased many defense compounds like H_2O_2 as well as peroxidase activity in *M. Javanica* infested cucumbers roots. Roldi *et al.* (2017) found that silicates caused decreasing the (RKN) population and lowest eggs, juveniles and galls number. 50 uM of SA caused 95% as seed treatment and 78% as soil drenching reduction in number of egg masses that form of tomato plants. Salicylic acid has potential to lower (RKN) reproduction in tomato and seed priming is a fairly easy method to work with. All SA application treatments reduced all (RKN) parameters and increased cucumber growth criteria as well as antioxidant enzymes Bakr and Hewedy (2018); Ding and Ding (2020); Taher and Ami (2022).

The objective of the present investigation was to determine the ability of SA as inducer chemical resistant in eggplant against *M. incognita* as seedling roots dipping solutions of 100,150,200,250 and 300 uM.

MATERIALS AND METHODS

Nematode inoculum: The *M. incognita* (RKN) s eggs were isolated and extracted using 1% NaOCL solution for 2 min eggplant roots (*Solanum melongena* L.). Using 37 sieve, eggs were collected and washed by distilled water prior usage according to (Hussey and Baker, 1973).

Pot experiment: The current experiment was carried out under greenhouse conditions during 2022-2023 growing season at Agric, Research Station, Ismailia, Agric. Res. center to investigate the salicylic acid (SA) effects on eggplant (c.v Balady) infected with (*M. incognita*). Plastic pots (30 cm diam) were filled with 3 kg loamy sand soil collected from the Experimental Farm of Faculty of Agriculture Environmental Science, El Arish, Egypt.

Five salicylic acid (SA) dosages *i.e.* 100,150,200,250 and 300 uM were used as seedling roots dipping solutions for 2 hours directly before transplanting as one seedling /pot, the 10ml of a suspension contains 1000 eggs of (*M. incognita*) (RKN) were added in three holes in the soil around the plant roots . All treatment were replicated 4 times in completely randomized block design. Four pots were treated with (RKN) (*M. incognita*) only as negative control treatment. In addition to RKN inoculation, 4 pots were treated with 0.3 ml /plant of Oxamyl 24% SL chemical nematicide as a positive control treatment. All seedlings of positive and negative control treatments were dipped in distilled water for 2 hours before transplanting. Plants were managed through the growing period by standard recommended agriculture practice.

After sixty days from inoculation, plants were uprooted, number of galls egg masses , eggs/egg mass ,developmental stages and females were recorded on the roots while juvenile (J2) per 250 g soil was recorded. Roots were stained with acid fuchsin in lactic acid (Byrd *et al.,* 1983) and then examined for recording the number of developmental stages, females, eggs/egg mass, egg masses and galls per root. Juveniles in soil were extracted using sieving and modified Baerman technique (Goody, 1957). The nematode suspension was examined in Hawksley counting slide with dissecting microscope to quantify the juveniles numbers. Final population (PF) was recorded as a sum number of juveniles in soil and developmental stages and females in root. Reproductive factor (RF) was calculated as follows: RF= Final population (Pf)/initial population (Pi). Plant growth attributes *i.e.* fresh shoot and root length (cm) per plant and shoot and root fresh weight and dry shoot weights (g/plant) were recorded.

Biochemical analysis: Antioxidant enzymes (peroxidase, polyphenoloxidase and catalase) were determined. Crude extracts of both peroxidase and polyphenol oxidase was prepared as described by Aluko and Ogbadu (1986). Peroxidase activity was determined according to the method of Fehrmanand and Dimond (1967).
Polyphemoloxidase activity was measured according to (Broesch, 1954) while catalase activity was determined according to the method of Bach and Oparin (1968).
Total and free phenols were determined using the method of (Simons and Ross, 1971). Free amino acids (mgg^{-1}) were determined according to (Rosen, 1957). Proline (mgg^{-1}) was determined according to (Bates *et al.*, 1973). Photosynthetic pigments of leaves were determined using the method described by (Pareek *et al.*, 2017)
Statistical analysis: Statistical analysis of the collected data was performed using 6.303 of Computer program Cost (2005). Statistically significant differences between means were compared using analysis of variance (ANOVA) with the least significant differences (L.S.D.) and standard error at probability of 0.05.

RESULTS AND DISCUSSION

Effect of salicylic acid application on (RKN) (*M. incognita*) infection parameters:

Results in Table (1) showed that all salicylic acid application treatments were significantly ($P \leq 0.05$) reduced all studied (RKN) infection parameters with different magnitudes. Generally, all treatments decreased all studied (RKN) parameters *i.e.* number of galls, egg masses, egg /egg mass, developmental stages females/root system and J2 s /250 g soil, final population (Pf) and reproduction factor (RF) compared to control treatment. Results clear that the highest application dosage of salicylic acid (300 uM) was the most effective one in reducing nematode parameters followed by 250 uM application treatment. The lowest number of galls/root system recorded with the application rate of 300 uM salicylic acid with reduction percentage of (64.33%) followed by250uM application treatment (46.75 %) lower than control treatment. Obtained data in Table (1) cleared that all salicylic acid application. Treatments decreased the mean number of both egg masses and eggs /egg mass /root system compared to untreated plants. Salicylic acid application treatment at 300 uM resulted in the highest effect in reducing egg masses and eggs /egg mass with percentages reduction of 62.03 and 66.04 % lower than control treatment, respectively. Results revealed that 300 uM of salicylic acid application was the best treatment in inhibiting number of both developmental stages and females /root system with decreasing percentages of 67.30 and 61.45 % lower than that of control treatment, respectively.

Table (1): Effect of salicylic acid (SA) abiotic chemical resistance inducer on the root knot infection parameters.

Treatment	Galls /root system	Egg masses / root system	Egg/Egg mass	J2s/250 g soil	females / root system	Developmental stage / root system	Pf	Rf
100	160.2 (17.25%)	19.9 (9.13%)	38.2 (7.28%)	1121.1 (19.70%)	130.9 (18.34%)	74.9 (16.69%)	1326.9 (19.39%)	1.33 (19.39%)
150	141.3 (27.01%)	17.3 (21.00%)	36.1 (12.38%)	1038.6 (25.61%)	122.1 (23.83%)	61.2 (31.92%)	1221.9 (25.77%)	1.22 (26.06%)
200	119.6 (38.22%)	15.6 (28.77%)	34.2 (16.99%)	989.1 (29.16%)	113.6 29.13	57.9 (35.60%)	1160.6 (29.49%)	1.20 (27.27%)
250	103.1 (46.75%)	12.2 (44.29%)	25.1 (39.8%)	871.6 (37.57%)	101.7 (36.56%)	50.6 (43.72%)	1023.9 (38.80%)	1.02 (38.18%)
300	71.2 (63.22%)	8.1 (63.01%)	16.2 (60.68%)	421.4 (69.82%)	61.8 (61.45%)	29.4 (67.30%)	513.1 (68.83%)	0.51 (69.09%)
Oxamyl 24% SL	30.9 (84.04%)	4.3 (80.36%)	8.1 (80.34%)	221.7 (86.60%)	21.1 (86.84%)	10.6 (88.30%)	253.4 (84.61%)	0.25 (84.46%)
Control	193.6	21.9	41.2	1396.3	160.3	89.6	1646.1	1.65
L.S.D. at (0.05)	5.93	1.72	1.34	43.22	6.53	2.71	76.91	0.01

Values in parenthesis are percentages of reduction in comparison to control treatment.

The least effective treatment was 100 uM salicylic acid treatment on reducing developmental stages and females /root system with percentages decreasing of 16.69 and 18.34 % lower than that of control treatment, respectively. Regarding the effect of SA application treatments on both final population (Pf) and reproduction factor (RF), obtained data in Table (1) cleared that the lowest two values were recorded at 300 uM SA treatment with decreasing percentages of 68-83 and 69.09 % compared to control treatment, respectively. These findings were consistent with those of Bakr and Hewedy (2018) who found that SA application resulted in the highest reduction in number of galls, egg masses and females/ tomato roots and number of Js2 in the soil. According Walters *et al.*, 2013, SA treatment effects on reduction in infection parameters could be due to its effect as a signaling molecule involved in reactions at infection sites.

Roldi *et al.* (2017) and Zhan *et al.* (2018) found that number of eggs, juveniles and galls were lowered under Si application treatment.

Debona *et al.* (2017) cited that the silicon effects could be due to it is deposition in cell walls which avoid pathogenic penetration via physical barrier. According to Inanaga and Okasaka (1995), the most mechanism of using Si to control of nematodes is due to a double silica layer on cell walls which improving lignification of epidermal cells as well as making the cell walls more rigid and hence high resistance to penetration of parasite (Bakhat *et al.*, 2018; Khan and Siddiqui, 2020). This silicon -promoted physical barrier makes it more difficult for the styles to penetrate which resulting in reducing the number of galls as well the nematode population and multiplication in the plant roots (Silva *et al.*, 2010; Khan and Siddiqui, 2020).

Bozbuga (2020), studied SA exposure time of submergence tomato roots to 1000uM for 1 minute, 5 minute, 30, 60, 120 and 240 minutes and found that submergence plant roots at 60, 120 and 240 minutes plays negative effect on nematode reproduction rate. Nematode number was decreased in SA treated plants which could be related to genes increased level of PRS genes during early time of infection. Sorial *et al.* (2020), found that 100, 200 and 300 uM SA dipping plant solutions and soil drenching were reduced root galling and nematode population in soil as well as the number of egg masses and developmental stages per root system.

Effect of Salicylic acid application on biochemical parameters:

Obtained data in Table (2) illustrated that the activity of peroxidase, phenoloxidase and catalase antioxidant enzymes increased in all application rate of SA compared to control. The highest activity of peroxidase caused by300 uM salicylic acid treatment (0.49OD g^{-1}) compared to control treatment (0.31 O.D $g^{-1)}$ with an increase percentage of (38.7) higher than control treatment.

The addition of the highest rate of salicylic acid (300 uM) resulted in the highest activity of polyphenol oxidase antioxidant enzyme (0,30OD) with percentage increasing of (62, 50%) higher than that of control treatment. Also, plants treated with the same highest salicylic acid application treatment showed the highest activity of catalase antioxidant enzyme (0.29 OD) compared to control treatment (0.20 OD) with an increasing percentage of 45.00% higher than control treatment.

Sorial *et al.* (2020), found that 100, 200 and 300 uM of SA as dipping plant solutions and soil drenching increased the activity of antioxidant enzymes (peroxidase, polyphenol oxidase and catalase and total phenols .

The content of total and free phenols were increased with highest content at 300 uM SA application rate with an increasing percentages of 68.00 and 80.98% higher than control treatment, respectively.

The highest application rate 300 uM of SA resulted in the highest content of both caratinoids and total chlorophyll content in eggplant which recorded 200.53 and 93.72 % over control treatment, respectively. Free amino acids content was increased with 189.59 % higher than control treatment as a result of 300 uM application rate of SA treatment.

On the other hand, application of 300 uM of SA treatment resulted in the highest decrease of proline content with percentage reduction of 251.67% lower than that of control treatment.

According to Banci (1997), peroxidase catalyzes the oxidation of substrates via H2O2 as an acceptor of electrons. Such vital compounds play an important effect in biological processes, like lignin biothenhes degradation pathways in addition to defense mechanisms (Passardi *et al.,* 2005; Davies *et al.*, 2008).

The nature of peroxidase, catalyzed reactions, which involved a wide species of compounds with the help of H_2O_2 resulted in different peroxidases based biochemicals. Catalase acts in specific guaiacol peroxidase which protecting cell from toxicity of H_2O_2 (Ben Amor *et al.,* 2005). Korayem *et al.* (2012) cleared that most sugar beet genotype infected with *M, incognita* indicated significant increase in the catalase activity. Also, plants treated with *M. Javania* and SA showed high activity of catalase, Kesba and El-Beltagi (2012) found that catalase activity increases 46. 97, 57. 97 and 68.25% in three grape rootstocks when infected with *M. incognita* compared with healthy plants.

In addition to the benefit effect on *M. incognita* infection decreasing in eggplant, the endogenous chemical effect was found as result of increased activity of antioxidant enzymes *i.e.* catalase, peroxidase and polyphendoridase as well as antioxidant compounds *i.e.* total and free phenols.

Such effects could be due- that Si can activate genes and signals of biosynthesis of these defense compounds via acquired systemic resistance (ASR) (Fawe *et al.*, 2001).

Table (2) Effect of salicylic acid (SA) abiotic chemical inducer resistance on eggplant biochemical parameters.

SA treatments ,uM	Peroxidase (O.DL 6min)	Phenol oxidase(O.D 2 min)	Catalase (O.D)	Total phenols	Free phenols	Chlo (a)	Chol (B)	Total Chol (a+b)	Caratenoids (mg 100 g FW)	Proline (mg g-1 FW)	Free amino acids (mg- g - 1 FW)
100	0.22 (3.23)	0.27 (9.13%)	0.21 (5.00%)	33.6 (9.13%)	28.77 (21.34%)	7.72 (8.58%)	2.91 (5.43%)	10.63 (7.70%)	2.10 (10.53%)	2.45 (158.37%)	5.33 (29.06%)
150	0.37 (25.81%)	0.29 (20.38%)	0.23 (15.00%)	41.11 (33.52%)	35.23 (48.59%)	8.11 (14.6%)	3.01 (8.06%)	11.12 (12.66%)	2.97 (56.32%)	2.11 (200.00%)	6.97 (68.77%)
200	0.39 (38.22%)	0.31 (29.17%)	0.25 (25%)	43.21 (40.34%)	37.63 (58.71%)	9.03 (27.00%)	3.86 (39.86%)	12.89 (30.60%)	3.33 (75.26%)	1.99 (218.09%)	8.28 (100.48%)
250	0.44 (41.94%)	0.33 (37.50%)	0.27 (35.00%)	47.11 (53.00%)	40.21 (69.59%)	11.52 (62.03%)	4.11 (48.91%)	15.63 (58.36%)	4.21 (121.58%)	1.93 (227.99%)	9.33 (125.91%)
300	0.49 (58.06%)	0.39 (62.50%)	0.29 (45.00%)	51.73 (68.00%)	42.91 (80.98%)	13.30 (87.06%)	5.28 (110.87%)	19.12 (93.72%)	5.71 (200.53%)	1.80 (251.67%)	11.96 (189.59%)
Oxamyl 24% SL	0.43 (38.71%)	0.36 (50.00%)	0.25 (25.00%)	48.73 (58.27%)	41.82 (76.38%)	12.91(81.58%)	5.11 (85.14%)	18.02 (82.57%)	5.91 (211.05%)	1.70 (272.35%)	9.89 (139.47%)
Control	0.31	0.24	0.20	30.79	23.71	7.11	2.76	9.87	1.91	6.33	4.13
L.S.D. (0.05)	0.01	0.013	0.012	1.34	1.11	1.20	0.63	0.33	0.21	0.23	1.04

Values in parameters are percentages of increasing comparison to control treatment with exception of proline which represent reduction percentage lower than control treatment.

Obtained results showed that the higher Si rate application, the higher the phenol content (total and free) in eggplant. Ohri and Pannu (2010) cited that the raise of the number of phenolic substances caused more defenses and reducing infection criteria via resistant against nematodes attack.

The increase of free phenol compounds upon the infection of (RKN)s could be due to highly decomposition of such compounds resulting in polymer formation which play an important role in reaction of resistance. Farkas and Kiraly (1962) indicated that the metabolism of phenolic compounds increased as a result of injury or invasion by different pathogens. Mahajan *et al.* (1992) stated that the increasing in phenolic compounds contents were associated with highly nematocidal activity.

Giebel (1970) pointed out that the phenols occur in the form of glycosides which convert to free phenols via decomposing process as a result of plant stress response.

The application of SA to nematode infected eggplant found correlated with some physiological aspects like chlorophyll (a+b) which recorded the highest content at 300 uM application treatment.

According to Hayat *et al.* (2010), SA can play a role as phytohormones which have a role in photosynthesis which are agreed with our results. Lopez- Gomez *et al.* (2015), found that chlorophyll contents are decreased with increasing the density of nematode population.

Chlorophyll decreasing due to (RKN) interferes with both water and nutrient uptake and transportation via plant infection, (Kirkpatrick *et al.,* 1991).The high content of chlorophyll with SA application, the reduction the nematode effect on eggplant.

The decreasing significant effects of nematode infection on chlorophyll (a+b) in eggplant are in consistent with that of (Korayem *et al.*, 2012 and Labudda *et al.,* 2018).

Ahmed *et al.* (2009), indicated that the lack of photosynthetic pigments may be due to that nematode caused oxidative, bursts in the cells and failure to carry out photosynthetic process.

According to McElory and Kopsell (2009) carotenoids play a vital role as antioxidant on singlet O2 and dissipation of excess light energy as well as harvesting of light energy .On the other hands obtained data that the higher SA application rate , the lower than proline content of eggplant. Santhil-Kumar and Mysore (2012) pointed out that proline is a multi-functional amino acid which increase plant tolerance under abiotic stress conditions. According to Cecchini *et al.* (2011) the catabolism of proline could enhance at early stages for defense against pathogenic infection.

Effect of SA application on eggplant growth attributes:

Data presented in Table (3) showed that the influence of SA application treatments the highest application rate on eggplant growth attributes. Eggplant growth attributes were significantly ($P \leq 0.05$) increased as the result of increasing SA application rates compare to control treatment of SA resulted in the highest increase of fresh and dried shoot weights which recorded 20.15 and 8.11 g / plant compare to control treatment which recorded 9.11 and 6.22 g/plant , respectively. The lowest increase in both fresh and dry shoot of eggplant was found at 100 uM of SA which recorded 9. 62 and 6.10 g/plant, respectively .Data in Table (3) revealed that the fresh root weight (g) of eggplant was significantly ($P \leq 0,05$) increased with increasing SA dosage from 100 to 300 uM .The highest increase of fresh root weight was recorded under 300 uM of SA application 3,71 g/plant compare to control treatment which recorded (2.62 g /Plant).

Data presented in Table (3) showed that both shoot and root lengths (cm) of eggplant were significantly increased ($P \leq 0.05$) with the increasing of SA application rates. The highest increase in both shoot and root lengths were recorded under 300 uM of SA dose which recorded 38.33 and 12.62 cm, respectively. While the lowest increase in shoot and root lengths were recorded with plants treated with 100 uM of SA which recorded 27.91 and 10.11 cm, respectively. The overall increase of all eggplant growth attributes were recorded when plants treated with Oxamyl nematicide which recorded the first rank followed by 300 uM of SA application treatment.

Sorial *et al.* (2020) found that 100, 200 and 300 uM SA dipping plant solutions resulted in significant increase in plant growth attributes as comparing with control.

Table (3) Effect of salicylic acid (SA) abiotic chemical inducer resistance on eggplant growth attributes.

SA treatment	Fresh shoot weight (g/plant)	Fresh root weight (g/plant)	Dry shoot weight (g/plant)	Shoot length (cm)	Root length (cm)
100	9.62 (5.60%)	3.33 (11%)	2.81 (7.25%)	27.91 (6.00%)	10.11 (10.98%)
150	11.31 (24.15%)	4.50 (50%)	2.01 (11.07%)	31.33 (18.99%)	10.72 (17.67%)
200	14.96 (64.22%)	5.29 (76.33%)	2.99 (14.12%)	36.11 (37.14%)	10.99 (20.64%)
250	16.22 (78.05%)	6.73 (124.33%)	3.32 (26.72%)	37.21 (41.32%)	11.33 (24.37%)
300	20.15 (121.19%)	7.41 (147%)	3.71 (41.60%)	38.33 (45.58%)	12.62 (38.53%)
Oxamyl 24% SL	25.97 (176.07%)	8.76 (192%)	4.11 (56.87%)	40.11 (52.34%)	13.71 (50.49%)
Control	9.11	3.00	2.62	26.33	9.11
L.S.D.(0.05)	0.31	0.11	0.12	0.10	1.01

The obtained our data results showed that all SA application treatments resulted in increasing of all eggplant growth attributes compared to control .Salicylic acid(SA) at 300 uM treatment significantly (P<0.05) recorded the highest increase in all studied eggplant growth attributes compare to control treatment. Such obtained findings are in good line with that of (Bozbuga, 2020 and Sorial *et al.,* 2020).

Salicylic acid can resulted in decreasing of parasitism between nematode and plant. While, such effects could be due the capability ofnematodes to suppress the SA pathway (Uehara *et al.,* 2010).The results of eggplant growth attributes are corresponding with many studies which indicated that SA significantly increase the dry weight of shoots, length of roots, weight of shoots and roots fresh weight (Bakr *et al.*, 2018).

Conclusion

The SA solution concentration of 300 uM seedling roots dipping solution treatment as an abiotic resistance inducer was able to suppress and reduced reproduction of *M. incognita* in addition to improving eggplant growth attributes. This could be due to stimulate the activity of antioxidant enzymes and increasing content of plant resistance biochemical related compounds in eggplant which protect and immunize plants *M. incognita* infection. This approach not only decreases the use of nematicides which are harmful for human health as well as environmental pollution, but also could help the plant breeders for developing to resistance cultivars against, *M. incognita*.

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