

Induction of Systemic Resistance in Tomato by some Abiotic Compounds Against *Meloidogyne javanica*

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Abstract

The comparative of some abiotic compounds (Ascorbic acid, Citric acid, DL-Aspartic acid, Indole-3Acetic acid, Indole-3Butyric acid, DL-leucine, Gibberllic acid, L-Arginine and Sulfosalicylic acid), as resistance inducing for managing *Meloidogyne javanica* infecting tomato (Super strain B Australian cv.) was studied under greenhouse conditions. The results showed that all tested compounds reduced all nematode parameters compared with untreated control. Application of Citric acid caused a superior effect in the reduction percentage of J2 in soil (95.2%) followed by L-arginine (92.9%) and then Gibberllic acid (92.2%). Also, DL-Aspartic acid gave the highest effect in the reduction percentage of root galls (95.6%) followed by Citric acid and then L-Arginine acid as following (94.3% and 93.02%) respectively compared with untreated control. The treatments of L-arginine followed by Gibberllic acid were recorded the best in plant growth response of tomato plants compared with the untreated control. Tested abiotic agent inducers activators had the potential to suppress *M. javanica* infection through the stimulation of tomato tolerance. Most of the treatments showed a significant reduction in the percentage values of total and non-reducing sugars comparing with infected and healthy control, while Sulfosalicylic acid and L-arginine recorded high amounts in total and non-reducing sugars.

On the other hand, DL-Aspartic acid, Indol-3Acetic acid and Citric acid showed a high percentage of total phenols comparing with untreated control. Among all treatments, only DL-leucine showed a significant increment in proline comparing with untreated control and healthy control. The level of Polyphenol oxidase and peroxidase activity increased in the treated plants compared with untreated plants.

Keywords: Chemical inducers, induced resistance, root knot nematodes, tomato, sugars, phenols, Proline, peroxidase, polyphenol oxides.

Introduction

Tomato (*Solanum lycopersicum*) is the most popular and widely consumed vegetable crop. Tomato ranks second in priority after Potato in the world. Egypt ranks sixth in the production of tomato, China, USA, Italy, Turkey, India and Egypt are the

important tomato production countries (FAO, 2019).

The estimated production and area of tomato for Egypt are about 6751856 tons and 428182 feddan respectively, in 2019/2020 (FAO, 2020). The production and area of tomato for New Valley are about 2536 tons and 317 feddan,

respectively (Bulletin the Agricultural Statistics, 2020).

Tomato yields are reduced by plant-parasitic nematodes (PPN) that can act as pests on a wide range of important agricultural crops. Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive agricultural pests globally. They have a wide host range of plants, causing changeable yield losses especially in tropical and sub-tropical agriculture (Sikora and Fernandez, 2005). In Egypt, root-knot nematodes, *Meloidogyne* spp. are becoming a real threat to almost all vegetable crops, especially in the newly reclaimed areas and they have been considered as limiting factors in crop production (Ibrahim et al., 2000).

Control of root-knot nematodes is very challenging (Karssen et al., 2013). Multiple methods control such as regulatory, cultural, physical, biological and chemical methods used to nematode control on host plants with different advantages and disadvantages (Vigila and Subramanian, 2018). Currently, the use of nematicides is being limited, which are expensive, given the increasing concern for human health as well as the environment. Scientists are also looking for other nematode management strategies that aim to reduce pesticide use and promote non-chemical management practices as much as possible. One of the proposed environmentally friendly options is Induced Systemic Resistance (ISR). It is accepted as one of the most promising methods for controlling plant diseases. Induced resistance (IR) offers a natural defense mechanism of plants as an alternative nontraditional and eco-

friendly control methods. It is also promising to control soil-borne pathogens (Okubara and Paulitz, 2005). There are a wide number of abiotic and biotic agents which can induce host resistance to the pathogen (Walters et al., 2013). Ascorbic acid, previously used for induction of plant resistance in plants (Abd-El-Kareem et al., 2013), can control different fungal diseases (Abdel-Kader et al., 2012 and Shahda, 2000) and plant parasitic nematodes, such as root rot and Root-knot nematodes (Anter et al., 2014 and Arrigoni et al., 1979). BABA is a non-protein amino acids that has been used as chemical inducers against a wide spectrum of pathogens in various plants (Oka et al., 1999; Lee et al., 2000). Salicylic acid (SA) is one of the most essential signal molecules involved in activator defense responses and in sensitizing plant cells for a response to pathogen infection (Kariola, 2006). Gibberellins can play a role in regulating plant immunity (Denance et al., 2013). Defense enzymes polyphenol oxidase and peroxidase secreted by the host responsibility were evaluated for possible enhancement of the systemic acquired resistance (Bakr and Omar, 2018). The enzymatic actions such as peroxidase could lead to scavenging the accumulation of H₂O₂ in the tissue (Tripathi, 2006). Application of plant hormones and abiotic stimulations can increase the amount or activity enzymes, such as peroxidase and polyphenol oxidase, leading to systemic resistance (Garcion et al., 2014).

The aims of the present study are to evaluate the comparative suppressive effect of some chemical in-

ducers on tomato resistance to *M. javanica* infection. Moreover, determine the growth indices of tomato in the treated and untreated plants under glasshouse conditions. Also, study the effect of chemical inducers on sugars, phenols and proline content of treated plants. Finally, study the enzymatic activity of peroxidase and polyphenol oxidase.

Materials and Methods

Plant material:

A Meloidogyne-susceptible cultivar of the tomato plant (Super strain B Australian) was selected for this experiment. Tomato seeds (*Solanum lycopersicum*) were obtained from the Horticulture Research Institute (Agriculture Research Center (ARC), Giza, Egypt). Seeds were planted in trays containing composite.

Pure culture:

Root-knot nematode (*M. javanica*) isolated from tomato roots was reared on tomato plants in the greenhouse. Individual egg-masses were removed from the small galls of the infected plants by a needle, put in Petri dishes and put in an incubator at $25\pm 2^{\circ}\text{C}$ for a week for hatching. The hatched juveniles were collected daily and stored at 15°C . The pure cultures were used for all further studies in this work.

Evaluation of some chemical inducers on tomato infected with *M. javanica*:

Six-week old seedlings were transplanted in 25 cm diameter pots containing a mixture of 1:2 sterilized clay/sandy soil each pot containing three plants infected with 1,000 J2s by making deep holes around the plant (Hartman and Sasser, 1985; Eisenback and Triantaphyllou, 1991).

This experiment was conducted to explore the effectiveness of nine treatments (Ascorbic acid, Citric acid, DL-Aspartic acid, Indole-3Acetic acid, Indole-3Butyric acid, DL-leucine, Gibberellic acid, L-Arginine and Sulfosalicylic acid) with a concentration 5ppm. which were previously filled with soil. Pots were divided into eleven groups (nine groups for treatments and two groups for control infected and healthy) each comprises of three replicates. Plants were treated with 150 ml of tested chemicals as a soil drenching. Three pots saved as untreated control, and the pots watered twice weekly. After 60 days from tomato transplantation, the developed plants in each pot were uprooted. The stems were cut off and the soil was gently washed with tap water from the root system and then taken to the laboratory. The Length and weight of roots and shoots were determined. The roots were then washed to get rid of adhering soil particles to determine the number of galls, the number of egg-masses, developmental stages in roots also the nematode population in soil were counted according to Taylor and Sasser (1978).

Nematode parameters were counted as follow:

Root = 1g of root system x average of root weights

Reproduction factor (Rf) = final population / initial population

Number of root galls and nematode egg masses were counted and plants were rated on root gall index (R.G.I) and egg masses index (E.I) on a scale of 1-5

where 1= 0-2, 2= 3-10, 3= 11-30, 4= 31-100 and 5= more than 100 galls or egg masses/root system per plant.

Estimation of sugars, phenols, proline and enzymatic activity (peroxidase and polyphenol oxidase):

A Fresh plant sample (10g) from each replicate of each treatment was cut into small pieces and immediately macerated into 95% boiling ethanol for 10 min. The macerated were transferred into soxhlet units containing 75% ethanol as an extraction solvent. The extract process resumed for 12 hrs. Ethanol extracts were filtrated and evaporated until the complete removal of the ethanol. The dried residue was dissolved in 5ml isopropanol 50% and kept in the freezer till analysis the extracts were used, later for analysis of sugars and phenols.

Determination of sugar contents:

Total soluble sugars and non-reducing sugars were spectrophotometricall determined at 540 nm using the picric acid technique as described by Thomas and Dutcher (1924).

Total soluble sugars:

A volume of 0.5ml of each extract was placed in test tubes containing 5ml of distilled water and 4ml picric solution was added. The mixture was boiled for 10 min. After cooling, 1ml sodium carbonate solution (20%) was added and the mixture was boiled again for 15 min. After cooling the tubes were filled up to 10 ml with distilled water. Thereafter, the developed color was determined at 540 nm using a spectrophotometer in the presence of blank and using glucose as a standard.

Non-reducing sugars:

The same described procedure for total sugars was used except that picric solution and sodium carbonate-solution were added together at the same time. The same spectrophotometer and wavelength were used.

Determination of total phenols:

Total phenols determination was carried out as described by Snell and Snell (1953). Concentrate hydrochloric acid (0.25 ml) was added to 0.2 ml of the sample extract in a test tube and mixed. The mixture was then boiled for about 10 min. After cooling, 1ml Folin reagent and 5ml sodium carbonate solution (20%) were added and diluted to 10 ml using distilled water. After 30 min the intensity of the developed blue color was determined at 520 nm using chatichole as standard.

Determination of free phenols:

Free phenols determination was carried out using the same described method with some exception, since, 1 ml Folin reagent and 3 ml sodium carbonate solution (20%) were added to 0.2 ml of the sample extract dilution with distilled water to 10 ml. After 30 min the intensity of the developed blue color was determined at same wavelength.

Determination of proline:

Proline was determined by a colorimetric method (Bates *et al.*, 1973, Marín *et al.*, 2009) based on the interaction of proline with ninhydrin. For proline colorimetric determinations: a solution of proline, ninhydrin acid and glacial acetic acid (1:1:1) were incubated at 100°C for 1 hour. The reaction was arrested in an iced bath and the chromophore was

extracted with 4ml toluene and its absorbance was determined at 520 nm.

Determination of peroxidase activity:

The crude enzyme was prepared and peroxidase activity was determined according to Allan and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogallin in the presence of H₂O₂ at 425nm using a UV spectrophotometer. Fresh leaves of the different treatments were cut into small pieces and macerated in per-chilled mortar after adding 2.0 ml of (0.2M) sodium phosphate buffer at pH7.0 per one gram sample. The extracts were filtered, and the filtrates were centrifuged at 3000 rpm for 20 min and the clear supernatants were used as the crude enzymes. A portion of the crude enzymes was boiled to inhibit the enzyme activity to serve as a control then the following procedures were conducted to estimate the activity of each enzyme.

=buffer pH 7.0, 0.3 ml enzyme extract, 0.3ml (0.05M) pyrogallol, 0.1ml (1.0%) H₂O₂, and distilled water to bring cuvette contents to 3.0ml

Determination of polyphenol oxidase activity:

The crude enzyme was prepared as aforementioned determination of peroxidase activity. Reaction mixtures contained 0.5ml enzyme extract, 0.5ml (0.2N) sodium phosphate buffer pH 7.0 and 0.5ml (10⁻³N) catechol brought to a final volume of 3.0 ml with distilled water. The activity of polyphenoloxidase was expressed as the change in the absorbency of 1.0 ml of extract per min. at 495 nm. using a UV spectrophotometer.

Statistical analysis:

The differences between the mean values of various treatments were compared by Duncan's multiple range test Duncan (1955).

Experimental Results

The effects of nine chemical compounds on the induction of local resistance in tomato plants infected with *M. javanica* were investigated under greenhouse conditions. Data in Table (1) indicated that all the tested treatments showed a significant decrease in the number of second stage juveniles (j2s) in soil, galls, egg masses and females, except Indol-3-butyric acid increased the number of females in root system and inhibited the nematode reproduction factor (RF). The best results attributed to the treatment with Citric acid followed by L-arginine and Gibberellic acid which reduced the number of nematodes in soil by 95.2%, 92.9% and 92.6% respectively. Same treatments gave a reduction percentage of females as following Citric acid caused the highest effect (85.9%) followed by L-arginine (72.9%) and Gibberellic acid (64.7%) while the greatest number of females was observed with Indol-3-butyric acid (-135.3%). Results also showed that DL-Aspartic acid was the highest effect in the reduction percentage of root galls (95.6%) followed by Citric acid and then L-Arginine as follows (94.3% and 93.02%) respectively compared with control. Citric acid gave the highest reduction in reproduction factor of nematode (Rf) to 0.152 followed by L-arginine and Gibberellic acid (0.242 and 0.251) whereas DL-leucine followed by Ascorbic acid and Indol-3-butyric acid showed a low impact in reducing Rf value

(1.98, 1.864 and 1.84, respectively) comparing with untreated control (3.181).

Data presented in Table (2) showed that most of the treatments affected the plant growth parameters with significant differences. Shoot and root lengths were significantly higher in plants treated with the most treatments when compared with untreated/infected control. Results showed that L-arginine was recorded the best shoot and root Lengths (32.00cm and 17.75cm) followed by Dl-Aspartic acid and Gibberellic acid in shoot length (28.13cm, 28.75cm

and 30.50cm, respectively and Gibberellic acid in root length (17.5). While using Indol-3-butyric acid recorded low values of shoot and root lengths (19.25cm and 14.00cm, respectively). On the other hand, the lowest value of root length was recorded by Dl-leucine (12.75 cm) when compared with infected control (13.75cm). Citric acid followed by L-arginine and Gibberellic acid caused a significant increase in fresh shoot and root weights comparing with infected control.

Table 2. Impact of certain abiotic agents as resistance inducers on the plant growth response of tomato cv. Super strain B Australian infected with *M. javanica* under greenhouse conditions.

Treatments	Plant growth response			
	Shoot Length (cm)	Root Length (cm)	Shoot fresh wt.(g)	Root fresh wt.(g)
Ascorbic acid	22.63 ^{CD}	15.88 ^C	3.30 ^D	1.925 ^{BC}
Citric acid	26.50 ^{BC}	16.13 ^{BC}	7.30 ^A	4.92 ^A
DI-Aspartic acid	28.13 ^{AB}	14.25 ^D	5.525 ^{ABC}	3.65 ^{AB}
Indol-3Acetic acid	20.25 ^D	14.75 ^{CD}	3.375 ^D	3.77 ^{AB}
Indol-3-Butyric acid	19.25 ^D	14.00 ^{DE}	4.40 ^{CD}	4.775 ^A
DI-leucine	28.75 ^{AB}	12.75 ^E	5.750 ^{ABC}	3.45 ^{ABC}
Gibberellic acid	30.50 ^{AB}	17.50 ^{AB}	7.0 ^{AB}	3.825 ^{AB}
L-arginine	32.0 ^A	17.75 ^A	7.025 ^{AB}	3.625 ^{AB}
Sulfosalicylic acid	26.63 ^{BC}	16.0 ^C	5.20 ^{BCD}	2.425 ^{BC}
Control infected	25.50 ^{BC}	13.75 ^{DE}	4.6 ^{CD}	1.650 ^C
Control healthy	32.0 ^A	14.25 ^D	5.875 ^{ABC}	3.325 ^{ABC}

The effect of abiotic agents on total and non-reducing sugars was determined in tomato infected with *M. javanica*. Data in Table (3) revealed that most of the treatments showed a significant reduction in the percentage values of total and non-reducing sugars comparing with infected and healthy control, while Sulfo-salicylic acid and L-arginine recorded a high amounts in total and non-reducing sugars (92.2%, 83.2%, 91.4%, and 89.1 %, respectively) comparing with infected control (86.7% and 81.1%).

Also the percentage of total phenols values showed a significant

reduction with the most of treatments, while DI-Aspartic acid followed by Indol-3Acetic acid and then Citric acid showed high percentage of total phenols (138.7%,123.3% and 110.3%, respectively) comparing with untreated/infected control (106.26%).

Also, among all treatments, only DI-leucine showed a significant increment in proline (363.1%) in tomato infected with *M. javanica* compared with untreated/ infected control (255.2%) and healthy control (100%).

Table 3. Effect of abiotic agents on chemical analysis of tomato Super strain B Australian infected with *M. javanica*.

Treatments	Sugars		Phenols		Proline %
	Total %	Non-Reducing %	Total %	Free %	
Ascorbic acid	58.0	44.2	76.9	47.79	185.5
Citric acid	67.2	61.1	110.3	103.9	159.2
DI-Aspartic acid	66.2	55.3	138.7	90.7	110.5
Indol-3Acetic acid	56.1	46.2	123.3	114.5	57.9
Indol-3-Butyric acid	52.03	49.4	93.7	92.8	47.4
DI-leucine	58.5	51.4	99.5	97.8	363.1
Gibberellic acid	74.7	71.7	90.4	84.7	40.8
L-arginine	91.4	89.1	103.5	98.2	50
Sulfosalicylic acid	92.2	83.2	97.1	95.6	197.4
Control infected	86.7	81.1	106.26	93.4	255.2
Control healthy	100	100	100	100	100

Determination of Enzymatic Activities

Data in Fig (1) showed that using of the previous treatments were significantly effective in stimulation peroxidase (PO) and polyphenol oxidase (PPO) activities. The plant analysis showed a high level of PO activity in all treatments comparing with untreated control except DI-Aspartic acid and Sulfosalicylic acid, which had no effect in PO activity. The Highest peroxidase activity was

recorded for DI-leucine followed by Gibberellic acid compared to the untreated control. The same trend was observed with polyphenol oxidase activity which recorded a very a high level of PPO activity in all treatments compared to the untreated control. PPO activity showed the highest stimulation with DI-leucine followed by Indol-3-Butyric acid and Ascorbic acid compared to the untreated control.

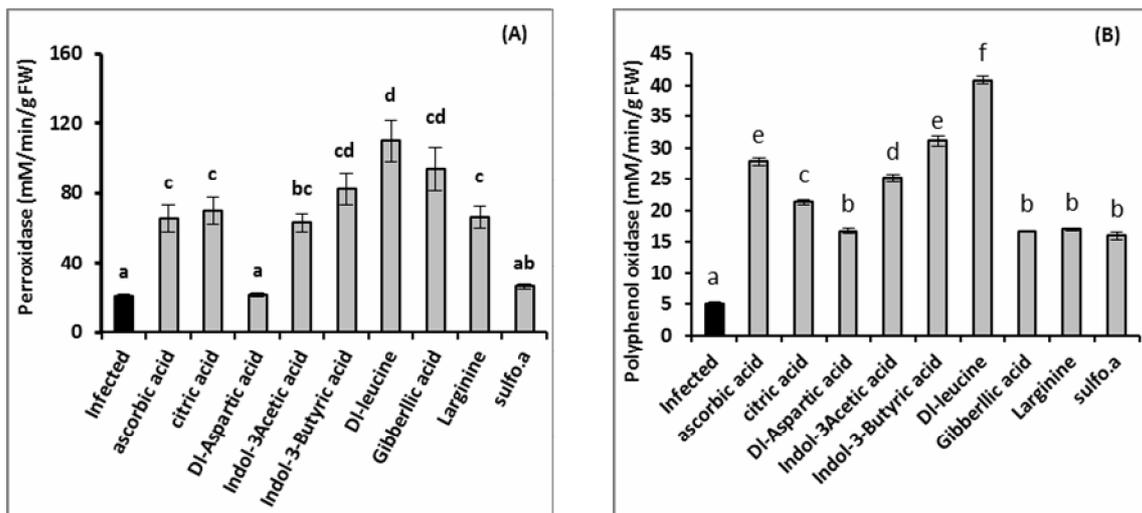


Fig.1: Effect of certain abiotic agents as resistance inducers on peroxidase(A) and polyphenol oxidase (B) activities in leaves of tomato plants (cultivar super strain B Australian) infected with *M. javanica* under greenhouse conditions. The data are means \pm SD (n = 6). Different letters indicate statistically significant differences according to one way ANOVA; Tukey HSD- post-hoc.

Discussion

Plants have evolved complex mechanisms to defend themselves against pathogens, and thus a great deal of attention has been directed towards elucidating the molecular nature of resistance (Bakr and Omar, 2018).

Induced Systemic Resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signaling pathways as well as to its potential use in plant protection (Attia, 2019 and Sahebani and Hadavi, 2011).

The tested abiotic agent inducers may affect directly or indirectly the stimulus to hatching, on juveniles ability to find, penetrate and settlement of the feeding site as well as on the development of nematodes (Cook, 1991 and Dale *et al.*, 2013).

From the obtained results, it was noticed that one application of all the tested organic acids either as soil drenching induced tolerance to *M. javanica* in terms of the reduction in the tomato gall numbers and 2nd juveniles in the soil, these results are in partial agreement with (Anter *et al.*, 2014 and Al-Ghonaimy and Zawam, 2016) who found that soil drench of all inducers used on tomato roots reduced *M. javanica* reproduction.

Regarding the effect of the tested chemical compounds on tomato growth characteristics all treatments significantly promoted the root length compared to the control treatment. There are several possible mechanisms for how organic acids may promote plant growth and increase resistance against nematode infestation. Increasing these two

growth parameters may be due to these treatments as growth promoters.

Ascorbic acid, Citric acid, DL-Aspartic acid, Indole-3Acetic acid, Indole-3Butyric acid, DL-leucine, Gibberellic acid, L-Arginine and Sulfofosalicylic acid may have a direct or indirect role in this respect. Most of the induced compounds reduced the reproduction of *M. javanica* on tomato proving their potentiality in controlling this serious pest compared with untreated control. This effect against *M. javanica* could be attributed to the role of the used compounds in the induction of plant resistance as reported by Molinari and Baser (2010).

In this study, the best effect of all nematode parameters was treatment with Citric acid followed by L-arginine and Gibberellic acid.

Gibberellins play a role in regulating plant immunity (Denancé *et al.*, 2013). Gibberellins can also stimulate or suppress plant defense responses depending on the plant-pathogen combination; gibberellins apparently enhance resistance to biotrophs and susceptibility to necrotrophs (Bari and Jones, 2009). Results in this study showed that, Salicylic acid (SA) has been tested as an inducer of resistance against *M. javanica*. Salicylic acid (SA) was found to reduce the number of 2nd juveniles and other developmental stages of *M. javanica* in tomato plants Osman (1993). The shoots weight of infected plants with nematodes and treated with SA were increased compared with infected plants without treatment, they also showed that numbers of root galls and eggs/g root were decreased when plants were

treated with SA (Mukherjee *et al.*, 2012). Contrary, application of Salicylic acid as soil drench did not show any enhancement of tomato resistance against *M. javanica* (Oka *et al.*, 1999).

The application of β amino butyric acid (BABA) as a resistance chemical inducer in tomato plants as soil drenching caused asystemic induction of plant defense mechanisms and decreased the root galls, number of eggs (Oka *et al.*, 1999).

The fresh weights of both shoot and root significant increased by most different treatments. These results confirmed by (El-Sherif *et al.*, 2015) how reported that, the activation of plant growth parameters may be due to the ability of treatments in reducing the nematode infection on the root. Healthy or low infected roots can translocate the water and nutrients from the soil via phloem and xylem in the tomato root system, which affected the growth of tomato plants.

Since some of the used chemicals are growth regulators, they may enhance some metabolic cycles and pathways that cause accumulation of some metabolites that resist pathogenic organisms. The effect of the tested chemicals on the phenolic, sugars and proline was determined. Regarding sugars content, the obtained data show a positive correlation between the amounts of total or reducing sugars and the used compounds. Also, the amounts of free phenols showed the same trend with regard to the used compounds.

Most of the treatments showed significant reduction in the percentage values of total sugars and non-

reducing compared with infected and healthy control. These findings are in accordance with the results of Bird and Loveys (1975) and McClure (1977) who reported that the total sugar concentrations in root exudates collected from uninfected apical roots were enhanced. This response to infection could be due to the observed morphological changes. In the present study, it was determined that untreated plants showed elevated levels of phenolic compounds which can be supported by the findings of Mahajan *et al.* (1992). Increased the levels of total phenols may serve as defense compounds against such pathogens (Kosuge, 1969).

A high amount of proline was produced in DI-leucine. The proline contents of tomato showed a vast difference in different treatments. It may be concluded here that proline is an indicator of environmental stresses imposed on plants. Proline is important to protect plant cells against oxidative damage by stabilizing key cellular detoxification mechanisms (Székely *et al.*, 2008).

Using the previous treatments were significantly effective in stimulating peroxidase (PO) and polyphenol oxidase (PPO) activities. The plant analysis showed a high level of PO and PPO activity in treatments compared with untreated control. Activation of plant defenses related the enzymes against pathogens and accumulation of plant defense metabolites is a vital mechanism of chemical inducers. Previous researchers reported that application of plant with abiotic or biotic stimulators or hormones can increase the activity of defending enzymes, such as polyphenol

oxidase and peroxidase steering to induce systemic resistance (Ananieva *et al.*, 2004 and Odjakova and Hadjiivanova, 2001). These enzymes play a key role in important biological processes, such as biosynthesis of lignin, degradation pathways, and host-defense mechanisms (Passardi *et al.*, 2005; Davies *et al.*, 2008 and Regalado *et al.*, 2004).

Our results give an approach to control *M. javanica* in tomato using compounds that are safer than nematicides. These results should be considered when designing an integrated pest management program for root-knot nematodes or other nematode pathogens in cucumber and other crops.

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استحثاث المقاومة في نباتات الطماطم باستخدام بعض المركبات غير الحيوية ضد نيماتودا تعقد *Meloidogyne javanica* الجذور

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المخلص

عند عمل دراسة مقارنة بين بعض المركبات غير الحيوية (حمض الاسكوريك، حمض الستريك، حمض الأسبرتك، أندول حمض الخليك، أندول حمض البيوترك، حمض الجبريليك، الأرجنين، حمض سلفوساليليك) لاستخدامها كمستحثات لمقاومة نباتات الطماطم صنف سوبر استرين بي الاسترالي لمرض تعقد الجذور المتسبب عن *M. javanica* تحت ظروف الصوبة.

أشارت النتائج إلى ان جميع المركبات المستخدمة أدت إلى إنخفاض في جميع القياسات النيماتودية مقارنة بالكنترول المعدي غير المعامل. كان تأثير مركب حمض الستريك هو الأفضل في تقليل أعداد اليرقات في التربة بنسبة (٩٥,٢%) ويليه مركبي الأرجنين والجبريليك بنسبة ٩٢,٩% و ٩٢,٢%. أيضا كان لهذه المركبات ذات التأثير في تقليل النسبة المئوية لأعداد العقد علي الجذور كالتالي حمض الاسبرتك بنسبة ٩٥,٦% ويليه حمض الستريك والارجنين بنسبة ٩٤,٣% و ٩٣,٠٢% علي التوالي.

سجل كلا من مركبي الأرجنين وحمض الجبريليك الأفضل في استجابة نباتات الطماطم للنمو مقارنة بالكنترول المعدي. هذه المنشطات الكيماوية لها القدرة علي مقاومة *M. javanica* من خلال تحفيز قدرة الطماطم علي التحمل.

أظهرت معظم المركبات انخفاضا معنويا في النسبة المئوية للسكريات الكلية وغير المختزلة مقارنة بالكنترول المعدي غير المعامل. بينما سجل كلا من حمض سلفوساليليك والأرجنين كميات عالية من السكريات الكلية وغير المختزلة.

في حين كانت مركبات حمض الاسبرتك واندول حمض الخليك وحمض الستريك أظهرت زيادة في النسبة المئوية لمحتوي نباتات الطماطم من الفينولات.

كذلك زاد نشاط إنزيمي البيروكسيديز والبولي فينول اوكسيديز كنتيجة لاستخدام هذه المركبات في النباتات المعاملة مقارنة بالكنترول المعدي غير المعامل.