Induced resistance in pepper plants against root knot nematode by some inducers in relation to the histological changes

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ABSTRACT

Biochemical and histological studies were made on roots of pepper (*Capsicum annuum*) infected with root-knot nematode and treated by certain chemical resistance inducers (salicylic acid (SA), indol acetic acid (IAA),ethylene (Eth), Jasmonic acid (JA), in addition to a biofertilizer named (Halex-2). These resistance inducers were tested at three concentrations 100, 200 and 300 µM for SA, IAA, and Eth and 15, 20 and 25 µM for JA while the biofertilizer was tested at 7g/L as biotic resistance inducers against root-knot nematode *Meloidogyne incognita* under greenhouse conditions at the Faculty of Agriculture Shebin El-Kom, Menoufia University in season 2016. All concentrations of the tested inducers significantly reduced root-galling and nematode population in soil as well as the number of egg masses and developmental stages per root system. The greatest inhibition effect on reproduction of nematode was recorded with Eth at 300µM, which did not significantly differ from that of a nematicide-treated plants. Also, all treatments significantly enhanced all vegetative plant growth characters compared with the infected plants. Biochemical analysis showed a significant increase in the activity of antioxidant enzymes (peroxidase, polyphenoloxidase and catalase), total and reducing sugars, and total phenols at the highest concentration of the tested inducers, especially, Eth compared with the nematode-infested plants. Histological studies indicated that less giant cells were observed in almost all treatments compared to the nematode-infected control plants. However, the highest concentrations of IAA, Eth (300µM) and Halex-2 treatments showed a good performance, with no giant cells found. Sixty days after nematode inoculation, all treatments (except SA and Halex-2) showed a poor formation of regular giant cells divided from cytoplasm and contained less number of nuclei compared to the infected plants. It seems that the tested inducers could be recommended to control *M. incognita* on pepper plants. Thereby, decreases the costs and side effects of using nematicides.

Key words: *Capsicum annuum*, salicylic acid (SA), indol acetic acid (IAA), ethylene (Eth), Jasmonic acid (JA), *Meloidogyne incognita*

INTRODUCTION

Pepper (*Capsicum annuum*) is one of the most important vegetable crops in Egypt. Most common pepper varieties are susceptible to the southern root-knot nematode *M. incognita* (Ibrahim *et al.*, 2011). Root-knot nematodes are the most economically important nematodes worldwide. Several species of root-knot nematodes are serious pathogens that cause severe damage to major crops. This nematode has been managed by soil fumigants, some chemicals and alternative methods.

The application of salicylic acid (SA), or chemicals with similar action, reduces the root infection by *M.*

incognita and nematode reproduction (Hari et al., 2011 and Hagag et al., 2016). Treatment of soil with SA clearly improved the control of M. Javanica and M.incignita (Naserinasab et al., 2011 and Bakr and Hewedy,2018). Salicylic acid plays an important role in the reduction of parasitism between nematode and plant (Wubben et al., 2007). In many cases, the effect has been explained by the capability of the nematodes to suppress the SA pathway (Uehara et al., 2010 and Shukla et al., 2018).

Foliar application of SA on plants induces a systemic effect that can suppress root knot nematode infection (Selim et al., 2014 and Zebire, 2017). Several complex gene expression and subsequent hormone signaling like JA and SA signaling pathway during incompatible interactions between plants and nematode are considerable aspects to understand the mechanism of resistance.

Jasmonic acid (JA) is a phytohormone with essential role in plant defense against pathogenesis and herbivorous arthropods. (Kazunori et al., 2015). Salicylic acid, JA and ethylene (Eth) regulate the resistance gene mediated and induced defense responses. The signaling of JA and Eth generally showed synergistic interactions while negative pathway may occur between the JA and SA (Bellafiore et al., 2008). Plants have a variety of antioxidant enzymes (e.g. peroxidase, catalase and phenoloxidase) to scavenge excessive reactive oxygen species (ROS) in plant tissue to prevent self-damage. Moreover, other enzymes superoxide dismutase (SOD) detoxify ROS produced by the plant cell and induce plant resistance (Bellafiore et al., 2008, Ali et al., 2018 and Roze et al., 2008).

Godelieve G. and Melissa G. M. (2019) reported that, application of JA

on tomato, rice and soybean invariably reduces root-knot nematode (RKN) infection (Cooper et al., 2005; Nahar et al., 2011 and kyndt et al., 2017), while inhibitors of JA biosynthesis enhance infection (Nahar et al., 2011 and Zhao et al., 2015). Davies (2010) reported that Eth probably having a restraining role by activating nematode repellents and JA, biosynthesis, facilitating radial expansion of the giant cells. Ethylene and auxin are important plant hormones involved in the regulation of many important plant processes. For instance, cell differentiation, cell expansion and responses of plant to biotic stresses. Nahar et al. (2011) and Mantelin et al. (2013) cited that Eth inhibits RKN infection, possibly through a decrease in nematode attraction to the roots. Consistent with Eth playing a role in plant defense to RKN infection, resistant plants showed more up-regulation of Eth biosynthesis and response genes than susceptible plants (Kumari et al., 2016 and Shukla et al ., 2018).

Grunewald et al. (2009) found that, auxin manipulation is well-known to be an important process during initiation and easily development of highly specialized feeding sites (NFS) of sedentary plant parasitic nematodes. Auxin is known for its role in cell expansion via the up-regulation of cell wall modifying proteins and plasma membrane proton pumps that regulate acid growth (Majda and Robert, 2018). Jasmonic acid and SA are the principal plant defense hormones and can have different effects depending on the specific host-nematode interaction, nematodes have evolved plant peptide hormone effect or mimics to facilitate parasitism. Mur et al. (2006). Ali et al. (2013) reported that the Eth-responsive RAP 2.6 gene is down regulated in syncytia and its over expression leads to enhanced resistance which the authors suggest is

a result of activated JA pathway. Yuan et al., (2016) reported that, auxin and Eth cooperatively lead to the development of cell wall during transfer cell formation.

Li et al., (2006) reported that three biological compounds including SA, JA and Eth regulate the resistance gene inducing basal defense responses. Plant hormones like Eth and JA have the ability to interfere with tomato SA inducible potato cyst nematode (PCN) resistance pathway in susceptible cultivars (Uehara et al., 2010).Nahas et al., (2011) and Fudali et al., (2013) reported that treating tomato plants by Eth inhibited gall development and giant cells. Nina et al., (2015) suggested that there is a positive role of Eth during nematode attraction, whereas JA triggers early defense responses against H. schachtii. Salicylic acid seems to be a negative regulator during female development.

The objective of the present study was to determine the ability of some inducers i.e. SA, JA, indol acetic acid (IAA), Eth and a biofertilizer) to induce systemic resistance in pepper plants against the root-knot nematode M. incognita, with a special reference to biochemical changes in antioxidant enzymes, sugars, phenols as well as root histology.

MATERIALS AND METHODS

Plastic pots (30-cm-diam.) were filled with 7 kg sandy-loam soil (2:1; v/v). Seedlings were dipped into solutions of SA, IAA, JA and Eth at three concentrations, 100, 200 and 300 µM for SA and IAA;15, 20 and 25 µM for JA, and 7g/L for a biofertilizer named (Halex-2) [which contains a mixture of growth promoting N_2-fixing bacteria of genera Azospirillum, Azotobacter and klebsiella, which was supplied by the Biofertilization Unit, Plant Pathology Dept., Alexandria University], for two hours before planting and then applied as a soil drench by drenching 20 ml/plant of each inducer concentration once every two days for two weeks. Three days after treatment, seedlings were inoculated with 5000 second-stage juveniles (J2s) of M. incognita/individual seedling (one seedling/pot). Three pots inoculated with 5000 J2s in tap water served as a control (Sahebani and Hadavi, 2009). Each treatment was replicated three times, with seedlings soaked and drenched with tap water served as control. Three pots treated with the nematicide vydate at 0.3 ml/plant served as a positive control. Pots were randomly arranged on a greenhouse bench at 25±2ºC, and watered as needed. Sixty days after nematode inoculation, plants were uprooted and number of galls, egg masses, and developmental stages were recorded per root, and J2 was counted per 250 g soil. Final population and reproduction factor (RF) were calculated according to the equation: RF= Pf (final population) / Pi (initial population) (Sasser et al., 1984). Plant growth parameters i.e. fresh shoot and root weights (g), dry shoot weight (g), shoot and root length (cm) were also recorded. Data of chemical analysis were recorded, namely antioxidant enzymes activity (peroxidase, polyphenoloxidase, and catalase), total and reducing sugars, and total and free phenols. Histological studies were also conducted in roots.

Physiological and biochemical analysis:

1. Antioxidant enzymes activity

Crude enzyme extracts of peroxidase and polyphenoloxidase were prepared according to Aluko and Ogbadu (1986). One gram of the homogenized pepper fresh leaves was extracted with 3 ml of 0.1 M phosphate buffer (pH 7). The homogenate was

filtered and then centrifuged at 3000 rpm for 15 min. at 4ºC. The supernatant filtered and collected as an enzyme extract. Enzyme extracts were stored at 2-5ºC and aliquots of these were assayed for enzymes activity using Milton Roy Spectronic 601 spectrophotometer. Enzymes activity were determined as follows:

A) Peroxidase

Peroxidase activity was determined according to the method of Fehrman and Dimond (1967). The increase in absorbance was determined using spectrophotometer (Milton Roy Spectronic 601) at 430 nm from 60-120 second after substrate was added. Peroxidase activity was expressed as O.D/g fresh weight (fw)/min.

B) Polyphenoloxidase

Polyphenoloxidase activity was measured following the method described by Broesh (1954). The enzyme activity was measured as the change in absorbance after 45 minutes at 495 nm and expressed as O.D./g fw/45 min.

C) Catalase

Catalase activity was determined as described by Bach and Oparin (1968). The decomposition of H_2O_2 was measured by titration of the remaining substrate with 0.0052 N potassium permanganate after stopping the enzymatic reactions with 5ml of 2% (v/v) sulphoric acid and the readings were replaced in a standard equation to find the final results.

2. Sugars and phenols determination

Sample preparation

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

A) Total and reducing sugars

Total soluble sugars and reducing sugars were spectrophotometric determined using the picric acid technique as described by Thomas and Dutcher (1924). A volume of 0.5 ml of each extract was placed in a test tube; containing 5 ml of distilled water and 4 ml picric solution were added. The mixture was boiled for 10 min. After cooling, 1 ml sodium carbonate solution (20%) was added and the mixture was boiled again for 15 min. After which, it was cooled and the tubes were completed to 10 ml with distilled water. Thereafter, the density of developed color was determined at 540 nm using spectrophotometer (Milton Roy Spectronic 601) in presence of a blank and using glucose as a standard.

Reducing sugars

The same described procedure for total sugars was used except that the picric- and sodium carbonate-solutions were added together at the same time. The same spectrophotometer and wavelength were used. Sugars concentration was expressed as mg/g fw.

B. Phenol compounds (Total and Free phenols)

Total phenols was determined as described by Simons and Ross (1971). 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, 1 ml Folin reagent

and 5 ml sodium carbonate solution (20%) were added and diluted to 10 ml using distilled water. After 30 min., the density of the developed blue color was determined at 520 nm using catechol as a standard.

Free phenols were determined 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, diluted with distilled water to 10 ml. After 30 min., the density of the developed blue color was determined at same wavelength. Total and free phenols concentration was measured as mg /g fw.

3. Histological studies

Samples for anatomical studies were taken from nematode-infected roots of each treatment, at 60 days from nematode inoculation. Samples were cut into suitable pieces not more than 5-mm thick in order to facilitate the exchanges of different solutions. Samples were placed in formalin-ethyl alcohol-acetic acid (F.A.A.) for 36-48 hr and samples were washed by tap water, then dehydrated using ascending concentrations of ethyl alcohol. Before infiltration, samples were passed into increasing concentrations of xylol in absolute alcohol. Infiltration was completed in oven when pure melted wax was added. Samples were embedded in paraffin wax (O'Brien and Mccully, 1981). Sections were microtomed at 15 micron and the combination of safranin-light green stain was used. Photomicrography was obtained histometry in order to observe the areas of different tissues, and good transverse sections were drawn by means of camera lucida.

Statistical analysis

The collected data were subjected to statistical analysis using the F-test and means were compared by LSD at level of probability as described by (Snedecor and Cochran 1972) and using (Costat software 1985).

RESULTS

Data presented in Table (1) show that all the tested abiotic inducers were effective in reducing the nematode root galling and inhibiting the nematode reproduction on pepper. All tested concentrations reduced all the related nematode parameters. The positive effect of such treatments was increased as dosage of the inducers increased. The highest concentration of Eth (300µM) was the most effective one, followed by SA, biofertilizer, IAA, and JA in reducing the root galling, number of juveniles in soil as well as number of egg-masses and developmental stage/root system. The lowest reduction was obtained by the lowest concentration of JA (15µM) compared to plants treated with nematode alone. Results also show that the highest concentration of all inducers were the most effective in reducing the nematode population and reproduction factor compared to the other two concentrations as shown in Table (1) and Fig. (1).

Data in Table (2) showed that treating pepper plants with abiotic and biotic inducers significantly enhanced all vegetative plant growth characters, i.e., fresh shoot and root weight (g), dry shoot weight (g), plant height and root length(cm) compared with the untreated control plants. The greatest effect was recorded with the application of Eth at 200 µM, followed by the highest concentration of JA, SA, IAA and biofertilizer. On the other hand, the lowest effect was recorded with the lowest concentrations of the inducers compared with plants treated with nematode alone.

Table (1): Effect of certain abiotic and biotic inducers for management of *Meloidogyne incognita* in pepper plants.

| Treatment	Conc. (µM)	Galls/ root system	Egg masses/root system	J2/250 g soil	Developmental stages/root system	Final population (PF)	Reproduction factor (RF)
Salicylic acid	100	8	9	68	17	102	0.020
	200	7	5	48	6	66	0.013
	300	5	2	11	4	22	0.004
Jasmonic acid	15	37	19	91	26	173	0.034
	20	14	11	56	18	99	0.019
	25	13	7	14	6	40	0.008
Indol acetic acid	100	17	15	93	45	170	0.034
	200	10	9	65	33	117	0.023
	300	9	5	54	10	78	0.015
Ethylene	100	13	11	50	21	95	0.019
	200	3	7	43	13	66	0.013
	300	1	2	17	7	27	0.005
Biofertilizer (Halex-2)	7g/L	8	6	130	39	183	0.036
Vydate (nematicide)	0.3ml/ plant	5	4	72	9	90	0.018
Nematode alone		114	98	483	212	907	0.181
LSD5%		1.254	2.103	11.04	2.11	-	-
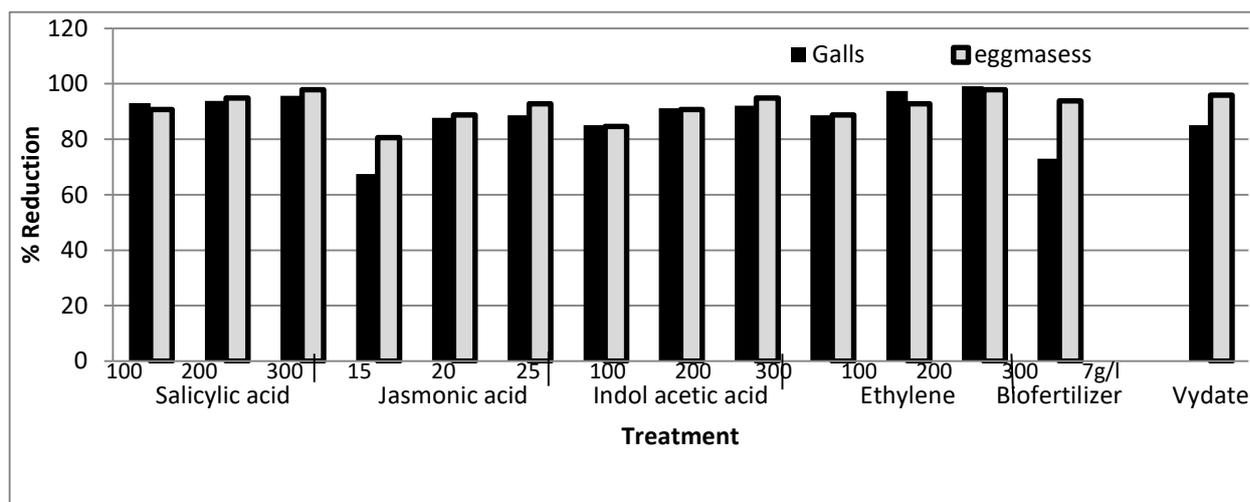
Fig.1A: Effect of certain abiotic and biotic inducers at different concentrations on the reduction of galls, egg masses /root system.
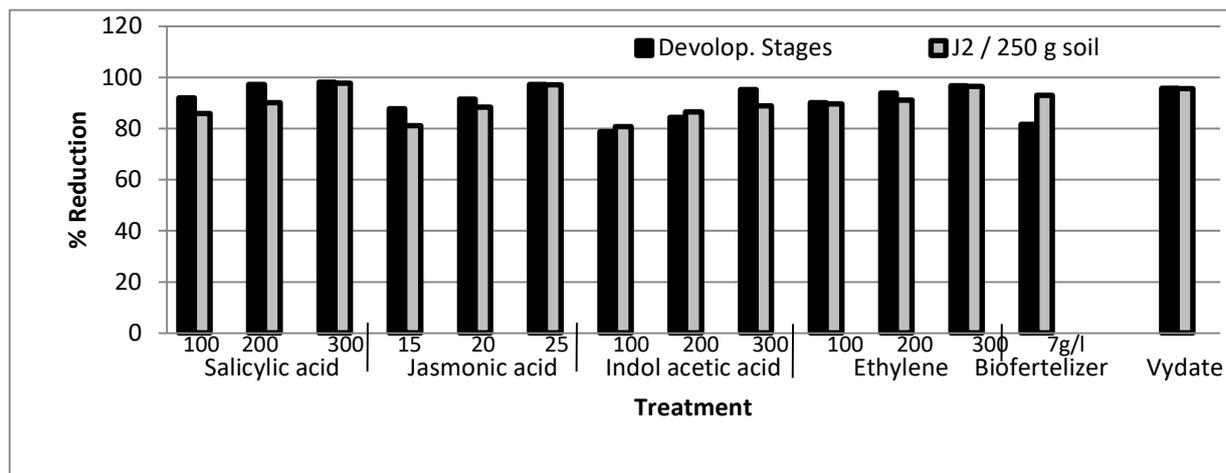
Fig.1B: Effect of certain abiotic and biotic inducers at different concentrations on the reduction of developmental stages /root system and J2/250 g soil.
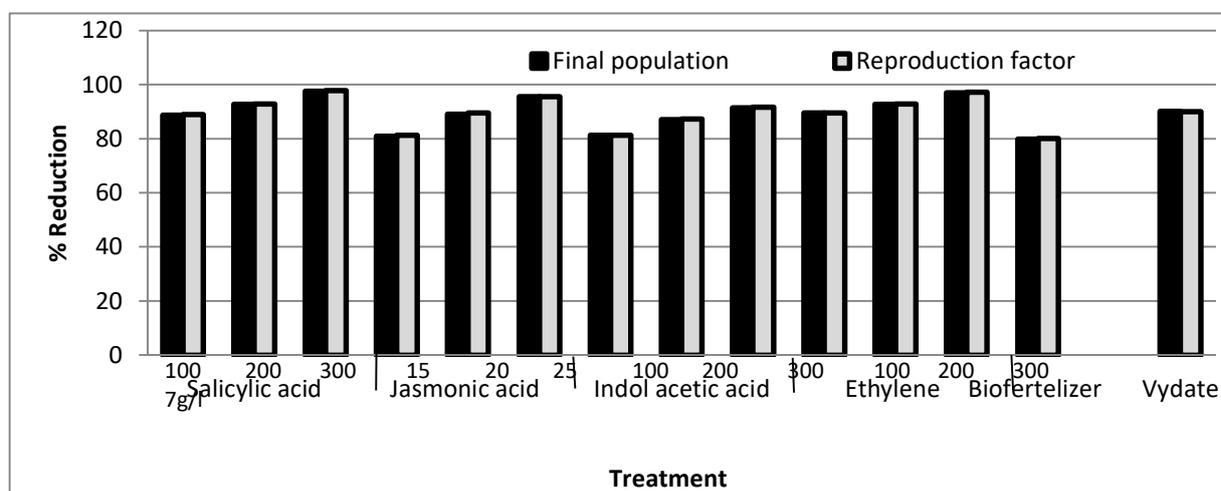

Fig.1C: Effect of certain abiotic and biotic inducers at different concentrations on the reduction of final population and reproduction factor.

Table (2): Effect of certain abiotic and biotic inducers on growth characters of pepper plants infected with *Meloidogyne incognita*.

Treatment	Conc. (*µM*)	Fresh shoot weight (g)	Fresh Root weight (g)	Dry shoot weight (g)	Shoot length (cm)	Root length (cm)
Salicylic acid	100	8.3	2.0	1.8	31.3	8.5
	200	9.8	2.1	2.0	32.0	9.0
	300	10.8	2.4	2.1	33.0	11.3
Jasmonic acid	10	7.2	2.4	2.1	34.6	9.0
	15	10.2	2.6	2.2	37.3	8.6
	25	11.7	2.7	2.7	39.3	8.6
Indol acetic acid	100	8.3	2.2	2.0	30.6	10.3
	200	9.8	2.8	2.1	32.6	11.6
	300	10.8	3.3	2.5	35.0	13.3
Ethylene	100	12.5	3.1	2.6	37.6	9.0
	200	15.5	3.9	3.1	42.3	10.0
	300	13.7	2.3	2.8	39.6	12.0
Biofertilizer (Halex-2)	7g/L	13.4	2.9	2.5	36.3	11.5
Vydate (nematicide)	0.3ml/plant	11.7	2.2	2.1	29.3	8.3
Nematode alone		7.4	3.3	1.6	20.6	7.0
Healthy plants		11.3	3.5	2.4	34.6	10.8
LSD5%		0.166	0.726	0.412	2.064	0.066

The effect of adding the inducers at different concentrations on antioxidant enzyme activity of pepper plant treated with the root-knot nematode M. incognita is given in Fig. (2). It can be illustrated that IAA and Eth at 300µM significantly increased PO compared to healthy plants. The best treatment in increasing PO and PPO activity was 300µM of Ethylene, flowed by SA, IAA, and Biofertilizer. However, the least increase in enzymes activity was obtained by 15µM of JA. On the other hand, Eth. at300µM was the best treatment in increasing the activity of catalase.

Data presented in Fig. (3) recorded the effect of some abiotic and biotic inducers on reducing sugars content in pepper plants. It can be concluded that all treatments under study significantly increased sugar content. The most effective treatment in increasing the total and reducing sugars was Eth at 300µM, and JA at 25µM. However, the least sugar content was recorded in the nematode-alone treated plants. Data presented in Fig. (4) showed the effect of some inducers on phenol content in pepper plants. It was found that all treatments significantly increased phenols content. The highest effective treatment in increasing the total and free phenols was Eth and SA at 300µM. The lowest level of total and free phenol concentrations was recorded in nematode-alone treated plants.

Histological studies

Treated and untreated infected pepper roots were processed for histological examination via making transverse sections, 60 days after nematode inoculation using a microscope. It can be noticed that M. incognita induced alterations in cells of cortical and the main region in pepper roots (Fig. 5). Giant cells were found prolonged in vascular parenchyma cells with different shapes from circular to irregular shape. Clusters were present as a result of forming giant cells and the growth of the nematode females. Moreover, it can be recognized the hypertrophied nuclei were aggregated in the cytoplasm with number ranged from 13-15 and induced more disruption in xylem and cortex layers (Fig. 5).

As for histological alterations in pepper plant infected with root knot nematode M. incognita but treated with SA (300µM), the number of giant cells was less than that in the control (treatment of nematode alone) 60 days after nematode inoculation, which ranged from 8-10 with less numbers of hypertrophy and disruption in xylem and cortex layers as a result of presence of giant cells (Fig. 6).

Regarding the effect of JA at 25µM, after 60 days from nematode inoculation, it was found about 4-5 irregular shape cells and hypertrophied nuclei cells (Fig. 7). Pepper plants infected with nematode and treated with IAA at 300µM showed better performance at 60 days after nematode inoculation, it can be noticed that giant cells formed in feeding sites and disruption in xylem and cortex layers as a result of the presence of less number of giant cells and growing female nematodes (Fig. 8). It can be noticed also, root transverses section become irregular due to inducing compressed cells. In this concentration it can be noticed that pepper plants infected with nematode and treated with Eth at 300µM after 60 days from nematode inoculation did not show any giant

cells and the root section almost similar to that of the healthy control plants (Fig. 9 & Table 2).The root transverse sections of pepper plants infected with nematodes and treated by the biofertilizer Halex-2 at 7g/L showed were similar to those less number of giant cells (ranged from 2-3) (Fig.10).

Infected roots of pepper plants with nematode M. incognita and treated with nematicide vaydate showed a poor formation and irregular giant cells divided from cytoplasm and contained less number of nuclei (Fig. 11) compared to the healthy plants (Fig. 12).
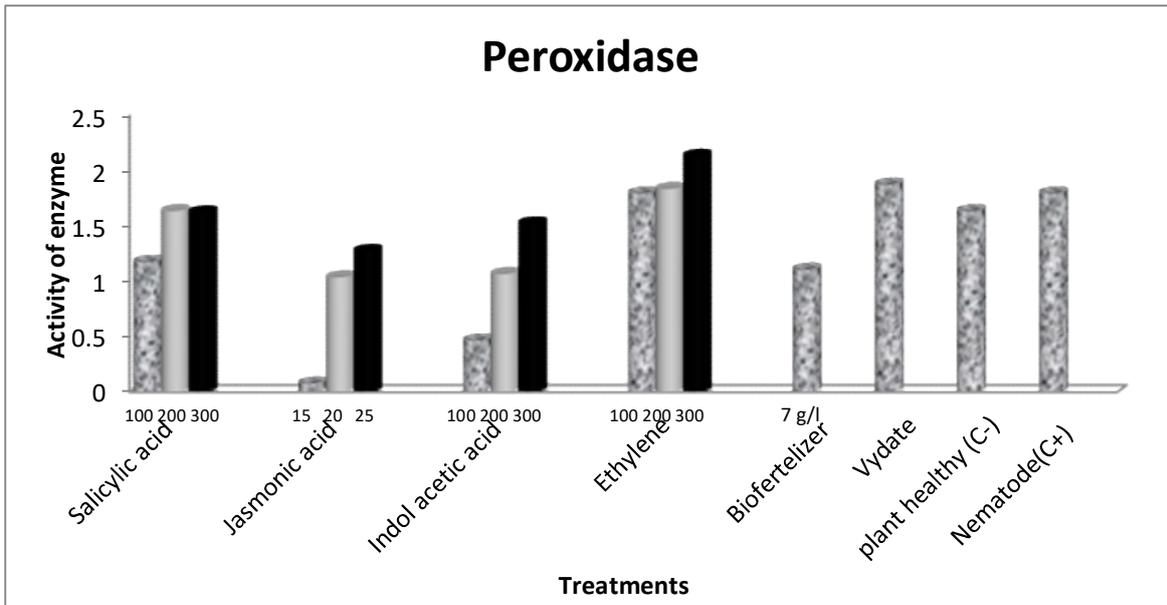
Fig. 2 (A): Effect of certain abiotic and biotic inducers on peroxidase enzyme activity of pepper plants infected with *Meloidogyne incognita*.
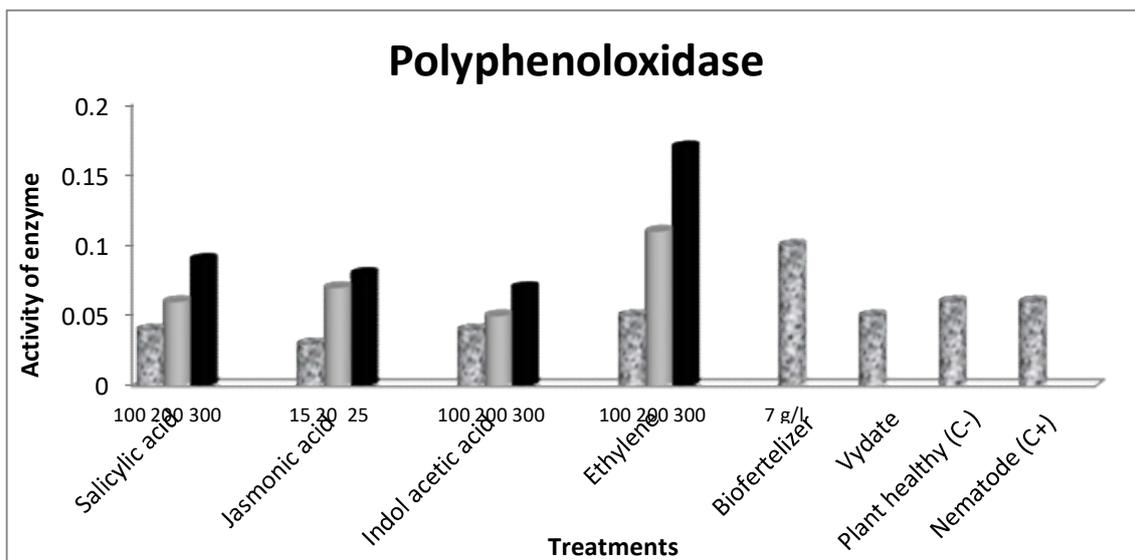

Fig. 2 (B): Effect of certain abiotic and biotic inducers on polyphenoloxidase enzyme activity of pepper plants infected with *Meloidogyne incognita*.
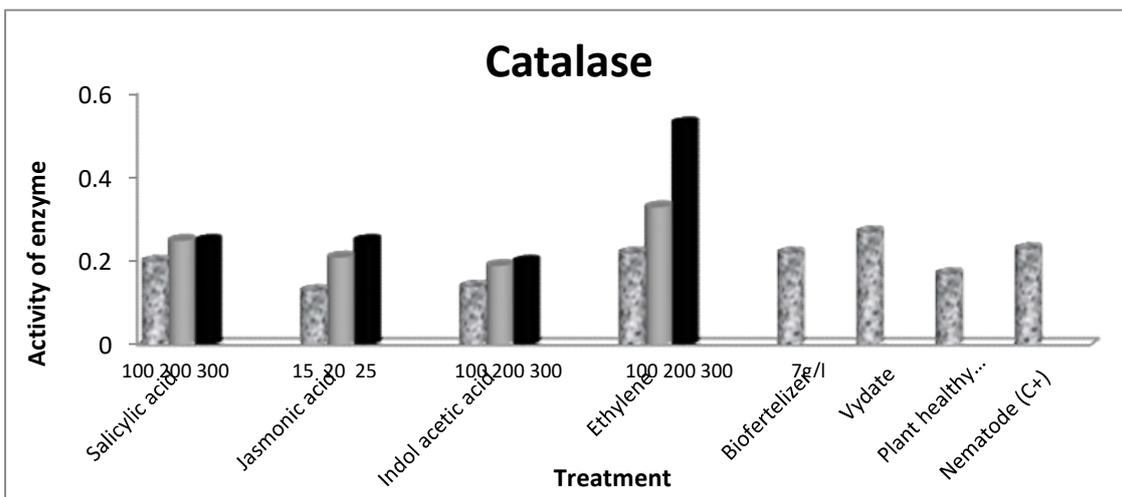
Fig. 2 (C): Effect of certain abiotic and biotic inducers on catalase enzyme activity of pepper plants infected with *Meloidogyne incognita*
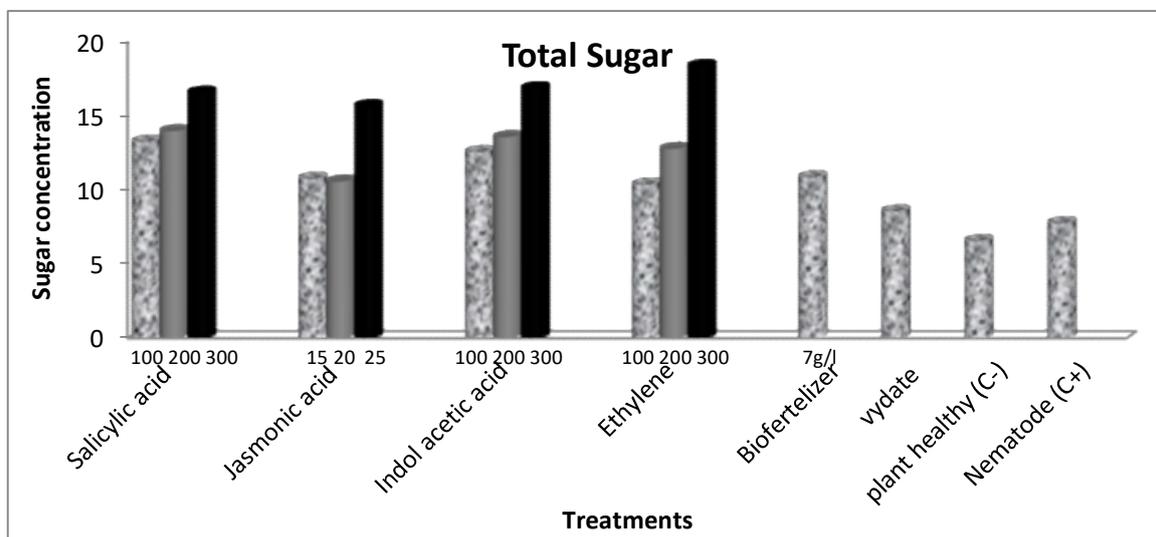

Fig. 3 (A): Effect of certain abiotic and biotic inducers on total sugar content of pepper plants infected with *Meloidogyne incognita*.
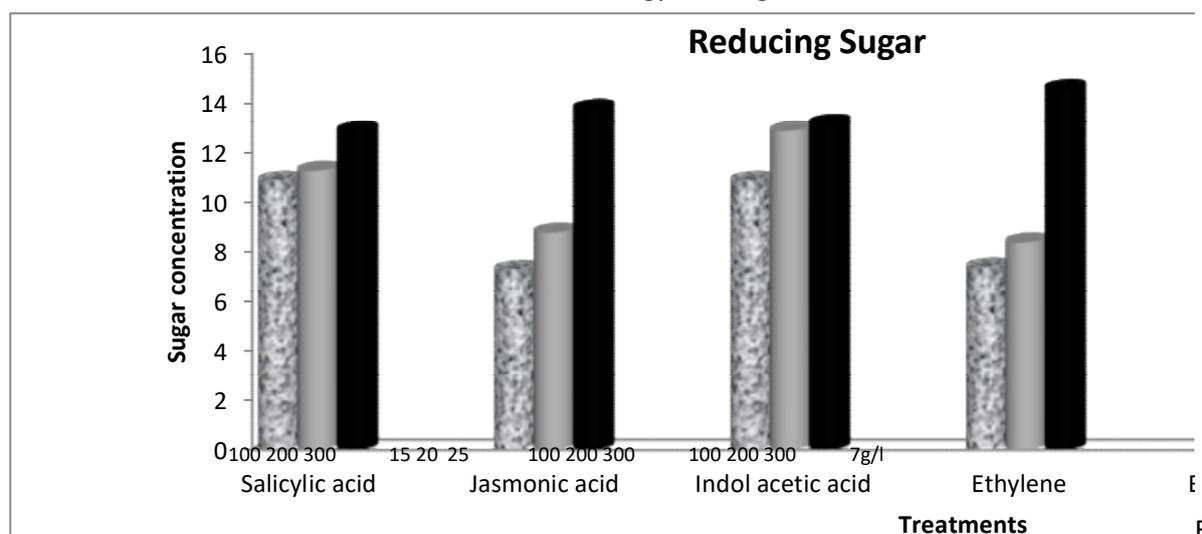
ig. 3 (B): Effect of certain abiotic and biotic inducers on sugar content of pepper plants infected with *Meloidogyne incognita*.
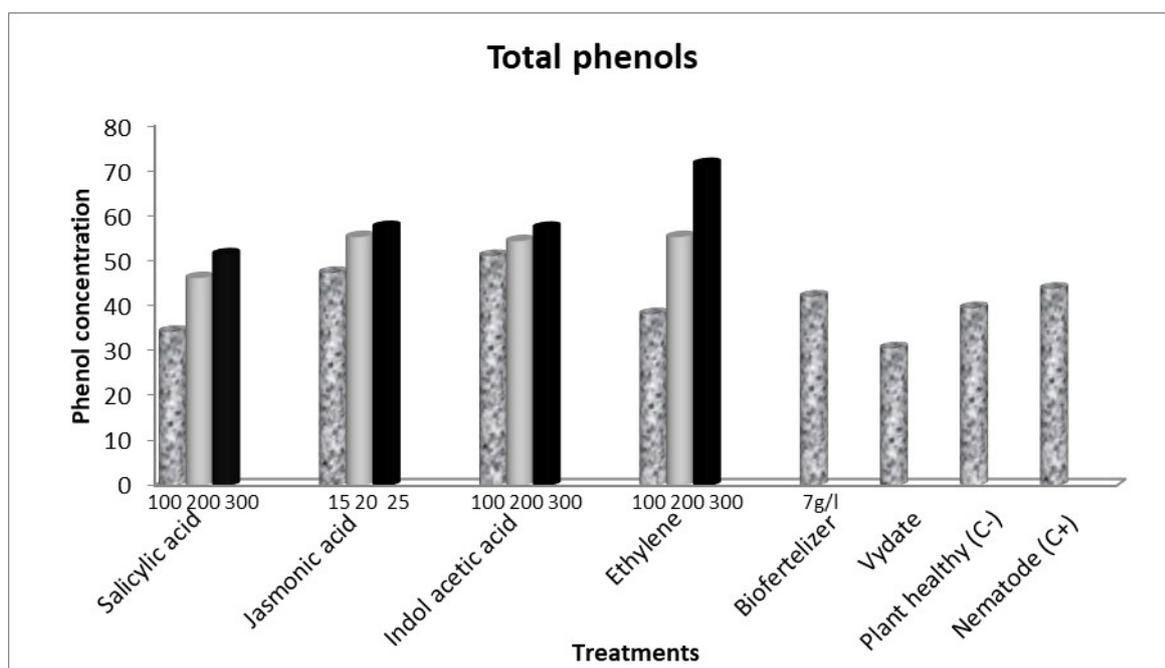
Fig. 4 (A & B): Effect of certain abiotic and biotic inducers on total phenols concentrations in pepper plants infected with *Meloidogyne incognita*.
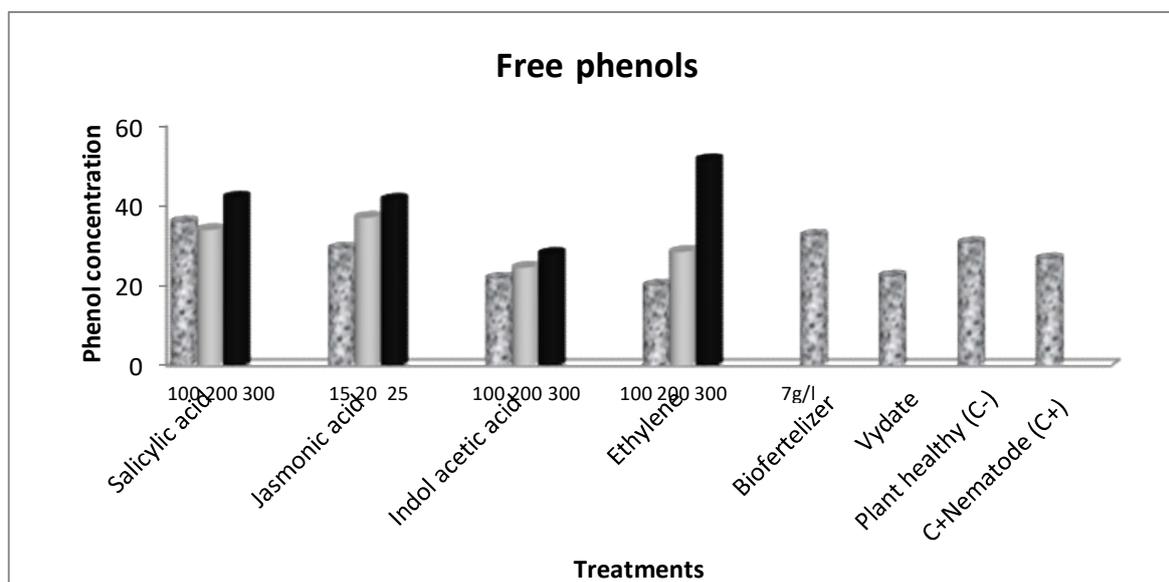

Fig. 4 (A & B): Effect of certain abiotic and biotic inducers on free phenols concentrations in pepper plants infected with *Meloidogyne incognita*.

DISCUSSION

All inducers used in this experiment have reduced nematode population at all concentrations used. This finding may refer to understanding that phytohormone involvement in the establishment and maintenance of the feeding site is critical to the development.

During nematode infection, the pre-parasitic second stage juveniles (J2) penetrate the roots of a suitable host, then migrate intercellularly towards the vascular cylinder to find a competent

plant   cell   for   the   induction   of   multinuclear feeding cell complex.
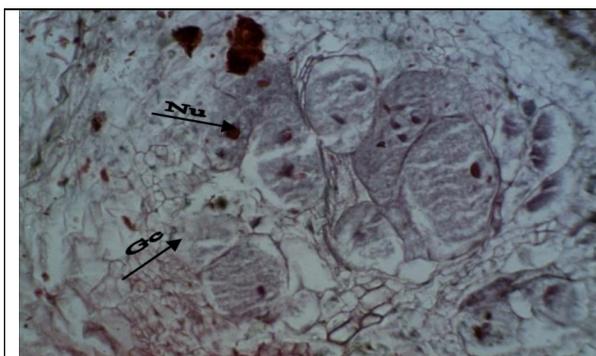
Fig. (5): Root transverse section of pepper plants infected with *M. incognita*.
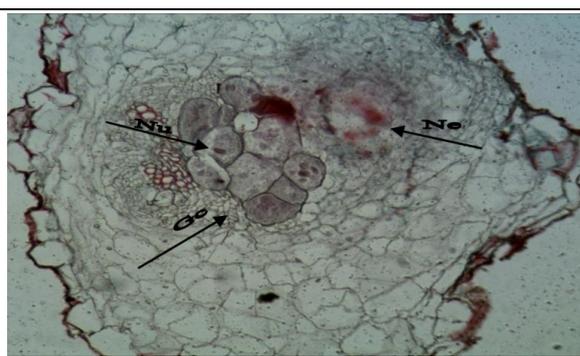
Fig. (6): Root transverse section of pepper plant infected with *M. incognita* and treated with SA (300µM).
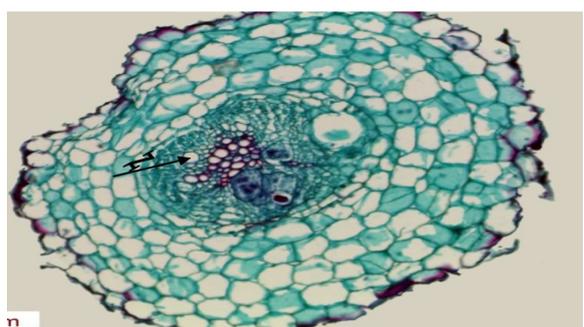
Fig. (7): Root transverse section of pepper plants infected with *M .incognita* and treated with JA (25µM).
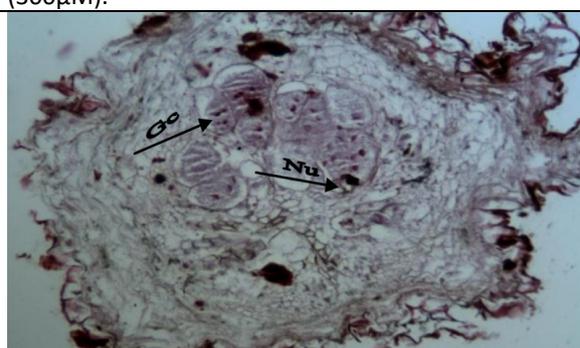
Fig. (8): Root transverse section of pepper plants infected with *M. incognita* and treated with IAA (300µM).
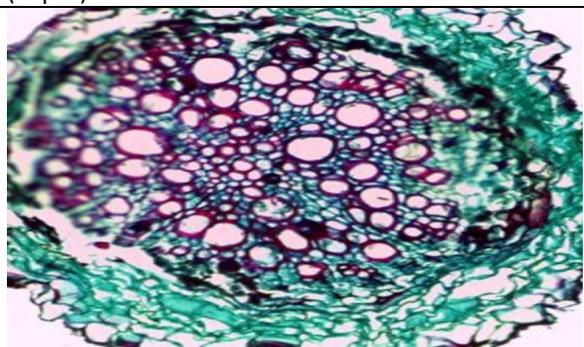
Fig. (9): Root transverse section of pepper plants infected with *M. incognita* and treated with ethylene (300µM).
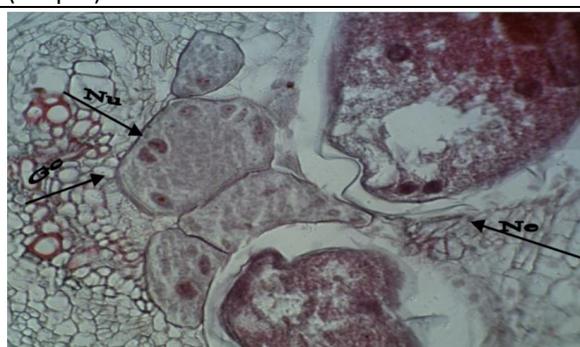
Fig.(10): Root transverse section of pepper plants infected with *M. incognita* and treated by the biofertilizer Halex-2 at 7g/L.
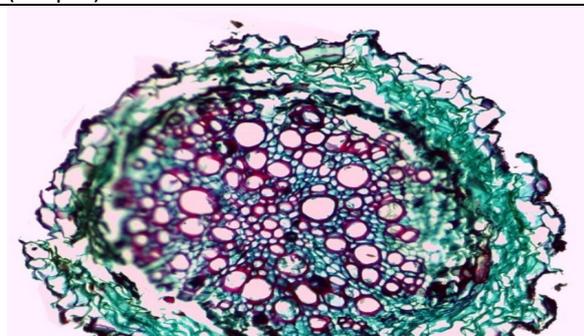
Fig. (11): Root section of pepper plants infected with *M. incognita* and treated with the nematicide Vydate
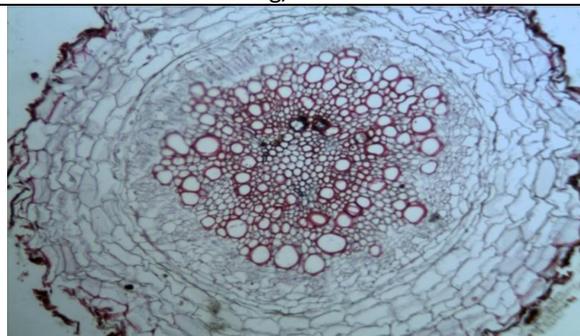
Fig.(12): Root section of healthy plant roots as a control

Root knot nematodes induce giant cells as a feeding site. Vallad and Goodman (2004) showed that at least two forms of induced resistance, systemic acquired resistance (SAR) and induced systemic resistance (ISR) as distinct phenomena based on the type of inducing agents and host signaling pathways that result in resistance expression. Ethylene and auxin are important plant hormones involved in the regulation of many important plant processes. For instance, cell differentiation, cell expansion, and responses of plant to abiotic stresses (Davies, 2010). Moreover, these hormones play vital roles in many plant pathogen interactions, including manipulation of plant defense responses and development of symptoms. Ethylene induced plant resistance by genes activation. Meanwhile, plant hormones like Eth and JA have the ability to interfere with tomato SA inducible resistance pathway in susceptible cultivars. Thus, phytohormones involved in many processes of plant nematode parasitism like the involvement of invasion plant cell and induction of syncytium (Uehara et al., 2010).

Wang *et al.*, (2007) reported that SA causes global repression of auxin-related genes, which repress proteins and inhibit auxin response SA mediated disease resistance mechanism due to the inhibitory effect on auxin signaling.

Oka and Spiegel (1999) indicated that induced resistance can be local or systemic. Local induced resistance refers to cases where the response is local whereas systemic induced resistance describes resistance that is induced in a part of the plant that is specially separated from the point of induction. Although they differ, both local and systemic resistance requires some time to develop after application of the inducing treatment and both are non-specific in nature. (Hammerschmidt 1999) reported that reduced population growth of the pathogen in locally induced resistance may be due to the production of PR proteins (defensive plant proteins specifically induced in pathological or related situations) and cell wall alterations that prevent or inhibit growth and development of the pathogen as well as challenge pathogens. (Conrath *et al.*. 2002) described that resistance-activating treatment in systemic resistance results in a change in cells at a distance from the induction site that allows rapid defense activation called priming.

Huang (1998) reported that plant defense mechanisms can be either preformed or induced only after the plant is attacked or otherwise injured. The general understanding is that plants can actively guard themselves and have induced resistance against virulent pathogens.

We document here a different tactic to reduce plant damage due to nematodes. Using novel inducers i.e., SA, IAA, JA, Eth and a biofertilizer, which have broad spectrum effects, including benefits to plant growth and enhancement of plant resistance. Programmed cell death (PCD) and hypersensitive response (HR) are the main results of ROS (free radicals) signaling in plants (Jonathan et al., 2004). It has been reported that the production of ROS is one of the earliest events in molecular plant-nematode interaction (Grundler *et al.*, 1997). R proteins frequently lead to the up regulation of genes involved in ROS production, which result in HR. Plants have a variety of antioxidant enzymes (e.g. peroxidase, catalase and polyphenoloxidase) to scavenge excessive ROS in plant tissue to

prevent self-damage. Moreover, another enzyme; superoxide dismutase (SOD), detoxifies ROS produced by the plant cell and induces plant resistance (Bellafiore *et al*., 2008, Roze *et al*., 2008, and Ali *et al*., 2018).

Peroxidase represents a large group of oxidoreductases that catalyzes the oxidation of substrate molecules using hydrogen peroxide as electron acceptor. These enzymes play a key role in important biological processes, such as lignin degradation pathway and host defense mechanisms (Davies *et al*., 2008). Mostafenezhad *et al*. (2014) reported that treatment with SA promoted the highest activity of peroxidase, 4 days after treatment. Catalase plays the role of a specific peroxidase protecting cell from toxic effects of H2O2 (Ben Amor et al., 2005).

Devrajan and Srenivasan (2002) reported that peroxidase and polyphenoloxidase (catechol oxidase) were synthesized in the root of banana (*Musa* sp.) due to infection with M. incognita. They also concluded that biochemical and molecular changes were associated with resistance reaction of banana against root-lesion incited by root-knot nematodes. Moreover, (Singh *et al.* 2013) reported that the accumulation of phenolic acid was also increased after nematode infection. (Patel *et al.* 2001) reported that *Meloidogyne* spp. have ability to induce synthesis of peroxidase, polyphenoloxidase and total phenols in roots of chickpea.

Our results indicated that the inducers used in our study significantly increased antioxidant enzymes i.e., peroxidase, polyphenoloxidase as well as catalase, which promoted synthesis of chemical components i.e., sugars and phenols, which reflected a good performance of pepper plants under nematode infection conditions.

According to the results of many authors, it is possible that phenolics play the main role: (i) Browning and slow formation of wide necrosis in plants susceptible to the migratory nematodes. (ii) Quick browning and formation of non-expandable necrosis in plants resistant to migratory parasites. (iii) IAA oxidase inhibition, which may favor auxin decomposition and formation of necrosis in plants resistant to nematodes.

Histological changes in pepper plant roots infected with root-knot nematode *M. incognita* as affected by the addition of some electors

Our investigation of histological alterations in pepper roots infected with *M. incognita* and treated with some electors revealed that SA (300µM) poorly induced formation of giant cells with limited hypertrophy. However, JA treatment incited less numbers of giant cells, little damage in cortex and xylem layers, and less number of hypertrophied giant cells. IAA at 300µm showed a good performance at the beginning; noticed as no formation of any giant cells. While Eth (300µM) treatment did not induce formation of any giant cells and the root section has no difference from the healthy control root section. The biofertilizer (Halex-2) treatment recorded less number of giant cells and irregular formation and disruption in xylem layer.

Glazer *et al*. (1983 & 1985) reported that chemical blocking ethylene production inhibited gall development in tomato and that treatment of plants with Eth- precursor led to better giant cells enlargement. This is an important role of Eth-responses in activation of JA-dependent defense against RNC (Nahas *et al*., 2011; Fudali *et al*., 2013). Eth probably plays different roles at different stages of the nematode infection process: having a restraining role by activating nematode repellents

and JA biosynthesis, facilitating radial expansion of the giant cells. Auxin manipulation is well-known to be an important process during initiation and development of nematode feeding sites (NFS) of sedentary plant parasitic nematodes (Grunewald *et al.*, 2009).

Saeed (2005) studied the anatomical alterations in roots of M. incognita-infected soybean plants due to application of some resistance inducers. He found that histological alterations in root tissue of soybean plants revealed normal appearance of the nematode, which succeeded in developing giant cells with normal shape, size and contents. Apparently, nematode status seemed to be normal inserting its head region in a cluster of normal giant cells, laying in stellar region, extending in cortical layer. At least six giant cells were noticed in close vicinity to the nematode frontal part and encircled with hyperplastic cells. Also, giant cells contained several darkly stained nuclei with prominent nuclei. Interestingly, no necrotic areas were found around or near the nematode or giant cells.

CONCLUSION

It can be concluded that 300 μM of all inducers is the best concentration to controlling root knot nematode and preventing the formation of giant cells. It has a similar effect as the nematicide vydate and also increases the content of sugars, phenols and antioxidant enzymes activity. Moreover, ethylene at 300μM was the most effective in controlling M. incognita in pepper plant. This novel and promising results may lead to a reduction of the use of nematicides, which are harmful to human and the environment. It can also help the plant breeders to create new resistant cultivars against M. incognita.

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