CHANGES IN ZUCCHINI DEFENSE RESPONSES AGAINST *MELOIDOGYNE JAVANICA* (RHABDITIDA: MELOIDOGYNIDAE) INDUCED BY *POCHONIA CHLAMYDOSPORIA*

Motahareh Lalezar*, Mohammad Reza Moosavi* and Abdoreza Hesami**

* Department of Plant Pathology, Marvdasht Branch, Islamic Azad University, Marvdasht, IRAN. E-mail: rmmoosavi@yahoo.com
** Department of Organic Chemistry, Marvdasht Branch, Islamic Azad University, Marvdasht, IRAN.

[Lalezar, M., Moosavi, M. R. & Hesami, A. 2016. Changes in Zucchini defense responses against *Meloidogyne javanica* (Rhabditida: Meloidogynidae) induced by *Pochonia chlamydosporia*. Munis Entomology & Zoology, 11 (1): 151-159**]**

ABSTRACT: *Meloidogyne javanica* causes serious damage to many crops and its management is not easily achievable. *Pochonia chlamydosporia* var. *chlamydosporia* (Pcc) is a potent biocontrol agent whose ability in stimulating plant defense has been ambiguous. This study was designed to analyze the kinetics of some defense-related enzymes in the roots of zucchini plants after inoculation with either one or both of Pcc and *M. javanica*. Activity of phenylalanine ammonia-lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO), and catalase (CAT) in root samples were examined in a week with one day interval beginning from the next day following inoculation with *M. javanica*. When the plants were inoculated with both Pcc and nematode, the activity of PAL, POX and CAT was significantly improved during the experiment compared with other treatments. The PPO activity in such plants was more than PPO activity in nematode-infected plants only on the 5th and 7th day after inoculation. Individual application of Pcc frequently resulted in enhanced activity of PAL, POX and CAT compared with these enzymes activity in control treatment. This is the first report on the ability of *Pochonia chlamydosporia* var. *chlamydosporia* for inducing or improving the plant innate defense.

KEY WORDS: Catalase, nematophagous fungi, peroxidase, phenylalanine ammonia lyase, plant defense induction, polyphenol oxidase, root-knot nematode

Root-knot nematodes (*Meloidogyne* spp.) can annually destroy about 5% of agricultural products worldwide (Agrios, 2005). *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 is an economically important nematode that can parasitize more than 2000 different plant species (Perry et al., 2009). This nematode has a widespread distribution in Iran (Moosavi, 2012) and often imposes a considerable loss to many crops including zucchini (*Cucurbita pepo*) (Ghaderi et al., 2012).

Nowadays, chemical nematicides are the main controlling method of plant-parasitic nematodes (PPNs) (including *M. javanica*) which adversely impact the environment and human health (Moosavi & Zare, 2015). These harmful effects have intensified the search for finding safer, environmentally friendly control alternatives (Moosavi & Askary, 2015) such as biological control (Davies & Spiegel, 2011). Biocontrol of plant diseases will be more successful if the potent biocontrol agent (BCA) could also stimulate the plants innate immunity systems (Walters & Bennett, 2014).

It has been demonstrated that many microorganisms have good potential in the management of PPNs, however their significance is not similar (Cumagun & Moosavi, 2015). Fungi are one of the most important antagonistic groups among them the species of *Pochonia* have been considered as one of the top four BCAs against PPNs (Moosavi & Zare, 2012). *Pochonia* spp. are facultative egg parasites of cyst and root-knot nematodes that penetrate into their hosts' eggshell via producing appressorium and extracellular enzymes (Manzanilla-López et al.,

2013). The fungus can successfully colonize the root epidermis and cortex (Bordallo et al., 2002; Macia-Vicente et al., 2009) but there is no information on its ability to induce plant defense mechanisms.

Many enzymes in plants are induced in response to biotic or abiotic stimulator leading to systemic resistance. Increasing in the amount or activity of these enzymes is usually considered as a sign of plant defense activation. These enzymes include phenylalanine ammonia-lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO), and catalase (CAT) (Garcion et al., 2014). PAL is an important enzyme involves in the phenylpropanoid biosynthetic pathway. This enzyme is also responsible for the synthesis of the polyphenol compounds such as phenylpropanoids, flavonoids and lignin (MacDonald & D'Cunha, 2007).

Peroxidases (POX) catalyze many important biological processes of plant-defense mechanisms (Passardi et al., 2005; Gupta, 2010). For example, they play a significant role in the strengthening of cell wall structures by catalyzing the suberin polymerization (Arrieta-Baez & Stark, 2006), lignin biosynthesis (Almagro et al., 2009) and cross-linkage of the structural proteins like extensions (Jackson et al., 2001). Plant peroxidases also facilitate the formation of diferulic acid linkages (Fry, 2004) and production of hydroxyl radical (Schweikert et al., 2000).

Plant PPOs oxidize polyphenols into quinines which are considered as antimicrobial compounds. It is suggested that they are also involved in the lignifications of plant cell wall during the attack of pathogens (Constabel & Barbehenn, 2008; Tran et al., 2012). Catalase (CAT) involves in the antioxidative defense system of plants. This enzyme detoxifies H_2O_2 when the level of hydrogen peroxide elevates in cell (Bilgin, 2010).

This study was designed to determine whether *P. chlamydosporia* could induce the defense mechanism of zucchini plants by itself or could improve the defense responses of zucchini plants to *M. javanica*.

MATERIALS AND METHODS

Fungal isolate and inoculum preparation

One indigenous isolate of *Pochonia chlamydosporia* var. *chlamydosporia* (Pcc; IRAN 1212 C) was selected for this experiment whose efficiency in controlling *M. javanica* was previously confirmed (Moosavi et al., 2010). The fungus was grown on PCA (potato-carrot agar) medium to stimulate the production of conidia (Zare & Gams, 2004). The plates were inoculated by streaking the surface of culture media in parallel lines with the fungal inoculum. Ten days later, the conidia were collected by sterile distilled water and their concentration was estimated by average of three counts. The concentration of the propagule was finally adjusted to 10^6 propagule per mL distilled water.

Preparation of nematode inoculum

The needed inoculum was prepared on tomato plants (cv. Early-Urbana) starting from a single nematode egg mass formerly identified as *M. javanica* (Moosavi et al., 2011). The eggs were isolated from the 0.5 to 1 cm pieces of galled roots by agitating for 2 to 3 min in 0.5% sodium hypochlorite solution. The suspension was then rinsed over 60- and 20-µm sieves (Hussey & Barker, 1973) and the inoculum on 20-µm sieve was transferred to a beaker. The number of eggs and second stage juveniles (J2s) were estimated by means of three counts and adjusted to 100 eggs and J2s per mL.

Plant material, inoculation and experimental design

Seeds of zucchini (cv. Tees F1-801, Samyer, USA) were surface sterilized with 1% NaOCl for 5 minutes and planted in 500 g plastic pots. The pots (15 cm diameter, 15 cm depth) had been filled with sterile sandy loam soil (sand 67.3%, clay 12.1%, silt 20.6%, organic matter 3.5% with pH 7.5). There were 4 sets of

treatment including 1) zucchini plants inoculated with fungal inoculum, 2) zucchini plants inoculated with nematode inoculum, 3) zucchini plants inoculated with both fungal and nematode inoculum, and 4) not- inoculated zucchini plants (control). The seedlings were inoculated with Pcc when they had developed the first set of true leaves. The inoculation was done by drenching the soil around the crown of the seedlings with 20 mL of conidial suspension at a concentration of 10^6 conidia / mL. After one week, nematode inoculum was added to soil around the roots of zucchini plants at a rate of two eggs and J2s / g soil. Each treatment had five replications and pots were arranged in a completely randomized design in a greenhouse.

Determination of enzymatic activity

Activity of phenylalanine ammonia-lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO), and catalase (CAT) in root samples of treatments were evaluated in a week with one day interval beginning from the next day following inoculation with *M. javanica*. Fresh roots were homogenized with liquid nitrogen after being cleaned in tap water and dried with a filter paper. The same volume of 10 mM sodium phosphate buffer (pH 6 at 4°C) was mixed with the homogenized tissue and was then filtered into a centrifuge tube through a 0.2 mm nylon filter. The mixture was centrifuged at 12,000 *g* for 20 min at 4°C and the supernatant was stored at −80°C until being examined (Chen et al., 2000). Soluble protein concentration of the supernatant was measured by the standard Bradford assay (Bradford, 1976) using crystalline bovine serum albumin as a reference.

PAL activity

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.24) activity was determined by the method of Ochoa-Alejo & Gomez-Peralta (1993). One mL of 50 mM Tris-HCl buffer (pH 8.8 containing 15 mM of β-mercaptoethanol) was mixed with 0.5 mL of 10 mM L-phenylalanine, 0.4 mL of deionized water and 0.1 mL of enzyme extract and the reaction mixture was incubated at 37°C for 1 h. The reaction was terminated by adding 0.5 mL of 6M HCl and the product was extracted with 15 mL diethyl ether. The extraction solvent was evaporated at 22 °C under reduced pressure. The solid residue was suspended in 3 mL of 0.05M NaOH. The concentration of trans-cinnamic acid in the mixture was quantified with the absorbance at 290 nm. One unit of PAL activity is equal to 1 µmol of cinnamic acid produced per min.

POX activity

Total peroxidase (POX; EC 1.11.1.7) activity was detected according to Mohammadi and Kazemi (2002). 25 mM citrate–phosphate buffer (pH 5.4) was added to root's enzymatic extract contained 20 µg protein and 1 mM guaiacol (as an electron donor) to make a final volume of 1 milliliter. The reaction was started by adding 10 µL of 30% H_2O_2 (Merck Co., Germany). The rate of increase in absorbance at 475 nm was measured over 30 s at 25 °C using Perkin Elmer lambda-45 spectrophotometer. Potassium cyanide (7 µM) was used as an inhibitor for POX. The results were expressed as changes in absorbance (*A*) / min / mg protein.

PPO activity

Polyphenol oxidase (PPO; EC 1.14.18.1) activity was determined by mixing 25 mM citrate–phosphate buffer (pH 6.4) with the root's enzyme extract contained 30 µg protein and 5 mM L-proline to make a final volume of 1 mL. The samples were ventilated in a test tube for 2 min and the reaction was then initiated by addition of pyrocatechol (1, 2-dihydroxybenzene) as the substrate at a final concentration of 20 mM. The initial rate of increase in absorbance at 515 nm was measured over a time period of 1 min at 25°C. The PPO activity was expressed as the changes in absorbance (*A*) / min / mg protein. Ascorbic acid prepared in the same buffer solution (7 mM final concentration) was used as an inhibitor of PPO activity (Mohammadi & Kazemi, 2002).

CAT activity

Total catalase (CAT; EC 1.11.1.6) activity was calculated by following the decline in A240 as H_2O_2 was catabolized in a 3 mL reaction mixture containing 10 mM potassium phosphate buffer (pH 7.0), appropriate amount of root extract containing 30 µg protein, and 35 µL H_2O_2 (3%). The activity of CAT on consumption of H_2O_2 was measured using the extinction coefficient (40 mM^{-1} cm^{-1}) and stated as changes in absorbance at 240 nm / min / mg protein (Kato & Shimizu, 1987).

Statistical analysis

Statistical analysis was carried out using SAS software (version 9.1.3; SAS Institute, Cary, NC) (1990). All data were subjected to one-way analysis of variance (ANOVA), and treatment means were separated using Duncan's Multiple Range Test.

RESULTS

PAL activity

PAL activity in various treatments was significantly different (F=64.24; df=15; P< 0.0001). The highest PAL activity was recorded in the plants treated with both Pcc and *M. javanica*. PAL activity had a rapid increase when the plants treated by both fungus and nematode from the first upto the fifth day after inoculation (DAI), and then decreased. The lowest activity of PAL was detected in untreated control plants. The PAL activity on the first DAI was similar in the plants treated only with Pcc or nematode. PAL activity in nematode-infected plants was higher than in fungus-infected ones on the third and fifth DAI, however a lower activity was observed for the same comparison on the 7th DAI (Figure 1).

POX activity

Compared with control treatment and at similar time span, POX activity significantly increased when the zucchini plants were inoculated with either one or both of Pcc and *M. javanica* (F=124.06; df=15; P< 0.0001). The enzymatic activity in all treatments was increased until the fifth DAI, and then decreased. The highest POX activity was observed in the roots that were inoculated with both Pcc and *M. javanica*. The fungus could stimulate the POX activity in roots; however except for the first DAI, the enzymatic activity was lower compared with the nematode-inoculated plants. The POX activity in the control plants remained around a constant level during the experiment (Figure 2).

PPO activity

There was a significant difference between treatments in stimulation of PPO activity in zucchini roots (F=91.8; df=15; P< 0.0001). Though the differences were not so distinguishable at the first DAI, the PPO activity in nematode-infected plants was slightly higher than other treatments. The enzymatic activity on the third DAI was greatest in the plants which were inoculated with *M. javanica*, but no significant difference was seen between the activity of enzyme in the plants that were inoculated with either PCC or with both PCC and nematodes. A prominent rise in PPO activity was seen at the fifth DAI when the greatest activity was recorded for the plants inoculated with both fungus and nematode. Afterward, the PPO activity declined until 7th DAI (Figure 3).

CAT activity

Changes in catalase activity over the studied days was different among treatments (F=89.5; df=15; P< 0.0001). Enzyme activity in control treatment was similar from the first DAI till the end of experiment. When the plants were only inoculated with Pcc, catalase activity was more than control treatments except for

the 7th DAI. The greatest enzyme activity was recorded when the nematode-infected plants had Pcc around their roots. CAT activity in inoculated plants was increased until the 5th DAI and then declined. The activity of CAT in each treatment was similar on the first and 7th DAI apart from the plants that were inoculated with both fungus and nematode (Figure 4).

DISCUSSION

Plants can resist against pathogen infection using several layers of constitutive and induced defense mechanisms (Walters, 2011a; Sholevarfard & Moosavi, 2015). Many plant enzymes like phenylalanine ammonia lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO) and catalase (CAT) are involved in defense responses against plant pathogens (Anderson et al., 2006; Dubey, 2010).

Rise in concentration of defense enzymes in nematode-infected plants (especially *Meloidogyne*-infected plants) have been repeatedly reported (Zacheo et al., 1997; Walters, 2011b; El-Beltagi et al., 2012; Pourjam et al., 2015). It has also been reported that many microorganism could induce plant systemic resistance against pathogens such as phytonematodes (Ramamoorthy et al., 2001; Sharon et al., 2011; Pieterse et al. 2013; Walters & Bennett, 2014), but it was not clear whether *Pochonia chlamydosporia* has the ability to stimulate or improve the plant-defense mechanisms.

Stimulation of the plants innate immunity systems is a new approach in crop protection (Reglinski et al., 2014). Pcc was applied to soil one week before nematode inoculation to provide enough time for the fungus to establish itself successfully in rhizosphere and cortex. Our results showed that inoculating the plants with both Pcc and nematode would significantly improve the activity of PAL, POX and CAT during the experiment compared with other treatments. The PPO activity in such plants was more than PPO activity in nematode-infected plants only on the 5th and 7th DAI. Inoculating the plants merely with Pcc often resulted in enhanced activity of PAL, POX and CAT compared with these enzymes activity in control treatment. This is the first report on the ability of Pcc in stimulating the plant defense.

M. javanica is exposed to a variety of plant defense responses since most stages of its life cycle occur in their host plant. A rapid and temporary increase in defense-enzymes activity occurs following second stage juveniles (J_2s) penetration, however the activity quickly declines in susceptible hosts (Melillo et al., 2006; Gao et al., 2008). Maintaining the enzymatic activity at higher level during longer time can benefit the host plant in protecting itself. Several biocontrol fungi (Sahenani & Hadavi, 2008; Malek Ziarati et al., 2012; Mostafanezhad et al., 2014) and bacteria (Chen et al., 2010; Siahpoush et al., 2011; Tavakol Norabadi et al., 2014) can enhance the activity of defense-related enzymes and induce plant defense.

At nematode presence, the enzymes activity increased till the 5th DAI and then decreased. The same trends were observed when the plants were inoculated with either Pcc or both Pcc and nematode. The pattern of changes in activity of defense-related plant enzymes in current research are similar to many previous studies in which the enzymes activity increased upto 4th or 5th DAI and then declined (Sahebani & Hadavi, 2008; Chen et al., 2010; Siahpoush et al., 2011; Mostafanezhad et al., 2014).

The results of current study showed that presence of Pcc in rhizosphere or cortex of host plant could elevate the plant defense enzymes activity over a longer time and thus may leading to induced resistance in plants. It can be consequently concluded that Pcc can use both direct (parasitism) and indirect (induce resistance) mechanisms to control *M. javanica*. It means that the fungus could induce the resistance mechanisms at least at the early stages of plant infection by

nematode, and may lessen J2 penetration and establishment of feeding cells. Then the fungus parasitizes the nematode eggs at their emergence. Further studies are required to examine defense-enzyme activity in above-ground parts of the plant after soil application of Pcc to see whether the fungus has the ability of inducing systemic resistance. As well, study the enzymatic changes over the growth period will provide better information on the indirect effect of Pcc in controlling nematode.

ACKNOWLEDGEMENT

This project is supported by the Marvdasht Branch, Islamic Azad University. The financial help are sincerely acknowledged by the authors.

LITERATURE CITED

Agrios, G. N. 2005. Plant Pathology (5th edition). Academic Press, New York, USA, 922 pp.

Almagro, L., Gómez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barceló, A. & Pedreño, M. A. 2009. Class III peroxidases in plant defence reactions. Journal of Experimental Botany, 60: 377-390.

Anderson A. J., Blee, K. A. & Yang, K.-Y. 2006. Commercialization of plant systemic defense activation: Theory, problems and successes. In: Tuzun, S. & Bent, E. (Eds), Multigenic and Induced Systemic Resistance in Plants. Springer Science + Business Media, Dordrecht, Netherlands, pp: 386-414.

Arrieta-Baez, D. & Stark, R. E. 2006. Modeling suberization with peroxidase-catalysed polymerization of hydroxycinnamic acids: cross-coupling and dimerization reactions. Phytochemistry, 67: 743-753.

Bilgin, D. D. 2010. ROS, oxidative stress and engineering resistance in higher plants. In: Gupta, S. D. (Ed), Reactive Oxygen Species and Antioxidants in Higher Plants. CRC Press, Boca Raton, USA, pp. 205-227.

Bordallo, J. J., Lopez-Llorca, L. V., Jansson, H. B., Salinas, J., Persmark, L. & Asensio, L. 2002. Colonization of plant roots by egg-parasitic and nematode-trapping fungi. New Phytologist, 154: 491-499.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

Chen, C., Belanger, R., Benhamou, N. & Paulitz, T. C. 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Phytium aphanidermatum*. Physiological and Molecular Plant Pathology, 56: 13-23.

Chen, F., Wang, M., Zheng, Y., Lou, J., Yang, X. & Wang, X. 2010. Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber *Fusarium* wilt by *Bacillus subtilis* B579. World Journal of Microbiology and Biotechnology, 26: 675-684.

Chitwood, B. G. 1949. Root-knot nematodes – Part 1. A revision of the genus *Meloidogyne* Goeldi, 1887. Proceedings of the Helminthological Society of Washington, 16: 90-104.

Constabel, C. P. & Barbehenn, R. V. 2008. Defensive roles of polyphenol oxidase in plants. In: Schaller, A. (Ed.), Induced Plant Resistance to Herbivory. Springer, New York, USA, pp. 253-269.

Cumagun, C. J. R. & Moosavi, M. R. 2015. Significance of biocontrol agents of phytonematodes. In: Askary, T.H. & Martinelli, P.R.P. (Eds.), Biocontrol Agents of Phytonematodes. CABI Publishing, Wallingford, UK, pp: 50-78.

Davies, K. G. & Spiegel, Y. 2011. Biological control of plant-parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms, Progress in Biological Control 11. Springer Science+Business Media, Dordrecht, Netherlands, 311 pp.

Dubey, R. S. 2010. Metal toxicity, oxidative stress and antioxidative defense system in plants. In: Gupta, S. D. (Ed), Reactive Oxygen Species and Antioxidants in Higher Plants. CRC Press, Boca Raton, USA, pp. 177-203.

El-Beltagi, H. S., Farahat, A. A., Alsayed, A. A. & Mahfoud, N. A. 2012. Response of antioxidant substances and enzymes activities as a defense mechanism against root-knot nematode infection. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40: 132-142.

Fry, S. C. 2004. Oxidative coupling of tyrosine and ferulic acid residues: intra- and extraprotoplasmic occurrence, predominance of trimers and large products, and possible role in inter-polymeric cross-linking. Phytochemistry Reviews, 3: 97-111.

Gao, X., Starr, J., Göbel, C., Engelberth, J., Feussner, I., Tumlinson, J. & Kolomiets, M. 2008. Maize 9-lipoxygenase ZmLOX3 controls development, root-specific expression of defense genes, and resistance to root-knot nematodes. Molecular Plant-Microbe Interactions, 21: 98-109.

Garcion, C., Lamotte, O., Cacas, J.-L. and Métraux, J.-P. 2014. Mechanisms of defence to pathogens: biochemistry and physiology. In: Walters, D.R., Newton, A.C. & Lyon, G.D. (Eds.), Induced Resistance for Plant Defense: A Sustainable Approach to Crop Protection, Second Edition. John Wiley & Sons, Oxford, UK, pp. 106-136.

Ghaderi, R., Kashi, L. & Karegar, A. 2012. The Nematodes of Iran, Based on the Published Reports until 2011. Agricultural Training and promotion Publishing, Tehran, 217 pp.

Gupta, S. D. 2010. Reactive Oxygen Species and Antioxidants in Higher Plants. CRC Press, Boca Raton, USA, 362 pp.

Hussey, R. S. & Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter, 57: 1025-1028.

Jackson, P. A. P., Galinha, C. I. R., Pereira, C. S., Fortunato, A., Soares, N. C., Amanqio, C. B. Q. & Ricardo, C. P. 2001. Rapid deposition of extension during the elicitation of grapevine callus cultures specifically catalysed by a 40-kilodalton peroxidase. Plant Physiology, 127: 1065-1076.

Kato, M. & Shimizu, S. 1987. Chlorophyll Metabolism in Higher Plants. VII. Chlorophyll Degradation in Senescing Tobacco Leaves; Phenolic-dependent Peroxidative Degradation. Canadian Journal of Botany, 65: 729-735.

MacDonald, M. J. & D'Cunha, G. B. 2007. A modern view of phenylalanine ammonia lyase. Biochemistry and Cell Biology, 85: 273-282.

Macia-Vicente, J. G., Jansson, H. B., Talbot, N. J. & Lopez-Llorca, L. V. 2009. Real-time PCR quantification and live-cell imaging of endophytic colonization of barley (*Hordeum vulgare*) roots by *Fusarium equiseti* and *Pochonia chlamydosporia*. New Phytologist 182: 213-228.

Malek Ziarati, H., Sahebani, N. & Etebarian, H. R. 2012. Biological Control and Systemic Induction of Peroxidase and Polyphenoloxidase by *Trichoderma harzianum* in Tomato Plants Infected with Nematode *Meloidogyne javanica*. Biological Control of Pests and Plant Diseases, 1: 61-72.

Manzanilla-López, R. H., Esteves, I., Finetti-Sialer, M. M., Hirsch, P. R., Ward, E., Devonshire, J. & Hidalgo-Díaz, L. 2013. *Pochonia chlamydosporia*: Advances and challenges to improve its performance as a biological control agent of sedentary endo-parasitic nematodes. Journal of Nematology, 45: 1-7.

Melillo, M. T., Leonetti, P., Bongiovanni, M., Castagnone-Sereno, P. & Bleve-Zacheo, T. 2006. Modulation of reactive oxygen species activities and H_2O_2 accumulation during compatible and incompatible tomato–root-knot nematode interactions. New Phytologist, 170: 501-512.

Mohammadi, M. & Kazemi, H. 2002. Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Science, 162: 491-498.

Moosavi, M. R. 2012. Nematicidal effect of some herbal powders and their aqueous extracts against *Meloidogyne javanica*. Nematropica, 42: 48-56.

Moosavi, M. R. & Askary, T. H. 2015. Nematophagous fungi- Commercialization. In: Askary, T.H.& Martinelli, P.R.P. (Eds.), Biocontrol Agents of Phytonematodes. CABI Publishing, Wallingford, UK, pp: 187-202.

Moosavi, M. R. & Zare, R. 2012. Fungi as biological control agents of plant-parasitic nematodes. In: Merillon, J.M. & Ramawat, K.G. (Eds.), Plant Defence: Biological Control. Progress in Biological Control 12. Springer Science + Business Media, Dordrecht, Netherlands, pp: 67-107.

Moosavi, M. R. & Zare, R. 2015. Factors affecting commercial success of biocontrol agents of phytonematodes. In: Askary, T.H. & Martinelli, P.R.P. (Eds.), Biocontrol Agents of Phytonematodes. CABI Publishing, Wallingford, UK, pp: 423-445.

Moosavi, M. R., Zare, R., Zamanizadeh, H. R. & Fatemy, S. 2010. Pathogenicity of *Pochonia* species on eggs of *Meloidogyne javanica*. Journal of Invertebrate Pathology, 104: 125-133.

Moosavi, M. R., Zare, R., Zamanizadeh, H. R. & Fatemy, S. 2011. Pathogenicity of *Verticillium epiphytum* isolates against *Meloidogyne javanica*. International Journal of Pest Management, 57: 291-297.

Mostafanezhad, H., Sahebani, N. & Nourinejhad Zarghani, Sh. 2014. Control of root-knot nematode (Meloidogyne javanica) with combination of *Arthrobotrys oligospora* and salicylic acid and study of some plant defense responses, Biocontrol Science and Technology, 24: 203-215.

Ochoa-Alejo, N. & Gomez-Peralta, J. E. 1993. Activity of enzymes involved in capsaicin biosynthesis in callus tissue and fruits of chili pepper (*Capsicum annuum* L.). Journal of Plant Physiology, 141: 147-152.

Passardi, F., Cosio, C., Penel, C. & Dunand, C. 2005. Peroxidases have more functions than a Swiss army knife. Plant Cell Reports, 24: 255-265.

Perry, R. N., Moens, M. & Starr, J. L. 2009. Root-Knot Nematodes. CABI Publishing, Wallingford, UK, 447 pp.

Pieterse, C. M. J., Poelman, E. H., Van Wees, S. C. M. & Dicke, M. 2013. Induced plant responses to microbes and insects. Frontiers in Plant Science, 4: 475. doi:10.3389/fpls.2013.00475

Pourjam, E., Kamali, N. & Sahebani, N. 2015. Elicitation of defense responses in tomato against *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* wilt complex. Journal of Crop Protection, 4: 29-38.

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. & Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protection, 20: 1-11.

Reglinski, T., Dann, E. & Deverall, B. 2014. Implementation of Induced Resistance for Crop Protection. In: Walters, D.R., Newton, A.C. & Lyon, G.D. (Eds.), Induced Resistance for Plant Defense: A Sustainable Approach to Crop Protection, Second Edition. John Wiley & Sons, Oxford, UK, pp. 249-200.

Sahebani, N. & Hadavi, N. 2008. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Soil Biology & Biochemistry, 40: 2016-2020.

SAS. 1990. SAS/STAT user's version 6, 4th edition. Vol. 1 & 2. Cary: SAS Institute Inc.

Schweikert, C., Liszkay, A. & Schopfer, P. 2000. Scission of polysaccharides by peroxidase-generated hydroxyl radicals. Phytochemistry, 53: 565-570.

Sharon, E., Chet, I. & Spiegel, Y. 2011. *Trichoderma* as a biological control agent, In: Davies, K. & Spiegel, Y. (Eds.), Biological Control of Plant-Parasitic Nematodes: Building Coherence between Microbial Ecology and Molecular Mechanisms, Progress in Biological Control 11. Springer Science+Business Media, Dordrecht, Netherlands, pp. 183-201.

Sholevarfard, A. R. & Moosavi, M. R. 2015. The potential of separate and combined application of some plant extracts and defense inducer molecules for controlling *Meloidogyne javanica*. Nematropica, 45: 82-91.

Siahpoush, S., Sahebani, N. & Aminian, H. 2011. Change of some defense compounds of cucumber treated with *Bacillus cereus* and salicylic acid against *Meloidogyne javanica*. African Journal of Plant Science, 5: 829-834.

Tavakol Norabadi, M., Sahebani, N. & Etebarian, H. R. 2014. Biological control of root-knot nematode (*Meloidogyne javanica*) disease by *Pseudomonas fluorescens* (Chao). Archives of Phytopathology and Plant Protection, 47: 615-621.

Tran, L. T., Taylor, J. S. & Constabel, C. P. 2012. The polyphenol oxidase gene family in land plants: Lineage-specific duplication and expansion. Genomics, 13: 395 (12 pages).

Walters, D. R. 2011a. Sounding the alarm: Signaling and communication in plant defense. In: Walters, D.R. (Ed.), Plant Defense: Warding off Attack by Pathogens, Herbivores, and Parasitic Plants. Blackwell Publishing, Oxford, UK, pp. 77-124.

Walters, D. R. 2011b. What Defenses Do Plants Use? In: Walters, D.R. (Ed.), Plant Defense: Warding off Attack by Pathogens, Herbivores, and Parasitic Plants. Blackwell Publishing, Oxford, UK, pp. 15-76.

Walters, D. R. & Bennett, A. E. 2014. Microbial induction of resistance to pathogens. . In: Walters, D.R., Newton, A.C. & Lyon, G.D. (Eds.), Induced Resistance for Plant Defense: A Sustainable Approach to Crop Protection, Second Edition. John Wiley & Sons, Oxford, UK, pp. 149-170.

Zacheo, G., Bleve-Zacheo, T. & Melillo, M. T. 1997. Biochemistry of plant defence responses to nematode infection. In: Fenoll, C., Grundler, F.M.W. & Ohl, S.A. (Eds.), Cellular and Molecular Aspects of Plant-Nematode Interactions. Springer Science+Business Media, Dordrecht, Netherlands, pp. 201-213.

Zare, R. & Gams, W. 2004. A monograph of *Verticillium* section Prostrata. Rostaniha, 3: 1-188.

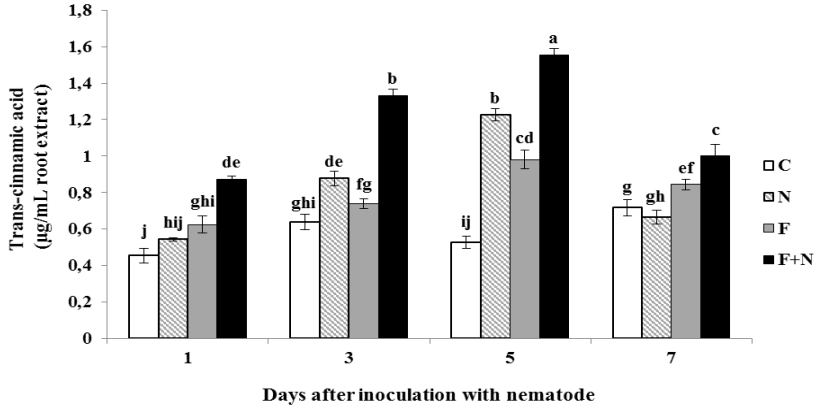

Figure 1. Effect of *Pochonia chlamydosporia* var. *chlamydosporia* (F) separately and in combination with *Meloidogyne javanica* (N) on the activity of phenylalanine ammonia lyase in the roots of zucchini in comparison with control (C) plants. PAL specific activity was estimated by releasing of trans-cinnamic acid from phenylalanine. Same letters above bars (mean ± SE) indicate no statistical significance ($P < 0.05$).

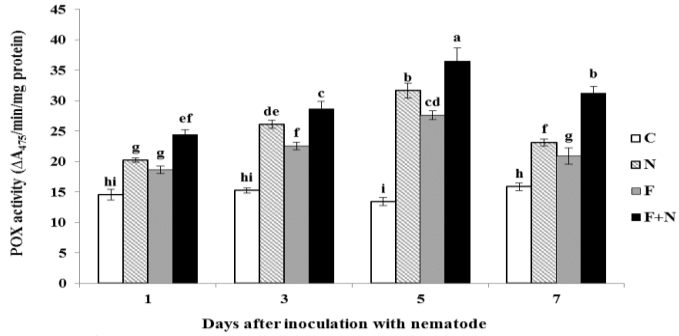

Figure 2. Activity of peroxidase (POX) in the roots of zucchini plants inoculated with either one or both of *P. chlamydosporia* var. *chlamydosporia* (F) and *M. javanica* (N) compared with control (C) plants. Same letters above bars (mean ± SE) indicate no statistical significance ($P < 0.05$).

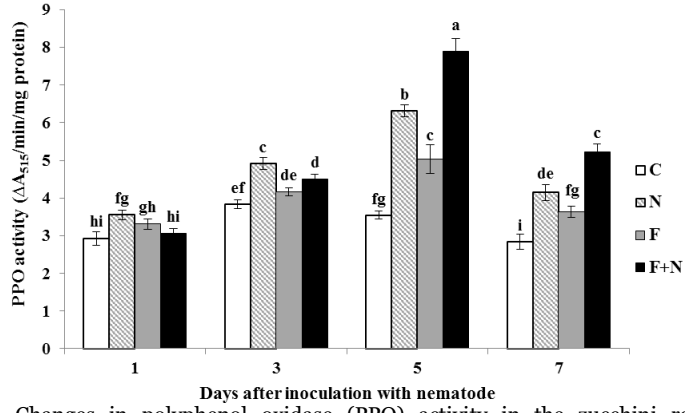

Figure 3. Changes in polyphenol oxidase (PPO) activity in the zucchini roots after inoculation with *P. chlamydosporia* var. *chlamydosporia* (F) and *M. javanica* (N) or without inoculation (C). Same letters above bars (mean ± SE) indicate no statistical significance ($P < 0.05$).

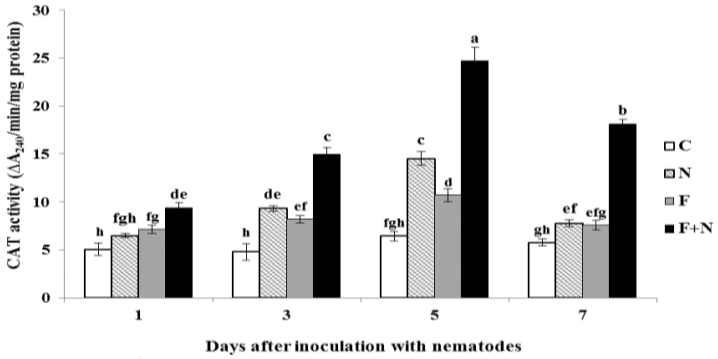

Figure 4. Activity of catalase in the roots of zucchini plants inoculated with *P. chlamydosporia* var. *chlamydosporia* (F) and *M. javanica* (N) or non-inoculated plants (C). Columns with unlike letters above their bars (mean ± SE) are significantly different ($P < 0.05$).