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Quantitative changes of chitinase and ß 1, 3 glucanase in cucumber roots pre-colonized by VAM fungus against *Meloidogyne incognita*

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Abstract

Chitinase and ß 1, 3 glucanase activities in susceptible and tolerant cucumber roots pre-colonized with *Funneliformis mosseae* against root knot-nematode *Meloidogyne incognita* were studied. Mycorrhizal plants which pre-colonized for 7 weeks inoculated with 1500 J_2 per 1 kg soil. The quantitative activity of chitinase and ß 1, 3 glucanase enzymes was assessed on 2, 4, 6 and 8th days after *M. incognita* inoculation based on split- plot in time design. Also the results showed that AMF pre-inoculation caused a significant decrease in RKN pathogenicity factors (number of galls, eggs, egg sacs and J_2) in both tolerant and susceptible cultivars. Inoculation of susceptible roots with AMF significantly reduced the nematode pathogenicity factors. Also the results indicated that the activity of both enzymes increased in the plant with AMF compared with the cucumber roots inoculated with *M. incognita* alone. Cucumber mycorrhizal roots showed the highest mean activity of chitinase and ß 1, 3 glucanase respectively on the 4th and 6th days after M.i inoculation. Preinoculation of AMF increased significantly the activity of both enzymes in cucumber cultivars inoculated with nematode. Although, the level of chitinase and ß 1, 3 glucanase enzymes in tolerant cucumber was significantly higher than susceptible on different days. We concluded that 'increasing cucumber tolerance to root-knot nematode can be related to involvement of chitinase and beta-1,3-glucanase. So vesicular arbuscular mycorrhizae can be considered as a suitable option for biocontrol of the root-knot nematodes.

Key words: Chitinase, ß 1, 3 glucanase, *Funneliformis mosseae, Meloidogyne incognita, Cucumis sativus*

The cucumber (*Cucumis sativus*), is a widely cultivated plant in the gourd family, Cucurbitaceae which 75000 ha of Iran's agricultural land is under cultivation of cucumbers and has an average production of 300 tons per hectare. Greenhouse production has a total area of 5800 ha and has a total of 1454218 tons of production (FAO, 2015). Root-knot nematodes (RKN), *Meloidogyne* spp. are one of the most important plant parasitic nematodes in the world which has a wide range of hosts (Hussey & Janssen, 2002; Oka *et al*., 2000). Damage caused by these pathogens includes weakening, formation of galls and preventing root growth. RKN alter the vascular system and disrupt the transfer of food from the soil (Vovlas *et al*., 2005). The physiological changes occurring throughout the host plant and it has been reported to cause an annual loss of $ 547.5 million in cucurbits (Jain *et al*., 2007). Due to the geographical expansion, host domain and the importance of RKN, their control is inevitable. Use of resistant plant prevent or restrict the reproduction of the nematode by activating defense mechanisms that have been able to restrict the penetration of the second-generation larvae of the nematode or prevent

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the formation of a nutrition site for the nematode and its reproduction (Silva *et al*., 2013). Recently the use of biological agents for control of plant-parasitic nematodes has been investigated (Nguyen *et al*., 2007). The use of biocontrol organisms, such as arbuscular mycorrhizal fungi (AMF) is an environmentally favorable alternative to manage plant parasitic nematodes (Bajaj *et al*., 2015; 2017). AMFs are principal elements of the soil microflora which form a mutualistic symbiosis with most plant species. AMFs have been involved in increasing the availability and uptake of soil phosphorus and trace elements, therewith elevating host plant growth (Ceballos *et al*., 2013; Hart *et al*., 2014). They directly limit the growth of the pathogen by creating a physical barrier on the root or producing substances, such as antibiotics and other compounds, and thus increase plant resistance. AMFs increase the antioxidant activity in plants (Baslam & Goicoechea, 2012). AMFs and RKN compete with each other for the same site in rhizosphere of host plants (Elsen *et al*., 2003; De la Pena *et al*., 2006). Reduce the severity of disease caused by *Pratylenchus* and *Meloidogyne* is reported by the mycorrhiza symbiosis (Li *et al*., 2006; De la Pena *et al*., 2006). Studies have shown that among arbuscular mycorrhizal fungi, *Glomus intraradices*, *G. etunicatum* and *Funneliformis mosseae* are able to reduce root-knot nematodes damage (Hol & Cook, 2005). Zhang *et al*., (2008) observed that inoculation cucumber plants with three species of AMF, *Glomus mosseae*, *Glomus intraradices* and *Glomus versiforme* were reduced number of galls and eggs in roots. Also Elsen *et al*., (2003a, b) reported that arbuscular mycorrhizal fungi decreased *Radopholus similis* populations in roots of different genotypes of banana. Tomato roots inoculated with *Funneliformis coronatus* stimulates plant growth and significantly reduced root-knot nematode infection (Diedhiou *et al*., 2003). Studies showed that the populations of *Radopholus similis* were reduced in soils with mycorrhizal fungi (Elsen *et al*., 2001). Reduced nematode disease because of mycorrhizal fungi has been shown in many studies (Elsen *et al*., 2001; Li *et al*., 2006; De la Pena *et al*., 2006; Rumbos *et al*., 2009). Hence, AM fungi can be used as biocontrol agents to reduce infestation by root-knot nematodes (Wani *et al*., 2017). AMF colonized plants show enhanced production of defence-compounds such as phenolics (López-Ráez *et al*., 2010), β-1, 3-glucanase (Pozo *et al*., 1999) and chitinolytic enzymes (Benhamou *et al*., 1994). In various studies it has been referred to increase chitinase level in AMF plants (Lambais & Mehdy, 1995; Pozo *et al*., 1996; 2002; Spanu *et al*., 1989). This issue has been confirmed in the control of *M. incognita* nematode by the *Glomus versiforme* in grapevine (Li *et al*., 2006). Chitinases, glucanase and protease are considered as the most important enzymes for biological control of plant parasitic nematodes (PPN) (Sharon *et al*., 2001; Safari-Motlagh & Samimi, 2013). Chitinase was an important toxicity factor in biological management of root-knot nematodes, because the nematode eggshell and the cuticle are composed of a chitin layer, and can be degraded by chitinases (Chen *et al*., 2015; Jung *et al*., 2002). Greenhouse and field experiments demonstrated defensive effects against PPN by AMF in plants such as banana, coffee and tomato (Calvet *et al*., 2001; Vos *et al*., 2012; Alban *et al*., 2013; Koffi *et al*., 2013). In view of the above studies, the aim of this study was to evaluate chitinase and ß 1, 3 glucanase activities in susceptible and tolerant cucumber roots pre-colonized with *Funneliformis mosseae* against root-knot nematode *Meloidogyne incognita*.

Materials and Methods

Preparation of Nematode and VAM inoculum: A pure isolate *M. incognita* race 1 was obtained from the Department of Plant Protection, Guilan University. In order to replicate the nematode population, a susceptible tomato cultivar (Early Urbana Y) were prepared

from Falat agricultural company in Iran. Tomato seeds were surface-sterilized in 10% sodium hypochlorite (NaOCl) for 2 minutes and then washed several times with distilled water. Then the seeds were cultured in pots with a mixture of soil and sand (2:1) and were irrigated every day. After four leaflets 4 holes to a depth of approximately 3 cm created around the plant crown then 4 egg sacs placed in the holes and were covered with soil and then watered. Pots were kept for 2 months in ideal greenhouse conditions (temperature 25-27 ° C). Inoculum of *F. mosseae* which consisted of spores, hyphae, colonized root fragments and potting soil was obtained from Zist Fanavar Tooran Company in Shahrood, Iran.

Evaluation of enzyme activity: In this assay we used of tolerance (Superdominos) and susceptible (Danito) cultivars to *M. incognita* which has been studied in previous research (Sadegh Mousavi *et al.*, 2006). Cucumber seeds were cultured as described above in 2.1 sections. This experiment was performed in a split-plot in time design with four treatments and four replications for each treatment. Treatments were as follows: plants without *F. mosseae* and *M. incognita* (control), plants inoculated with *M. incognita* (Mi), plants inoculated with *F. mosseae* and plants inoculated with *M. incognita* + *F. mosseae* (Mi + Fm). Mycorrhizal plants were mixed with 75 g (3750 spores) of the Fm inoculum per kg soil. The twice autoclaved inoculum of Fm was added into non-mycorrhizal plants. After one month, 1500 J_2 of *M. incognita*, per kg soil were inoculated in nematode treatment. Plants were uprooted in 2, 4, 6 and 8 days after inoculation (DAI) with nematodes.

Evaluation of reproductive factors of *M. incognita*: Plants were uprooted 28 days after inoculation (DAI) with nematodes. The average number of galls and egg sacs on roots was evaluated. For this purpose, in the nematode treatments, from each treatment 3 samples (one gram of root) were prepared. And after counting the number of galls by stereomicroscope, the mean was calculated. And by multiplying the average root galls per one gram on total weight of roots, the number of galls in total roots was calculated. Similarly, the average number of egg sac per one gram root and total root system were counted. Five egg-masses per plant were randomly taken using forceps. The egg-masses were obtained based on the procedure of Hussey & Barker (1973). From the total suspension, 1 ml suspension was pippeted on counting slide. The numbers of eggs were counted using stereomicroscope at magnification of 50x. The number of eggs/egg-mass was evaluated. Final nematode population/pot was extracted from soil using a modified Jenkins (1964) technique and from roots by using Hussey & Barker method (1973). From each soil sample, approximately 100 g of soil and 3 g of cucumber roots were used. Oostenbrink's (1966) reproduction factor (R = final nematode population/initial nematode population) is used to measure the reproductive capacity of nematodes Nematode suspensions were adjusted to 10 ml by withdrawing water and then agitated by blowing through a pipette. Immediately, a 1ml of sub sample was transferred to a counting slide. Nematodes enumerated under a microscope then returned to suspension and the process was repeated five times. All counts were multiplied by 10 and averaged to estimate the number of *M. incognita* per gram of roots and 100 g of soil. Data were analyzed by using SAS 9.4 software. Mean comparison of all factors was performed by least significant difference (LSD) at 1% probability level (Table 1).

Preparation of chitinase enzyme extract: Samples (0.5 g) of cucumber root tissue were collected at various times after nematode inoculation and immediately squeezed and crushed using liquid nitrogen. Then, 1 ml of 0.1% sodium phosphate buffer was added and mixed thoroughly. The resulting mixture was immediately transferred to 2 ml microtube and centrifuged at 13000 rpm at 4°C for 20 minutes. The supernatant was kept separate for testing at -20°C (Reuveni, 1995).

Table 1. Mean comparison of pathogenicity factors changes in the susceptible and tolerant roots at 28 days after inoculation with *M. incognita.*

Treatment		No. of nodes/g of root	No. of egg sacs/g of root	No. of eggs/g of root	Number of J_2/100g of soil	RF=Pf/Pi
Superdominos	*M. incognita*	8.75 a	7.5 a	3068.5 a	235.7 a	1.02 a
	M. incognita+ F. mosseae	4.00 b	3.5 b	1452. 5 b	94.0 b	0.47 b
	LSD	3.01	1.29	176.22	49.8	0.92
Danito	*M. incognita*	36.25 a	32.5 a	8347.3 a	487.5 a	2.8 a
	M. incognita + F. mosseae	15.5 b	10.0 b	3031.3 b	207.0 b	1.0 b
	LSD	9.35	9.08	5591.5	292.67	1.93

Data are means of four replications. Data followed by the same letter are not significantly different according to least significant difference (LSD) at 1% probability level

Chitinase assay: One gr of cucumber root tissue was mixed with 3 ml of 1 M sodium acetate buffer (pH 6) and centrifuged at 13000 rpm at 4°C for 15 minutes. The supernatant containing chitinase enzyme was stored at -20° C before testing. 0.3 µl of 1 M sodium acetate buffer (pH 4.7), 1 ml of enzyme solution and 0.2 ml of colloidal chitin were pipetted into eppendorf tubes. After incubation for 2h at 37° C the solution was centrifuged at 12225 g at 6° C for 5 minutes. After centrifugation, 0.75 ml of supernatant and 0.25 ml of nitric salicylic acid solution mixed with 0.7% NaOH and 0.1 ml of 10 M NaOH and were placed for 5 minutes at 100^{o} C (Jin *et al.*, 2005). N-acetyl glucosamine (GlcNac) was used as a standard and then the light absorption at 582 nm is measured (Miller, 1959). The enzyme activity was expressed as µ mol of N acyl glu min^{-1} mg^{-1} of protein.

Preparation of ß-1, 3- glucanase enzyme extract: The total activity of β1, 3 glucanase was assayed by Abels and Forrence (1970) method with a few changes. Some 0.75 g cucumber root tissue was collected at the above mentioned times after nematode inoculation and the samples were immediately extracted with 1.5 ml of 0.05 M sodium acetate (pH 5.0) buffer by grinding at 4° C using a pestle and mortar. The enzyme extract was dialyzed against two changes of water and then two changes of 0.01 M sodium acetate (pH 5.0) buffer overnight. These extracts were used as crude enzyme.

ß-1, 3- glucanase assay: A 30 µl enzyme extract was added to 30 µl 4% laminarin and incubated at 40°C for 10 min. The reaction was stopped by adding 187 µl of dinitrosalicylic reagent (Miller, 1959) and heating for 5 min in a boiling water bath. The resulting colored solution was diluted with 4.5 ml of water vortexes and its absorbance at 500 nm was determined. The blank was the crude enzyme preparation mixed with laminarin with zero time incubation. Enzyme activity was expressed as µmol of equivalent glucose released h^{-1} mg^{-1} of protein.

Results and Discussion

The effect of *F. mosseae* on nematode pathogenicity factors: In present study, pre-inoculation of AMF fungus (*F. mosseae*) decreased the number of galls, eggs and egg sacs compared to non mycorrhizal treatments in both cultivars. The results indicate that AMF fungus can increase tolerance of susceptible cultivar to nematode. Zhang *et al*., (2008) observed that

inoculation cucumber plants with three species of AMF, *G. mosseae*, *G. intraradices* and *G. versiforme* were reduced number of galls and eggs in roots. Also Elsen *et al*., (2003 a, b) reported that AMF fungi decreased *Radopholus similis* populations in roots of different genotypes of banana. Tomato roots inoculated with *F. coronatus* stimulates plant growth and significantly reduced root-knot nematode infection (Diedhiou *et al*., 2003). In this study number of J_2 in soils were lower in the treatment with *M. incognita* added with *G. mosseae* compared to *M. incognita* alone treatment. Dos Anjos *et al*., (2010) found that prior inoculation of AMF before adding nematode inoculum reduced soil infection. Studies showed that the populations of *R. similis* reduced in soils with AMF (Elsen *et al*., 2008). Reduced nematode disease because of mycorrhizal fungi has been shown in many studies (Elsen *et al*., 2001; Li *et al*., 2006; De la Pena *et al*., 2006; Rumbos *et al*., 2009).

Results of Sequential Changes of Chitinase Enzyme: Changes in Chitinase activity in resistant and susceptible cucumber cultivar pre inoculated with *F. mosseae* were studied in response to *M. incognita* race 1. In the roots of tolerant cucumber, the maximum percentage increase of chitinase activity was recorded in plants inoculated with AMF fungus *F. mosseae* (Fm) than uninoculated Fm. In plants inoculated with root-knot nematode (*M. incognita*) alone, the activity of chitinase increased from the 4th day after inoculation (DAI) of nematode and peaked on the 6th DAI and then decreased at 8th DAI so that its amount reached the control plant (Fig. 1A). In tolerant plants (susceptibe) the most level of chitinase was reported in Mi+ Fm, Fm alone, Mi alone and control (without Fm and Mi) plants respectively (Fig. 1A). In the roots of susceptible cucumber, the highest percentage increase of chitinase activity was observed respectively in plants inoculated with both Mi + Fm and plants inoculated with Fm alone. Overall, the highest level of chitinase was reported in Mi+ Fm, Fm alone, Mi alone and control (without Fm and Mi) plants respectively (Fig. 1B). The results showed that plants inoculated with Fm alone, the highest level of enzyme activity was observed at 4 days after inoculation and a decrease was recorded at 6 and 8 days after inoculation. Sequential development of chitinase enzyme in nematode inoculated (Mi alone) susceptible and tolerant cucumber cultivar was similar to control plants (without Mi and Fm). The maximum percentage increase of chitinase activity in AMF plants was observed at 4th days after inoculation and then decreased (Fig. 1A, B). Our results are similar to the results of previous researchers. They demonstrated that mycorrhizal tomatoes, inoculated with *M. javanica* chitinase activity had the highest amount at 4th days after inoculation and had a significant difference with other measurement times (Sohrabi *et al*., 2012). The maximum percentage of chitinase activity was recorded in the Fm inoculated resistant and susceptible cucumber when compared to uninoculated with Fm (Fig. 1A, B). These are in accordance with Sohrabi *et al*., (2012) who stated that the percentage of chitinase enzyme in the control plant (without *G. mosseae* and *G. intraradices* and *M. javanica*) and the inoculated plant with the nematode alone was not significant at all measuring time, and the nematode could not stimulate the chitinase activity alone, but when the *G. mosseae* and *G. intraradices* was present, activation of chitinase significantly increased in the early stages of nematode attack. The previous studies have proven that application of AMF, *G. mosseae*, increase systemic resistance to tomatoes against *M. incognita* and *Pratylenchus penetrans* (Vos *et al*., 2012). It has proved that chitinase enzymes can play a very important role in resistance against pathogens. Chitinase gene is a group of genes responsible for defense responses, and usually after the pathogens attack, the level of gene expression in the plant cells increases (Ahangar *et al*., 2015). The role of chitinase enzyme in controlling pathogens has been shown (Li *et al*., 2006). Chitinase was an important toxicity factor in biocontrol of nematodes, since the nematode eggshell and the cuticle is composed of a chitin layer, and can be degraded by chitinases (Chen *et al*., 2015; Jung *et al*., 2002; 2006).

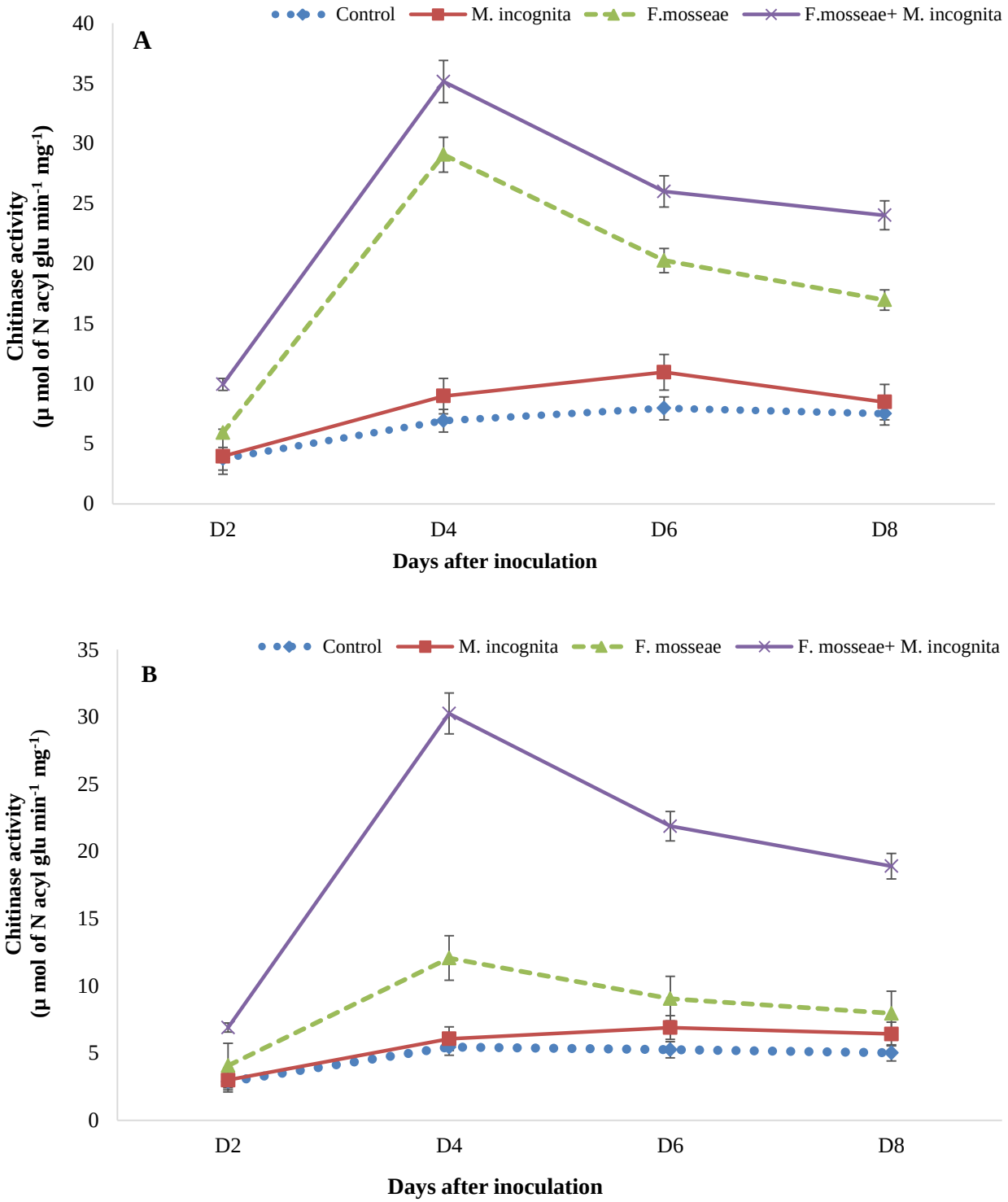

Fig. 1. Changes of chitinase activity at different days after nematode (*M. incognita*) inoculation in roots of cucumber A: tolerant (superdominos) B: susceptible (danito). Each number is mean of 4 replications.

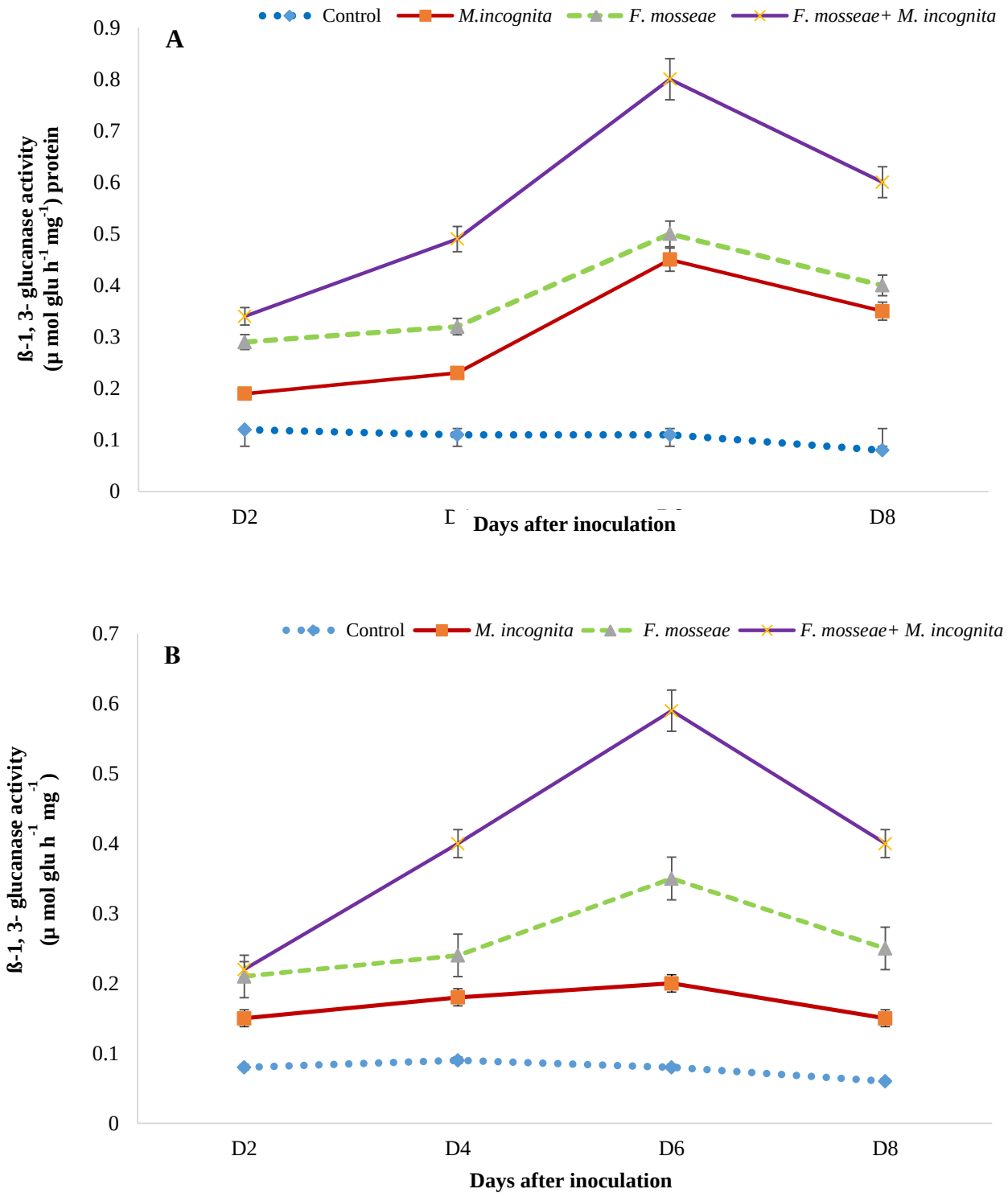

Fig. 2. Changes of ß-1, 3- glucanase activity at different days after nematode (*M. incognita*) inoculation in roots of cucumber A: Tolerant (superdominos); B: susceptible (danito). Each number is mean of 4 replications.

Results of Sequential Changes of β-1, 3-glucanase Enzyme: Changes in ß-1, 3-glucanase activity was studied after inoculation with *M. incognita* race 1 in resistant and susceptible cucumber pre inoculated with *F. mosseae* as shown in Fig. 2. The highest percentage of ß-1, 3-glucanase activity was found on 6th day after inoculation in all treatments (Fig. 2A, B). ß-1, 3-glucanase activity in both tolerant and susceptible cucumber inoculated with Mi alone, increased gradually and reaching its peak on the sixth day and then declining (Fig. 2A). The maximum percentage of ß-1, 3-glucanase activity in susceptible and tolerant cucumber was recorded on the 6th day after inoculation in Mi+Fm inoculated plants (Fig. 2A, B). In the control treatment (without Fm and Mi), the enzyme activity did not change much and was the same as the 2nd day after inoculation (Fig. 2A, B). The activity of β 1-3 glucanase in nematode-inoculated plants were less than plants inoculated with Fm alone (Fig. 2B). Studies have shown that β -1, 3 glucanase enzyme are inducible protein that are produced by the plant in response to pathogens (Kini *et al*., 2000). A study was conducted on resistance and susceptible Alfalfa (*Medicago sativa* L.) to the root-lesion nematode, *Pratylenchus penetrans* and the results showed that the levels of mRNA of β-1, 3 glucanase enzyme were similar in the roots of both susceptible and resistant cultivars, but in the roots of the resistant plant the accumulation of nematodes was much faster than susceptible plants after inoculation (Baldridge *et al*., 1998). β-1, 3 glucanase is able to break down the pathogenic cell walls. β- 1, 3 glucanase causes hydrolysis of related substrates, then releases active biological oligosaccharides (elicitors and suppressors), which regulates the safety of plant tissues (Zinoveva *et al*., 2001). Recently in one study, induction of chitinase and β-1, 3 glucanase activity in resistant and susceptible clones of sugar beet inoculated with *P. zeae* was investigated and the results showed that in resistant clones inoculated with a nematode, chitinase and β-1, 3 glucanase activity increased but in non-inoculated clones decreased (Sundararaj & Kathiresan, 2012).

Conclusion

Our results indicated that pre-colonized roots with *F. mosseae* increased the activity of the β -1, 3 glucanase and chitinase enzyme significantly and also reduced nematode pathogenicity factors. This confirms the positive effect of *F. mosseae* on decreasing nematode damage and increasing tolerance of susceptible plants through activation of defensive enzymes. Comparison of tolerant and susceptible cultivars showed that probably the rate of accumulation of defense enzymes in tolerant cultivar was higher than susceptible cultivar. Therefore, current study implies that control of plant nematodes with the help of AMF will be a safe method for human and environment for managing the root-knot nematode. Overall, AMFs increase tolerance of crops by combined action of defense enzymes against plant parasitic nematodes and can be as a bio protector agent may replace pesticides and fertilizers in the near future.

References

Abeles, F. B. & Forrence, L. E. (1970). Temporal and hormonal control of β-1, 3-glucanase in *Phaseolus vulgaris* L. Plant Physiology, 45, 395-400. doi: 10.1104/pp.45.4.395.

Abeles, F. B., Bosshart, R. P., Forrence, L. E. & Habig, W. E. (1970). Preparation and purification of glucanase and chitinase from bean leaves. *Plant Physiology*, 47, 129-134.

Ahangar, L., Babaeezad, V., Ranjbar, G., Najafizarini, H. & Biabani, A. (2015). Expression profile of defense-related genes in susceptible and resistant wheat cultivars in response to powdery mildew infection. *J. Mod. Genet*, 10**,** 33-46.

Alban, R., Guerrero, R. &Toro, M. (2013). Interactions between a root-knot nematode (*Meloidogyne exigua*) and arbuscular mycorrhizae in coffee plant development (*Coffea arabica*). *American Journal of Plant Sciences*, 4, 19-23.

Bajaj, R., Hu, W., Huang, Y., Chen, S., Prasad, R., Varma, A. & Bushley, K. (2015). The beneficial root endophyte *Piriformospora indica* reduces egg density of the soybean cyst nematode. *Bioll Control*, 90, 193-199.

Bajaj, R., Prasad, R., Varma, A. & Bushley, K. E. (2017). The role of arbuscular mycorrhizal fungi and the mycorrhizal-like fungus *Piriformospora indica* in biocontrol of plant parasitic nematodes. In: *Mycorrhiza* (Ed. by) Varma, A., Prasad, R. & Tuteja, N. Springer International Publishing AG, Cham, pp 43-56.

Baldridge, G. D., O'Neill, N. R. & Samac, D. A. (1998). Alfalfa (*Medicago sativa* L.) resistance to the root-lesion nematode, *Pratylenchus penetrans*: defense-response gene mRNA and isoflavonoid phytoalexin levels in roots. *Plant Mol. Biol*., 38, 999-1010.

Baslam, M. & Goicoechea, N. (2012). Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. *Mycorrhiza*, 22, 347-359.

Benhamou, N., Fortin, J. A., Hamel, C., St-Arnaud, M. & Shatilla, A. (1994). Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum f.sp. chrysanthemi*. *Phytopathology*, 85, 958-968.

Calvet, C., Pinochet, J., Herna´ndez-Dorrego, A., Estau´n, V. & Camprubi´, A. (2001). Field micro plot performance of the peach-almond hybrid GF-677 after inoculation with arbuscular mycorrhizal fungi in a replant soil infested with root-knot nematodes. *Mycorrhiza*, 10, 295-300.

Ceballos, I., Ruiz, M., Fernández, C., Peña, R., Rodriguez, A. & Sanders, I. R. (2013). The *in vitro* mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava. *Plos One*, 8, e70633.

Chen, C., Liu, S., Liu, Q., Niu, J., Liu, P. & Zhao, J. (2015). An ANNEXIN-like protein from the cereal cyst nematode *Heterodera avenae* suppresses plant defense. *PLOS ONE*, 10, e0122256. https://doi.org/10.1371/journal.pone.0122256

De la Pena, E., Rodriguez-Echevarria, S., van der Putten, W. H., Freitas, H. & Moens, M. (2006). Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass, *Ammophila arenaria*. *New Phytol*., 169, 829-840.

Diedhiou, P. M., Hallmann, J., Oerke, E. C. & Dehne, H. W. (2003). Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation on tomato. *Mycorrhiza*, 13, 199-204.

Dos Anjos, E. C. T., Cavalcante, U. M. T., Gonçalves, D. M. C., Pedrosa, E. M. R., dos Santos, V. F. & Maia, L. C. (2010). Interactions between an arbuscular mycorrhizal fungus (*Scutellospora heterogama*) and the root-knot nematode (*Meloidogyne incognita*) on sweet passion fruit (*Passiflra alata*). *Braz Arch Biol Technol*., 53, 801-809.

Elsen, A., Baimey, H., Swennen, R. & De Waele, D. (2003a). Relative mycorrhizal dependency and mycorrhiza mycorrhiza nematode interaction in banana cultivars (*Musa* spp.) differing in nematode susceptibility. *Plant and Soil*, 256, 303-313.

Elsen, A., Beeterens, R., Swennen, R. & De Waele, D. (2003b). Effects of an arbuscular mycorrhizal fungus and two plant-parasitic nematodes on *Musa* genotypes differing in root morphology. *Biology and Fertility of Soils*, 38, 367-376.

Elsen, A., Declerck, S. & De Waele, D. (2001). Effects of *Glomus intraradices* on the reproduction of the burrowing nematode (*Radopholus similis*) in dixenic culture. *Mycorrhiza*, 11, 49-51.

Elsen, A., Gervacio, D., Swennen, R. & De Waele, D. (2008). AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp. a systemic effect. *Mycorrhiza*, 18, 251-256.

FAO. (2015). Major Crops – By Countries/Regions, Rankings; Choose

Cucumber and Gherkins, World. Food and Agricultural Organization, FAOSTAT.

Hart, M., Ehret, D. L., Krumbein, A., Leung, C., Murch, S., Turi, C. & Franken, P. (2014). Inoculation with arbuscular mycorrhizal fungi improves the nutritional value of tomatoes. *Mycorrhiza*, 25, 359-376.

Hol, W. H. G. & Cook, R. (2005). An overview of arbuscular mycorrhizal fungi–nematode interactions. *Basic and Applied Ecology*, 6, 489-503.

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

Hussey, R. S. & Janssen, G. J. W. (2002). Root-knot nematodes: *Meloidogyne* species In: Plant resistance to parasitic nematodes. (Ed. by) Starr, J. L., Cook, R. & Bridge, J. CAB International, Wallingford, UK, pp. 43-70.

Jain, R. K., Mathur, K. N. & Singh, R. V. (2007). Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian Journal of Nematology*, 37, 219-220.

Jenkins, W. R. (1964). A rapid centrifugel flotation technique for separating nematodes from soil. *Plant Diseases*, 48, 692.

Jin, R. D., Suh, J. W., Park, R. D., Kim, Y. W., Krishnan, H. B. & Kim, K.Y. (2005) Effect of chitin compost and broth on biological control of *Meloidogyne incognita* on tomato (*Lycopersicom esculentum* Mill.). *Nematology*, 7, 125-132. https://doi.org/ 10.1163/1568541054192171

Jung, W. J., Jung, S. J., An, K. N., Jin, Y. L., Park, R. D., Kim, Y. K., Shon, B. K. & Kim, T. H. (2002). Effect of chitinase-producing *Paenibacillus illinoisensis* KJA-424 on egg hatching of root-knot nematode (*Meloidogyne incognita*). *Journal of Microbial Biotechnology*, 12, 865871.

Jung, W. J., Kuk, J. H., Kim, K. Y., Jung, K. C. & Park, R. D. (2006). Purification and characterization of exo-B-D-glocosamidinase from *Aspergillus fumigatus* S-26. *Protein Expr. Purif*., 45, 125-131.

Kini, K. R., Vasanthi, N. & Shetty, H. S. (2000). Induction of ß-1, 3-glucanase in seedlings of pearl millet in response to infection by *Sclerospora graminicola*. *European Journal of Plant Pathology*, 106, 267-274.

Koffi, M. C., Vos, C., Draye, X. & Declerck, S. (2013). Effects of *Rhizophagus irregularis* MUCL 41833 on the reproduction of *Radopholus similis* in banana plantlets grown under in vitro culture conditions. *Mycorrhiza*, 23, 279-288.

Lambais, M. R. & Mehdy, M. C. (1995). Differential expression of defense-related genes in arbuscular mycorrhiza. *Canadian Journal of Botany*, 73, 533-540, Supplement 1.

Li, H. Y., Yang, G. D., Shu, H. R., Yang, Y. T., Ye, B. X., Nishida, I. & Zheng, C. C. (2006). Colonization by the arbuscular mycorrhizal fungus *Glomus versiforme* induces a defense response against the root-knot nematode *Meloidogyne incognita* in the grapevine (*Vitis amurensis* Rupr.), which includes transcriptional activation of the class III chitinase gene *VCH3*. *Plant Cell Physiology*, 47, 154-163.

López-Ráez, J. A., Flors, V., García, J. M. & Pozo, M. J. (2010). AM symbiosis alters phenolic acid content in tomato roots. *Plant Signal. Behav*., 5, 1138-1140.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Annual Chemistry*, 31, 426-428.

Nguyen, N. V., Kim, Y. J., Oh, K. T., Jung, W. J. & Park, R. D. (2007). The role of chitinase from *Lecanicillium antillanum* B-3 in parasitism to root-knot nematode *Meloidogyne incognita* eggs. *Biocontrol Science and Technology*, 17, 1047-1058.

Oka, Y., Koltai, H., Bar-Eyal, M., Mor, M., Sharon, E., Chet, I. & Spiegel, Y. (2000). New strategies for the control of plant parasitic nematodes. *Pest Management Science*, 56, 983-988.

Oostenbrink, M. (1966). Major characteristics of the relation between nematode and plants. *Medad. Landbouwhogesch. Wageningen*, 66, 1-46.

Pozo, M. J., Azcón-Aguilar, C., Dumas-Gaudot, E. & Barea, J. M. (1999). B-1, 3-glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. *Plant Science*, 141, 149-157.

Pozo, M. J., Cordier, C., Dumas-Gaudot, E., Gianinazzi, S., Barea, J. M. & Azcón-Aguilar, C. (2002). Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. *Journal of Experimental Botany*, 53, 525-534.

Pozo, M. J., Dumas-Gaudot, E., Slezack, S., Cordier, C., Asselin, A., Gianinazzi, S., Gianinazzi-Pearson, V., Azcón-Aguilar, C. & Barea, J. M. (1996). Induction of new chitinase isoforms in tomato roots during interactions with *Glomus mosseae* and/or *Phytophthora nicotianae* var *parasitica*. *Agronomie*, 16, 689-697. DOI: 10.1051/agro:19961014

Reuveni, R. (1995). Biochemical markers as tools for screening resistance against plant pathogens, In: Novel Approaches to Integrated Pest Management (Ed. by) Reuveni, R. CRC Press, Boca Raton, FL, pp. 21-45.

Rumbos, C. H., Reimann, S., Kiewnick, S. & Richard, A. (2009). Interactions of *Paecilomyces lilacinus* strain 251 with the mycorrhizal fungus *Glomus intraradices*: Implications for *Meloidogyne incognita* control on tomato. *Biocontrol Science and Technology*, 16, 981-986.

Sadegh- Mousavi, S., Karegar, A. & Deljoo, A. (2006). Responses of some common cucumber cultivars in Iran to root-knot nematode, *Meloidogyne incognita*, under greenhouse condition. *Iranian Journal of Plant Pathology*, 42, 241-252.

Safari-Motlagh, M. R. & Samimi, Z. (2013). Evaluation of *Trichoderma* spp. as biological agents in some of plant pathogens. *Annals of Biological Research*, 4, 173-179.

Sharon, E., Bar Eyal, M., Chet, I., Herrera, E., Strella, A., Klelfeld, O. & Splege, Y. (2001). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology, 91, 687-693.

Silva, R. V., Oliveira, R. D. L., Ferreira, P. S., Ferreira, A. O. & Rodrigues, F. A. (2013). Defense responses to *Meloidogyne exigua* in resistant coffee cultivar and non-host plant. *Tropical Plant Pathology*, 38, 114-121. DOI: 10.1590/S1982-56762013000200004

Sohrabi, F., Fadaei-Tehrani, A. A., Rezaee Danesh, Y. & Jamalli-Zavareh, A. (2012). Study on interaction between arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) and root-knot nematode (*Meloidogyne javanica*) in tomato. *Journal of Plant Pathology*, 48, 131-134.

Spanu, P., Boller, T., Ludwig, A., Wiemken, A., Faccioa, A. & Bonfante-Fasolo, P. (1989). Chitinase in roots of Mycorrhizal *Allium Porrum* regulation and localization. *Planta*, 177, 447-550.

Sundararaj, P. & Kathiresan, T. (2012). Induction of ß-1, 3-glucanase and chitinase activities in resistant and susceptible sugarcane clones inoculated with *Pratylenchus zeae*. *International Journal of Nematology*, 22, 47-56.

Vos, C., Schouteden, N., van Tuinen, D., Chatagnier, O., Elsen, A. & De Waele, D. (2013). Mycorrhiza-induced resistance against the root-knot nematode *Meloidogyne incognita* involves priming of defense gene responses in tomato. *Soil Biology Biochemistry*, 60, 45-54.

Vos, M., Tesfahun, A. N., Panis, B., De Waele, D. & Elsen, A. (2012) Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode *Meloidogyne incognita* and the migratory nematode *Pratylenchus penetrans*. *Applied Soil Ecology*, 61, 1-6.

Vovlas, N., Mifsud, D., Landa, B. Vb. & Castillo, P. (2005). Pathogenicity of the root-knot nematode *Meloidogyne javanica* on potato. *Plant Pathology*, 54, 657-664.

Wani, K. A., Manzoor, J., Shuab, R. & Lone, L. (2017). Mycorrhiza - Nutrient Uptake, *Biocontrol, Ecorestoration*, Springer International Publishing AG.

Zhang, L., Zhang, J. & Christie, P. (2008). Pre-inoculation with arbuscular mycorrhizal fungi suppresses root-knot nematode (*Meloidogyne incognita*) on cucumber (*Cucumis sativus*). *Biol Fertil Soils*, 45, 205-211.

Zinoveva, S. V., Vasyukova, N. I., Udalova, Zh. V. & Ozeretskovskaya, O. L. (2001). PR Proteins in Plants Infested with the root-knot nematode *Meloidogyne incognita* (Kofoid et White, 1919) Chitwood 1949, *Dokl. Biol. Sci*., 379, 393-396.