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SOIL AMENDMENT WITH OAK DEBRIS ENHANCES THE BIOLOGICAL ACTIVITY OF *METARHIZIUM ANISOPLIAE*, AGAINST *MELOIDOGYNE JAVANICA*

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ABSTRACT

Biocontrol potential of *Metarhizium anisopliae* on some phytonematodes has been reported earlier. Considering the importance of organic soil amendments as well as the use of bioagents in sustainable agriculture, this research aimed to reduce the pesticide use in control of root-knot nematode, *Meloidogyne javanica* in tomato. Three separate experiments were performed to determine the effects of *M. anisopliae* IMI 330189 and different levels of oak tree debris on *M. javanica*. In the first and the second experiments, the effective method of application of *M. anisopliae* IMI 330189 and the effect of different rates of debris of oak trees were evaluated, respectively. In third experiment which was replicated two times, the combination effects of both of the factors, the fungus as well as the different rates of soil amendments were determined. Pots were filled with pasteurized soil mixture and the six leaves tomato seedlings were inoculated with 3000 second stage larvae of *M. javanica*/kg of soil. Eight weeks after treatment, plant growth and nematode reproduction factors were compared. Based on the results of the first experiment, soil drench application of *M. anisopliae* IMI 330189 suspension was selected for use in the other treatment. In the second experiment, statistical analysis revealed that, those inoculated tomato plants which were cultivated in pots with 150g oak debris/kg soil, received the minimum nematode damage. Both replicates of experiment three had the same results. In both runs of the experiment three, combination of *M. anisopliae* IMI 330189 and oak debris at the rates of 100 and 150g/kg soil, were the best treatments, which caused more than 90% reduction in reproduction factor of nematode ($P \leq 0.05$). As compared to control, reductions in number of galls were 55%, 50% and 76%, in experiments 1, 2 and 3 respectively. It was 71%, 72% and 86% for nematode reproduction factor, showing the significance of combined effect of both tested agents.

Keywords: Biological control, nematode management, organic soil, *Quercus branti*, root knot nematode, soil amendment

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular, widely grown and the second most important vegetable crop, after potatoes. Iran is the sixth top producer of tomato in the world with an approximate production of 6000000 tons per year (FAOSTAT, 2015). Cultivation of off-season tomato is an important source of income in Iran, especially for smallholder farmers. Nematodes have been identified as one of the major pests affecting tomato production throughout the world, particularly, in tropical and subtropical regions (Kunwar *et al.,* 2015). Over 60 different species of plant parasitic nematodes, representing 19 genera, affect tomato that in between, the most destructive nematodes are the genus *Meloidogyne* Göldi (Norman, 1992), the root-knot nematodes. Most widespread and devastating species of this genus are *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood. These species can cause complete crop loss under adverse growing conditions (Kunwar *et al.,* 2015).

There are several potential methods for management of the root knot nematodes. These methods are grouped into two main categories, chemical and non-chemical. Although the chemicals are widely used against the phytonematodes, but because of hazardous effects of these compounds on non-target organisms and on the environment, there

is a need to develop other control strategies (Akhtar and Malik, 2000; Anastasiadis *et al.,* 2008). The economic costs of investigation and registration are a major obstacle for producing a new nematicide (Chitwood, 2002). Nowadays, non-chemical measures are widely used to control the plant parasitic nematodes. Biocontrol of phytonematodes is one of the most important approaches in nematode management and moving towards a sustainable agriculture (Mokhtari *et al*., 2009). There are some soil inhabiting fungi that has biocontrol potential on phytonematodes, which can be used in nematode management program (Tian *et al*., 2007). Green muscardine, *M. anisopliae*, is a soil dwelling fungus with worldwide distribution. It first reported as *Entomophthora anisopliae* by Metschnikoff (1879), then renamed to *M. anisopliae* by Sorokin (1883). This is one of the most important entomopathogenic fungi with unique characteristics, hence widely used in insect control program (Richards and Rogers, 1990; Medonica, 1992). The fungus has mass growth on artificial culture media and produces abundant conidia, but the conidia only germinates in contact with their host (Farashiani *et al*., 2011). There are little reports on impact of *M. anisopliae* on nematodes. The effect of this fungus against *Rotylenchulus reniformis* was investigated by Tribhuvaneshwar *et al.* (2008). They have reported that application of this bioagent was

reduced the final population of this plant parasitic nematode as well as some species of free-living nematodes. Based on their reports, the growth of infected tomato plants has been improved after application of *M. anisopliae*. In a survey in Boyer-Ahmad region in Iran, some naturally infected nematodes to *M. anisopliae* were observed (Ghayedi and Abdollahi, 2013). They purified the isolated fungus and also they showed the biocontrol potential of the isolate on *Heterodera avenae*, with a power of 47.1% of larval mortality. Biocontrol potential of *M. anisopliae* against some species of root knot nematodes has been shown (Jahanbazian *et al.,* 2014b; Khosrawi *et al.,* 2014; Jahanbazian *et al.,* 2015).

Organic soil amendment can improve the fertility as well as the physical properties of soil (Boehm *et al.,* 1993; McSorley and Gallaher, 1995). By adding some plant materials to the soil, the water holding capacity will increase, and subsequently the biological condition of the soil will be improved (Akhtar and Mahmood, 1993). Different kinds of waste materials have been used against plant parasitic nematodes. In a study, combination of wasted papers and chitin has reduced the population density of root-knot nematode (Culbreath *et al.,* 1985). Oka and Yermiyahu (2002) were applied the composted agricultural waste materials to the soil and reduced total number of root knot nematode. Soil composting and application of green and animal manure were also effective

measures in management of soil borne plant pathogens (Hoitink and Boehm, 1999). In such organic amended soils, there are lots of beneficial microorganisms like fungi and bacteria which are able to parasitize or prey on nematodes (Belair and Benoit, 1996; Perry and Wesemael, 2008).

Besides improving the fecundity and physical properties of the soil, some of the soil amendments suppress the nematode population and has nematicidal effects also. Elmi and Abdollahi (2015) successfully used the licorice residue and spent mushroom compost of oyster mushroom (*Pleurotus ostreatus*) against *M. javanica.* There are many plant species in 57 families, which their parts have nematicidal components (Sukul, 1992). Effects of some medicinal plants against phytonematodes have been studied by El-Nagdi and Mansour (2003). In their study, *Jasminum multiflorum* showed more nematicidal activity as compared to *Mimosa pudica*. Oilcakes, sawdust, urea and bagasse were successfully used to control nematodes (Sikora and Fernandez, 2005). Effect of cabbage leaf (Ghazalbash *et al.,* 2011) and root bagasse of *Glycyrrhiza glabra* L. (Abdollahi and Ramezani, 2012) on *M. javanica,* have been approved. The effects of composted sawdust against M. javanica on tomato plant have been studied by Prakash and Singh (2014). Based on their findings, with the application of composted sawdust, especially in the soil containing 30% of soil amendment the plant growth has been enhanced.

Because of great diversity of climate and geographical conditions, more than 700 species of medicinal plants grow in Iran (Sharafzadeh and Alizadeh, 2012). Some of these medicinal plants have nematicidal effects. In Iran, use of nematicidal plants against phytonematodes has been started by Abivardi (1971). In recent years, Ghazalbash and Abdollahi (2011), Abdollahi and Ghazalbash (2012) and Ghazalbash and Abdollahi (2013) have studied the nematicidal as well as the antifungal activity of *Ferulago angulata* and *Zataria multiflora*. According to the latter, application of plant materials from both plants, improved the development of tomato plant. In their study, different parts of these medicinal plants reduced nematode reproduction and gall formation in all treatments that inoculated with *M. javanica* alone and in combination with *F. oxysporum* f.sp. *lycopersici*. The Zagros Mountains in the center and west of Iran is mainly covered by *Quercus branti*, the Iranian oak tree. Antimicrobial effects of oak fruits against bacterial pathogens have been proved (Sadeghian *et al.,* 2012). The effect of Iranian oak tree debris on biological activity of *Pseudomonas fluorescens* and *Trichoderma vierns* against *M. javanica,* has been studied (Moradi *et al.,* 2015). Based on their results, the maximum reduction in reproduction factor was observed in treatments with one or both of the biocontrol agents. The rate of reduction in root galling was more than 56% for treatments

which received *T. virens*, as compared to unamended treatments. Considering the antimicrobial effects of oak trees and the hazards of the chemical pesticides, this research aimed to study the combined effects of debris of oak trees and the biocontrol agent, *M. anisopliae,* on *M. javanica*.

MATERIALS AND METHODS

Preparation of plant debris

Rotten leaves of under canopy of oak trees were collected from the oak forest of Boyer-Ahmad, Kohgiluyeh and Boyer-Ahmad Province, Iran, with geographical coordinates of 30°41'26.35"N 51°35'36.43"E.

Inoculum preparation

Eggs of *M. javanica* were extracted from infected roots of a susceptible tomato plant (*Lycopersicon esculentum* cv. Early Urbana) by grinding in NaOCl solution for three minutes. Second-stage juveniles emerging from eggs were daily collected on a 30 um sieve, stored at 25°C and used in experiments within 5 days (Hussey and Barker, 1973).

Preparation of *Metarhizium anisopliae*

Green Muscle TC$^{®}$, a biopesticide with spores of *M. anisopliae* var *acridum* (IMI 330189), purchased from the market and used in the experiments.

Raising tomato plants

Seeds of susceptible tomato cultivar (Early Urbana) were sown in plastic pots which filled with sterilized soil mixture. Three weeks after germination, uniform healthy seedlings of tomato were selected and transplanted to other

plastic pots containing sterilized sandy clay-loam soil (60% sand: 40% mixture of silt and clay).

Experiment designs

The effects of two methods of application of *M. anisopliae* and the effects of different rates of oak debris on *M. javanica,* as well as the combination effect of oak debris and *M. anisopliae* under glasshouse condition, were studied in three separate pot experiments.

Experiment I: Two methods, soil drench with spore suspension and root dip in spore suspension were compared to investigate the better application method of *M. anisopliae* against *M. javanica*. Two treatments, inoculated with nematode and uninoculated, were used as controls. The study was set up in a completely randomized design with four replicates. Tomato seedlings were transplanted to the main pots. In root dip method, the washed soil-off bare roots of plants were dipped into a suspension of 10^7 spores/ml for 20 min, prior to transplanting. In soil drench treatment, two centimeters of surface soil was pushed aside and 10 ml of a suspension of 10^7 spores/ml was added and then covered with soil (Naserinasab *et al.,* 2011). The better method of application was used for the other experiment.

Experiment II: In this experiment, eight treatments, each with four replicates were set up as follows:

T1: Uninoculated control without oak debris (Control)

T2: Inoculated control without oak debris (N)

T3: Uninoculated, with 50g oak debris/kg soil (OD_{50})

T4: Inoculated, with 50g oak debris/kg soil ($OD_{50} \times N$)

T5: Uninoculated, with 100g oak debris/kg soil (OD_{100})

T6: Inoculated, with 100g oak debris/kg soil ($OD_{100} \times N$)

T7: Uninoculated, with 150g oak debris/kg soil (OD_{150})

T8: Inoculated, with 150g oak debris/kg soil ($OD_{150} \times N$)

To meet the objectives of this study, four soil mixtures that varied in volumetric proportions of sand and oak debris but constant proportion of clay and silt, were prepared. In order to keep the soil texture uniform across all of the treatments, the coarse sand fraction was inversely varied with oak debris fraction (silt, clay, sand+oak debris with a proportion of 20:15:65 v/v, respectively). Plastic pots with 15 cm diameter were filled with one kg of sterile soil mixture. Six-leaf seedlings of tomato were transplanted to the pots and they were inoculated with 3000 J2/kg of soil, at the time of transplanting, then they were placed in a completely randomized arrange in the glasshouse and watered daily. Plants were grown under natural light conditions, relative humidity ranging from 55-68% and temperature ranging from 26-31°C. Eight weeks after inoculation, plants were gently removed from pots and the growth factors of

plant (length and weight of shoot and root) and the reproduction rates of the nematode (No. of galls/root, No. of egg masses/root, No. of J2s/kg soil and reproduction factor) were determined (Jacquet *et al.,* 2005).

Experiment III: In this experiment, 16 treatments, each with four replicates were set up as follows:

T1: Uninoculated control without oak debris, without *Metarhizium* (Control)

T2: Uninoculated control without oak debris, with *Metarhizium* (F)

T3: Inoculated control without oak debris, without *Metarhizium* (N)

T4: Inoculated control without oak debris, with *Metarhizium* (N × F)

T5: Uninoculated, with 50g oak debris/kg soil, without *Metarhizium* (OD_{50})

T6: Uninoculated, with 50g oak debris/kg soil, with *Metarhizium* (OD_{50} × F)

T7: Inoculated, with 50g oak debris/kg soil, without *Metarhizium* (OD_{50} × N)

T8: Inoculated, with 50g oak debris/kg soil, with *Metarhizium* (OD_{50} × N × F)

T9: Uninoculated, with 100g oak debris/kg soil, without *Metarhizium* (OD_{100})

T10: Uninoculated, with 100g oak debris/kg soil, with *Metarhizium* (OD_{100} × F)

T11: Inoculated, with 100g oak debris/kg soil, without *Metarhizium* (OD_{100} × N)

T12: Inoculated, with 100g oak debris/kg soil, with *Metarhizium* (OD_{100} × N × F)

T13: Uninoculated, with 150g oak debris/kg soil, without *Metarhizium* (OD_{150})

T14: Uninoculated, with 150g oak debris/kg soil, with *Metarhizium* (OD_{150} × F)

T15: Inoculated, with 150g oak debris/kg soil, without *Metarhizium* (OD_{150} × N)

T16: Inoculated, with 150g oak debris/kg soil, with *Metarhizium* (OD_{150} × N × F)

In treatments with *M. anisopliae* var *acridum* IMI 330189, two centimeters of surface soil was pushed aside and a suspension of 2 g of biopesticide mixed in 20 ml of sterile distilled water was added to the soil (20 ml of distilled water was used in control treatments) and then covered with soil. For other treatments which were without application of *Metarhizium*, the procedure was same as the experiment II.

Statistical analysis

Data were tested for homogeneity of variance and normal distribution. The collected experimental data were organized and analyzed by using a one-way analysis of variance (ANOVA). General linear model procedures were used to perform the analysis of variance using SPSS 20 for Windows computer software package (SPSS Inc. Chicago, USA). Where F-value was found to be significant, least significant difference (LSD) was used to compare the means at $P \leq 0.05$ levels of significance.

RESULTS

Results of Experiment I

Based on data analysis, both of application methods significantly reduced the rate of nematode reproduction and improved the plant growth. There was no significant difference

between uninoculated control and both of the application methods ($P \leq 0.05$). As compared to control treatment, the rate of decrease in number of galls, egg masses, eggs, J2s and reproduction factor, were 36%, 94%, 360%, 137% and 350%, respectively (Table 1). Because the soil drench application of the tested biopesticide is more practicable, this method has selected for use in experiment III.

Results of Experiment II

Different rates of oak debris had significant effects on growth factors of tomato. Among those treatments that were inoculated with nematode, maximum length as well as dry and fresh weight of shoot and root has been observed in treatments with 150 g oak debris/kg of soil. For shoot and root length and also fresh weight of shoot and dry weight of root the treatment with the use of 150 g oak debris/kg of soil was at par with the treatment by applying 100 g oak debris ($P \leq 0.05$) (Fig. 1). Fig. 2 presents the significant effects of soil amendment on nematode related parameters ($P \leq 0.05$). Any increase in oak debris amount, caused significant and step wise reduction in number of J2s in soil, number of galls and egg masses in root, and nematode reproduction factor. Significant reduction in number of eggs/egg mass was observed only in treatment with 150 g/kg of soil ($P \leq 0.05$). In most cases, there were no significant difference between soil amendment with 100 g of oak debris and both of 50 g and 150 g of oak debris per kg of soil. Effect of oak debris on rates of reduction

in nematode-related factors has been shown in Table 1. For instance, rate of reduction in number of galls were 36%, 81% and 134% for 50 g, 100 g and 150 g of oak debris, respectively.

Results of Experiment III

Results of the both runs of experiment III has been shown in Figs. 3 & 4. Based on the results, by adding oak debris and the bioagent to the soil, fresh weight and length of shoot and root significantly increased in uninoculated treatments, which they were at par with inoculated treatments, with 100 and 150 g/kg of oak debris plus *Metarhizium* ($P \leq 0.05$). Maximum length and also weight of shoot and root belonged to those uninoculated treatments that received more than 100 g of oak debris /kg of soil, with applying *Metarhizium* to the soil, which were even more than the uninoculated control. Almost in all of the inoculated treatments, there was a significant increase in plant growth, as compared to inoculated control ($P \leq 0.05$). By increasing the amount of oak debris, up to 150 g/kg of soil, significant decrease in number of galls has been observed. In this regard, comparing the rate of effect, the role of *Metarhizium* in nematode inhibition was not that much prominent as individual use of this bioagent. Number of eggs/egg mass and number of J2s/kg soil significantly decreased by increasing the percentage of oak debris in the presence/absence of *Metarhizium*. The rate of decrease in number of eggs was not similar

to decrease in J2s population in the soil. In this case, the important point was the effect of *Metarhizium* on eggs of nematode. Where the fungus added to the soil, number of eggs significantly decreased ($P \leq 0.05$) (Fig. 2, Table 1). Minimum reproduction factor was recorded in treatments with 100 g and 150g of oak debris /kg of soil, as well as in the treatment of 50 g of oak debris with *Metarhizium* application ($P \leq 0.05$).

DISCUSSION

In our first and third experiments, we successfully used the fungus *M. anisopliae* against the root knot nematode, *M. javanica*. Results of experiment II, showed that application of oak debris can significantly reduce the rate of nematode reproduction and improve the growth of tomato plants as well. In any treatment with the fungus, the growth of plant was improved and the reproduction rate of the nematode has been decreased. In case of growth parameters of tomato, in interaction of *M. anisopliae* with nematode (N × F), the biomass of tomato plants significantly increased, as compared to inoculated treatment (N) and even as comparing to the healthy plants (Control) ($P \leq 0.05$). Maximum shoot and root length, as well as fresh and dry weight of shoot and root was obtained in treatment in interaction of the fungus and oak debris (OD_{150} × F) which was significantly above the normal growth in control plants ($P \leq 0.05$). Inoculation with nematode didn't cause a significant decrease in

growth parameters, as compared to uninoculated control, but application of oak debris or/and the fungus *M. anisopliae*, had promotive effects on plant growth and it was at the highest level for interaction of OD_{150} × F. In case of inoculated tomatoes, best results were taken in treatments with 150g of oak debris. Comparing to treatments with the same rate of oak debris, the interaction of OD_{150} × N × F was more successful in grown promotion, than the interactions N × F and OD_{150} × N, which is showing the enhancement of the bioactivity of the fungus in the presence of oak debris.

Some microorganisms are able to promote the growth of some plants. The capability of microorganism to colonizing the roots of plant is an important factor to have the promoting power (Schroth and Hancock, 1982). According to Hussey (1985), an increase in shoot weight is due to health of roots that can have a good uptake and transport of water and nutrients. Some species of *Metarhizium* are attracted to roots of certain plant species (Wang and St Leger, 2007) and has root colonization ability (Bruck, 2005). Conidial germination and different rates of root colonizing by *M. anisopliae* isolates, has been reported (Elena *et al.*, 2011; Sassan *et al.*, 2012). Even some isolates of *M. anisopliae* have endophytic behavior (St. Leger, 2008). Bio-priming effects of *M. anisopliae* on germination and seedling growth of flax seed have been shown by Bakhit *et al.* (2015).

Based on their study, bio-priming treatments improved germination characteristics of flax seed as well as the seedlings vigor. In current study, all of growth-related factors of tomato plants were improved by soil amending, using oak debris. By adding the oak debris (OD, OD × F; OD × N × F), growth related parameters had significantly increased, showing the significance of the interaction of oak debris with fungus in improving the rate of plant growth ($P \leq 0.05$). By growing the tomato plants in an organic soil, Sterrett *et al.* (1982) had obtained a significant increase in fresh and dry biomass.

Reduction in nematode population varied in different treatments. As compared to inoculated control (N), interaction of nematode with fungus (N × F) significantly decreased the nematode related factors. The rate of eggs/root and the reproduction factor most affected than the other factors (more than three times reduction). According to Ghayedi and

Abdollahi (2003), the fungus produces sticky conidia that attach to nematode cuticle. The conidia germinate, parasitize and kill the cadaver, by direct penetration and producing the infective hyphae inside the nematode body. The fungus produces some cyclopeptides and destruxins which may play an important role in its pathogenicity (Kershaw *et al.*, 1999). The lethal effect of *M. anisopliae* culture extract has been also reported (Mohanty *et al.*, 2008; Jahanbazian *et al.*, 2014a). Prior to any direct attack to the host, the fungus produces destruxin A and destruxin B that can kill the host (Roberts, 1966). In our studies, number of galls, egg masses and eggs were reduced by soil application of *M. anisopliae* spore suspension. So, it can be concluded that the tomato roots has been colonized by *M. anisopliae* and in this way, the rate of nematode penetration to the roots was declined.

Table 1: Comparison of percentage of decrease in nematode-related factors on tomato plants infected with *Meloidogyne javanica*, treated with debris of oak trees and *Metarhizium anisopliae*, as compared to untreated control

Experiment	Treatment Factor	*Metarhizium*	50g oak debris	50g oak debris + *Metarhizium*	100g oak debris	100g oak debris + *Metarhizium*	150g oak debris	150g oak debris + *Metarhizium*
		% of decrease as compared to control						
Exp1	Galls/root	36%	-	-	-	-	-	-
	Egg masses/root	94%	-	-	-	-	-	-
	Eggs/root	360%	-	-	-	-	-	-
	J2s/soil	137%	-	-	-	-	-	-
	RF	350%	-	-	-	-	-	-
Exp2	Galls/root	-	36%	-	81%	-	134%	-
	Egg masses/root	-	108%	-	229%	-	234%	-
	Eggs/root	-	150.95%	-	260.4%	-	396.48%	-
	J2s/soil	-	152.83%	-	401.87%	-	1038.49%	-
	RF	-	150.79%	-	263.84%	-	406.48%	-
Exp3a	Galls/root	39%	35.49%	47%	77%	92%	129%	147%
	Egg masses/root	88%	97.4%	105%	211%	223%	241%	258%
	Eggs/root	355%	147.5%	425%	271%	556%	406%	691%

	J2s/soil	144%	161%	183%	385%	409%	972%	1002%
	RF	341.6%	147.67%	409.46%	273.35%	548.16%	414%	696.2%
Exp3b	Galls/root	37.7%	31.8%	51.7%	78.9%	95.2%	130.6%	144.1%
	Egg masses/root	81.7%	108.2%	99.4%	223.6%	215%	262%	249%
	Eggs/root	372%	140.5%	442.6%	267.44%	566.4%	385.1%	709.7%
	J2s/soil	132.5%	145.5%	200.5%	404.2%	430.7%	974%	980.5%
	RF	353.1%	140.4%	426.6%	270.4%	558.9%	393.7%	714.4%

Exp3a=first run of experiment 3; Exp3b=second run of experiment 3
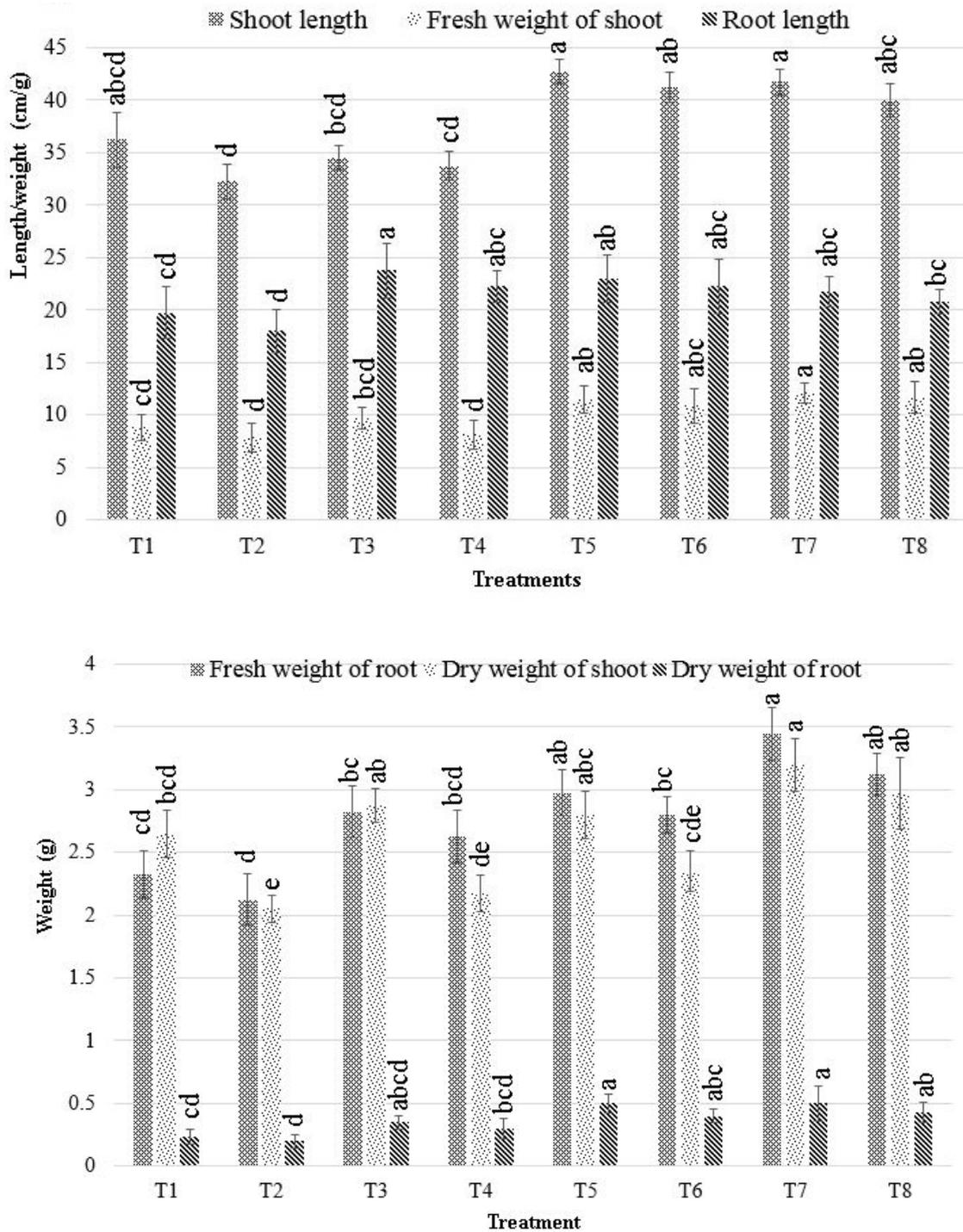
Fig. 1. Effect of different rates of oak tree debris on growth parameters of tomato, inoculated with *Meloidogyne javanica*
T1: Uninoculated control without oak debris; T2: Inoculated control without oak debris; T3: Uninoculated, with 50g oak debris/kg soil; T4: Inoculated, with 50g oak debris/kg soil; T5: Uninoculated, with 100g oak debris/kg soil; T6: Inoculated, with 100g oak debris/kg soil; T7: Uninoculated, with 150g oak debris/kg soil; T8: Inoculated, with 150g oak debris/kg soil
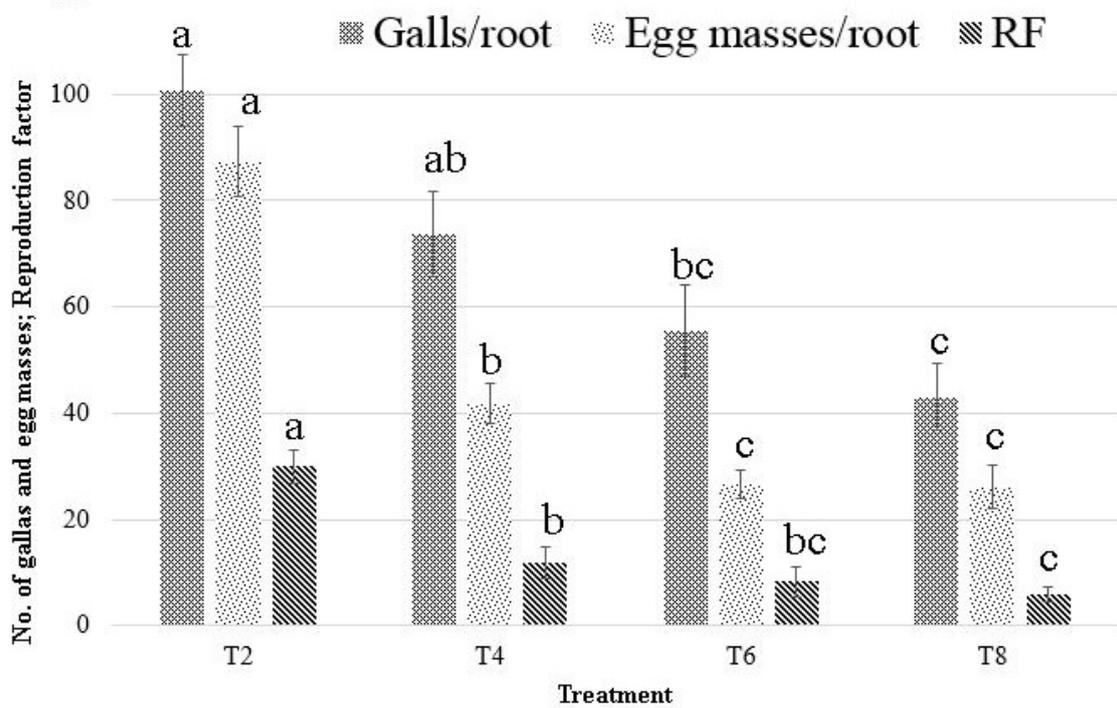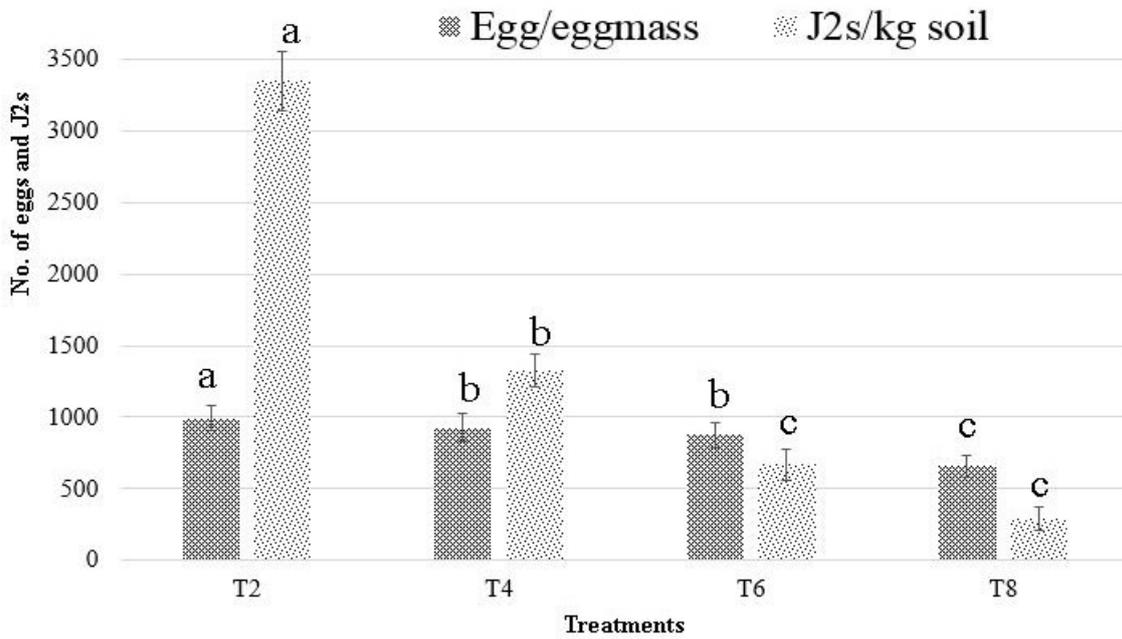
Fig. 2. Effect of different rates of oak tree debris on reproduction of *Meloidogyne javanica* in tomato
T2: Control, without oak debris; T4: Treated with 50g oak debris/kg soil; T6: Treated with 100g oak debris/kg soil; T8: Treated with 150g oak debris/kg soil
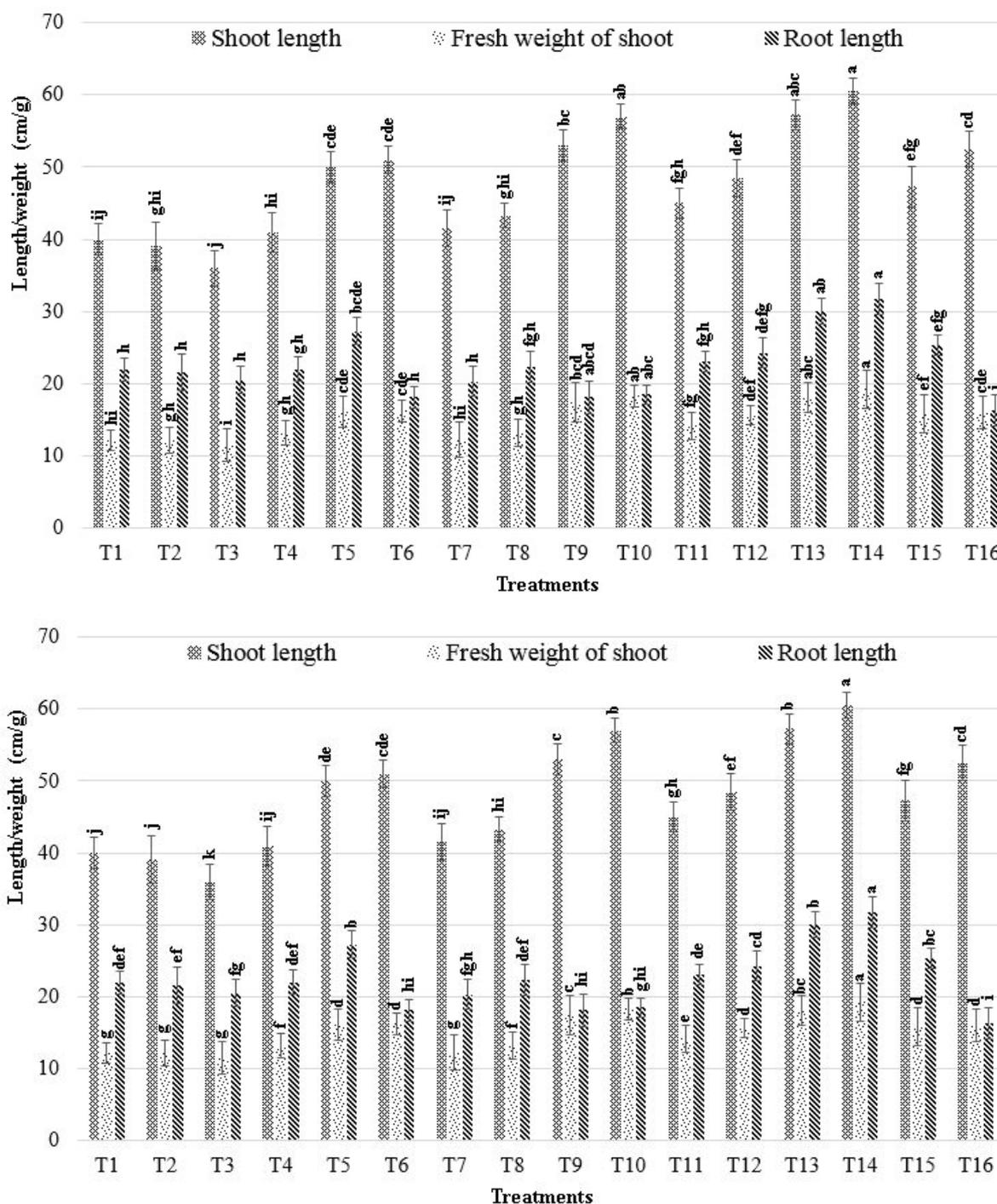
Fig. 3a. Effect of different rates of oak tree debris and *Metarhizium anisopliae* on growth parameters of tomato (shoot and root length, fresh weight of shoot), inoculated with *Meloidogyne javanica* (top: first run; down: second run)
T1: Uninoculated control without oak debris, without *Metarhizium*; T2: Uninoculated control without oak debris, with *Metarhizium*; T3: Inoculated control without oak debris, without *Metarhizium*; T4: Inoculated control without oak debris, with *Metarhizium*; T5: Uninoculated, with 50g oak debris/kg soil, without *Metarhizium*; T6: Uninoculated, with 50g oak debris/kg soil, with *Metarhizium*; T7: Inoculated, with 50g oak debris/kg soil, without *Metarhizium*; T8: Inoculated, with 50g oak debris/kg soil, with *Metarhizium*; T9: Uninoculated, with 100g oak debris/kg soil, without *Metarhizium*; T10: Uninoculated, with 100g oak debris/kg soil, with *Metarhizium*; T11: Inoculated, with 100g oak debris/kg soil, without *Metarhizium*; T12: Inoculated, with 100g oak debris/kg soil, with *Metarhizium*; T13: Uninoculated, with 150g oak debris/kg soil, without *Metarhizium*; T14: Uninoculated, with 150g oak debris/kg soil, with *Metarhizium*; T15: Inoculated, with 150g oak debris/kg soil, without *Metarhizium*; T16: Inoculated, with 150g oak debris/kg soil, with *Metarhizium*
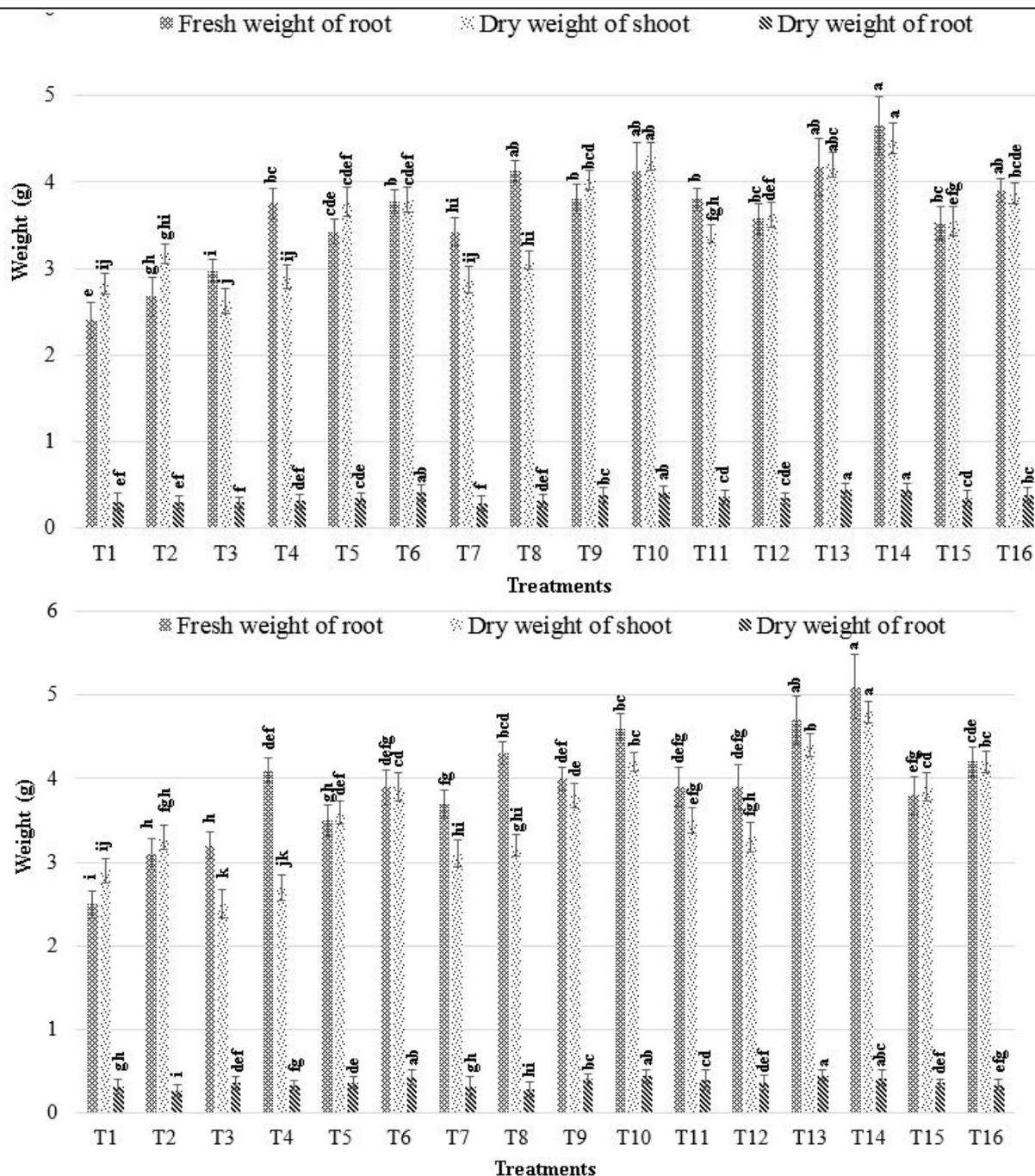
Fig. 3b. Effect of different rates of oak tree debris and *Metarhizium anisopliae* on growth parameters of tomato (fresh weight of root, dry weight of shoot and root), inoculated with *Meloidogyne javanica* (top: first run; down: second run)
T1: Uninoculated control without oak debris, without *Metarhizium*; T2: Uninoculated control without oak debris, with *Metarhizium*; T3: Inoculated control without oak debris, without *Metarhizium*; T4: Inoculated control without oak debris, with *Metarhizium*; T5: Uninoculated, with 50g oak debris/kg soil, without *Metarhizium*; T6: Uninoculated, with 50g oak debris/kg soil, with *Metarhizium*; T7: Inoculated, with 50g oak debris/kg soil, without *Metarhizium*; T8: Inoculated, with 50g oak debris/kg soil, with *Metarhizium*; T9: Uninoculated, with 100g oak debris/kg soil, without *Metarhizium*; T10: Uninoculated, with 100g oak debris/kg soil, with *Metarhizium*; T11: Inoculated, with 100g oak debris/kg soil, without *Metarhizium*; T12: Inoculated, with 100g oak debris/kg soil, with *Metarhizium*; T13: Uninoculated, with 150g oak debris/kg soil, without *Metarhizium*; T14: Uninoculated, with 150g oak debris/kg soil, with *Metarhizium*; T15: Inoculated, with 150g oak debris/kg soil, without *Metarhizium*; T16: Inoculated, with 150g oak debris/kg soil, with *Metarhizium*
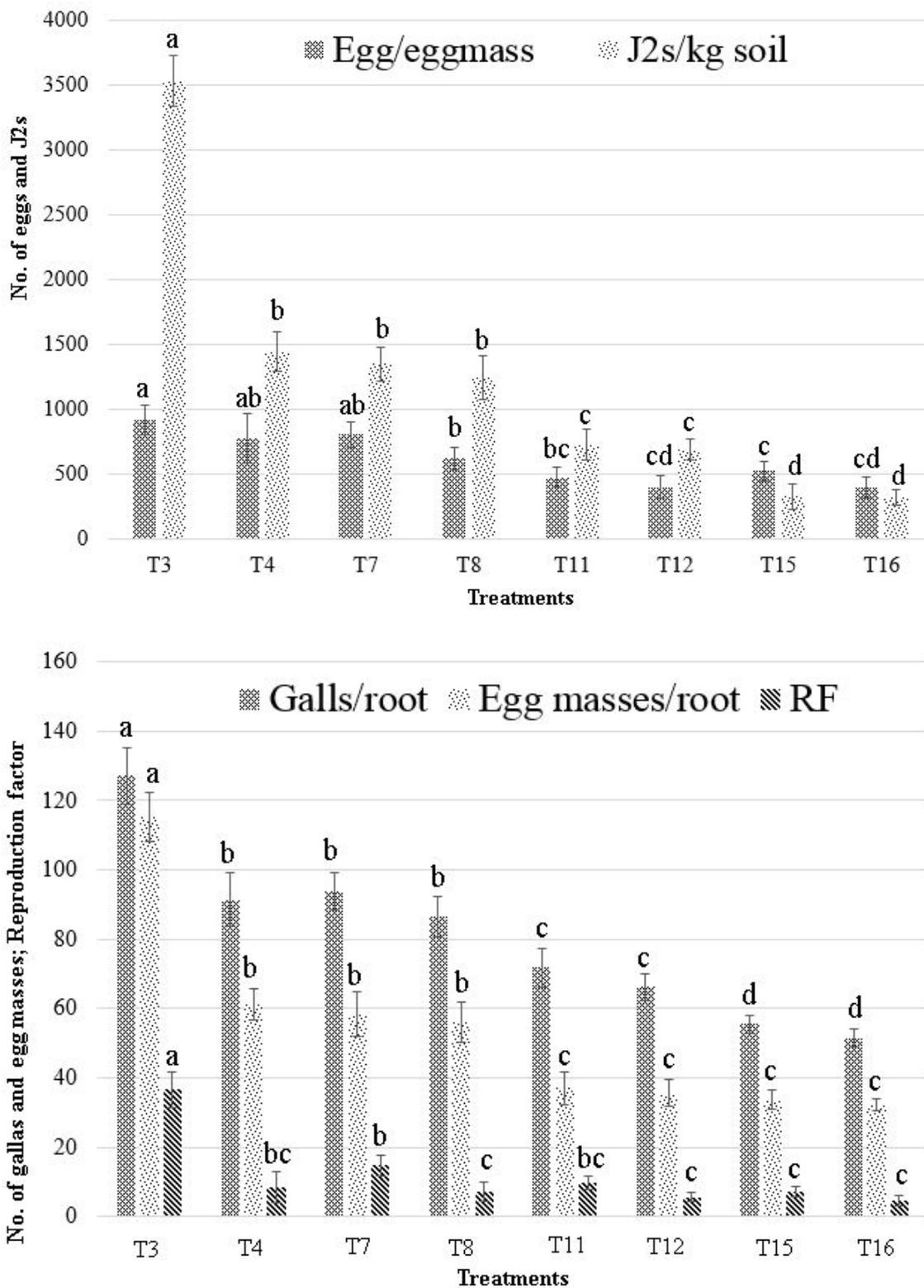
Fig. 4a. Combined effect of different rates of oak tree debris and *Metarhizium anisopliae* on reproduction of *Meloidogyne javanica* in tomato(first run)
T3: Treated without oak debris, without *Metarhizium* (control 1); T4: Without oak debris, with *Metarhizium* (control 2); T7: Treated with 50g oak debris/kg soil, without *Metarhizium*; T8: Treated with 50g oak debris/kg soil, with *Metarhizium*; T11: Treated with 100g oak debris/kg soil, without *Metarhizium*; T12: Treated with 100g oak debris/kg soil, with *Metarhizium*; T15: Treated with 150g oak debris/kg soil, without *Metarhizium*; T16: Treated with 150g oak debris/kg soil, with *Metarhizium*
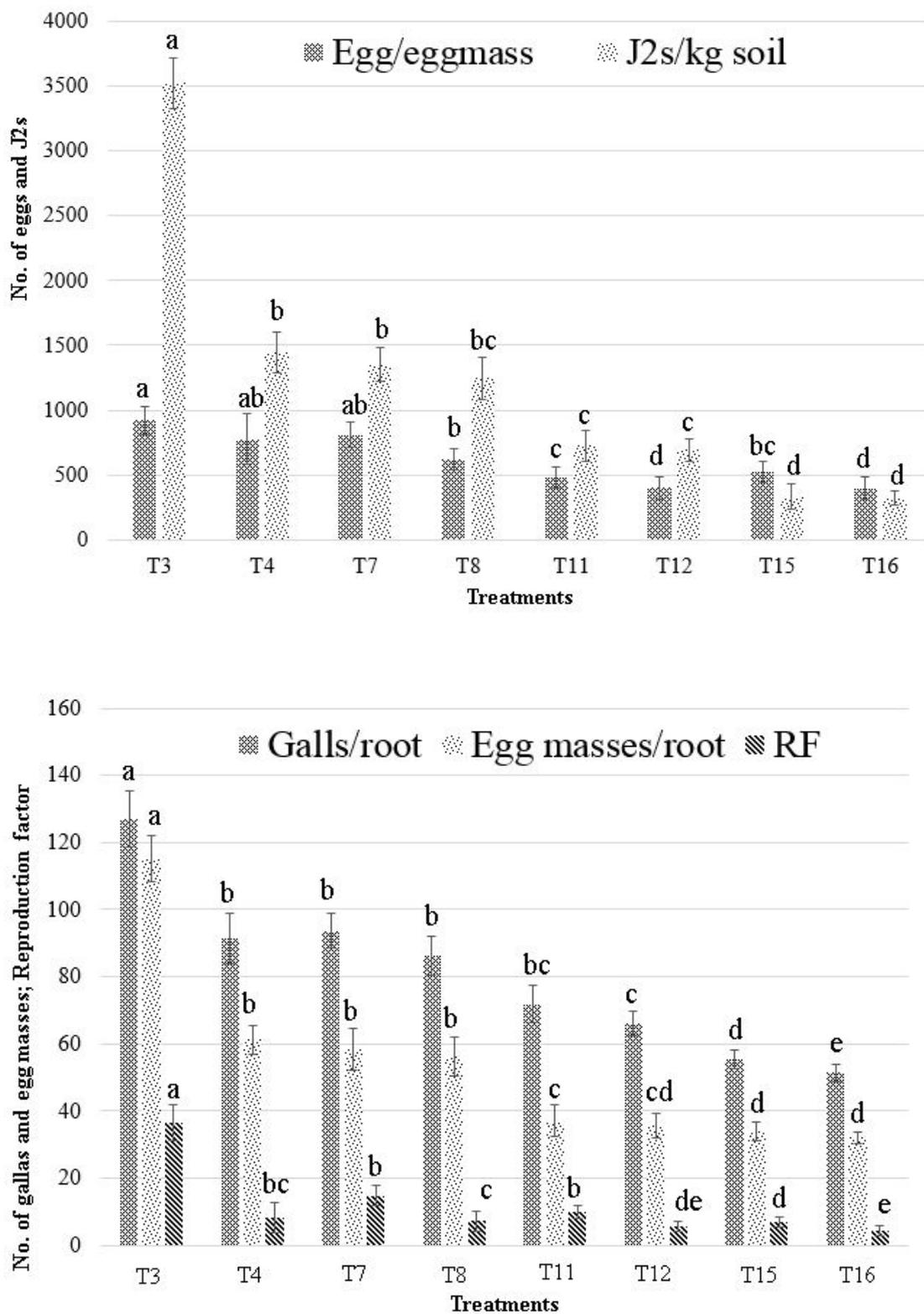
Fig. 4b. Combined effect of different rates of oak tree debris and *Metarhizium anisopliae* on reproduction of *Meloidogyne javanica* in tomato(second run)
T3: Treated without oak debris, without *Metarhizium* (control 1); T4: Without oak debris, with *Metarhizium* (control 2); T7: Treated with 50g oak debris/kg soil, without *Metarhizium*; T8: Treated with 50g oak debris/kg soil, with *Metarhizium*; T11: Treated with 100g oak debris/kg soil, without *Metarhizium*; T12: Treated with 100g oak debris/kg soil, with *Metarhizium*; T15: Treated with 150g oak debris/kg soil, without *Metarhizium*; T16: Treated with 150g oak debris/kg soil, with *Metarhizium*

The rate of reduction in nematode population in the system of "N × OD" at the rates of 50 and 100 g/kg of soil was lesser than the interaction of nematode and fungus (N × F). By adding the oak debris to interaction of nematode and fungus, the nematicidal activity of the used bioagent significantly increased ($P \leq 0.05$). It has found that the interaction of "OD_{150} × N × F" was most effective treatment with seven times reduction of number of eggs/root and reproduction factor and ten times reduction in number of J2s/soil. Any increase in amount of oak debris, enhanced the nematicidal activity of the tested bioagent. Reductions in nematode population have been reported as a result of compost application in the soil (McSorley and Gallaher, 1996).

Significant control of soil-borne plant pathogens, including root-knot nematodes, has been provided by using the organic soil amendments (Mannion *et al.,* 1994). As reported by McSorley and Gallaher (1995), some changes in chemical and physical properties of the soil may induce plant response and also increase their tolerance against plant parasitic nematodes. In our study, the greatest reduction in nematode-related factors were observed in those plants were grown in soils which treated with 100g of oak debris/kg of soil, with applying the bioagent, *Metarhizium*. Our results also showed that there is no significant increase in nematode control by adding more than 100g of oak

debris. This result is supported by findings of Habicht (1975), who suggested that relatively high amount of this organic amendment is required to control the root-knot nematode.

Our results on application of oak debris in the soil are similar to the findings of Siddiqui and Alam (2001). They have reported that plant parts of *Azadirachta indica* and *Melia azadirach* have nematicidal properties. In their experiments, development of *M. incognita* has been inhibited, when soil was amended with different parts of these plants. In a greenhouse experiment, application of neem cake at the rate of 1%, reduced the number of *Pratylenchus penetrans* and *M. hapla* in tomato roots, by 67% and 90%, respectively (Abbasi *et al.,* 2009). In Italy, olive pomace has controlled the root knot nematode (D`Addabdo and Sasanelli, 1996). Bello *et al.* (2002) successfully used olive against *Meloidogyne*, fungi, insects, and weeds, in Uruguay. They have indicated that bio-fumigation which is based on application of local organic sources, can be used as an alternative measure for nematode control. According to them, bio-decomposition of organic materials can improve the plant vigor and its final yield as well. They also expressed that because they improve all of the chemical, physical and biochemical characteristics of soil, these materials are even more effective than the conventional fumigants.

In some cases, the nematicidal effects of some botanicals is due to their biologically active constituent (Chitwood, 2002; Ameer-Zareen *et al.,* 2003) and in some other cases it is characterized by the lipophilic properties of botanicals that enable them to dissolve the cell membrane of nematode, results in interfering of their functional groups with the enzyme protein structure (Knoblock *et al.,* 1989). Organic amendments probably release ammonia with nematicidal properties related to increase of carbon dioxide and nitrogen levels (Akhtar, 1998). Some phenolic compounds and terpenoids with nematicidal activity are also known in organic waste materials (Shaukat *et al.,* 2004). Soil amendment with plant materials such as chopped stem and leaf is a low cost method which can use in nematode management. Because this is a very safe and inexpensive method, it can be easily achieved by farmers. According to Adegbite and Adesiyan (2005), nematostasis activities of some botanicals are related to their ovicidal properties. They have also suggested that some botanicals may affect the embryonic development.

In our study, a good control of *M. javanica* has obtained by adding oak debris and/or *Metarhizium*. Some studies have been carried out to investigate the antimicrobial properties of oak. Analysis of the extract components of oak fruits have been given by Sadeghian *et al.* (2012). According to their study, tannins and phenolic compounds could be responsible for antimicrobial activities. In a study on physical and chemical properties of debris of oak trees (Owliaie *et al.*, 2011), it has proved that the decayed debris contain considerable amounts of organic carbon, total nitrogen, available phosphorus, absorbable potassium, EC, micro-nutrients such as iron, manganese and zinc. In their study, significant decrease in pH, calcium carbonate and copper in decayed debris, have been observed. In the time course of decomposition of plant debris, some substances such as phenol components or toxic materials including free ammonium gases, nitrate, sulfur gases and organic acids are produced. Such materials kill nematodes directly or reduce egg hatching. It may also make some chemical and physical changes in the soil and subsequently increase the amount of phosphorus, potassium and sodium of soil, improving the plant growth (Dropkin *et al.,* 1958). Oka *et al.* (2006) showed that soil amendment can considerably reduce the population density of *M. javanica* by altering the soil pH.

Co-application of two or more antagonistic agents can provide a good tool for use of different capabilities from different sources, against the target pathogen and make a broad spectrum of biocontrol by simulating the natural condition (Janisiewicz, 1996). Soil condition is a main factor for enhancing the bioefficacy of useful microorganisms. It is necessary to provide a favorable environmental condition to enhance the

effectiveness of bioagents. There is a positive correlation between the percentage of organic matter obtained from decomposition of plant debris in the rhizosphere and the population density of bioagents (Nelson *et al.,* 1983). In our study, it has been observed that in amended soil, the tested fungus was more effective on nematode, than the unamended soil. In a same study, survival of *Trichoderma* sp. has been studied (Davet *et al.,* 1981). Based on their study, soil condition including the amounts of organic materials was a very important factor affecting the population density and survival of *Trichoderma* and its success in biocontrol of plant pathogens. According to Ahmadzadeh (2012), some environmental and nutritional factors have critical roles in success of antagonistic microorganisms to establish in a new environment. He also expressed that activities of bioagents against the plant pathogens, can increase in those soils with organic materials, which release some carbohydrates to the soil. Some reasons of increase in plant susceptibility to plant pathogens in poor soils, has been studied by Morgan and Whipps (2001). It is suggested that providing a favorable condition for activating of antagonistic agents is required for having a successful biocontrol program against the plant pathogens (Copping, 1998). Some conditions of rhizosphere, such as temperature, moisture, pH and abundance of organic materials of soil play critical roles in

Trichoderma survival (Rouhani, 2004). Effect of soil condition on bioactivity of antagonistic bacteria has also discussed by Ahmadzadeh (2012). Enhancing the biocontrol activity of *Pseudomonas fluorescens* and *Trichoderma vierns* against *Meloidogyne javanica*, by application the oak debris in tomato, has been demonstrated by Moradi *et al.* (2015). Based on their study, soil amendment had significant effects on activity of tested bioagents. As compared to unamended soil, 56.3% reduction in root galling was observed in those soils that were amended with *T. virens*, and maximum increase in dry weight of root was respectively obtained by 68.2% and 56.1%, in treatments with *P. fluorescens* or *T. virens*. Sharon *et al.* (2001), showed that combination of *T. harzianum* and *T. vierns* with *P. fluorescens*, caused an increase in growth factors of tomato plants that were infected with root-knot nematode.

In our study, the role of plant debris in increasing the activity of the tested biopesicide has been shown. It was also proved that there was no adverse effect of oak debris, which potentially has antimicrobial activity, on antagonistic power of applied bioagent. Complementary studies are required to investigate the effects of different rates of *Metarhizium* in combination with oak debris, and also studying the effects of different environmental conditions on controlling the nematode is suggested.

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