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Research Article

Plant debris of oak forest as soil amendment, to improve the biocontrol activity of *Pseudomonas fluorescens* and *Trichoderma vierns* against *Meloidogyne javanica*, in tomato

Rojin Moradi[1], Faranak Moradi[2], Kioumars Mirehki[2] and Mohammad Abdollahi[2*]

1. Department of Plant Protection, College of Agriculture, Kurdistan University, Sanandaj, Iran.
2. Department of Plant Protection, College of Agriculture, Yasouj University, Yasouj, Iran.

Abstract: Application of antagonistic agents in the rhizosphere of plants is an important approach in control of soil-borne pathogens. Establishment and persistence of biocontrol agents in the soil is a major concern in biological control. This study aimed to determine the effect of plant debris of oak forests on activity of *Pseudomonas fluorescens* and *Trichoderma vierns* against *Meloidogyne javanica* in tomato in green house conditions. In different treatments, both or one of the bioagents were applied to the soil inoculated with *M. javanica* and amended or unamended with oak plant debris. Based on the results, the growth–related factors of tomato improved in those treatments, in which plant debris were mixed with the soil. In case of nematode-related factors, the number of galls on roots decreased significantly in pots amended with plant debris. Maximum reduction in reproduction factor was observed in treatments with one or both of the biocontrol agents. In comparison to unamended treatments, the rate of reduction in root gall was 56.3% for treatments receiving *T. virens* and maximum increase in dry weight of root was observed in soils treated with *P. fluorescens* or *T. virens,* 68.2% and 56.1%, respectively.

Keywords: Biocontrol, Food security, Fungi**,** Nematode management, Organic matter

Introduction

Despite development of information and achieving remarkable improvement in the management of plant diseases, plant pathogens still cause many losses in greenhouse products. Soil-borne pathogens are affected by physical, chemical and biological properties of the soil. Root-knot nematode, *Meloidogyne javanica* is considered as one of the major plant pathogens infecting some strategic plant products, such as tomato (Hillocks, 2002). Biological control of soil-borne pathogens is one of the most effective methods that have been studied in many countries. Biological control of root knot nematode by applying antagonistic fungi and bacteria, have been reported and is supposed to reduce the pesticide use (Starr *et al.,* 2002). In Iran, some research works have been done using antagonistic agents against *Meloidogyne* sp. It has been shown that those biocontrol agents are successful which have the ability to overcome unsuitable environmental conditions. They are compatible to particular ecological conditions and can settle in natural conditions (Heydari and Pessarakli, 2010).

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[*] **Corresponding author**, e-mail: abdollahi@yu.ac.ir
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Pseudomonas fluorescens and *Trichoderma vierns*, are soil dwelling microorganisms that can be found in most of the soil ecosystems. Due to their low population in normal conditions of the soil, often they are unable to interfere with plant pathogens. In many cases, because of lacking the ability of establishment and conformance with the soil conditions, these bioagents become ineffective (Copping, 1998). By colonization in the rhizosphere, *P. fluorescens* creates significant protection against plant parasitic nematodes. Producing some metabolites, volatile organic compounds and antibiotics, may be noted as important mechanisms in colonized bacteria (Siddiqui *et al.,* 2006).

Cook and Rovira (1976) showed that *P. fluorescens* can decrease the severity of soil-borne diseases by root colonizing and biofilm formation. Environmental conditions and nutritional composition of the soil such as phosphorus, nitrogen, iron, organic carbon, divalent cations, metal salts and pH, are the key factors affecting biofilm formation and establishing the bacteria (Danhorn and Fuqua, 2007). *Trichoderma* species have interactions with different microorganisms in rhizosphere environment. Because of high potential in utilizing the different sources of food, *Trichoderma* has high biocontrol potential against plant parasitic nematodes. It also has some antagonistic mechanisms such as competition, parasitism and antibiotic production (Sharon *et al.,* 2001). These properties may somewhat change under different soil conditions. In most cases, soil amended with plant debris and organic matter, increases the stability of antagonist agents in rhizosphere and induces plant defense system. Soil amendment adds some nutritional components to the soil and changes the soil chemical properties (Heydari and Pessarakli, 2010). The nematicidal effects of some plant debris on plant parasitic nematodes have been investigated by Linford *et al.* (1938). Application of organic materials such as the soil of under canopy of trees which contains plant debris, have positive effects on plant growth and also improves the defense system of plant. Because the plant debris contains important elements and chemical components, they provide a good nutritional as well as chemical condition during decomposition. Such condition is favorable for natural enemies of plant pathogens (Moreno *et al.*, 2007). The success of such plant materials in nematode control depends on the source of plant material, chemical composition, complexity of the process, environmental conditions and the nematode species (Oka *et al.,* 2002).

El-Nagdi and Mansour (2003) studying the nematicidal effects of some medicinal plants demonstrated that *Jasminum multiflorum* had more nematicidal effect as compared to *Mimosa pudica*. Hasabo and Noweer (2005) showed the effects of the leaf extract of *Ocimum basilicum*, marigold, China berry and seed extract of neem against the juveniles of root knot nematode. Effect of cabbage leaf (Ghazalbash *et al.,* 2011) and root bagasse of *Glycyrrhiza glabra* (Abdollahi and Ramezani, 2012) on *M. javanica* have been demonstrated. The nematicidal as well as the fungicidal effects of *Ferulago angulata* and *Zataria multiflora* have been studied on root knot nematode, *M. javanica* (Ghazalbash and Abdollahi, 2013; Abdollahi and Ghazalbash, 2012). This study was implemented in glasshouse conditions to determine the effects of oak tree debris on biocontrol potential of *P. fluorescens* and *T. vierns,* against root knot nematode, *M. javanica* on tomato.

Materials and Methods

Preparation of plant debris

Plant debris including rotten leaves of oak trees were collected from the oak forest at depth of 15-20 cm, located near Javanrood region of Kermanshah Province with geographical coordinates of 34°47'30"N, 46°20'6"E.

Preparation of nematode population

Pure culture of nematode was prepared according to Hussey and Barker (1973). Identification of nematode species was based

on morphological and morphometrical characters of second stage juveniles and females, especially the characteristics of perineal pattern (Jepson, 1987). A single egg-mass was reared on tomato cultivar Red Cloud. Sufficient infestation was provided by successive planting of tomato seedlings into the soil containing nematode. The infected roots were blended in a solution of sodium hypochlorite 1% to obtain the eggs and second stage juveniles. The concentration of eggs and J2s in the suspension was counted in one ml of suspension.

Isolation, purification and identification of antagonistic strains of *Pseudomonas sp*.

Bacterial strains were isolated from the rhizosphere of cucumber and tomato infected by root-knot nematode, by culturing the diluted soil suspension (Rahanandeh *et al.,* 2012). Dilutions of 10 to 10^9, of each soil sample were prepared in sterile distilled water and 0.5 ml of each dilution were cultured on King's-B medium and incubated at 25 °C, in five replicates. The isolates then were purified using the fluorescent response method. A portable UV lamp was passed under Petri dishes and those bacterial colonies with more illumination were selected. Single colony of each strain was cultured on S1 medium and final colonies were purified after 48 to 72 h. In total, ten isolates were purified from the rhizosphere of cucumber and tomato. Some biochemical tests including catalase, oxidase, anaerobic growth, gram, arginine dihydrolase, starch hydrolysis, gelatin hydrolysis, levan production from sucrose were done. Nitrate reduction and fluorescent pigments production tests were carried out using King's-B medium. Tests on use of various sources of sugar were also performed for isolated colonies (Schaad *et al.,* 2001).

To select the suitable strains, antagonistic tests on second stage juveniles of nematode were performed using Cayrol (1989) method. Suspension of bacterial isolates was prepared using spectrophotometer in absorbance of1 at 600 nm (Burr, 1978). One ml of suspension of each bacterial strain at concentration of 10^9 cfu/ml were added to the Petri dishes that contained newly hatched second stage larvae of nematode in five replicates and distilled water used as control. Petri dishes were incubated at 28 °C and numbers of inactivated larvae were counted 48 and 72 h after treatment. Percentage of larval mortality was recorded for each treatment. To determine the effect of bacterial isolates on the rate of egg hatching, in five replicates, an average of 200 eggs of nematode transferred to Petri dishes with one ml of a suspension of each isolate at the concentration of 10^9cfu/ml and sterile distilled water was used as control. The Petri dishes were incubated at 28 °C for one week and percentage of unhatched eggs was recorded (Siddiqui *et al.,* 2008).

Isolation of *Trichoderma* sp.

Trichoderma species were isolated from the soil samples of tomato fields collected from an average depth of 25 cm. Soil dilution method was done using Davet selective medium (Davet, 1981) and then the fungus was purified with single-spore method by culturing on water–agar medium. The isolates were grown on PDA (potato dextrose agar) at 24 °C. In order to identify the *Trichoderma* species, Bissett (1991) identification key was used. *T. virens* were identified based on micro-morphology of sporulation and the color and morphology of its sporulating structures and conidia (Ubalua and Oti, 2007). Cultures of *T. virens* were maintained on PDA slant and stored at 4°C for further use. The spore suspension was prepared in distilled water, at concentration of 10^6 spores/ml with the help of a Neubauer hemocytometer (Lumsden *et al*., 1990).

Greenhouse experiment

The experiment was done in a completely randomized design with eight treatments in greenhouse conditions with five replications. Treatments were as follows:

1- Tomato seedlings inoculated with *M. javanica*, amended with plant debris

2- Tomato seedlings inoculated with *M. javanica*, plus suspension of *T. vierns,* amended with plant debris
3- Tomato seedlings inoculated with *M. javanica*, plus suspension of *P. fluorescens,* amended with plant debris
4- Tomato seedlings inoculated with *M. javanica*, plus suspension of *T. vierns* and *P. fluorescens,* amended with plant debris
5- Tomato seedlings inoculated with *M. javanica*, without adding the plant debris
6- Tomato seedlings inoculated with *M. javanica*, plus suspension of *T. vierns,* and no plant debris
7- Tomato seedlings inoculated with *M. javanica*, plus suspension of *P. fluorescens,* and no plant debris
8- Tomato seedlings inoculated with *M. javanica*, plus suspensions of *T. vierns* and *P. fluorescens,* without plant debris

Surface sterilized seeds of Red Cloud cultivar of tomato were planted in containers with sterilized soil. Two weeks after sowing the seeds, four leaf tomato plants were transplanted to pots containing two kg of steamed soil. For those treatments with plant debris, 300gr sterilized forest plant debris was added to steamed soil. For treatments with *T. vierns*, spore suspension prepared at concentration of 10^6 spores per ml in sterile distilled water was used. Three days after transplanting, soil of pots was inoculated with *T. vierns* using soil drench method (Sharon *et al.,* 2001). Soil drench, applying *P. fluorescens* was done using 50 ml of bacterial suspension at a concentration of 10^9/ml, three days after transplanting. In those treatments in which both bioagents were used, four days after applying suspension of *T. vierns*, bacterial suspension was added.

One week after soil treatment with biological agents, when the seedlings were at six-leaf stage, each one of pots were inoculated with 4000 eggs and second stage juveniles of *M. javanica*. The pots were maintained in a greenhouse with uniform temperature of 28 °C and humidity of 65% and were irrigated every 48 h. Assessment of treated plants was carried out three months after inoculation. Growth-related factors of tomato (length of shoot and root, fresh weight of shoot and root and dry weight of shoot and root), and nematode-related factors (number of galls/root, number of egg masses/ root, number of J2s/pot) were recorded (Sasser and Freckman, 1987). The reproduction factor was then calculated. Data were analyzed using SPSS20 software. Analysis of variance and mean comparison was done by Duncan's multiple range test.

Results and Discussion

Identification of *Trichoderma* and *Pseudomonas* species

The isolated fungi were identified as *T. vierns* by their morphological characteristics of colony as well as phialide, conidiophore, conidia, chlamydospore and hyphae. On PDA medium, colonies grew rapidly and yellow to green-blue in color. Aerial mycelia were floccose, white to greyish. Sporulation of the isolated fungus was sporadic and did not cover all the surface of colony. Chlamydospores were abundant terminal or intercalary, mostly oval in shape, colorless to pale yellow, measured 5-9 × 6-12 micrometer. Conidia formed on macronematous, colorless conidiophore. Conidiophore branches were irregular arising singly or in opposite pairs, in right angle or reflexed toward the apex. Conidiophore branches gave rise to 3-6 lageniform to ampulliform phialides, about 8 micrometer in length. The phialides were constricted at the base, swollen in the middle and attenuated to the apex. Obovoid conidia measured 3-4×4-5 micrometer. The conidia were smooth-walled, dark green in color and often with large gloeoid mass.

On the basis of the identification key by Schaad *et al*. (2001), regarding biochemical tests, nitrate reduction, production of fluorescent pigments on King's-B medium and usage of different sources of sugar, bacterial isolates were identified as *P. fluorescens* biotype I. Antagonistic assessment test showed that among the bacterial strains, strain K122 was most effective against egg hatching and caused the most mortality in second stage larvae, so this isolate was selected for further use.

The effect of treatments on nematode-related factors in tomato plants infected with *M. javanica*

The effects of treatments on nematode-related factors in *M. javanica*-infected tomato plants are shown in Tables 1 and 2. Analysis of variance (Table 1) showed significant differences among treatments ($P \leq 0.01$). According to data showed in table of mean comparison (Table 2), the treatments with plant debris greatly reduced nematode activity and there were significant differences between amended and unamended soil as for number of galls in root system. Compared to its unamended control, the rate of reduction in root galling was 44.9%, 54.1%, 56% and 43.2% for treatments without any biocontrol agent, treated with *P. fluorescens*, treated with *T. virens* and treated with both bioagents, respectively ($P \leq 0.05$).

Number of egg masses and eggs in root system in all treatments was significantly lesser than those in the unamended inoculated control. Comparing to corresponding unamended control, the rate of reduction in number of egg mass was 55.3%, 26.7%, 26.8% and 5.2% and reduction in number of eggs was 58.6%, 27.0%, 56.0% and 63.2% for treatments with no bioagent, treated with *P. fluorescens*, treated with *T. virens* and those treated with both bioagents, respectively ($P \leq 0.05$). There were significant differences in the number of second stage juveniles between the amended and unamended soil for all the supplied bioagents., The number of second stage juveniles were reduced 50.3%, 31%, 45% and 19% in treatments with no bioagent, treated with *P. fluorescens*, treated with *T. virens* and treated with both bioagents, respectively, as compared to unamended controls ($P \leq 0.05$). Reproduction factor was also significantly affected in amended soil. Compared with corresponding unamended control, the reproduction factor was reduced at the rates of 53.6%, 31.6%, 45.0% and 23.1% for treatments with no bioagent, treated with *P. fluorescens*, *T. virens* and both bioagents, respectively ($P \leq 0.05$).

Our findings are in agreement with the findings of Sahebani *et al.* (2006) and Mirehki *et al.* (2013). Significant control of root-knot nematodes has been achieved by applying organic soil amendments (Mannion *et al.*, 1994). It has been indicated that bio-decomposition of organic materials can improve the plant vigor and final yield as well (Bello *et al*., 2004). Siddiqui and Alam (2001) reported that plant parts from *Azadirachta indica* and *Melia azadirach* have nematicidal properties. In their experiments, development of *M. incognita* was inhibited when soil was amended with parts of these plants. In a greenhouse experiment in three soil types, application of neem cake at the rate of 1%, reduced the number of *Pratylenchus penetrans* and *M. hapla,* by 67% to 90% in tomato roots (Abbasi *et al.*, 2009). Organic amendments probably release ammonia with nematicidal properties related to increase of carbon dioxide and nitrogen levels (Akhtar, 1998). Presence of some phenolic compounds and terpenoids with nematicidal activity has also been reported in organic waste materials (Shaukat *et al*., 2004).

Table 1 Analysis of variance of nematode-related parameters of tomato plants inoculated with *Meloidogyne javanica*, treated with antagonistic agents and plant debris.

Sources of variance	df	Mean Square				
		No of galls/root	No of egg mass/root	No of egg/root	No of J2s/soil	Reproduction factor
Treatments	7	341.745 **	32.702 **	1308073.246*	13881274.890 **	1.463 **
Error	24	12.660	11.720	468791.906	7446.615	0.031
CV%	-	8.260	10.670	11.060	9.210	8.83

* and **: Significant at 5% and 1% level of probability, respectively.

Table 2 Nematode-related parameters on tomato plants treated with antagonistic agents and plant debris.

Treatments	Agents[1]	Nematode-related parameters (Mean ± CV%)[2]				
		No of gall/ root	No of egg mass/root	No of egg / root	No of J2 / soil	Reproduction factor
Unamended soil	*Mj*	106.9 ± 12.2a	34.0 ± 10.1a	2172 ± 14.1a	8875 ± 2.8a	2.8 ± 10.5a
	Mj + Pf	100.2 ± 8.5ab	15.0 ± 12.4b	1130 ± 9.3ab	6415 ± 7.9b	1.9 ± 15.1b
	Mj + Tv	104.0 ± 9.6a	14.9 ± 11.2b	1652 ± 10.9ab	6225 ± 6.3b	2.0 ± 6.6b
	Mj + Tv + Pf	71.0 ± 6.1ab	11.6 ± 9.6b	850 ± 8.4ab	4415 ± 9.8cd	1.3 ± 8.3c
Amended soil	*Mj*	58.9 ± 5.5b	15.2 ± 8.2ab	900 ± 10.1ab	4415 ± 11.9cd	1.3 ± 7.4c
	Mj + Pf	46.0 ± 10.2c	11.0 ± 7.3ab	825 ± 7.3ab	4425 ± 13.2cd	1.3 ± 12.4c
	Mj + Tv	45.5 ± 11.1c	10.9 ± 10.2ab	726 ± 13.1ab	3425 ± 10.9cd	1.1 ± 14.6c
	Mj + Tv + Pf	40.3 ± 12.6c	11.0 ± 14.3b	313 ± 11.8b	3575 ± 12.5d	1.0 ± 9.4c

[1]. Mj: *Meloidogyne javanica*, Pf: *Pseudomonas fluorescens*, Tv: *Trichoderma virens.*
[2]. Means followed by dissimilar letters in a column are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$).

The effect of treatments on growth-related factors of tomato plants infected by *M. javanica*

According to analysis of variance of growth-related factors of tomato plants (Table 3), treatments had different effects on growth parameters of the plant ($P \leq 0.05$ for dry weight of shoot as well as root and $P \leq 0.01$ for other growth parameters). Based on the table of mean comparison (Table 4), shoot length significantly increased in all amended treatments. As compared to its unamended control, the rates of increase in shoot length were 12.4%, 7.4%, 9.2% and 3.5% for treatments without any biocontrol agent, treated with *P. fluorescens*, *T. virens* and both bioagents, respectively ($P \leq 0.05$). In case of root length, there was significant increase in all amended as well as in unamended soils with bioagents. The rates of increase in amended soils comparing to unamended ones, were 15.9%, 19.4%, 21.9% and 15.9% for treatments without any biocontrol agent, treated with *P. fluorescens T. virens* and both bioagents, respectively ($P \leq 0.05$). Comparing to its unamended control, the fresh weight of shoot were significantly increased at the rates of 17.3%, 10.2%, 10.3% and 10.9% for treatments without any biocontrol agent, treated with *P. fluorescens*, *T. virens* and both bioagents, respectively ($P \leq 0.05$), but in case of dry weight of shoot, only the treatment without applying any biocontrol agent in unamended soil was significantly different from all other treatments, indicating the effects of bioagents in both amended and unamended soils ($P \leq 0.05$). For fresh weight of root, other than the treatment with both bioagents that showed 19.2% increase in pots was amended ($P \leq 0.05$), there were no significant differences between treatments. Maximum increase in dry weight of root was observed in soils treated with *P. fluorescens* or *T. virens,* at the rates of 68.2% and 56.1%, respectively.

Our results showed that not only there is no inhibitory effect between *T. vierns* and *P. fluorescens*, but also they had synergistic effects in both nematode-related as well as plant growth-related factors, in reducing the harmful effects of *M. javanica*. Sharon *et al.* (2001), demonstrated that the application of *T. harzianum* and *T. vierns* in combination with *P. fluorescens*, cause an increase in growth factors of root-knot nematode infected tomato. Knowledge of compatibility or competition between different bioagents is important for combination of antagonists against a particular pathogen (Davet, 2004).

Table 3 Analysis of growth-related parameters variance of tomato plants inoculated with *Meloidogyne javanica*, treated with antagonistic agents and plant debris.

Sources of variance	df	Mean Square					
		Shoot length	Root length	Fresh weight of shoot	Fresh weight of root	Dry weight of shoot	Dry weight of root
Treatments	7	32.281 **	32.281 **	15.508 **	2.269 **	0.498 *	0.599 *
Error	24	6.344	1.281	0.695	0.414	0.029	0.019
CV%	-	4.450	4.960	4.350	8.280	8.360	3.760

* and ** Significant at 5% and 1% level of probability.

Table 4 Growth-related parameters of tomato plants inoculated with *Meloidogyne javanica*, treated with antagonistic agents and plant debris.

Treatments	Agents[1]	Growth related parameters (Mean ± CV%)[2]					
		Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
Unamended soil	*Mj*	51.0 ± 6.6c	18.3 ± 5.2c	16.2 ± 6.2b	5.2 ± 4.6c	4.2 ± 3.9b	1.0 ± 5.9b
	Mj + Pf	53.7 ± 2.2bc	19.8 ± 2.5c	16.7 ± 3.0b	6.1 ± 7.5bc	4.5 ± 7.7a	1.1 ± 3.6b
	Mj + Tv	54.5 ± 3.2bc	18.8 ± 4.7c	16.5 ± 5.9b	6.2 ± 4.8bc	4.5 ± 11.5a	1.1 ± 4.8b
	Mj + Tv + Pf	57.5 ± 6.2ab	21.2 ± 8.3b	16.5 ± 3.0b	6.5 ± 8.9b	4.7 ± 8.7a	1.6 ± 2.1a
Amended soil	*Mj*	58.2 ± 2.6ab	21.7 ± 7.9b	19.0 ± 4.3a	6.2 ± 4.9bc	4.7 ± 9.2a	1.3 ± 4.5ab
	Mj + Pf	58.0 ± 3.7ab	24.5 ± 3.8a	18.4 ± 3.9a	7.2 ± 11.7ab	5.1 ± 11.2a	1.8 ± 3.5a
	Mj + Tv	60.0 ± 8.1a	24.0 ± 3.4a	18.2 ± 4.6a	6.4 ± 11.5b	5.0 ± 6.8a	1.7 ± 2.1a
	Mj + Tv + Pf	59.6 ± 3.0a	25.2 ± 3.9a	18.3 ± 3.9a	7.8 ± 12.4a	5.1 ± 7.9a	1.9 ± 3.6a

[1] Mj: *Meloidogyne javanica*, Pf: *Pseudomonas fluorescens*, Tv: *Trichoderma virens.*
[2] Means followed by dissimilar letters in a column are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$).

One of the basic concerns in biological control is to maintain the population density of the applied bioagents at an effective level. According to Davet *et al.* (1981), maintaining the population and survival of the propagules of *Trichoderma* sp. in soil, is an important factor in success of biological control of plant pathogens. Such properties are affected by soil conditions, including the amounts of organic materials in the soil. Application of organic materials to the soil can increase bioagents activities against plant pathogens. Such kinds of amendments release some carbohydrates and other substances which are required for activity of the biocontrol agent. Moreover, the loss of organic materials in plant, may make plant susceptible to pathogen attack (Morgan and Whipps, 2001).

P. fluorescens is one of the most active bacteria in the rhizosphere which is able to colonize the whole root system and cause considerable limitation in nematode activity (Siddiqui and Mahmood, 2001). Nematicidal activity of *P. fluorescens* against *M. javanica* has been demonstrated under laboratory and greenhouse conditions (Ali *et al.*, 2002). Conformation to the rhizosphere, is a key character for successful biological control of root pathogens (Siddiqui *et al.*, 2006). By producing diacetylphloroglucinol, *P. fluorescens* (Fll3) causes significant inhibition in egg hatch and also has mortality effect on the

second juvenile larvae of *Globodera rostochiensis* (Cronin *et al*., 1997). Sharon *et al.* (2001) and Siddiqui *et al.* (2008) have studied the effects of *P. fluorescens* and *T. vierns*. Their studies support our findings, but in our study the role of plant debris of oak trees increased the antagonistic effects of applied bioagents. Supporting our results, Ahmadzadeh (2012) has expressed that each biocontrol agent requires environmental and nutritional factors to be successful in antagonistic action and establishment to the new environment. According to Copping (1998), the most important factor, is providing favorable conditions for antagonistic activities of bioagent. The studies of Rouhani (2004) showed that the conditions of rhizosphere including moisture, temperature, pH, amount of organic matters and biological activity, play direct roles in survival of *Trichoderma* species. These conditions will also affect the activity, population density and biofilm formation of bacteria (Ahmadzadeh, 2012).

In a study on physical and chemical properties of soil of the under canopy of oak trees, it has been shown that the decayed plant debris contain considerable amounts of organic carbon, total nitrogen, available phosphorus, absorbable potassium, EC, micro-nutrients such as iron, manganese and zinc (Owliaie *et al*., 2011). In their study, significant decrease in pH, calcium carbonate and copper, 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.

Ability of an antagonist microorganism to colonize the plant root is an important factor in biological control (Schroth and Hancock, 1982). It is necessary to provide a favorable environmental condition to enhance the effectiveness of bioagents. Rouhani (2004) showed that the physical, chemical and nutritional conditions of soil, play an important role in biological activity, population changes and survival of *Trichoderma* species. 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). The discussed literature can explain the results of our study in which the addition of plant debris improved the activities of bioagents. Altering the soil condition in favor of antagonistic microorganisms, may prolong the effectiveness of biological control. In this study it has been shown that plant debris can increase the antagonistic activities of applied bioagents. Complementary studies are required to investigate the effects of amending and sowing time on effectiveness of plant debris.

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افزودن بقایای گیاهی جنگل بلوط به منظور افزایش فعالیت عوامل کنترل زیستی *Pseudomonas fluorescens* و *Trichoderma vierns*، علیه *Meloidogyne javanica* در گوجه‌فرنگی

روژین مرادی[1]، فرانک مرادی[2]، کیومرث میره‌کی[2] و محمد عبدالهی[2*]

۱- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه کردستان
۲- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه یاسوج
* پست الکترونیکی نویسنده مسئول مکاتبه: abdollahi@yu.ac.ir
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چکیده: افزودن عوامل آنتاگونیست به محیط ریشه گیاهان ازجمله رویکردهای مهم در مهار زیستی بیمارگرهای خاکزاد است. استقرار و دوام عوامل کنترل زیستی در خاک از دغدغه‌های مهم در مهار زیستی است. این مطالعه به‌منظور تعیین اثر بقایای گیاهی جنگل بلوط بر فعالیت آنتاگونیستی *Pseudomonas fluorescens* و *Trichoderma vierns* علیه نماتد ریشه گرهی *Meloidogyne javanica* در گوجه‌فرنگی انجام گردید. این آزمایش در قالب طرح کاملاً تصادفی با هشت تیمار و پنج تکرار در گلخانه گروه گیاهپزشکی دانشگاه کردستان انجام شد. در تیمارهای آزمایش از یک یا هر دو عامل آنتاگونیست در دو حالت با و بدون افزودن بقایای گیاهی استفاده گردید. براساس نتایج به دست آمده، شاخص‌های رشدی گوجه‌فرنگی در تیمارهایی که بقایای گیاهی به خاک افزوده شده بودند، به‌طور چشم‌گیری بهبود یافت. در مورد شاخص‌های تکثیری نماتد هم تعداد گال در ریشه در تیمارهایی که دارای بقایای گیاهی بودند به طور معنی‌داری کم شد که بیشترین کاهش در تیمارهای با یک یا دو عامل آنتاگونیست مورد بررسی، صورت پذیرفت. در مقایسه با تیمارهای بدون بقایای گیاهی، میزان کاهش گال‌زایی در تیمار دارای *T. virens* حدود ۶۱ درصد و میزان افزایش وزن خشک ریشه در تیمار دارای *P. fluorescens* یا *T. virens* به ترتیب حدود ۶۸ درصد و ۵۶ درصد بود.

واژگان کلیدی: کنترل زیستی، امنیت غذایی، قارچ‌ها، مدیریت نماتد، مواد آلی