Insight Plant Disease 1 (1): 1-11, 2011
ISSN 2198-1948 / DOI: 10.5567/PLANTDIS-IK.2011.1.11

The Effects of Some Biocontrol Agents and their Combination on Root-knot Disease on Tomato

[1]Hadi Golzari, [2]Masuod Ahmadzadeh, [1]Naser Panjehkeh, [1]Mohammad Salari and [3]Ebrahim Sedaghati-khoravi
[1]Department of Plant Protection, University of Zabol, Zabol 98615-538, Iran
[2]Department of Plant Protection, University of Tehran, Karaj 31587-77871, Iran
[3]Department of Plant Protection, University of Rafsanjan, Rafsanjan, 7818744167, Iran

Abstract: The effects of *pseudomonas* sp., *Glomus* sp., their combination and *Trichoderma harzianum* on root-knot disease reduction were investigated. In this experiments, combination of bacterial strains UTPF86 (P2) and 7NSK2 (p1) with species *G. mosseae* (G1) and *G. intraradices* (G2) (G2P2M, G1P2M and G1P1M) gave the best results. These treatments reduced the gall number, gall diameter and nematode penetration, followed by 78, 70, 60, 79, 69 and 60%, respectively ($p<0.05$). Bacterial strains 7NSK2 and UTPF86 produced the maximum of salicylic acid (inducing resistance agent) and hydrogen cyanide (agent of neurosis and maim) in *in vitro* ($p<0.01$) and mycorrhizal fungi induces the resistance. Therefore, it was expected that the combination of mention two agents would give the best effect in enhancement of plant growth and nematode biocontrol. Among combined treatments, $T2Mt_1$, P2M and G1M gave the best results. These treatments reduced the gall number, gall diameter and nematode penetration, followed by 59, 48, 32% and 71, 60, 49% and 58, 48, 32%, respectively so that their effects were not significant ($p<0.05$). $T1Mt_2$ was the worst treatment. However, this treatment reduced the gall number, gall diameter and nematode penetration, followed by 16, 12 and 17%, respectively so that its effects against the contaminated control was significant ($p<0.05$). The present study demonstrated that AM fungus and bacteria can coexist without adversely affecting one another. In fact, suitable combinations of biocontrol agents can further increase the plant growth and resistance to pathogens.

Key words: Combination, *Meloidogyne javanica*, mycorrhizal fungi, *Pseudomonas* sp. *Trichoderma harzianum*

INTRODUCTION

Plant parasitic nematodes cause significant damage to agriculture within the EU and throughout the world (Sasser and Freckman, 1987).The root-knot nematode, *Meloidogyne* spp., is one of the most important Plant parasitic nematodes. *Meloidogyne* spp. is able to infect more than 2000 plant species (Jung and Wyss, 1999). The damage caused by the root-knot nematode is much higher in tropical and sub-tropical countries (Taylor and Sasser, 1978). Studies have shown that root-knot nematodes can cause suppression in yield of tomato as high as 85% (Sasser, 1979; Taylor and Sasser, 1978).

Beneficial pseudomonads can antagonize soil-borne pathogens through various mechanisms (Bakker *et al*., 1991). For example, bacterial siderophores inhibit plant pathogens through competition for iron, antibiotics suppress competing microorganisms or hydrogen cyanide (Ahl *et al*., 1986) and chitinases and glucanases lyse microbial cells; and these compounds have been implicated in the reduction of deleterious and pathogenic rhizosphere microorganisms, creating an environment more favorable for root growth (Leong, 1986). Recent studies have demonstrated that some rhizobacteria can also act indirectly by inducing systemic resistance in the plant towards soil-borne fungi and plant-parasitic nematodes (Siddiqui and Shaukat, 2002a, b).

The improvement and eventual commercialization of fluorescent pseudomonads as biocontrol agents depends in part on understanding and exploiting the mechanisms involved in these antagonistic interactions among bacteria, pathogens and their plant hosts.

The establishment of the AM in the roots of more than 80% of all land plants is the result of a complex exchange of signals between the host plant and AMF. Classically, four major groups of mycorrhizal mode of action mechanisms that mediated bioprotection have been considered: (1) direct competition, (2) mechanism mediated by alteration in plant growth, nutrition and morphology, (3) biochemical and molecular changes in

Corresponding Author: Hadi Golzary, Department of Plant Protection, University of Tehran, Karaj 31587-77871, Iran
Tel: +98-913279204, +98-345-4225648 Fax: +98-2612819254

mycorrhizal plants that induce pathogen resistance and (4) alterations in the soil microbiota and development of pathogen antagonism (Vierheilig *et al*., 2008). *Arbuscular mycorrhizal* fungi are of great value in promoting uptake of phosphorus, nitrogen, minor elements and water and also increase plant growth and yield of several crops (Hayman, 1982; Zambolim and Schenck, 1983; Allen, 1996).

Weindling (1932) over 75 years ago, demonstrated the antagonistic nature of *Trichoderma* fungal species from the genus, Trichoderma were demonstrated over 75 years ago. The genus, *Trichoderma* is common filamentous imperfect fungi (Deutromycetes, Dematiaceae), the most common saprophytic fungi in the rhizosphere and found in almost any soil. *Trichoderma* species are free-living fungi that are common in soil and root ecosystems. They are opportunistic, avirulent plant symbionts, as well as parasites of other fungi. Some strains establish robust and long-lasting colonizations (colonies) of (on) root surfaces and penetrate into the epidermis and a few cells below this level. Biocontrol activities of *T. asperellum*-203 and *T. atroviride* IMI 206040 (both fungi were previously defined as strains of *T. harzianum*) have been reported against *M. javanica* in soil (Sharon *et al*., 2001). Other *Trichoderma* species and isolates have also exhibited significant biocontrol activity against *M. javanica* in growth chamber experiments (Spiegel *et al*., 2006). Most studies on nematodes concurred that the promising fungal antagonists *Trichoderma* spp., had different and in fact multiple modes of action. For example, *Trichoderma virens* invaded, ramified, grooved and vacuolated the root-knot nematode eggs. Eapen *et al*. (2005) reported easy staining of eggs for microscopy due to the increased permeability of eggshell. The antagonistic action of *Trichoderma* spp. was chiefly attributed to chitinolytic activity of the fungi on cellular structure of nematodes which is rich in chitin. Early researchers identified key concepts and developed tactics for multiple option management of nematodes. Although, the emphasis on integrated pest management over the past three decades has promoted strategies and tactics for nematode management, comprehensive studies on the related soil biology ecology are relatively recent. Traditional management tactics include host resistance (where available), cultural tactics such as rotation with nonhosts, sanitation and avoidance and destruction of residual crop roots and the judicious use of nematicides. There have been advances in biological control of nematodes but field-scale exploitation of this tactic remains to be realized (Barker and Koenning, 1998).

This study is aimed on determination of effects of different antagonistic agents separately and their combination in decrease of the disease indices and increase of growth indices also decrease of damage due to *Meloidogyne javanica* nematode on tomato seedling to have a safe environment. With the increasing cost of inorganic fertilizers, the environmental and public health hazards associated with pesticides and pathogens resistant to chemical pesticides. AM fungi may provide a further suitable and environmental alternation for sustainable agriculture.

MATERIALS AND METHODS

Individuation, identification and reproduction of Nematode: Mature females were dissected out from large galls on the roots of tomato plants. Perineal patterns slides (10-20) from each sample or locality were prepared and examined under microscope to study their characteristics Perennial patterns of mature females of root-knot nematode were prepared for identification. At least 10 perennial patterns of each sample of nematode species were examined to make more accurate identification (Jepson, 1987). For reproduction of nematode, single egg mass was separated from terminal of the females' body and were inserted to the soil by making three holes around the tomato seedlings (Race of Early Urbana) in greenhouse conditions.

Nematode inoculums: *Meloidogyne javanica* nematode was obtained from pure cultures was maintained on roots of tomato. The entire root system was dipped in water and soil was removed gently without detaching egg sacs. Eggs were extracted by vigorous shaking of infested roots in a 1% sodium hypochlorite solution for 3 min. The resulting suspension was then passed through a range of different mesh-size sieves. The eggs were collected on a fine sieve (38 mm) and washed in tap water to remove all traces of sodium hypochlorite before use. Hatched juveniles of *M. javanica* were obtained by placing the eggs in sterile distilled water for 5 days at 28°C. The inoculum was used for the laboratory and glasshouse tests (Siddiqui *et al*., 2006).

Bacterial inoculums: The Fluorescent Pseudomonad bacterial strains (UTPF86, UTPF5, 7NSK2) that were used in this study were obtained from the Department of Plant Pathology, University of Tehran, in Iran. Bacterial Strains (1) were cultivated at 24°C for 24 h with shaking (150 rpm) in 250 mL Erlenmeyer flasks containing 100 mL of King's B medium. The bacterial culture was centrifuged at 2800 rpm for 20 min, the supernatant discarded and the pallet resuspended in $MgSO_4$ (0.1 M) (Siddiqui and Shahid, 2003).

Mycorrhizae inoculums: Two mycorrhizal species were used in this work. The air dried inoculum of *Glomus intraradice* and *G. mosseae* was obtained from the Soil and Water Research center in Iran, Tehran and prepared on Sudan grass (*Sorghum bicolor* L.) grown in sandy loam soil mixed with washed river sand in the ratio of 3:2 (v/v) respectively for four-month-old then spores of fungi were isolated by sieving and decanting and were counted under stereomicroscope to the help of a fine hair brush. There were 32 *G. mosseae* spores and 26 *G. intraradices,* spores per soil gram.

Trichoderma inoculums: Two *Trichoderma harzianum* strains (T1 and T2) were used in this work (Table 1). Mention strains were obtained from the Department of Plant Pathology, University of Tehran, in Iran. Two strains of *T. harzianum* that had been evaluated for their nematicide activity in vitro beforehand, was cultured in Agar water medium. Plates was incubated in 25°C for 5 days because the fungi growth as well as. A mixture of peat and wheat bran (1:1, v/v) has been used extensively as a medium for delivery of *T. harzianum* preparations (Sivan *et al*., 1984). In this modified preparation, the pH remains constant and low (5.5) during the entire growth period, thus preventing bacterial contamination. The mention mixture was autoclaved for 15 min, twice in two days continuously. The fungi suspension (10 mL, 10^8 CFU g^{-1} per mL) was inserted to the steriled mixture in the Erlen. The mention mixture was incubated for 18 days in 25°C because the fungi growth as well as (Spiegel and Chet, 1998).

Primary investigation of the effective metabolite production in bacterial antagonistic specifics: Some tests (hydrogen cyanide, protease, Salicylic acid and siderofor) was did to measure of the ability and secondary metabolite production meter in Fluorescent pseudomonad bacteria for primary screen of the thirty bacterial strains (Schaad *et al*., 2001). Eleven strains were chief in mention tests that selected for survey of the nematicide activity.

Biocontrol of root-knot nematode by Flourescent Pseudomonad bacteria (In greenhouse): Three-week-old tomato seedlings (Early Urbana) were planted in plastic pots (12 cm diameter) filled with 700 g unsterilized sandy-loam (pH 8.1; moisture retaining capacity 38%) and cultivated in a glasshouse (19-24 and 29-33°C day and night temperatures, respectively). After one week, the plants were treated with one of the following bacteria (three bacterial strains that had been evaluated for their nematicidal activity *in vitro* beforehand) by pipetting 30 mL of the bacterial suspension (diluted to OD600 = 0.5 which corresponds to 2.1×10^9 CFU mL^{-1}) into soil around the root system. Each treatment was replicated 5 times. Control plants received 30 ml of one-fourth concentration Ringer solution. Two days after bacterial application, 2000 freshly hatched juveniles of *M. javanica* were inserted to the soil by making three holes around the seedlings (Siddiqui and Shaukat, 2002c). The plant samples were taken eight-week-old after nematode inoculation. Tomato roots were carefully rinsed in tap water, separated from the shoot, blotted dry and weighed. The numbers of galls produced on the entire root system were counted using a hand lens and their diameter was measured. Final larva mortality in percent and population densities of the nematode in the roots were estimated following the method outlined by Siddiqui and Shaukat (2002b).

Biocontrol of root-knot nematode by mycorrhizal (in greenhouse): Plants were prepared and cultivated similar to the experiment of eight. After one week, the plants were treated with one of the following fungi, 40 g soil (per gram 32 spores inclusive 1200 *G. mosseae* spores) and 50 g soil (per gram 26 spores inclusive 1200 *G. intraradices* spores) into soil around the root system for each treatment separately. Each treatment was replicated 5 times. Two-week after fungi application, 2000 freshly hatched juveniles of M. javanica were inserted to the soil by making three holes around the seedlings (Bhat and Mahmood, 2000). The plant samples were taken eight-week-old after nematode inoculation and shoot and root dry weight, number of galls per root system, gall diameter, nematode population in soil and root and percent root colonization of AM fungus were measured and recorded.

Biocontrol of root-knot nematode by *Trichoderma* (In greenhouse): Plants were prepared and cultivated similar to eight-experiment. After one week, the plants were treated with one of the following fungi. The *trichoderma* inoculum was mixtured (1% w/w) with soil in each pot (Sharon *et al*., 2001). Each treatment was replicated 5 times. In this section, three-time-factor was used in consist: In one-time-fictor (t_1), Trichoderma fungi and 2000 j_2 (juvenile larva) was inserted to the soil by making three holes around the seedlings and three-week-old tomato seedlings was transplanted after 18 days. In two-time factor (t_2), seedlings was transplanted and inoculated with fungi and nematode simultaneity. In three-time factor (t_3), seedlings was transplanted and inoculated with fungi then inoculated with nematode after 18 days (Spiegel and Chet, 1998). The plant samples were taken

eight-week-old after nematode inoculation and shoot and root dry weight, number of galls per root system, gall diameter, nematode population in soil and root were measured and recorded.

Biocontrol of root-knot nematode to integrate flourescent pseudomonad bacteria and mycorrhzal fungi (In greenhouse): Plants were prepared and cultivated similar to mentioned experiment. After one week, the plants were treated with one of the following fungi, 40 g soil (per gram 32 spores inclusive 1200 *G. mosseae* spores) and 50 g soil (per gram 26 spores inclusive 1200 *G. intraradices* spores) into soil around the root system for each treatment separately (Shafi and Mahmood, 2000). Each treatment was replicated 5 times. Plants were treated with one of the following bacteria (three bacterial strains that had been evaluated for their nematicide activity in vitro beforehand) by pipetting 30 mL of the bacterial suspension (diluted to OD600 = 0.5 which corresponds to 2.1×10^{9} CFU mL^{-1}) into soil around the root system. Control plants received 30 mL of one-fourth concentration Ringer solution. Two-week after fungi and bacteria application, 2000 freshly hatched juveniles of *M. javanica* were inserted to the soil by making three holes around the seedlings. The plant samples were taken eight-week-old after nematode inoculation and shoot and root dry weight, number of galls per root system, gall diameter, nematode population in soil and root and percent root colonization of AM fungus were measured and recorded.

The evaluation of nematode population in root: The number of juveniles, eggs and females in the roots were also estimated. The roots were cut into small pieces and mixed.; gr root was macerated for 45s in blender to recover nematode eggs, females and larvae. The total root population of nematodes was determined with the number of larvae and females present in 1 g root and by multiplying it with total weight of root.

The Larva mortality of nematode in percent: The Larva mortality of nematode in percent was determined in infected roots against control roots via Abbott's formula (Abbott, 1925):

$$\text{The Larva mortality of nematode (\%)} = 100\times[1-(Tn/Cn)]$$

Where:

Tn = Nematode population after inoculation
Cn = Nematode population in control
n = Nematode population
T = Treatment (fungi or bacteria)
C = Control (non treatment)

The evaluation of root colonization percentage by mycorrhizal fungi. The proportion of root colonized by *G. mosseae* was determined by the grid line intersecting method (Giovannetti and Mosse, 1980) after clearing the roots with KOH (Phillips and Hayman, 1970) and staining the roots in 0.05% trypan blue lactophenol.

Statistical analyses: Data were subjected to one-way analysis of variance (ANOVA) followed by the Complete Randomized Design (CRD) test using SAS software (ver. 9.1, USA). Meanwhile each treatment was replicated 5 times.

RESULTS

Primary investigation of the effective metabolite production in bacterial antagonistic specifics (*In vitro*): Its Information has been shown in under Table 1.

Investigation of nematicide activity (*In vitro*): Its Information has been shown in under Table 2 and 3.

Biocontrol of root-knot nematode by some antagonistic agents: The rate of growth and disease was different in plants of infected to nematode so that they were affected by antagonistic agents against the just nematode infected plants (as contaminated control). The differences were significant in all indices at level of five percent (Table 1, 2).

Growth indexes: Among all the treatments of infected to nematode and the antagonistic agents, root dry weight against contaminated control was in range of 29 to 76% and shoots dry weight was in range of 22 to 75%. The treatments of G2P2M and T1Mt2 in nematode infected plants had the best and worst effect in enhancement of the dry weight. Among the combined treatments, G2P2M and G2P3M increased the dry weight of the nematode infected plants, followed by 76 and 58 %, respectively so

Table 1: Investigation of the effective metabolite production in bacterial antagonistic

Bacterial strain	HCN (Color changing of picric paper)	Protease mm Diameter of halo	SA Absorption of light	Lipase mm Diameter of halo	Siderophore (cas) mm Diameter of halo	Amylase mm Diameter of halo	SIDEROPHORE (Abs 400 nm)	Siderophore (casad) mm Diameter of halo
UTPf5	3	2	0.043	-	16.5	8	1.037	10
UTPf86	2	3	0.287	-	15.5	13	0.855	12
UTPa95	3	5	0.022	-	18.0	6	2.404	15

P1 is the bacterial strain *P. æruginosa* 7NSK2 and P3 (UTPF5), P2 (UTPF86) is the bacterial strain *P. fluorescens*

Table 2: Mean comparative of Root-Knot nematode control by three strains of bacterial (*In vitro*)

Bacterial strain	Parasitism on eggs (%)	Parasitism on J2s (%)
UTPa95	8/4±6/68a	3/7±9/7a
UTPf5	1/3±4/46c	3/1±9/58c
UTPf86	7/6±4/44c	4/2±9/70b
Non treated (Control)	8/1±1/6e	6/2±9/8g

Means with the same letter are not significantly different at 1% level according to Dunkan. UTPa95 is the bacterial strain *P. aeruginosa* 7NSK2 and UTPF5, UTPF86 is the bacterial strain *P. fluorescens* that they were used from b collection, of University of Tehran, in Dep. Plan protection. Numbers is in percentage

Table 3: Mean comparative of Root-Knot nematode control by two strains of Trichoderma (*In vitro*)

Trichoderma isolate	Parasitism on eggs (%)	Parasitism on J2s (%)
T1	68.90b	83.87a
T2	88.36a	80.80a
M	7.30b	10.80c

Means with the same letter are not significantly different at 1 % level according to Dunkan. T1and T2 is two isolates of *Trichoderma harzianum* that they were used from mycology collection, of University of Tehran, in Dep. Plan protection. M is the plants inoculated by *Meloidogyne javanica*. Numbers is in percentage

Table 4: Means comparison of the effects of some antagonistic agents on the plant growth

Treatment	Shoot dry weight (gram)	Root dry weight (gram)	Root Colonization (%)
P1M	1.29f	0.59fghi	-
P2M	1.77cde	0.75def	-
P3M	1.01gh	0.50hi	-
G1M	1.14fg	0.57fghi	61.85f
G2M	1.05g	0.54ghi	60.92f
$T1Mt_1$	1.89bcd	0.82cde	-
$T1Mt_2$	0.78ij	0.42ij	-
$T1Mt_3$	1.68e	0.66efgh	-
$T2Mt_1$	1.99b	0.85cd	-
$T2Mt_2$	0.84hi	0.48hi	-
$T2Mt_3$	1.75cde	0.69defg	-
G1P1M	2.06b	0.97bc	69.56c
G1P2M	2.32a	1.12ab	71.14b
G1P3M	1.95bc	0.83cde	67.7d
G2P1M	1.73de	0.67efgh	66.18e
G2P2M	2.48a	1.26a	72.75a
G2P3M	1.66e	0.65efgh	65.32e
M	0.61j	0.30j	-

Means having common letters, of Duncan test at 5% level have not significant difference from each other treatment. M is the infected control that it has *M. javanica* nematode. P1 is the bacterial strain *P. aeruginosa* 7NSK2 and P3 (UTPF5), P2 (UTPF85) is the bacterial strain *P. fluorescens*. G is the Mycorrhizal species which include the *G. mosseae* (G1) and *G. intraradices* (G2). T is the *T. harzianum* isolate. t is the inoculated time factor. GPM is the integration of mycorrhizal species (G1 and G2), *P. fluorescens* strains and *M. javanica*.

that they had the highest and lowest effects, respectively. The difference was significant in these two treatments ($p<0.05$). Among the Pseudomonas bacteria and nematode treatments, P2M and P3M increased the dry weight of the nematode infected plants, followed by 63 and 40%, respectively, so that they were the highest and lowest in treatments. The difference was significant in these two treatments ($p<0.05$). Between mycorrhizal and nematode treatments, G1M and G2M increased the dry weight of the nematode-infected plants, followed by 46 and 43%, respectively. The difference was not significant between these two treatments ($p<0.05$). Among the *Trichoderma* fungus and nematode treatments, T2Mt1 and T1Mt2 increased the dry weight of the nematode-infected plants, followed by 67 and 26%, respectively so that they were the highest and lowest in treatments, respectively. The difference was significant in these two treatments ($p<0.05$). Amount of damage to the root and shoot dry weight of infected plants against control were in range of 66 to 74%. Reduction of the dry weight was significant in nematode-infected plants against other treatments ($p<0.05$) (Table 4).

Disease indexes: Among all the treatments of nematode infected plant and antagonistic agents, reduction of the gall number against control was in range of 16 to 78% and reduction in the gall diameter was in range of 12 to 77%. The treatments of G2P2M and T1Mt2 caused the highest and lowest reduction in the number and gall diameter gall. Their differences was significant against other treatments ($p<0.05$). Among the combined treatments, G2P2M and G2P3M decreased the gall diameter and gall number against the nematode infected plants, followed by 78, 39, 77 and 54%, respectively. These treatments had the highest and lowest effect in reduction of the gall number and gall diameter, respectively. The difference was significant in these two treatments ($p<0.05$). Among the Pseudomonas bacteria and nematode treatments, P2M and P3M decreased the gall number and gall diameter against the nematode infected plants, followed by 48, 23, 60 and 37%, respectively. These treatments had the highest and lowest effect in reduction of the gall number and gall diameter, respectively. The difference was significant in these two treatments ($p<0.05$). Among the treatments of the plants infected to mycorrhizal fungi and nematode, G1M and G2M decreased the gall diameter and gall number against the just nematode infected plants, followed by 32, 29, 49 and 48%, respectively. The difference was not significant in these two treatments Among the *Trichoderma* fungus and nematode treatments, T2Mt1 and T1Mt2 decreased gall diameter and gall number, followed by 59, 16 and 71 and 12%, respectively. These treatments had the highest and lowest effect in reduction of the gall number and gall diameter. The difference was significant in these two treatments ($p<0.05$). In addition, gall number and gall diameter was significant in treatments against contaminated control at the level of five percent (Table 5). Number of eggs and second-stage juveniles (J2) per gram root were significantly lower in fungal treatments ($p<0.05$).

Table 5: Mean's comparison of the effects of some antagonistic agent in reduction of nematode infection

Treatment	Gall number	Gall diameter (mm)	Penetration of nematode	Larva mortality in percent [(100-(1-nT/nC]
P1M	520cde	3.2fg	1049ed	33hi
P2M	402fgh	2.6hi	810gh	48ef
P3M	595bc	4.1d	1204bc	23jk
G1M	526cd	3.3fg	1060ed	32hi
G2M	545cd	3.4ef	1102cd	29ij
$T1Mt_1$	330hij	2.9fgh	676ijk	57cd
$T1Mt_2$	644b	5.7b	1293b	17k
$T1Mt_3$	533cd	3.7de	1071d	31hij
$T2Mt_1$	312ij	1.9k	652jk	58cd
$T2Mt_2$	551c	4.9c	1103cd	29ij
$T2Mt_3$	383ghi	2.6hi	778hi	50def
G1P1M	308j	2.1jk	622k	60c
G1P2M	231k	1.7kl	485l	69b
G1P3M	377hij	2.4ij	763hij	51de
G2P1M	450efg	2.9gh	911fg	42fg
G2P2M	166l	1.5l	337m	78a
G2P3M	472def	3.0fgh	952ef	39gh
M	768a	6.5a	1556a	-

Means having common letters, of Duncan test at 5% level have not significant difference from each other treatment. M is the infected control that it has *M. javanica* nematode. P1 is the bacterial strain *P. aeruginosa* 7NSK2 and P3 (UTPF5), P2 (UTPF85) is the bacterial strain *P. fluorescens*. G is the Mycorrhizal species which include the *G. mosseae* (G1) and *G. intraradices* (G2). T is the *T. harzianum* isolate. t is the inoculated time factor. GPM is the integration of mycorrhizal species (G1 and G2), *P. fluorescens* strains and *M. javanica*.

DISCUSSION

The nematode penetration to the root of plants so that treated with UTPF86 strain was more than *P. aeruginosa* 7NSK2 (P1) but total galls in inoculated roots with UTPF86 was lower than that may be caused by highest production of salicylic acid in UTPF86 strain (Table 1). Salicylic acid (SA) is known to play a critical signaling role in the activation of plant defense responses after attacking of pathogen (Klessig *et al*., 2000). This indicates that ISR by rhizobacteria is independent of in planet accumulation of SA. In recent literature, SA has been reported as an endogenous signal for the activation of certain plant defense responses, inclusion of expression of PR gene and enhanced resistance to pathogens (Conrath *et al*., 1995). In addition, the performance of UTPF86 strain in the biocontrol of root-knot nematode in greenhouses was better than the other strains. Also similar studies showed that production of salicylic acid by *Pseudomonas* sp. increases the defense mechanisms of host and the resistance will induce systemic or local defense and the pathogen indirectly dead. Application of SA is known to express resistance reaction in cowpea roots against *M. incognita* (Nandi *et al*., 2002) but the role of production of SA by rhizobacteria against *M. javanica* has not been investigated yet. Based on the presented results it is assumed that fluorescent pseudomonad mediate systemic resistance against root-knot nematode in tomato by pathway of SA-independent transduction (Siddiqui and Shaukat, 2004). The researches are shown that CAT extracts from leaves is found to be less sensitive to SA inhibition than root CAT SA (1 mM) completely inhibits root CAT, whilst the same concentration causes approx. 60% inhibition of CAT is in leaf (Molinari and Loffredo, 2006) Generally, SA treatment don't seem to limit the degree of infestation of J2 significantly, although, it may have been an inhibited effect on the reproduction of nematode index (Molinari, 2005).

In this research, *P. aeruginosa* 7NSK2 (P1) strain was cultured, the presence of hydrogen cyanide UTPF86 was shown and Pykrete paper changed from yellow to reddish brown color. It seems, there is a positive relationship between hydrogen cyanide by bacteria and the rate of nematode mortality. Nematode population in soil and rhizosphere treated to 7NSK2M was lower than other treatments. It is probably due to the production of volatile compound of hydrogen cyanide that it is highest. Gallagher and Manoil (2001) founded that production of HCN by *P. aeruginosa* plays a key role in killing the juveniles of *Meloidogyn* sp., as inhabiting soil against nematode (Siddiqui *et al.,* 2006). *P. aeruginosa* 7NSK2 (P1) strain UTPF86 strain produced hydrogen cyanide more than other strains in laboratory tests. On the other hand its nematicide also was higher in the laboratory. The penetration of nematode to the inoculated roots with *P. aeruginosa* 7NSK2 (P1) strain was lower than other strains. Siddiqui *et al*. (2006) investigated the role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of the root-knot nematode on tomato and suggested for effective control of *M. javanica*, there is a direct relation between production of HCN by CHA0 and mortality of nematode (Siddiqui *et al*., 2006). It cannot be stated with certainty whether HCN production by 7NSK2 (P1) strain is the only mechanism responsible for the suppression of root-knot nematode against other mechanisms (Bakker *et al*., 1991) such as, alteration of root exudates that reduce attraction of nematode, enhance host defense mechanism to systemic resistance and/or blockage of the potential entry sites for nematode following increase number of bacterial cells in the rhizosphere and roots can be involve in the reduction of invasion of nematode and development of ultimate root-knot (Siddiqui *et al*., 2003). Therefore, it is concluded DAPG, pyoluteorin and hydrogen cyanide play a critical role (but not primary) in nematode mortality through neuromuscular disorders (Siddiqui and Shaukat, 2004). Studies of human and animal cyanide poisoning indicate that the poison strongly affects neurological tissue

(Way, 1984) and it is possible that this poison causes the nematode mortality also reflects hypersensitivity of neuromuscular tissues (Gallagher and Manoil, 2001).

7NSK2 (P1) strain in laboratory tests produces significant amounts of protease and it had the highest percentage of egg non-hatching. The AprA protease of CHA0 strain contributes in biocontrol of *M. incognita* directly or indirectly. These data support the involvement of AprA protease in the inhibition of egg hatching and juvenile's mortality. However, AprA protease may not be the only antinematode factor of bacterial strain, antibiotic compounds produce under GacA control also have a role in biocontrol of nematode (Siddiqui *et al.*, 2005). Briefly, this results demonstrate that protease enzyme in strain 7NSK2 (P1) is effective to biocontrol of *M. javanica* directly or indirectly.

In plants inoculated by nematodes and mycorrhiza indicated very small primary galls because the endomycorrhiza interfered with the development of nematodes. In endomycorrhizal root system was observed significant reduction in the number of *M. incognita* larvae that they develop into adult. Inoculation of tomato plants with *G. mosseae* and *G. intraradises* 14 days after nematode infection, significantly reduced the number and size galls. Our results are in agreement with those of Kellam and Schenck (1980), who reported a similar effect of *G. mosseae* on tomato plants infected by root-knot nematode.

The growth's response of nematode-infected plant as a result of mycorrhiza assisted nutrition is commonly mentioned in the literature as the capacity of mycorrhiza to improve plant's health, confer protection, prophylactic effect, or increase host tolerance against the nematode pest and etc. (Elsen *et al.*, 2008). Increase in plant growth after colonization of root by AM fungi is due to improvement in the mineral nutrient status of host plant. During AMF colonization, there is little evidence that classic plant resistance responses occur at high levels. However, these responses are greatly stimulated when a subsequent challenge with a pathogen occurs (St-Arnaud and Vujanovic 2007; Gianinazzi-Pearson *et al.*, 1996) but a good colonization of AMF is a prerequisite for this response (Cordier *et al.*, 1998; Slezack *et al.*, 2000). It seems that AMF colonization acts as a priming system, immunize the plant against a pathogen (Elsen *et al.*, 2008).

In the present study, *G. intraradices* improved plant growth of nematode-infected plants by reduction in reproduction of nematode. This object has been shown for other AM fungus. We presumed inhibition of disease by *G. intraradices* that it might be related to the increase in content of phosphorus, because a significant increase in phosphorus and dry mass of roots was observed (data not shown). However, an increase in phosphorus may not be the sole cause of disease inhibition. In addition, change of nutrient uptake and root system, a mycorrhizosphere effect and activation of plant defense mechanisms are thought to be responsible for disease inhibition by AM fungi (Demir and Akkopru, 2005).

The inoculation of mycorrhiza and the number of spores was not significantly affected in infection of nematode. Inoculation of mycorrhizal before nematode intensifies and spores are formed more than other times. In this case, effective control of nematodes was done against inoculation of mycorrhizal and nematode simultaneity. In this research, the total spore of mycorrhiza was sufficient for inoculation. Based on references, 1200 spores inoculated for each plant. It is possible that higher initial inoculum density of nematode might affect on the initial colonization of the fungus. However, it is doubtful that enhancement of the fungus initial inoculum would exert such an effective agent on the number of penetrated nematodes to tomato seedling, because the nematode requires only a few hours to infection of root while the fungus requires at least 10 days to become established in the roots (Kellam and Schenck, 1980).

In this study, root colonization (%) by *G. mosseae* (G1) was 2% more than root colonization (%) by *G. interaradices* (G2).

If root colonization (%) is more each value the nematode penetration and gall number will reduce. Although, mycorrhizal plants have higher root systems than non-mycorrhizal plants, the total number of galls produces on each mycorrhizal plant is less than the number of produced galls on non-mycorrhizal plants. Fewer galls in mycorrhizal roots demonstrates reduction of ability of the nematode penetration or the presence of the fungus may influence the development of giant cells which can, in turn, interfere with development of nematode. Our results are in agreement with those of Kellam and Schenck (1980).

The time of inoculation is important in nematode biocontrol by *Trichoderma* sp. In this study, T2Mt1 was the best treatment to biocontrol of nematode. In this treatment, nematode responsed against *Trichoderma* sp. isolates for 18 days then transplanted three-week-seedling. The concentration of the acidic component(s) in the soil is very low and a certain period of time may therefore, was needed for its accumulation before its effect on J2 becomes significant. Thus in those experiments in which J2 were exposed to *T. harzianum* for 18 days prior planting, was observed maximum nematicidal efficacy; but when J2 were exposed to the fungus during planting. In treatments where *T. harzianum* had been

assigned to the root-ball, was not recorded nematicidal activity. For all studied species, the use of wheat bran increased the production of spore. However, the enhancement was extremely high for *T. harzianum* and *T. viride* nutritional supplementation needed production of high spores' (Cavalcante *et al*., 2008).

In this study, top wet weight in $T2Mt_1$ and $T2Mt_3$ treatments had not significant difference (in 0.05 levels) but its difference was significant in disease indexes (in 0.05 levels). Top wet weight had significant increase in pre-colonization plants by *T. harzianum* but they had not significant reduction in disease indexes (in 0.05 levels) also, Sharon *et al*. (2001) resulted that biocontrol of pre-colonization plants was not satisfactory but stem wet weight had significant increase in these plants (in 0.05 levels). Treatment of the soil by *T. harzianum* isolates at the time of transplanting caused the small reduction in nematode galling. However, total gall was reduced significantly when treatment was took place one week before transplanting-allowing to extend exposure of the nematode against the antagonist. Our results are in agreement with those Fattah and calleague (Al-Fattah *et al*., 2007).

In this research, larval mortality in percent and parasitism on egg's nematode was great by *T. harzianum* isolates that it may be because of proteolytic or production of chitinolytic enzyme by the fungus. The germ has also been found to trigger proteolytic and production of chitinolytic enzyme by the fungus (Sharon *et al*., 2007). This combination of enzymes is required to disrupt the eggshell (Tikhonov *et al*., 2002; Khan *et al*., 2004), although, chitinolytic capacity is probably the most important activity on the eggshells (Morton *et al*., 2004). While *T. atroviride* presented the greatest efficiency for parasitism of J2s, probably because of its high proteolytic activities (Sharon *et al*., 2007).

However, secondary metabolites from fungi also contain compounds which are toxic to plant parasitic nematodes (Al-Fattah *et al*., 2007). *Trichoderma* may be effective as an egg pathogen; and a pre-plant treatment when eggs are present, not J2, may give better results (Al-Fattah *et al*., 2007). Additional studies are needed to clarify the interaction of *Meloidogyne* spp. with *T. harzianum* as a biocontrol agent in terms of the physiological roles of enzyme activities in response to attraction of nematode and fungal colonization.

In this research, the nematode population was very high in the soil of T1Mt3 and T2Mt3 treatments. The nematode penetration in root was less in the treatment of *Trichoderma* fungi and nematode simultaneity. This order may be because of pre-colonization of root by fungi. Our results are in agreement with those of Chet *et al.* (2006). Some *Trichoderma* rhizosphere-competent strains colonize also entire the surface of root with morphological features reminiscent of those seen during mycoparasitism. Penetration to the root tissue is usually limited to the first or second layers of cells and only in the intercellular spaces (Chet *et al*., 2006).

The strain UTPF86 (P2) produced the maximum of salicylic acid in vitro thus its role is important in induced resistance (Table 1). In other hand, induced resistance is one of the biocontrol mechanisms by mycorrhizal fungus. Therefore, it was not far that combined use of *Glomus* sp. and UTPF86 (P2) strain that it had high percentage of colonization, caused the greatest increase and the plant growth with adverse effects on gall number and gall diameter than other treatments. Also 7NSK2 (P1) strain produced cyanide hydrogen more than other strains in vitro (Table 1). This material has neurosis and maims effect on nematode larva and reduces the nematode penetration. Combined use of *Glomus* sp. and 7NSK2 (P1) sitted in secondary station after combined use of *Glomus* sp. and UTPF86 (P2) strain against other treatment. Combined use of *Glomus* sp. and *Pseudomonas* sp. caused the greater increase and plant growth coinciding by adverse effects on nematode multiplication and morphometric. When *Pseudomonas* sp. and *Glomus* sp. were applied together, the increase in tomato growth was greater than when either agent was applied alone.

Pseudomonas sp. was better in reduction of galls and reproduction of nematode against *Glomus* sp. whereas use of the two together was better than when either agent was applied alone. Namely, combined application of the *Glomus* sp. and the *Pseudomonas* sp. was better than when either agent was applied alone. Root colonization by *Pseudomonas* sp. was increased when inoculated by *Glomus* sp. more than inoculation of single. Also inoculation of *Pseudomonas* sp. increased the root colonization by the AM fungus. The present study demonstrated that AM fungus and plant-growth-promoting rhizobacterium can coexist without adversely affecting one another. In fact, suitable combinations of these biocontrol agents can further increase the plant growth and resistance to pathogens.

These results will help to define optimal conditions for better growth of tomato as well as and for management of root-knot nematode in infested field.

ACKNOWLEDGMENTS

This research has been done by supporting of pole biological control of plant pests and diseases, College of Agriculture and Natural Resources, University of Tehran in the laborator of biological control of plant diseases.

REFERENCES

Abbott, W.S., 1925. A method of computing of the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Ahl, P., C. Voisard and G. Defago, 1986. Iron bound-siderophores, cyanic acid, and antibiotics involved in suppression of *Thielaviopsis basicola* by a *Pseudomonas fluorescens* strain. J. Phytopathol., 116: 121-134.

Al-Fattah, A., A. Dababat and R.A. Sikora, 2007. Use of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of meloidogyne incognita on tomato. Jordan J. Agric. Sci., 3: 297-309.

Allen, M.F., 1996. The ecology of arbuscular mycorrhizas: A look back into the 20th century and a peek into the 21st. Mycol. Res., 100: 769-782.

Bakker, P.A., H.M. Van, R. Peer and B. Schippers, 1991. Suppression of Soil-Borne Plant Pathogens by Fluorescent Pseudomonads: Mechanisms and Prospects. In: Biotic Interactions and Soil-Borne Diseases, Beemster, A.B., G.R. Bollen, M.J. Gerlagh, A.M. Ruissen and B. Schippers (Eds.). Elsevier, Amsterdam, pp: 217-230.

Barker, K.R. and S.R. Koenning, 1998. Developing sustainable systems for nematode management. Phytopathology, 36: 165-205.

Bhat, M.S. and I. Mahmood, 2000. Role of *Glomus mosseae* and *Paecilomyces lilacinus* in the management of root knot nematode on tomato. Arch. Phytopath. Pfl., 33: 131-140.

Cavalcante, R.S., H.L.S. Lima, G.A.S. Pinto, C.A.T. Gava and S. Rodrigues, 2008. Effect of moisture on *Trichoderma* conidia production on corn and wheat bran by solid state fermentation. Food Bioprocess Technol., 1: 100-104.

Chet, I., A. Viterbo, Y. Brotman and T. Lousky, 2006. Enhancement of plant disease resistance by the biocontrol agent *Trichoderma*. Life Sciences Open Day. Weizmann Institute of Science. http://www.weizmann.ac.il/Biology/open_day_2006/book/Abstracts/Ilan_Chet.pdf

Conrath, U., Z. Chen, J.R. Ricigliano and D.F. Klessig, 1995. Two inducers of plant defense responses, 2,6-dichloroisonicotinec acid and salicylic acid, inhibit catalase activity in tobacco. Proc. Natl. Acad. Sci. USA., 92: 7143-7147.

Cordier, C., M.J. Pozo, J.M. Barea, S. Gianinazzi and V. Gianinazzi-Pearson, 1998. Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Mol. Plant Microbiol., 11: 1017-1028.

Demir, S. and A. Akkopru, 2005. Using of Arbuscular Mycorrhizal Fungi (AMF) for Biocontrol of Soil-Borne Fungal Pathogens. In: Biological Control of Plant Diseases: Current Concepts, Chincholkar, S.B. and K.G. Mukerji (Eds.). The Howarth Press Inc., Binghamton, NY, USA.

Eapen, S.J., B. Beena and K.V. Ramana, 2005. Tropical soil microflora of spice-based cropping systems as potential antagonists o f root-knot nematodes. J. Invertebr. Pathol., 88: 218-225.

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

Gallagher, L.A. and C. Manoil, 2001. Pseudomonas aeruginosa PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. J. Bacteriol., 183: 6207-6214.

Gianinazzi-Pearson, V., E. Dumas-Gaudot, A. Gollotte, A.T. Alaoui and S. Gianinazzi, 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytol., 133: 45-57.

Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol., 84: 489-500.

Hayman, D.S., 1982. The physiology of vesicular arbuscular endomycorrhizal symbiosis. Can. J. Bot., 61: 944-963.

Jepson, S.B., 1987. Identification of Root Knot Nematodes. Commonwealth Agricultural Bureaux, Farnham Royal, UK..

Jung, C. and U. Wyss, 1999. New approaches to control plant parasitic nematodes. Appl. Microbiol. Biotechnol., 51: 439-446.

Kellam, M.K. and N.C. Schenck, 1980. Interactions between a vesicular-arbuscular mycorrhizal fungus and root-knot nematode on soybean. Phytopathology, 70: 293-296.

Khan, A., K.L. Williams and H.K.M. Nevalainen, 2004. Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biol. Control, 31: 346-352.

Klessig, D.F., J. Durner, R. Noad, D.A. Navarre and D. Wendehenne *et al*., 2000. Nitric oxide and salicylic acid signaling in plant defense. Proc. Nat. Acad. Sci. USA., 97: 8849-8855.

Leong, J., 1986. Siderophores: Their biochemistry and possible role in the biocontrol of plant pathogene. Ann. Rev. Phytopathol., 24: 187-209.

Molinari, S., 2005. Salicylic acid as an elicitor of resistance to root-knot nematodes in tomato. Proceedings of the 15th Meeting of the Eucarpia Tomato Working Group, September 20-23, 2005, Bari, Italy.

Molinari, S. and E. Loffredo, 2006. The role of salicylic acid in defense response of tomato to root-knot nematodes. Physiol. Mol. Plant Pathol., 68: 69-78.

Morton, C.O., P.R. Hirsch and B.R. Kerry, 2004. Infection of plant-parasitic nematodes by nematophagous fungi-a review of application of molecular biology to understand infection processes and to improve biological control. Nematology, 6: 161-170.

Nandi, B., N.C. Sukual, N. Banerjee, S. Sengupta, P. Das and P.S.B. Santi, 2002. Salicylic acid enhances resistance in cowpea against *Meloidogyne incognita*. Phytopathol. Medit., 41: 39-44.

Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158-161.

Sasser, J.N. and D.W. Freckman, 1987. A World Perspective on Nematology: The Role of the Society. In: Vistas on Nematology, Veech, J.A. and D.W. Dickson (Eds.). Society of Nematologists, Hyattsville, Maryland, United States, pp: 7-14.

Sasser, J.N., 1979. Economic Importance of *Meloidogne* in Tropical Countries. In: Root-Knot Nematodes (*Meloidogne* spp.) Systematics, Biology and Control, Lamberti, F. and C.E. Taylor (Eds.). Academic Press, London, UK., pp: 359-374.

Schaad, N.W., J.B. Jones and W. Chum, 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd Edn., APS Press, Saint Paul, MN., pp: 373.

Sharon, E., M. Bar-Eyal, I. Chet, A. Herra-Estrella, O. Kleifeld and Y. Spiegel, 2001. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology, 91: 687-693.

Sharon, E., A. Chet, A. Viterbo, M. Bar-Eyal, H. Nagan, G.J. Samuels and Y. Spiegel, 2007. Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. Eur. J. Plant Pathol., 118: 247-258.

Siddiqui, I.A. and S.S. Shaukat, 2002a. Mixtures of plant disease suppressive bacteria enhance biological control of multiple tomato pathogens. Biol. Fertility Soil, 36: 260-268.

Siddiqui, I.A. and S.S. Shaukat, 2002b. Resistance against the damping-off fungus *Rhizoctonia solani* systemically induced by the plant-growth-promoting Rhizobacteria *Pseudomonas aeruginosa* (IE-6S+) and *P. fluorescens* (CHA0). J. Phytopathol., 150: 500-506.

Siddiqui, I.A. and S.S. Shaukat, 2002c. Rhizobacteria-mediated Induction of Systemic Resistance (ISR) in tomato against *Meloidogyne javanica*. J. Phytopathol., 150: 469-473.

Siddiqui, I.A. and S.S. Shahid, 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: Importance of bacterial secondary metabolite, 2,4-diacetylpholoroglucinol. Soil Biol. Biochem., 35: 1615-1623.

Siddiqui, I.A., S.S. Shaukat, G.H. Khan and N.I. Ali, 2003. Suppresion of *Meloidogyne javanica* by *Psuedomonas aeruginosa* IE-6S^{+} in tomato: The influence of NaCl, oxygen and iron levels. Soil Biol. Biochem., 35: 1625-1634.

Siddiqui, I.A. and S.S. Shaukat, 2004. Systemic resistance in tomato induced by biocontrol bacteria against the root-knot nematode, *Meloidogyne javanica* is independent of salicylic acid production. J. Phytopathology, 152: 48-54.

Siddiqui, I.A., D. Haas and S. Heeb, 2005. Extracellular protease of Pseudomonas fluorescens CHA0, a biocontrol factor with activity against the root-knot nematode Meloidogyne incognita. Appl. Environ. Microbiol., 71: 5646-5649.

Siddiqui, I.A., S.S. Shaukat, I.H. Sheikh and A. Khan, 2006. Role of cyanide production by *Pseudomonas fluorescens* CAHO in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. Microbiol. Biotechnol., 22: 641-650.

Sivan, A., Y. Elad and I. Chet, 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. Phytopathology, 74: 498-501.

Slezack, S., G. Dumas, M. Paynot and S. Gianinazzi, 2000. Is a fully established arbuscular mycorrhizal symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches*? MPMI., 13: 238-241.

Spiegel, Y. and I. Chet, 1998. Evaluation of *Trichoderma* sp as a bio control agent against soil borne fungi and plant parasitic nematodes in Israel. Integr. Pest Manage. Rev., 3: 169-175.

Spiegel, Y., E. Sharon, M. Bar-Eyal, A. Van Assche, S. Van Kerckhove *et al*., 2006. Evaluation and mode of action of *Trichoderma* isolates as a biocontrol agent against plant-parasitic nematodes. Proceedings of IOBC Meeting, IOBC Bulletin, September 6-10, 2006, Spa, Belgium.

St-Arnaud, M. and V. Vujanovic, 2007. Effect of the Arbuscular Mycorrhizal Symbiosis on Plant Diseases and Pests. In: Mycorrhizae in Crop Production: Applying Knowledge, Hamel, C. and C. Plenchette (Eds.). Haworth Press, Binghampton, NY., USA., pp: 67-122.

Taylor, A.L. and J.N. Sasser, 1978. Biology, Identification and Control of Root-Knot Nematodes (*Meloidogyne* species). North Carolina State University, Raleigh, NC., USA., Pages:111.

Tikhonov, V.E., L.V. Lopez-Llorca, J. Salinas and H.B. Jansson, 2002. Purification and characterization of chitinases from the nematophagous fungi *Verticillium chlamydosporium* and *V. suchlasporium*. Fungal Genet. Biol., 35: 67-78.

Vierheilig, H., S. Steinkellner, T. Khaosaad and J.M. Garcia-Garrido, 2008. The Biocontrol Effect of Mycorrhization on Soil-Borne Fungal Pathogens and the Autoregulation of the AM Symbiosis: One Mechanism, Two Effects? In: MYCORRHIZA: Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics, Varma, A. (Ed.). Springer-Verlag, Heidelberg, Germany, pp: 307-320.

Way, J.L., 1984. Cyanide intoxication and its mechanism of antagonism. Annu. Rev. Pharmacol. Toxicol., 24: 451-481.

Weindling, R., 1932. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology, 22: 837-845.

Zambolim, L. and N.C. Schenck, 1983. Reduction of the effect of pathogenic, root infecting fungi on soybean by the mycorrhizal fungus, *Glomus mosseae* L. Phytopathology, 73: 1402-1405.