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MANAGEMENT OF *Meliodogyne incognita* IN POTATO BY CULTURES FILTRATES OF THE MICOPARASITIC FUNGI, *Trichoderma harzianum* OR *T. viride*.

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ABSTRACT

A greenhouse experiment was conducted to study the efficacy of the fungi cultures filtrates of *Trichoderma harzianum* or *T. viride* at 60%, 80% and 100% concentrations in comparison with oxamyl on the control of *Meloidogyne incognita* infecting potato plant cv. sponta. Results indicate that the tested filtrates obviously caused remarkable increase in potato plant growth characters and significantly reduced root galling, females, and eggmasses on root system as well as number of juveniles of *M. incognita* per cc soil. As the filtrate concentration of the micoparasitic fungi increased from 60% up to 100% , the percentage increase of plant growth characters increased from 7.69 to 37.7 % for shoot dry weight as well as from 17.3 to 56.9% for fresh weight of whole plant in case of *T. harzianum* while the increase in case of *T. viride* was recorded to be from 10.25 to 139.7% and from 13.75 to 77.33% for the former and latter criteria, respectively. *T. harzianum* at 100% achieved the highest percentage increase of tuber fresh weight with value of 93.3%, followed by *T. viride* at 100 % (66.6%) and then oxamyl (50.5%), respectively as compared to nematode alone. Moreover, oxamyl application achieved the highest percentage of increase for length of shoot plus root with value of 25 %, whereas both fungal filtrates at100% gave an equal value (10%) for this criterion. With respect to nematode development parameters, *T. harzianum* at the three concentrations tested surpassed those of *T. viride* in supressing number of galls, females, eggmasses and juveniles/ cc soil as compared to nematode alone. Oxamyl application gave a considerable reduction percentage values of nematode development parameters tested but not exceeding those of *T. harzianum* filtrate at 100% or 80% for number of females (69.5 or 69.0%) and eggmasses (69.2% or 68.7%), respectively. *T. harzianum* at 100% achieved the highest percentage of increase values of nitrogen (45.2%) phosphorus (98.2%) and potassium (101.0%) and recorded the highest percentage of reduction in total chlorophyll content with value of 27.62%, respectively, followed by *T. viride* at 100% with values of 44.5%, 105.3% 73.2% and 27.06%, respectively as compared to that of nematode alone . Oxamyl application accomplished the highest percentage reduction of total chlorophyll content value of 28.46 % comparing to nematode alone and other tested treatments.

Keywords : Fungi culture filtrates, *Trichoderma harzianum* , *T. viride* , Oxamyl, *Meloidogyne incognita*, potato cv. sponta, Management.

INTRODUCTION

Potato, *Solanum tuberosum* is a major food crop in many countries of the world including Egypt. Potato is subjected to be attacked by various plant parasitic nematodes , especially, root-knot nematodes, *Meloidogyne spp.* (Talavera *et al.*, 1998; Aboul-Eid and Youssef, 1998 ; Abd-Allah, 1999; Shady, 2002 and Salem, 2006). Their wide host range and favourable environmental conditions provoked suitable control measure to achieve reasonable results. Management of plant parasitic nematodes by *Trichoderma spp.* have been investigated by several researchers (Windham

et al. ,1989; Khan & Saxena, 1997; Khan *et al.* ,1997; Latha& Sivakumar, 1998; Aboul-Eid & Youssef, 1998 ; Amin & Mostafa, 2000 and Abdel-Bari *et al.*, 2000). The effects of six fungal filtrates *Aspergillus flavus, A. niger, A, ochrachus, A. tearus , Trichoderma harzianum* and *T. viride* against *Meloidogyne incognita* larvae were studied under laboratory conditions, revealed that all these fungi filtrates significantly affected nematode mortality after 24 hours and that fresh fungi filtrates, without storage, were most effective on percentage kill of *M. incognita* larvae (Abdel-Bari *et al.*, 2000). In the present study an attempt was carried out to determine the impact of the fungi culture filtrates of *Trichoderma viride* or *T. harzianum* at 60, 80 and 100% , separately in comparison with oxamyl at the recommended dose on controlling *Meloidogyne incognita* infecting potato plant cv. sponta under greenhouse conditions.

MATERIALS AND METHODS

Influence of certain fungal filtrates in comparison with oxamyl on *Meloidogyne incognita* infecting potato plant cv. Sponta under greenhouse condition 21± 3c^0 .

Potato tuber pieces cv. sponta with one sprout on each were surface sterilized and sown singly in black plastic bag with four small pores (two eachs side) filled with 900g stream-sterilized sandy soil each. Twenty seven plastic bags were used in this experiment. Inoculation with *M. incognita* was carried out 40 days after sowing by adding 10 eggmasses in holes around the base of each potato seedling and covered gently with sand . These eggmasses were collected from infected root of coleus plants, *Coleus blumei* with a pure culture of *M. incognita* formerly prepared and propagated on a bench in the greenhouse of Nematology Research unit of Agricultural Zoology Department, Faculty of Agriculture, Mansoura university, where this experiment was conducted . At the time of nematode inoculation, three concentrations of each fungal filtrates i.e. *Trichoderma viride* or *T. harzianum* at100, 80 and 60% were separately added per potato seedling at the level of 25ml/ bag as well as the recommended dose of oxamyl at the rate of 0.3ml/bag, respectively. Three seedling were only inoculated with nematode eggmasses at the level of nematode inoculum / each bag and another three seedling were left without nematode eggmasses and untreated that served as control.

Treatments were as follows:

1- N + *Trichoderma viride* 100%,
2- N + *Trichoderma viride* 80%,
3- N + *Trichoderma viride* 60%,
4- N + *Trichoderma harzianum* 100%,
5- N + *Trichoderma harzianum* 80%,
6- N + *Trichoderma harzianum* 60%,
7- N + Oxamyl 0.3ml,
8- N alone and
9-Plant free of N and untreated.

Each treatment was replicated three times. Plastic bags with potato seedlings received water as needed as well as NPK fertilizers at the recommended dose, placed on a greenhouse bench at temperature of 21 $\pm 3c^0$ and agronomically treated the same . Sixty days after inoculation, potato plants were carefully uprooted, plant growth criteria i.e. shoot dry weight, number of leaves and branches were determined and recorded. Infected roots of each plant were washed by tap water, fixed in 4% formalin for 24 hours and then stained in 0.01 lactic acid fuchsin method (Byrd *et al.*, 1983) and examined for recording number of galls, females and eggmasses. Number of *M. incognita* juveniles in 250g soil was also determined by extracting through sieve and modified Baermann-pan technique (Goodey, 1957) and recorded. Regarding N, P and K determination, 0.2g of dry weight of shoot was subjected to chemical analysis as follows: total nitrogen content was determined according to the improved Kjeldahl method (A.O.A.C.,1980) modified by distilling the ammonia into saturated boric acid solution and titration with (0.1 NaCl) standard. Total phosphorus was colorimetrically determined using chlorostannous reduced Molybdophosphoric Blue colour method, while total potassium was flam photometry estimated as described by Jakson (1967) . Chlorophyll content was spectrophotometrically measured in leaves of the harvested plant using Fadeel's method (1962). Chlorophyll concentrations were calculated according to Wellburn and Lichtenthaler (1984). The content of chlorophyll was then expressed in µg/g. F.Wt. of the leaves. Data were statistically subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan's multiple-range test to compare means (Duncan, 1955).

RESULTS

Data in Table (1) revealed the effect of fungal filtrates i.e. *Trichoderma viride* or *T. harzianum* at the concentrations of 100, 80 and 60% in comparison with oxamyl at the recommended dose 0.3 ml/ seedling on the growth of potato plant cv. sponta infected with *M. incognita* under greenhouse at $21\pm 3c^0$. Results indicated that all tested materials obviously caused remarkable increase in potato growth with various degrees. It is evident that the fungal filtrate of *T. Viride* at 100% ranked first over other treatments tested in improving plant growth parametes i.e. the fresh weight of whole plant and shoot dry weight with percentage increase values of 77.33 and 139.7% , respectively, followed by oxamyl treatment with values of 62.11 and 82.0% , for the same criteria, respectively, then *T. harzianum* at 100% with value of 56.92% for the first plant growth criterion. On the other hand *T. harzianum* at 100% gave the highest percentage of increase of tuber weight with value of 93.3 %, followed by *T. viride* at 100% with value of 66.6% and then seedling received oxamyl (50.5%), respectively. There were a positive correlations between the increments of each plant growth criteria tested and the concentrations of fungal filtrates.

As the concentration of such fungal filtrateincreased from 60% up to 100%, the percentage increase values of such plant growth parameter increased from 7.69 to 39.7 % for shoot dry weight as well as from 17.3 to 56.9 % for fresh weight of whole plant with respect to *T. harzianum* while those of *T. viride* were ranged from 10.25 to 139.7% for the first criterion and from 13.76 to 77.33 % for the latter criterion, respectively. Regarding number of leaves and branches of potato plant, plants received 100% of fungal filtrates of either fungi achieved the highest percentage of increase for both criteria with values of 121.4% and 46.6%, respectively. Likewise, similar results were obtained with length of shoot and root of potato plant where the concentration of 100% of either fungal filtrate gave an equal value of percentage increase which was amounted to 10% (Table 1), whereas oxamyl application achieved the highest percentage of increase with value of 25% for this criterion.

Data in Table(2) showed the impact of the fungal filtrates of *Trichoderma viride* or *T. harzianum* at 100, 80 and 60% singly in comparison with oxamyl on root galling, female and eggmasses on root system as well as number of juveniles of *M. incognita* per cc soil infecting potato cv, sponta under greenhouse conditions. Data indicated that all tested materials obviously reduced such nematode parameters recorded. Among tested concentrations of fungal filtrates , *T. harzianum* at the three concentrations i.e. 100, 80 and 60% surpassed those of *T. viride* in percentage reduction values of galls, female and eggmassas on root system of potato plants which were amounted to 66.5, 65.9 and 59.6% ; 69.5, 69.0 and 60.5 % , and 69.2, 68.7 and 60.3% , respectively whereas 64.2, 61.9 and 55.0% ; 62.5, 60.5 and 56.5 % and 62.4 , 60.8 and 56.3 %, respectively to the same criteria for *T. viride* concentrations tested (Table 2) when compared with the nematode alone. With respect to number of juvenile/ cc soil, oxamyl treatment accomplished the highest percentage reduction value of 66.6 % over the nematode alone, whereas all concentrations tested of both fungal filtrates showed similar results for this criterion that ranged from 56.8 to 64.3 for 60% up to 100% respectively. In addition, oxamyl treatment gave considerable values of percentage reduction for galls (67.1%), females (68.0%) and eggmasses (67.8), respectively. In general as the concentration of fungal filtrates of both fungi increased from 60%, 80% to 100% , the percentage reduction values of such nematode parameters tested increased (Table 2). Data in Table (3) showed the impact of two fungal filtrates i.e. *Trichoderma viride* and *T. harzianum* at the three concentrations i.e. 100, 80 and 60% in comparison with oxamyl on nitrogen (N), phosphorus (P) and potassium (K) concentrations in potato cv. sponta infected with *M. incognita* under greenhouse conditions. It is evident the N, P and K concentrations were obviously reduced by nematode infection. All tested treatments indicated remarkable increase in N, P and K concentrations exceeding those of nematode alone. Oxamyl application significantly ranked first in increasing N, P and K concentrations over all fungal filtrates concentrations tested and untreaeted uninoculated one except that of *T. harzianum* at 100% for (K) concentrations (29.83 ppm) where almost an equal value of (K) with that of oxamyl treatment was recorded (29.92 ppm)

Among the concentrations tested of the two fungal filtrates, *T. harzianum* at 100% achieved the highest increment in nitrogen , potassium and phosphorus concentrations contents that was amounted to 27.42 , 29.83 and 1.11 ppm followed by those of *T. viride* at 100% with value of 27.3 and 26.69 ppm while *T. viride* at 80% gave the highest value of percentage increase value for phosphorus (1.15ppm) as compared to that of nematode alone (Table3). Moreover, results also indicated that there was positive correlations between the concentrations of fungal filtrates tested and the concentrations of nitrogen and phosphorus in the dry shoot of potato plant as compared to nematode alone. As the concentrations of such fungal filtrates increased, the concentrations of N and P obviously increased. The impact of nematode infection to potato plant in plastic bags receiving either the tested concentration of *T. viride* or *T. harzianum* filtrates or oxamyl on total chlorophyll content , indicated that all tested components significantly decreased the total chlorophyll content of potato shoot comparing to nematode alone . Moreover, nematode infection without any treatment used showed the highest percentage of increase with value of 28.46% over the untreated uninoculated plant. However, as the concentrations of such fungal filtrates tested increased from 60% up to 100% values of percentage reduction of total chlorophyll content increased with range from 19.39 to 27.62% for *T. harzianum*, whereas those of *T. viride* ranged from 17.19 to 27.06%, respectively. Oxamyl treatment achieved the highest percentage reduction of total chlorophyll content value that was amounted to 28.46% comparing with nematode alone (Table 3).

Table 3: Nitrogen, Phosphorus and Potassium concentrations in dry shoot as well as chlorophyll content in fresh shoot of potato cv. Sponta as influenced by *Meloidogyne incognita* treated with certain fungal filtrates at three concentrations in comparison with oxamyl under greenhouse condtions 21 ±3C^0

Treatmeants	Plant Growth Parametes					
	N µg/mg	P ppm	K ppm	Chlorophyll content		
				A µg/ mg	B µg/mg	Total chlorophyll
T. viride 100% + N	27.3 b	1.07 bc	26.69 b	627.79 c	425.81 c	1053.60
T. viride 80% + N	24.91 d	1.15 b	25.74 b	635.52 c	458.62 c	1094.41
T. viride 60% + N	21.11 e	0.56 e	23.62 c	670.79 b	525.42 b	1196.21
*T.harzianum*100% +N	27.42 b	1.11 b	29.83 a	593.17 d	452.39 c	1045.56
*T.harzianum*80% + N	25.83 c	0.98 cd	23.83 c	597.41 d	543.01 b	1140.42
T.harzianum 60%+ N	24.77 d	0.92 d	24.20 c	624.36 c	540.12 b	1164.48
Oxamyl + N	28.24 a	1.29 a	29.92 a	522.69 f	510.69 b	1033.38
Control	25.5 c	0.65 e	24.07 c	545.58 e	425.36 c	970.94
N alone	18.89 f	0.56 e	14.84 d	823.05 a	621.55 a	1444.6

N= 10 egg-masses of *M. incognita*
Each figure presented the mean of three replicates.
Means in each column followed by the same letter(s) didn't differ at P<0.05 according to Duncan's multiple – range test.
T. viride* = *Trichoderma viride
T. harzianum* = *Trichoderma harzianum

DISCUSSION

Obviously, the present work showed the potential use of the fungal filtrates of both fungi i.e. *T. viride* or *T. harzianum* as a biofertilizer as well as it's thermostable toxin on root-knot nematode, *M. incognita* infecting potato cv. sponta and improving plant growth parameters. Such fungal filtrates at 100% gave remarkable percentage of increase values for total fresh weight of plant , shoot dry weight , fresh weight of tuber, number of leaves and branches as well as length of shoots and roots. These findings are in accordance with those reported by Amin and Mostafa (2000) who found that application of *Trichoderma viride* and *T. harzianum* singly or concomitanly showed better promotion in shoot growth and disc weight of sunflower cv. Baladey as compared with nematode alone .Moreover, higher increase indisc weight was also performed in sunflower growth in pots receiving *T. harzianum* (Amin and Mostafa, 2000). Supperession of eggmasses production by 69.2% in the present findings can also be supported by those reported by Amin and Mostafa (2000) with respect to percentage eggmasses reduction (39.13%) on roots of sunflower by *T. harzianum*. Moreover, the present findings also indicated that *T. harzianum or T. viride* filtrates at 100% showed significant results in suppressing number of galls, females, eggmasses and juveniles/cc soil as compared to nematode alone, a situation that strongly supported by the findings of Aboul-Eid and Youssef (1998) with respect to the filtrates of *Paecilomyces lilacinus* or *Trichderma viride* as fungal biocontrol agents against *M. incognita* and *Rotylenchulus reniformis* infections to four potato cultivars i.e. sponta, Nicola, Desiree and Diamant under greenhouse conditions. They also found that both fungal filtrates separately reduced the number of *M. incognita* galls and eggmasses on roots of the tested four potato cultivars. In conclusion due to the cost of chemical nematicide and hazards involved biological control of *M. incognita* on potato has been recorded as an alternative way of management .In the present investigation, it can be concluded that *T. harzianum* at 100% filtrate was found to be more effective in reducing nematode development parameters tested than that of *T. viride* on sponta at the same concentrations, however, *Trichderma spp.* are of the group of fungi which produce toxic metabolites to nematodes (Mankau, 1969) or affect hatching of larvae of *M. incognita* (Sharma and Saxena, 1992 and El-Hadidy, 1996). Moreover, more research is needed to be done on these group of fungi in this respect.

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مكافحة نيماتودا تعقد الجذور "ميليدوجين انكوجنيتا" بواسطة بعض مرشحات الفطريات والأوكساميل فى البطاطس بالاشارة للنيتروجين والفوسفور والبوتاسيوم ووضع الكلوروفيل

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اجريت تجربة فى الصوبة لدراسة تأثير مرشحات الفطريات "تريكوديرما فيردى وتريكوديرما هرزيانم" عند تركيزات ٦٠% و٨٠% و١٠٠% بالمقارنة مع مبيد الأوكساميل عند الجرعة الموصى بها لمكافحة نيماتودا تعقد الجذور "ميليدوجين انكوجنيتا" المصاحبة لنباتات البطاطس صنف سبونتا .اوضحت النتائج ان جميع المعاملات المستعملة ادت بوضوح إلى زيادة ظاهرة فى قياسات نمو البطاطس بدرجات مختلفة وخفضت بدرجة معنوية اعداد العقد النيماتودية والاناث وكتل البيض واعداد اليرقات لكل واحد جرام تربة لنيماتودا تعقد الجذور "ميليدوجين انكوجنيتا".واشارت النتائج الى انة كلما زاد تركيز المرشح الفطرى من ٦٠% إلى ١٠٠% زادت معدلات مقاييس نمو النباتات المختبرة من ٧,٦٩ إلى ٣٩,٧% الأوراق الجافة للمجموع الخضرى وكذا من ١٧,٣ إلى ٥٦,٩% للوزن الرطب الكلى للنبات بالنسبة لمرشح الفطر"تريكوديرما هرزيانم" عند تركيز ١٠٠% أما بالنسبة لتركيز ١٠٠% لمرشح فطر "تريكوديرما فيردى "لنفس الصفات السابقة فكان معدل الزيادة ١٠,٢٥ إلى ١٣,٩٧% و١٣,٧٥ إلى ٧٧,٣٣% على التوالى كما اظهر تركيز ١٠٠% لمرشح الفطر "تريكوديرما هرزيانم " أعلى معدل زيادة بقيمة ٩٣,٣% لوزن الدرنات يلية فى ذلك "يريكوديرما فيردى" عند نفس التركيز بقيمة قدرها ٦٦,٦% ثم المبيد الاوكساميل بقيمة قدرها ٥٠,٥ بالمقارنة بالنيماتودا بمفردها .كما اظهرت معاملة المبيد ايضاً اعلى معدل زيادة بقيمة قدرها ٢٥% فى ارتفاع النباتات (الساق+الجذر) اما مرشحات الفطر عند اعلى تركيز كانت هذه الزيادة نفسها وقدرها ١٠% لهذه الصفة النباتية كما تفوقت تركيزات مرشح الفطر "تريكوديرما هرزيانم " على مثيلتها من مرشح "تريكوديرما فيردى"فى معدلات نقص اعداد العقد النيماتودية والاناث وكتل البيض وعدد اليرقات فى جرام واحد تربة كما اعطت المعاملة بالمبيد معدلات نقض معنوية فى مقاييس النيماتودا المختبرة مقارنة بالنيماتودابمفردها لكن لم تزيد عن تلك الناتجة من المعاملة بمرشح الفطر "تريكوديرما هرزيانم" عند تركيز١٠٠% . كما اعطى مرشح الفطر "تريكوديرما هرزيانم" اعلى معدل زيادة فى النتروجين (٤٥,٢%)والفوسفور(٩٨,٢%) والبوتاسيوم(١٠١%) كما سجل اعلى معدل نقص فى المحتوى الكلى للكلوروفيل (٢٧,٦٢%)ويليها فى ذلك نفس التركيز لمرشح الفطر "تريكوديرما فيردى"بمعدلات زيادة ٤٤,٥% و ١٠٥,٣٥ و٧٣,٢% بالنسبة للنتروجين والفوسفور والبوتاسيوم وخفض المحتوى الكلى للكلوروفيل بمعدلات ٢٧,٠٦%على التوالى . كما ان المعاملة بالمبيد الاوكساميل اعطى اعلى معدل نقص فى المحتوى الكلى للكلوروفيل بقيمة قدرها ٢٨,٤٦% بالمقارنة بمعاملة النيماتودا بمفردها وغيرها من المعاملات .

Table 1: Effect of certain fungal filtrates in comparison with oxamyl on plant growth parameters of potato cv. Sponta infected with *Meloidogyne incognita* under greenhouse condtions 21 ±3c^0 .

Treatmeants	Plant Growth Parametes												
	Length (cm)		Fresh weight (g)		F. wt. whole pl. (g)	Increase %	Shoot dry weight(g)	Increase %	Number of tuber	Weight of tuber	Increase %	Number of leaves	Number of branch
	shoot	root	shoot	root									
T. viride 100 %+ N	19.33 ab	25.33 abc	14.6 ab	5.9 b	20.5 ab	77. 33	1.87 a	139.7	3 Abc	30 abc	66.66	82.66 bc	7.33 bcd
T. viride 80% + N	16.66 ab	23 abc	13 abc	4.96 b	17.96 abc	55.36	1.1 c	41.02	2.33 bc	22.83 abc	26.83	65.66 cd	6.33 cd
T. viride 60% +N	15.33 b	21.66 abc	9.24 d	3.73 b	13.15 d	13.75	0.86 c	10.25	2.66 bc	20.63 bc	14.61	51.33 de	5.33 d
T. harzianum 100% + N	22 a	22 abc	12.43 bc	5.7 b	18.14 abc	56.92	1.09 c	39.74	4.66 ab	34.8 a	93.33	93.33 b	9.66 a
T. harzianum 80% + N	19 ab	19 bc	11.23 cd	4.53 b	15.76 bcd	36.33	1.007 c	29.1	5.33 a	22.26 abc	23.66	56 de	8 abc
T. harzianum 60% +N	18.66 ab	17.16 c	9.43 d	4.14 b	13.57 cd	17.38	0.84 c	7.69	2.66 bc	20.36 bc	13.11	37.66 e	6.66 cd
Oxamyl + N	18.66 ab	27 ab	14.2 ab	4.54 b	18.74 abc	62.11	1.42 b	82.05	3.33 abc	27.1 abc	50.55	71.33 bcd	5.33 d
Control	20.5 ab	30 a	15.08 a	8.43 a	23.52 a	103.46	1.47 b	88.46	3.33 abc	32.7 ab	81.66	139.33 a	9.33 ab
N alone	15.66 ab	25 abc	12.59 bc	3.63 b	11.56 d		0.78 c		2 c	18 c		37.33 e	5 d

N = 10 eggmasses of *M. incognita*.
Each figure presented the mean of three replicates.
Means in each column followed by the same letter(s) didn't differ at P<0.05 according to Duncan's multiple –range test.

Table 2: Impact of certain fungal filtrates at three concentrations in comparison with oxamyl on number of root galls, eggmasses, females and juveniles in soil of *Meloidogyne incognita* infecting potato cv. sponta under greenhouse conditions 21 ± 3c^0.

Treatmeants	Number of galls	Reduction %	Rgi*	No. Of females	Reduction %	Number of eggmasses	Reduction%	Rgi*	Number j 2 /per 1cc soil	Reduction %
T. viride 100% + N	67 bc	64.2	4	75.0 b	62.5	74 bc	62.4	4	1.35 d	64.1
T. viride 80% + N	71.33bc	61.9	4	79.0 b	60.5	77 bc	60.8	4	1.42 c	62.4
T. viride 60% +N	84 b	55.0	4	87.0 b	56.5	86 b	56.3	4	1.62 b	56.8
T.harzianum 100% + N	64.66 bc	66.	4	61.0 c	69.5	60.66 c	69.2	4	1.343 d	64.3
T.harzianum 80% + N	63.66 c	65.9	4	62.0 c	69.0	61.66 c	68.7	4	1.423 c	62.4
T.harzianum 60% +N	75.66 bc	59.6	4	79.0 c	60.5	78 bc	60.3	4	1.62 b	56.8
Ox + N	61.66 c	67.1	4	64.0 c	68.0	63.33 c	67.8	4	1.35 d	66.6
N alone	187 a		5	200.0 a		196.66 a		5	1.76 a	

Each value is a mean of three replicates.
Means in each column followed by the same letter(s) didn't differ at P<0.05 according to Duncan's multiple –range test.
N= 10 egg-masses of *M. incognita*
*** Root- gall index (RGI) or egg-masses index (EI) was determined according to Taylor & Sasser (1978) as followes: 0 = no galls or egg-masses, 1=1-2 galls or egg-masses, 2=3-10 galls or egg-masses, 3 =11-30 galls or egg-masses, 4=31-100 galls or egg-massesand 5 = more than 100 galls or egg-masses.**