EFFECT OF SOME BIOLOGICAL AGENTS ON REPRODUCTION OF *MELOIDOGYNE INCOGNITA* ON TOMATO PLANTS

T.H. ABD EL-MOITY[1], E.M. ALI [1], M.H. EL-HAMAWI [1], T. EL-SHARKAWY [1], AND K. TIILIKKALA[2]

1 Institute of Plant Pathology, Agricultural Research Centre, Giza, Egypt.
2 Agricultural Research Centre of Finland 31600 Jokioinen, Finland.

(Manuscript received 26 January 1996)

Abstract

Three antagonists viz: *Trichoderma harzianum, Gliocladium virnes* and *Bacillus* sp. (B.15) isolated from Egyptian soil were used singly or in mixtures to control the root-knot nematodes *M.incognita* on tomato plants. The most effective isolate in controlling root-knot nematode was the isolate of *Bacillus* sp. (B. 15), whereas the least effective was the isolate of *Trichoderma harzianum* (T.S.).

No synergistic effect was obtained when different combinations from the antagonists were applied. Applying the antagonist to infested soil twice was always more effective compared with single treatment. Using *Bacillus* sp. twice was more effecttive than chemical treatment. Adding any of the antagonists separately or in combination resulted in a reduction in the number of egg-masses, number of eggs per plant and number of 2nd stage larvae in soil.

Adding any antagonist singly or in mixture increased the fresh weight of either the root or shoot system.

INTRODUCTION

Root knot nematode is considered as the most threatening and destructive pest in tomato (Houssny and Oteifa, 1956; Orton, 1973). Several investigators applied chemical or biological methods using single isolate of biocontrol agents (Adiko 1984; Abd El-Moity *et al.*, 1985; Baker, 1988; Dube, 1989; Jatala, 1982). Few attempts were made using mixtures of biocontrol agents to control this serious disease (Dube and Smart, 1987; Shahzad *et al.*, 1990).

Trichoderma harzianum (T.S.) was recorded as a biocontrol agent to control root knot nematode (Miller, 1976; Abd El-Moity *et al.*, 1985; Ali and Barakat, 1991; Ali1994). Some investigators used species of *Bacillus* to protect different crops against root knot nematode (Hanna *et al.*, 1995; Shahzad *et al.*, 1990; Sayre and Starr, 1986).

Gliocladium virnes (G.V) was also used to control *Meloidogyne arenaria* (Rodriguez - Kabana *et al.*, 1984).

In this study, three identified Egyptian isolates belonging to the previously mentioned antagonists were used singly or in mixtures according to their compatability with each other.

The aim of the present work was to study the effect of the different applications on M.incognita considering the following:

1. Root knot disease incidence in tomato plants.
2. The reduction of 2nd stage larvae (J_2) in soil.
3. The fresh weight of treated plants.

MATERIALS AND METHODS

I. Preparation of nematode inoculum :

A culture of *M.incognita* was maintained on tomato (*Lycopersicon esculentum* L.) grown in the green-house. Nematode inoculum was prepared by cutting the infected tomato roots to small pieces shreded in a blender for 40 seconds.

Inoculation procedure

Soon after the four-week old seedlings were transplanted individually in 20 cm diameter clay pots each containing 2.5 kg steam - sterilized soil, (4:3:2) loam, peat and sand respectively, each pot was inoculated with suspension of egg masses containing about 3000 eggs by making 3 holes at different depths (2-3 cm) around the roots and immediately after inoculation the roots were covered with soil.

Source of antagonists

Three different antagonists i.e. *Trichoderma harzianum*, (T.S.), *Gliocladium virnes* (G.V.) and *Bacillus* sp. (B.15) were used. All these isolates were obtained

from Egyptian wild isolates based on the method described by Abd-El-Moity *et al.* (1982).

Preparation of antagonistic inocula

Trichoderma harzianum (T.S.) and *Gliocladium virnes* (G.v.) were grown on a liquid gliotoxin fermentation media (Brian and Hemming, 1945) under complete darkness to stimulate toxin production (Abd El-Moity and Shatla, 1981). After nine days, inoculum were prepared by blending culture in an electric blender for 3 minutes. Spores were adjusted in the suspension to 32 x 10^7 spores/ml. *Bacillus* sp. was grown on nutrient glucose media for 72 h and then the bacterial suspension was adjusted to have similar concentration.

Previously prepared antagonist suspensions were added at the rate of 20 ml/ pot to the infested soil either as single isolates or in mixture. Biological treatments were added either once (one week before transplanting) or twice (one application before planting, and the second twenty one days after planting). Different mixtures were used : 1- *T.harzianum* (T.S.) + *Bacillus* sp. (B. 15) 2-*Gloicladium virnes* (G.v.) + *Bacillus* sp. (B. 15).

The effect of the different antagonists was compared with three other treatments i.e. : chemical nematicide (Vydate as Liquid 0.5%), infested soil with nematode only, and soil free from nematodes or antagonists.

All treatments received the same agricultural treatments such as amount of water, number of seedling/pot and amount of fertilizers.

All pots were arranged in a completely randomized design, and kept under greenhouse conditions at about 25-28°C.

After ninety days all plants were uprooted. Root and shoot systems were weighed and number of galls and egg-masses were counted. Number of second stage larvae (J2) was recorded/ 250 g soil taken from each pot.

All data were subjected to statistical analysis and means were compared using the least significant difference (L.S.D.) test at P = 0.05.

RESULTS AND DISCUSSION

Effect of different antigonists either as single additions or in mixtures were evaluted. Data in Table (1) show that the most effective isolate in controlling root-

knot nematode was the isolate of *Bacillus* sp. (B 15), whereas the least effective was the isolate of *Trichoderma harzianum* (T.S.). *Gliocladium virnes* (G.v.) occupied an intermediate position. The variation in efficacy may be due to the differences in type, quantity and activity of the secondary metabolites produced by the antigonist and their effect on the permeability of the parasitic nematodes. *Bacillus* sp. (B 15), *Trichodrma harzianum* (T.S.) and *Gliocladium virnes* (G.V.) were recorded as producing extracellular B-(1-3)-glucanase and chitinase. These enzymes play an important role in dissolving the outer shell of nematode eggs and consequently the inner content becomes subject to the effect of saprophytic organisms in soil (Stirling 1984; Poinar and Hansen, 1986; Starr, 1988, Abd El-Moity *et al.*, 1985; Chet *et al.*, 1979; Miller, 1976; Ali and Barakat 1991; Rodriquez *et al.*, 1984).

Table 1. Effect of the number of applications of individual antagonists or mixtures on root-knot disease incidence in tomato plants..

Treamtent	Number of applications	Root galing		Egg- masses		Eggs (in 1000 s)	
		No of galls/ plant	Rate of reduction	No of E.M. plant	Rate of reduction	No of eggs/ plant	Rate of reduction
G.V. + N	1	83	37.1	58	40.8	9.1	53.1
	2	(58)	(56.0	(40)	(59.2)	(5.4)	(72.2)
B. 15 + N.	1	60	45.5	28	71.4	2.9	85.1
	2	(31)	(76.5)	(14)	(85.7)	(1.8)	(90.7)
T.S. + N.	1	88	33.3	49	50	6.2	68
	2	(57)	(56.8)	(37)	(62.2)	(5.0)	(74.2)
G.v. + B. 15 + N.	1	75	43.2	36	63.3	4.0	79.4
	2	(44)	(66.6)	(31)	(68.4)	(3.9)	(79.9)
T.S + B. 15+N.	1	64	51.5	33	66.3	3.8	80.4
	2	(36)	(72.7)	(26)	(73.5)	(2.9)	(85.1)
Nematicide	1	42	68.2	18	81.6	2.6	86.6
Control (N.)	--	132	--	98	--	19.4	--
(A) L.S.D. (0.05)*	--	5.9	--	7.6	--	1.8	--

1 = Antagonist was added before planting.
2 = Antagonist was added before and after planting.
G.V. = *Gliocladium vines*
B. 15 = *Bacillus* sp.
T.H. = *Trichoderma harzianum*

These products could be Key factors in the mode of action of these microorganisms against target nematodes.

knot nematode was the isolate of *Bacillus* sp. (B 15), whereas the least effective was the isolate of *Trichoderma harzianum* (T.S.). *Gliocladium virnes* (G.v.) occupied an intermediate position. The variation in efficacy may be due to the differences in type, quantity and activity of the secondary metabolites produced by the antigonist and their effect on the permeability of the parasitic nematodes. *Bacillus* sp. (B 15), *Trichodrma harzianum* (T.S.) and *Gliocladium virnes* (G.V.) were recorded as producing extracellular B-(1-3)-glucanase and chitinase. These enzymes play an important role in dissolving the outer shell of nematode eggs and consequently the inner content becomes subject to the effect of saprophytic organisms in soil (Stirling 1984; Poinar and Hansen, 1986; Starr, 1988, Abd El-Moity *et al.*, 1985; Chet *et al.*, 1979; Miller, 1976; Ali and Barakat 1991; Rodriquez *et al.*, 1984).

Table 1. Effect of the number of applications of individual antagonists or mixtures on root-knot disease incidence in tomato plants..

Treamtent	Number of applications	Root galing		Egg- masses		Eggs (in 1000 s)	
		No of galls/ plant	Rate of reduction	No of E.M. plant	Rate of reduction	No of eggs/ plant	Rate of reduction
G.V. + N	1	83	37.1	58	40.8	9.1	53.1
	2	(58)	(56.0	(40)	(59.2)	(5.4)	(72.2)
B. 15 + N.	1	60	45.5	28	71.4	2.9	85.1
	2	(31)	(76.5)	(14)	(85.7)	(1.8)	(90.7)
T.S. + N.	1	88	33.3	49	50	6.2	68
	2	(57)	(56.8)	(37)	(62.2)	(5.0)	(74.2)
G.v. + B. 15 + N.	1	75	43.2	36	63.3	4.0	79.4
	2	(44)	(66.6)	(31)	(68.4)	(3.9)	(79.9)
T.S + B. 15+N.	1	64	51.5	33	66.3	3.8	80.4
	2	(36)	(72.7)	(26)	(73.5)	(2.9)	(85.1)
Nematicide	1	42	68.2	18	81.6	2.6	86.6
Control (N.)	--	132	--	98	--	19.4	--
(A) L.S.D. (0.05)*	--	5.9	--	7.6	--	1.8	--

1 = Antagonist was added before planting.
2 = Antagonist was added before and after planting.
G.V. = *Gliocladium vines*
B. 15 = *Bacillus* sp.
T.H. = *Trichoderma harzianum*

These products could be Key factors in the mode of action of these microorganisms against target nematodes.

When different combinations of these antagonists were applied to the soil, no synergistic effect was obtained. These data are contrary to those obtained by Abd El-Moity (1985) who reported synergistic effect in controlling some soil borne pathogen through mixtures of *Trichoderma harzianum* (T.S.) isolates. Such contradictory results could be explained by the fact that *T.harzianum* and *Gliocladium virnes* (G.v.) produce, in addition to chitinase, a wide range of antibiotic substances which may affect the bacterial growth of B.15 and consequently reduce its effect (Turner, 1971). Also synergism between different isolates of *Trichoderma* result from complementation of isolate metabolites. Data in the same table show also that applying antagonist to infested soil twice was allways more effective compared with only one treatment. This could be due to the fact that the efficacy of one biocontrol agent is related to its population and its establishment in soil (Ali, 1994; Enrique Capanillea *et al.*, 1989.

No doubt that adding the antigonist twice support its population in the soil. In addition, adding the antigonist after planting will provide the antagonist with a good chance to affect the 2nd stage larvae (J2) and consequently reduce infection.

The effect of these biological agents with the recommended nematicide was compared at the rate of 0.05%. The data showed *Bacillus* sp. (B 15), when applied twice was more effective than chemical treatment. At the same time, chemical treatment was superior to the other biological treatments. However, biological treatments when effective are safer, durable and cheaper.

The data also showed that in addition to the effect of biological treatment in controlling the disease, a clear reduction in the number of egg-masses and of eggs/plant was observed. This of course reduces the inoculum density in soil and the effect of this treatment will aslo extend to the next crop.

The effect of the different biological treatments on the inoculum potential of nematodes in soil, and number of 2nd stage larvae was also determined. Variation in the population is due to differences in percentage of egg hatching or the effect of the antagonist on the larvae itself. Data presented in Table (2) show that the highest percentage of reduction in population of the 2nd stage juveniles was obtained when the isolate of *Bacillus* sp. (B15) was used as a biocontrol agent. About 87% reduction in nematode population was obtained when compared with the control treatment. In this regard, no difference between chemical treatment and *Bacillus* was obtained. This high reduction in the 2nd stage larvae can be explained in the light of fact that

the outer layer of nematode eggs consists of chitin (Gabriel, 1968; Bird, 1976). Since *Bacillus* sp. (B 15) produce the enzyme chitinase, this might partially or completely destroy the outer shell of the egg. When the outer shell is destroyed the inner egg contents become exposed to soil saprophytic microorganisms and consequently reduce the percentage of egg hatching and number of 2nd stage larvae. No synergistic effects were observed against the number of larvae due to the use of mixtures of biocontrol agants. On the other hand, repeating the treatment twice was more effective than single application. This shows an additive effect.

Table 2. Effect of adding different antagonists in mixture or separately on the reduction of 2nd stage larvae (J2) in soil.

Treamtent	Number of application	Number of 2nd (J2) / 250 g.soil	% of Reduction
G.V. + N	1	250	30.5
	2	(180)	(50.0)
B. 15 + N.	1	175	51.4
	2	(45)	(87.5)
T.S. + N.	1	195	45.8
	2	(180)	(50.0)
G.v. + B. 15 + N.	1	160	55.5
	2	(135)	(62.5)
T.S + B. 15+N.	1	150	58.3
	2	(90)	(75.0)
Nematicide	1	45	87.5
Control (N.)	--	360	--
(A) L.S.D. (0.05)*	--	23.8	--

1 = Antagonist was added before planting.
2 = Antagonist was added before and after planting.
G.V. = *Gliocladium vines*
B. 15 = *Bacillus* sp.
T.S. = *Trichoderma harzianum*

Data also show that repeating the application with *T.harzianum* (T.S.) gave slight effect. This is because *T.harzianum* (T.S.) produce a wide range of antibacterial and antifungal substances (Turner, 1971; Abd El-Moity *et al.*, 1982). This wide range of such materials assist the antigonistic fungus for a repid establishment in the treated soil, so no clear effect was observed when this treatment was repeated.

It is suggested that repeating biological treatment for several crop rotations would lead to the increase of the inoculum level and consequently create what is called suppressive soil (Stirling, 1991), where nematode population maintains at low level and will not cause any considerable losses for the grower.

Since the main target for any research work is to increase the yield of the treated crop, the effect of biological treatment on some agronomical characteristics were investigated. Data in Table (3) show that adding any of the tested antagonists individually or in mixtures had led to an increase in the fresh weight of either root or foliage systems.

Treatments with *T.harzianum* (T.S.) in single form or in combination with *Bacillus* sp. were most effective in increasing fresh weight of treated plants. This phenomenon was explained by Abd El-Moity (1981) who explained the mode of action of *T.harzianum* (T.S.) on treated onion plants and attributed the increase of fresh weight to the increased availability of nutrient substances in soil in addition to the control of other minor-pathogens (Windham *et al.*, 1986) as well as the possibility of the production of some growth regulators.

Repeated applications, however, showed no or slight effect on the vigour of treated plants.

In the present work, biological treatments show less conspicious effect on nematode reproduction compared with chemical nematicides. It was reported that bioagents do not act suddenly or drastically (Lamberti and Ciancio, 1991), and some time is needed to develop and build up an effective population which can also be affected by environmental conditions.

Table 3. Effect of adding different antagonists in mixture or separately on the fresh weight of treated plants.

Treamtent	Number of application	Plant weight	
		Top fresh weight (g)	Root fresh weight (g)
G.V. + N	1	18.3	7.5
	2	(17.9)	(7.8)
G.V.	1	21	9.5
	2	(24.8)	(11.0)
B.15 + N	1	19.5	9.8
	2	(20.9)	(10.2)
B.15	1	26.6	11.9
	2	(26.8)	(12.9)
T.S. + N.	1	.19.1	8.2
	2	(19.6)	(8.8)
T.S.	1	25.1	10.4
	2	(25.3)	(12.3)
G.V. + B.15 + N.	1	18.9	10.1
	2	(19.6)	(10.9)
G.V. + B.15	1	24.2	11.6
	2	(25.4)	(12.8)
T.S. + B.15 + N.	1	20.9	10.7
	2	(23.6)	(12.5)
T.S. + B. 15	1	26.9	13.3
	2	(28.8)	(14.0)
Nematicide	--	22.7	9.2
Control (N)	--	16.6	6.6
L.S.D. (0.05)	3.7	--	1.3

1 = Antagonist was added before planting.
2 = Antagonist was added before and after planting.
G.V. = *Gliocladium vines*
B. 15 = *Bacillus* sp.
T.S. = *Trichoderma harzianum*

REFERENCES

1. Abd El-Moity, T.H. and M.N. Shatla. 1981. Biological control of white rot disease of onion *Sclerotium cepivorum* by *Trichoderma harzianum*. Phytopathology Z., 100: 29-35.
2. Abd El-Moity, T.H., G.C. papavizas and M.N. Shatla. 1982. Induction of new isolates of *Trichodrerma harzianum* tolerant to fungicides and experimental use for onion white rot control. Phytopathology, 72: 396.
3. Abd El-Moity, T.H., F.W. Raid, and S. El-Eraki. 1985. Biological control of root-knot nematode, *Meloidogyne javanica* of tomato. The 1st Conf. of Pests and Dis. of Veg. and Field Crop in Egypt. Ismailia, Oct. 1985 : 1013-1020.
4. Adiko, A. 1984. Biological control of *Meloidogyne incognita* with *Paecilomyces lilacinus*. M. Sc. Thesis, North Carolina State University, Raleigh., N.C., U.S.A.
5. Ali, E.M. 1994. Advanced studies to control plant parasitic nematodes by non-chemical methods. Ph.D. Thesis, Faculty of Agriculture, Cairo Univ. 118 pp.
6. Ali, H.H.A. and M.I.E. Barakat. 1991. Biocontrol and interaction with cantaloupe plants. Bull. Fac. of Agric. Univ. of Cairo, Vol. 42 : 279-292.
7. Baker, R. 1988. *Trichoderma* spp. as plant-growth stimulants. Reprinted from CRC Critical Review in Biotechnology, Vol. 7, Issue 2:97-106 .
8. Bird, A. 1976. The development and organization of skeletal structures in nematodes. In. N.A. Croll, (ed.) The organization of nematodes pp. 107-137. Academic Press, New York .
9. Brian, P.W. and H.G. Hemming. 1945. Gliotoxin a fungistatic metabolic product of *Trichoderma viride*. Annals Applied Biology, 32: 214-220.
10. Chet, I., Y. Elad, J. Katan and Y. Henis. 1979. Biological control of soil-borne plant pathogens by *Trichoderma harzianum*. Pages 585-592 In B.Schippers and W.Gams (eds). Soil-Borne Plant Pathogens. Academic Press, New York. 656 pp .
11. Dube, B.N. 1989. Biological control of *Meloidogyne javanica* using *Paecilomyces lilacinus* and an organic amendment. J. Nematol., 21 : 558.
12. Dube, B.N. and G.C. Smart. 1987. Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. J. Nematol., 19 : 222-227.

13. Enrique Capanilleas, E., K.R. Barket and L.N. Nelson. 1989. Growth of isolates of *Paecilomyces lilacinus* and their efficacy in biocontrol of *Meloidogyne incognita* on tomato. J. Nematol., 21: 164-172.

14. Gabriel, B.P. 1968. Enzymatic activities of some entomophagous harous fungi. J. Inveret. Pathol., 11:70-81 .

15. Hanna, A.I., T.H. Abd El-Moity and F.W. Riad. 1995. Evaluation of some biocontrol agents in controlling root knot nematodes and its effect on useful microorganisms (*Rhizobium legumonsarum* bv. *viceae*). Egyptian J. Appl. Sci., 10 (3) : 375-385.

16. Houssny, H.H. and B.A. Oteifa. 1956. Preliminary field tests for evaluating some tomato varieties for resistance to root knot nematodes. Hort. Abstr., 27 : 246. (No. 1575).

17. Jatala, P. 1982. Biological control with the fungus *Paecilomyces lilacinus*. pp. 172-176. In : International Meloidogyne Project. Proc. of the Res and Planning Conf on Root-Knot nematodes *Meloidogyne* spp., Octorber 25-29, 1982. Region III, Brazil.

18. Lamberti, F. and A. Ciancio. 1992. Biological control of plant parasitic nematodes. In E.C. Tjamos, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases. Progress and Challenges for the Future. Plenum Press New York, 17-20 pp.

19. Miller, P.M. 1976. Suppression of *Pratylenchus penetrans* and *Tylenchorhynchus dubius* by *Trichoderma viride*. J. Nematol., 9: 182-183.

20. Orton, W.K.J. 1973. *Meloidogyne incognita*. C.I.H. Description of plant-parasitic Nematodes. Set. 2, No. 18.

21. Poinar, G.O. and E.L. Hansen. 1986. Association between nematodes and bacteria. Helminthological Abstracts, 55: 61-81.

22. Rodriquez-Kabana, R., G. Morgan-Jones, G. Godoy, and B.O. Gintis. 1984. Effectiveness of species of *Gliocladium, Paecilomyces*, and *Verticillium* for control of *Meloidogyne arenaria* in field soil. Nematropica, 14 : 155.

23. Sayre, R.M. and M.P. Starr. 1988. Bacterial diseases and antagonisms of nematodes. In G.O. Poinar, and H.B. Jansson, (eds.). Diseases of nematodes, Vol. I. CRC. Press., Boca Raton, PP. 69-101.

24. Shahzad, S.S. Ehteshamul Haque and A.Ghaffar. 1990. Efficacy of *Pasteuria penetrans* and *Paecilomyces lilacinus* in the biological control of *Meloidogyne javanica* on mung bean. Int. Network Newsl., 7 (3) : 34-35.

25. Stirling. G.R. 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology, 74, 55.

26. Stirling, G.R. 1991. Biological control of plant parasitic nematodes. C.A.B. International, pp. 282.

27. Turner, W.B. 1971. Fungal Metabolites. Academic Press, London, New York, pp. 446.

28. Windham, M.T., Y. Elad and R. Baker. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology, 76 (5) : 518-521.

تأثير بعض الكائنات الحيوية على تكاثر نيماتودا تعقد الجذور على نباتات الطماطم

توفيق حافظ عبد المعطى ، الصاوى محمود على ، محمود حسن الحموى،
طه الشرقاوى، كارى تليكالا

١ معهد بحوث أمراض النبات بمركز البحوث الزراعية – الجيزة.
٢ معهد وقاية النبات بمركز البحوث الزراعية بفنلندا .

تم إختبار ثلاثة عوامل حيوية وهى : الفطر تريكودرما هارزيانم والفطر جلوكلاديم فيرنس ونوع من البكتريا باسيلس (السلالة ب ١٥) معزولة من التربة المصرية وأستخدمت فى معاملات منفردة أو فى خليط لمقاومة نيماتودا تعقد الجذور من النوع ميلودوجينى أنكوجنيتا على نباتات الطماطم.

كانت العزلة الأكثر تأثيراً فى مقاومة نيماتودا تعقد الجذور هى عزلة البكتريا من نوع الباسيلس سلالة (ب ١٥). بينما كانت العزلة من الفطر تريكودرما هارزيانم أقلهم تأثيراً.

كما وجد أن التأثير المشترك ظهر بوضوح عند إضافة خليط من هذه العوامل الحيوية المختلفة. ووجد أن إضافة أى عامل حيوى مرتين للتربة المصابة كان دائما أكثر تأثيراً مقارنة بالمعاملة لمرة واحدة.

كان استخدام الباسيلس مرتين أكثر تأثيراً من المعاملة الكيماوية. كما أن إضافة أى من العوامل الحيوية المستخدمة منفردا أو فى خليط مع العوامل الأخرى يؤدى إلى إنخفاض فى تعداد كتل البيض وعدد البيض علي النبات الواحد وكذلك تعداد اليرقات من العمر الثانى فى التربة.

أدى إضافة أى من العوامل الحيوية منفردة أو فى خليط مع العوامل الأخرى إلى زيادة فى الوزن الرطب لكل من المجموع الجذرى والخضرى.