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Management of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* by fungus culture filtrates and *Bacillus subtilis* on chickpea

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Summary – *Bacillus subtilis* and fungus filtrates of *Aspergillus niger, Curvularia tuberculata* and *Penicillium coryophilum* were used as seed treatments alone and in combination for the management of a root-rot disease complex of chickpea caused by the nematode *Meloidogyne incognita* race 3 and the fungus *Macrophomina phaseolina*. In general, treatments of all the four agents alone or in combination against plants inoculated with pathogens increase shoot dry weight, number of nodules and reduced nematode multiplication, galling and root-rot index. Increase in shoot dry weight and nodulation was greater when management agents were used against both pathogens compared to plants inoculated with either pathogen alone. Treatment of *B. subtilis* alone against *M. incognita* caused a similar increase in shoot dry weight as was caused by treatments with *A. niger* or *P. coryophilum* filtrates. Treatment of *B. subtilis* against *M. phaseolina* resulted in a larger shoot dry weight than caused by treatment with any of the filtrates used. However, use of *B. subtilis* on plants inoculated with both pathogens resulted in a similar shoot dry weight as was caused by *A. niger* filtrate treatment. Increase in shoot dry weight and reduction in nematode multiplication, galling and root-rot index were greater when plants inoculated with *M. incognita* or *M. phaseolina* or with both, were treated with *B. subtilis* plus *A. niger* or these two combined with one or two other culture filtrates. In general, fungus culture filtrates were less effective as management agents than *B. subtilis*.

Résumé – ***Protection du pois chiche contre* Meloidogyne incognita *race 3 et* Macrophomina phaseolina *grâce à des filtrats de culture de champignons et à* Bacillus subtilis.** – *Bacillus subtilis* et des filtrats de culture des champignons *Aspergillus niger, Curvularia tuberculata* et *Penicillium coryophilum* ont été utilisés, seuls ou en combinaison, comme traitement de semences pour protéger le pois chiche contre une maladie racinaire complexe associant le nématode *Meloidogyne incognita* race 3 et le champignon *Macrophomina phaseolina*. D'une manière générale, les traitements à l'aide de ces quatre agents, seuls ou en combinaison, accroissent le poids sec et le nombre de nodules de plantes inoculées avec les deux pathogènes, et réduisent la multiplication du nématode ainsi que le nombre de galles et la pourriture des racines. L'augmentation du poids sec et celle de la nodulation sont plus élevées si les agents de traitements sont utilisés contre les deux agents pathogènes en comparaison des plantes inoculées avec un seul des deux. Le traitement contre *M. incognita* à l'aide de *B. subtilis* seul provoque une augmentation de poids sec identique à celle causée par un traitement avec des filtrats de *A. niger* ou de *P. coryophilum*. Le traitement à l'aide de *B. subtilis* seul contre *M. phaseolina* provoque une augmentation de poids sec plus élevée que celle causée par aucun des filtrats fongiques utilisés. Cependant, l'utilisation de *B. subtilis* sur des plantes inoculées avec les deux pathogènes provoque une augmentation de poids sec semblable à celle causée par un traitement à l'aide de filtrat de *A. niger*. L'augmentation du poids sec, la réduction de la multiplication du nématode, celle du nombre des galles et celle de la pourriture des racines sont plus élevées si les plantes inoculées avec *M. incognita* et *M. phaseolina* – seuls ou en combinaison – sont traitées à l'aide de *B. subtilis* combiné à *A. niger*, ou à l'aide de ces deux agents additionnés de filtrats d'une ou deux autres cultures fongiques. D'une manière générale, les filtrats de cultures fongiques se révèlent des agents de contrôle moins efficaces que *B. subtilis*.

Key-words : *Bacillus subtilis, Cicer arietinum,* fungus culture filtrates, management, *Meloidogyne incognita* race 3, *Macrophomina phaseolina,* root-rot disease complex.

Chickpea, *Cicer arietinum* L., is one of the most important pulse crops of India and a chief source of protein for the large vegetarian population. There are several constraints in the successful cultivation of chickpea. One of these is the disease complex caused by the nematode *Meloidogyne incognita* and the fungus *Macrophomina phaseolina* (Tassi) Goid, which causes severe damage to this crop (Siddiqui & Husain, 1992).

Large numbers of fungi occur naturally in the rhizosphere and exert an influence on the other microorganisms. Some of these fungi produce toxic metabolites in culture media and fungus culture filtrates have shown potential as nematode management agents (Vaishnav *et al.*, 1985; Siddiqui & Husain, 1991). Bacteria are also capable of providing substantial disease control against pathogens (Weller, 1988). For example, *Bacillus subtilis* Cohn *emend.* Prazmowski inhibited other pathogens and was effective in increasing yields of several crops (Weller, 1988; Siddiqui & Mahmood, 1993). These results indicate that this bacterium warrants further study.

In the present study *Bacillus subtilis* and culture medium filtrates of three fungi; *Aspergilus niger* Van Tiegh, *Curvularia tuberculata* Jain and *Penicillium coryophilum* Dierckx were used alone and in various combinations as seed treatments for the management of the root-rot disease complex of chickpea which were generally found in the rhizosphere of this crop.

Materials and methods

The root-knot nematode *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* were used as test pathogens on chickpea, *Cicer arietinum* cv. P-256. A bacterium *Bacillus subtilis* and fungus culture filtrates of *Aspergillus niger, Curvularia tuberculata* and *Penicillium coryophilum* were used alone and in combination for the management of *M. incognita* and *M. phaseolina.*

Preparation of fungus culture filtrates

Three fungi, *Aspergillus niger, Curvularia tuberculata* and *Penicillium coryophilum* were separately cultured in Richards liquid medium (Riker & Riker, 1936) for 15 days at 25 °C. Mycelial mats were then removed and remaining medium was filtered through Whatman No. 1. Filtrates were centrifuged at 6000 rpm for 15 min. The supernatant was taken and the remainder was discarded. Centrifugation was done to remove the remaining hyphae and spores from the filtrates. To confirm that there were no spores or hyphae left, the filtrates were observed under the microscope. They were diluted ten times (S/10 concentration) and seeds were soaked for 4 h in the solution. Seed treatments with filtrates rather than soil treatment will be useful to reduce the quantity of filtrates required for application.

Inoculum of *Bacillus subtilis*

Bacillus subtilis culture was prepared on nutrient agar medium (Riker & Riker, 1936). Plates were incubated at 37 °C for 24 h and the bacteria were scraped from the plates and suspension prepared to contain 10×10^8 bacterial cells/ml as determined by serial dilution plating procedure (Cappucinno & Sherman, 1983). One hundred ml of the bacterial suspensions was poured into 100 g autoclaved soil and seeds were mixed in this soil. Seeds were dried for 1 h at room temperature. Seed coating with *B. subtilis* enabled us to reduce its quantity required for application compared with our earlier study (Siddiqui & Mahmood, 1993).

Inoculum of *Bradyrhizobium*

One hundred grams of a commercial culture of *Bradyrhizobium japonicum* (chickpea strain) was suspended in 1000 ml distilled water and 10 ml (equivalent to 1 g inoculum) was added around the seeds of each pot at the time of sowing.

Nematode inoculum

Meloidogyne incognita was collected from a chickpea field and multiplied on egg plant (*Solanum melongena* L.) using a single egg mass. The *M. incognita* was identified as race 3 using host differential tests (Taylor & Sasser, 1978). Egg masses were hand picked using sterilized forceps and placed in 9 cm diameter sieves of 1 mm pore size which were previously mounted with cross-layered tissue paper. For hatching, the sieves were placed in Petri dishes containing distilled water and kept in a 27 °C incubator. In the experiment 2000 freshly hatched second-stage juveniles were pipetted near fine roots of the chickpea seedlings which were exposed by removing the soil carefully and replacing it after inoculation.

Fungus inoculum

Macrophomina phaseolina was isolated from chickpea roots and maintained on potato dextrose agar (PDA). Fungus inoculum was prepared by culturing the isolate in Richards liquid medium (Riker & Riker, 1936) for 15 days at 25 °C. Mycelium was collected on blotting sheets to remove excess water and nutrients; 100 g mycelium was macerated in 1000 ml distilled water and 10 ml of this suspension containing 1 g fungus was inoculated into each pot. The inoculation procedure was similar to that used for nematodes.

Treatments and plant culture

Seeds of chickpea were surface sterilized with 0.1 % mercuric chloride for two minutes, washed three times in distilled water and treated with fungus culture filtrates and/or *B. subtilis* before sowing. Five seeds were sown in each of a series of 15 cm earthen pots containing 1 kg steam sterilized soil. After germination seedlings were thinned to one per pot. Filtrates and *B. subtilis* application had no adverse effect on the germination of seeds. There were sixteen potential treatments :

(1) control without *B. subtilis* or fungus culture filtrates,
(2) *B. subtilis* (BS),
(3) *C. tuberculate* filtrate (CT),
(4) *A. niger* filtrate (AN),
(5) *P. coryophilum* filtrate (PC),
(6) BS + CT,
(7) BS + AN,
(8) BS + PC
(9) CT + AN,
(10) AN + PC,
(11) CT + PC,
(12) BS + PC + AN,
(13) BS + CT + PC,
(14) CT + AN + PC,
(15) BS + AN + PC,
(16) BS + CT + AN + PC.

Each of these sixteen treatments was tested with three plant pathogen treatments. The three pathogen treat-

ments were *M. incognita, M. phaseolina* and *M. incognita* plus *M. phaseolina*. A control not treated with a pathogen was included with each of the sixteen treatments. Each of the 64 treatments was replicated four times and the experiment was conducted twice, i.e. in 1991 and 1992. Data presented in the paper were recorded in 1992 but both experiments showed a similar trend. In total, there were 256 pots which were arranged in split-disposition-plot design on a greenhouse bench. The pots of sixteen biocontrol agent treatments were arranged in sixteen different groups, each divided into four rows. Thus, there were 64 rows of different treatments. Pots were watered periodically and the experiment was terminated 90 days after inoculation.

OBSERVATIONS

Data recorded were shoot dry weight, number of nodules and galls, root-rot index and nematode density. Nematode in soil was extracted by Cobb's sieving and decanting technique followed by Baermann funnel (Southey, 1986). The number of juveniles, eggs and females in the roots were also estimated. Each root system was cut into small pieces and mixed; 1 g root was removed and macerated for 45 seconds in a Waring blender to recover nematodes eggs, females and larvae. A root-rot index was determined by scoring the severity of disease on a scale ranging from 0 (no disease) to 5 (severe root-rot). The data were analysed statistically under split plot design and critical differences were calculated at $P = 0.05$.

Results

Seed treatment with *B. subtilis* alone increased shoot dry weight more than the treatment with any of the fungus culture filtrates individually (Table 1). Among culture filtrates, seed treatment with *A. niger* culture filtrate resulted in the largest increase in shoot dry weight followed by *C. tuberculata* culture filtrate. The treatment of *B. subtilis* with *A. niger* or these two combined with one or two other tested culture filtrates resulted in the greatest increase in shoot dry weight. Use of *C. tuberculata* culture filtrate with *P. coryophilum* filtrate caused less increase in shoot dry weight compared to any combined treatment.

The treatment with *B. subtilis* alone on *M. incognita*-inoculated plants caused significant increase in shoot dry weight compared to *C. tuberculata* filtrate treatment (Table 1). Treatment with *A. niger* culture filtrate caused statistically the same increase in shoot dry weight to *M. incognita*-inoculated plants as was shown by *B. subtilis* or *P. coryophilum* culture filtrate treatments. The treatment of *B. subtilis* alone against *M. phaseolina*-inoculated plants caused high increase in shoot dry weight as compared to treatment with any of the tested culture filtrate. Out of three culture filtrates, treatment with *A. niger* filtrate against *M. phaseolina*-inoculated plants caused more increase in shoot dry weight than treatment with *P. coryophilum* filtrate. When any two or three culture filtrates were used against *M. phaseolina* except the treatment with *C. tuberculata* plus *A. niger,* the increase in shoot dry weight was statistically the same. Individual treatment with *B. subtilis* or *A. niger* culture filtrate caused significant increase in shoot dry weight against *M. incognita* plus *M. phaseolina*-inoculated plants compared to individual treatment with *C. tuberculata* or *P. coryophilum* filtrate. Combined treatment with any two culture filtrates against plants inoculated with either pathogen resulted in similar increase in shoot dry weight as shown by combined treatment with three filtrates. Increase in shoot dry weight was found greater when management agents were used against plants inoculated with both pathogens together compared to their use against a single pathogen.

Percentage increase in nodulation was significantly higher where *B. subtilis* was combined with any of the fungus culture filtrates compared to combined use of filtrates (Table 1). The combined use of culture filtrates resulted in similar increase in nodulation as was caused by the treatment with single culture filtrate treatment. Percentage increase in nodulation was significantly higher when management agents were used against plants inoculated with both pathogens compared to plants inoculated with single pathogen.

Treatment by management agents against *M. incognita*-inoculated plants caused significant increase in nodulation compared to plants without management agents but inoculated with *M. incognita* (Table 1). Treatment with management agents, except where *C. tuberculata* alone or *A. niger* plus *P. coryophilum* or three culture filtrates together were used, resulted in significant increase in nodulation of *M. phaseolina*-inoculated plants. Combined treatment by *B. subtilis* plus *A. niger* caused the highest increase in nodulation in *M. phaseolina*-inoculated plants compared with treatment by other management agents against the same pathogen. Use of *B. subtilis* with culture filtrates in different combinations caused significantly by greater increase in nodulation than when filtrates were used in combination on plants inoculated with both pathogens (Table 1).

Individually *B. subtilis* reduced nematode multiplication and galling of plants inoculated with *M. incognita* or *M. incognita* plus *M. phaseolina* more than when any fungus culture filtrate was used as treatment (Table 2). Treatment with *A. niger* filtrate reduced galling and nematode multiplication more than treatment with *C. tuberculata* or *P. coryophilum* filtrate. The treatment with *C. tuberculata* filtrate caused a similar reduction in nematode multiplication and galling to that caused by *P. coryophilum* treatment. Use of *B. subtilis* plus *A. niger* or these two combined with one or two other filtrates caused maximum reduction in galling and nematode multiplication either inoculated with *M. incognita* or *M. incognita* plus *M. phaseolina*. Use of filtrates in combination without *B. subtilis* caused less reduction in galling

Table 1. *Management of* Meloidogyne incognita *race 3 and* Macrophomina phaseolina *by fungus culture filtrates and* Bacillus subtilis *on chickpea.*

Potential management agent	Shoot dry weight (g)					No. of nodules per root system				
	Plant pathogen treatments				Mean per management agent treatment	Plant pathogen treatments				Mean per management agent treatment
	Control	*M. incognita* (MI)	*M. phaseolina* (MP)	MI + MP		Control	*M. incognita* (MI)	*M. phaseolina* (MP)	MI + MP	
No potential management agent	**7.92**	**5.78**	**6.02**	**3.44**	**5.79**	**46**	**28**	**32**	**15**	**30**
B. subtilis (BS)	2.02	27.34	24.25	68.60	24.01	4.3	32.1	25.0	93.3	30.0
C. tuberculata (CT)	0.76	20.42	11.63	55.23	16.58	2.2	28.6	9.4	80.0	20.0
A. niger (AN)	1.26	24.57	15.61	63.37	20.03	4.3	28.6	15.6	80.0	23.3
P. coryophilum (PC)	0.50	23.18	7.97	47.09	15.03	0.0	25.0	12.5	73.3	20.0
BS + CT	1.89	29.76	28.24	106.98	31.26	10.9	50.0	25.0	153.3	43.3
BS + AN	3.79	37.02	32.89	118.02	36.61	8.7	57.1	46.9	173.3	50.0
BS + PC	2.78	28.89	29.24	104.65	31.26	8.7	53.6	28.1	160.0	43.3
CT + AN	1.52	25.61	17.28	70.06	21.76	2.2	28.6	12.5	86.7	23.3
AN + PC	1.77	27.16	14.78	70.06	21.59	6.5	28.6	9.4	93.3	23.3
CT + PC	1.52	23.70	10.96	63.66	18.65	6.5	32.1	15.6	93.3	26.7
BS + CT + AN	4.80	37.54	32.23	116.57	36.79	8.7	42.9	21.9	146.7	36.7
BS + CT + PC	2.53	28.03	26.58	104.07	30.22	10.9	46.4	25.0	146.7	40.0
CT + AN + PC	1.89	25.26	14.62	69.77	21.07	10.9	25.0	3.1	100.0	23.3
BS + AN + PC	3.53	35.64	29.57	113.66	34.72	13.0	57.1	28.1	146.7	43.3
BS + CT + AN + PC	4.42	36.68	33.55	121.15	37.48	6.5	39.3	21.9	153.3	36.7
Mean per plant pathogen treatment	**8.09**	**6.34**	**7.26**	**6.26**	-	49	**38**	**38**	**32**	-

Critical difference (5 %) for		Shoot dry weight	Nodulation
Mean per plant pathogen treatment	=	**0.07**	**0.9**
Mean per potential management agent treatment	=	**0.13**	**1.9**
Mean per plant pathogen treatment at the same level of potential management agent treatment	=	**0.27**	**3.4**
Mean per potential management agent treatment at the same or different level of plant pathogen treatment	=	**0.29**	**3.8**

* Bold type shows actual observation on the plant. Normal type shows percentage decrease over respective controls.

and nematode multiplication compared to their use with *B. subtilis*. Treatment with *C. tuberculata* plus *P. coryophilum* caused less reduction in nematode multiplication and galling than when either of these filtrates were used with *A. niger*. Galling and nematode multiplication was greater with *M. incognita* alone than when plants were inoculated with *M. incognita* plus *M. phaseolina* (Table 2).

Root-rot index was 4 when plants were inoculated with *M. phaseolina* alone. The treatments of *M. phaseolina*-inoculated plants with *B. subtilis* plus *A. niger* filtrate or these two combined with one or two other filtrates resulted in lowest root-rot index (Table 2). Inoculation of both pathogens together resulted in the highest root-rot index. This root-rot index was greatly reduced to 2 when *B. subtilis* with *A. niger* or these two with one or two other filtrates were used on plants inoculated with both pathogens (Table 2).

Discussion

Bacillus subtilis reduced galling and nematode multiplication on plants treated with *M. incognita*, resulting in improved plant growth. This treatment also reduced the root-rot index of *M. phaseolina*-inoculated plants. Improvements in plant growth can be attributed to inhibitory effects of *B. subtilis* against pathogens (Yuen *et al.* 1988; Siddiqui & Mahmood, 1993). Previous studies indicated that treatments of *B. subtilis* increased yields of several crops (Merriman *et al.* 1974; Turner & Backman, 1986). Additionally, the bacterium improved plant growth by inhibiting non-parasitic root pathogens, producing biologically active substances, or by transform-

Table 2. *Management of* Meloidogyne incognita *race 3 and* Macrophomina phaseolina *by fungus culture filtrates and* Bacillus subtilis *on chickpea.*

Potential management agent	No. of galls per root system			Nematode population in 1000s			Root-rot index	
	Plant pathogen treatment		Mean per management agent treatment	Plant pathogen treatment		Mean per management agent treatment	MP	MI + MP
	M. incognita (MI)	*M. incognita* + *M. phaseolina* (MI + MP)		*M. incognita* (MI)	*M. incognita* + *M. phaseolina* (MI + MP)			
No potential management agent	**281**	**206**	**244**	**43.6**	**27.2**	**35.4**	**4**	**5**
B. subtilis (BS)	53.7	55.8	54.5	54.1	46.7	51.4	3	4
C. tuberculata (CT)	28.1	26.7	27.5	40.6	32.4	37.6	4	4
A. niger (AN)	36.3	39.8	37.7	45.9	39.7	43.5	3	4
P. coryophilum (PC)	24.9	26.7	25.8	36.0	27.2	32.5	4	4
BS + CT	70.1	67.0	68.9	63.8	60.7	62.4	2	3
BS + AN	77.9	79.1	78.3	76.8	74.6	76.0	1	2
BS + PC	66.5	67.9	67.2	62.6	61.0	62.1	2	3
CT + AN	48.0	49.5	48.8	52.8	41.5	48.3	3	4
AN + PC	44.8	48.1	46.3	52.1	39.3	47.2	3	4
CT + PC	43.1	45.6	44.3	47.0	34.9	42.4	3	4
BS + CT + AN	78.6	81.6	79.9	77.3	76.1	76.8	1	2
BS + CT + PC	71.9	76.2	73.8	64.4	61.0	63.3	2	3
CT + AN + PC	42.3	60.2	58.6	56.4	47.4	53.1	3	4
BS + AN + PC	80.1	82.0	81.1	76.1	75.7	75.9	1	2
BS + CT + AN + PC	79.4	84.5	81.6	79.4	76.1	78.2	1	2
Mean per plant pathogen treatment	**130**	**91**	-	**19.5**	**13.7**	-	-	-

Critical difference (5 %) for		Galling	Nematode population
Mean per plant pathogen treatment	=	3.3	0.3
Mean per potential management agent treatment	=	6.6	0.8
Mean per plant pathogen treatment at the same level of potential management agent treatment	=	12.1	1.1
Mean per potential management agent treatment at the same or different level of plant pathogen treatment	=	13.0	1.3

* Bold type shows actual observation on the plant. Normal type shows percentage decrease over respective controls

ing unavailable mineral and organic compounds into forms available to plants (Broadbent *et al.,* 1977). Moreover, non-cellular-extract of *B. subtilis* is also reported to have a high degree of larvicidal properties to root-knot and cyst nematodes (Gokte & Swarup, 1988). Our results concerning the efficacy of *A. niger* are in agreement with those of Mankau (1969 *a*); Siddiqui & Husain (1991) who demonstrated that filtrates of *A. niger* markedly reduced the number of nematodes in soil. The lethal effect of culture filtrates of *A. niger* on nematodes may be due to a toxic concentration of oxalic acid produced by the fungus. Moreover, autoclaved culture filtrate of *A. niger* also immobilized the nematodes indicating that the toxic principle is heat stable (Mankau, 1969 *a, b*). Efficacy of *C. tuberculata* and *P. coryophilum* culture filtrates can be attributed to their toxic secretions or excretions in the culture medium.

Use of *B. subtilis* with fungus culture filtrates was more effective at reducing effects of the pathogens than use of *B. subtilis* alone. This approach to management restricted the establishment of test pathogens. On the other hand combined use of different filtrates was found less effective because seed soaking in combined treatment was almost the same due to the same concentration and period of soaking. This study suggests that management of root-rot disease complex of chickpea by *A. niger* and *B. subtilis* will be best for the successful cultivation of this crop. Seed treatment by *A. niger* and *B. subtilis* will not be costly and it will be free from health hazards.

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References

BROADBENT, P., BAKER, K. F., FRANKS, N. & HOLLAND, J. (1977). Effect of *Bacillus* spp. on increased growth of seedlings in steamed and in non-treated soil. *Phytopathology,* 67 : 1027-1034.

CAPPUCINNO, J. G. & SHERMAN, N. (1983). *Microbiology : A laboratory manual.* California, USA, Addison Wesley Publ. Co., 466 p.

GOKTE, N. & SWARUP, G. (1988). On the potential of some bacterial biocides against root-knot and cyst nematodes. *Indian J. Nematol.,* 18 : 152-153.

MANKAU, R. (1969 *a*). Toxicity of cultures of *Aspergillus niger* to mycophagous nematode *Aphelenchus avenae. Phytopathology,* 59 : 13 [Abstr.].

MANKAU, R. (1969 *b*). Nematicidal activity of *Aspergillus niger* culture filtrates. *Phytopathology,* 59 : 1170.

MERRIMAN, P. R., PRINCE, R. D., KOLLMORGEN, J. F., PIGGOTT, T. & RIDGE, E. H. (1974). Effect of seed inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the growth of cereals and carrots. *Aust. J. Agric. Res.,* 25 : 219-226.

RIKER, A. J. & RIKER, R. S. (1936). *Introduction to research on plant diseases.* St. Louis, Chicago, New York & Indianapolis, John's Swift Co., 117 p.

SIDDIQUI, Z. A. & HUSAIN, S. I. (1991). Control of *Meloidogyne incognita* and *Macrophomina phaseolina* of chickpea by fungal filtrates. *Pakistan J. Nematol.,* 9 : 131-137.

SIDDIQUI, Z. A. & HUSAIN, S. I. (1992). Interaction of *Meloidogyne incognita* race 3, *Macrophomina phaseolina* and *Bradyrhizobium.* sp. in the root-rot disease complex of chickpea, *Cicer arietinum. Fundam. appl. Nematol.,* 15 : 491-494.

SIDDIQUI, Z. A. & MAHMOOD, I. (1993). Biological control of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* by *Paecilomyces lilacinus* and *Bacillus subtilis* alone and in combination on chickpea. *Fundam. appl. Nematol.,* 16 : 315-318.

SOUTHEY, J. F. (1986). *Laboratory methods for work with plant and soil nematodes.* London, Ministry of Agriculture Fisheries and Food, HMSO, 202 p.

TAYLOR, A. L. & SASSER, J. N. (1978). *Biology, identification and control of root-knot nematode* (Meloidogyne *spp.*). Raleigh, NC, USA, North Carolina State Graphics, 111 p.

TURNER, J. T. & BACKMAN, P. A. (1986). Biological cultures, tests control *Pl. Dis.,* 1 : 49.

VAISHNAV, M. V., PATEL, H. R. & DHRUJ, J. U. (1985). Effect of culture filtrates of *Aspergillus* spp. on *Meloidogyne arenaria. Indian J. Nematol.,* 15 : 116-117.

WELLER, D. M. (1988). Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopath.,* 26 : 379-407.

YUEN, G. Y., SCHROTH, M. N. & MCCAIN, A. H. (1988). Reduction in *Fusarium* wilt of carnation with suppressive soils and antagonistic bacteria. *Pl. Dis.,* 69 : 1071-75.