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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.

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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