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Biological control of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* by *Paecilomyces lilacinus* and *Bacillus subtilis* alone and in combination on chickpea

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Summary – *Paecilomyces lilacinus* and *Bacillus subtilis* were used for the biocontrol of the root-rot disease complex of chickpea caused by *Meloidogyne incognita* race 3 and *Macrophomina phaseolina*. Individually, *P. lilacinus* treatment was better against *M. incognita* while *B. subtilis* against *M. phaseolina*. The combined inoculation of *P. lilacinus* and *B. subtilis* improved dry shoot weight significantly when plants were simultaneously inoculated either with *M. incognita* or *M. phaseolina* or with both.

Résumé – ***Contrôle de* Meloidogyne incognita *race 3 et de* Macrophomina phaseoli *sur pois chiche par* Paecilomyces lilacinus *et* Bacillus subtilis, *seuls ou en combinaison*** – *Paecilomyces lilacinus* et *Bacillus subtilis* sont utilisés en vue du contrôle biologique d'une pourriture racinaire complexe du pois chiche causée par *Meloidogyne incognita* race 3 et *Macrophomina phaseoli*. Employés seuls, *Paecilomyces lilacinus* est meilleur contre *Meloidogyne incognita* et *Bacillus subtilis* contre *Macrophomina phaseoli*. L'inoculation combinée de *Paecilomyces lilacinus* et de *Bacillus subtilis* augmente de façon significative le poids sec des racines lorsque les plantes sont inoculées, au même moment, par *Meloidogyne incognita* et *Macrophomina phaseoli*, seuls ou en combinaison.

Key-words : Nemata, *Meloidogyne*, fungi, *Macrophomina*, *Paecilomyces*, bacteria, *Bacillus subtilis*, biological control.

Chickpea (*Cicer arietinum* L.) is an important pulse crop of India which is susceptible to root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood and *Macrophomina phaseolina* (Tassi) Goid. An interaction between *M. incognita* race 3 and *M. phaseolina* has been reported to cause severe damage on this important pulse crop (Siddiqui & Husain, 1992).

The use of micro-organism that can grow in the rhizosphere are ideal for use as biocontrol agents, since rhizosphere provides the initial barrier against pathogen attack of the root system (Weller, 1988). It is now a common belief that biological control can have an important role in agriculture.

Paecilomyces lilacinus (Thoms.) Samson has been reported to reduce nematode population densities (Jatala *et al.*, 1979; Morgan-Jones *et al.*, 1984; Jatala, 1986; Dube & Smart, 1987; Reddy & Khan, 1988) but in some places the results have been inconclusive (Dickson & Mitchell, 1985). Moreover, *Bacillus subtilis* Cohn *emend.* Prazmowski has also been used as successful biocontrol agent against plant pathogens (Broadbent *et al.*, 1977; Yuen *et al.*, 1985). In the present study these two biocontrol agents were used individually and simultaneously for the management of the root-rot disease complex of chickpea caused by *M. incognita* race 3 and *M. phaseolina*.

Materials and methods

Seeds of chickpea cv. P-256 were surface sterilized with 0.1 % mercuric chloride for 2 minutes and washed three times with sterile distilled water and treated with chickpea strain of *Bradyrhizobium* before sowing. Sucrose solution was used as sticker for the bacteria. Five bacteria treated seeds were sown in 15 cm earthen pots containing 1 kg steam sterilized soil. After germination the seedlings were thinned to one per pot. One week after germination the seedlings were inoculated with 2000 freshly hatched juveniles of *M. incognita* and 1 g *M. phaseolina*.

Meloidogyne incognita was called from the chickpea field and multiplied on eggplant (*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 was previously mounted with cross layered tissue paper. The sieves were placed for hatching in Petri dishes with distilled water in an incubator running at 27 °C. Two thousand freshly hatched second stage juveniles were pipetted near fine roots which were exposed by removing the soil carefully and replacing it after inoculation. The controls were inoculated with distilled water in the same way.

Macrophomina phaseolina was isolated from chickpea roots and maintained on potato dextrose agar (PDA). Fungus inoculum was prepared by culturing the isolate in Richard's 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 1 l distilled water and 10 ml of

this suspension containing 1 g fungus was inoculated in the same manner as were nematodes. *Paecilomyces lilacinus* was cultured and inoculated in the same manner as *M. phaseolina*.

Bacillus subtilis was inoculated 10 ml per plant as soil drench around the roots. In most studies it has been used as seed treatment but in the present study *Bradyrhizobium* was used with seeds. The culture of *B. subtilis* was prepared on nutrient agar medium (Riker & Riker, 1936). Plates were incubated at 37 °C for 24 h and bacterial growth was scraped and dissolved in distilled water. Suspension of bacteria were prepared to contain 10×10^8 bacterial cells/ml. The counting of bacterial cells in the suspension was done by preparing dilutions upto 10^{-7} and 0.1 ml suspension of each was carefully spread on nutrient agar plates (dilution 10^{-6} and 10^{-7} separately). Plates were incubated at 37 °C for 24 hours and bacterial colonies were counted. The inoculations were done in the same manner as *M. phaseolina*.

There were four experimental sets *(a)* without the treatment of biocontrol agents; *(b)* treated with *P. lilacinus* alone; *(c)* treated with *B. subtilis* alone; *(d)* treated with both biocontrol agents. All the experiment sets were having four treatments viz. control, *M. incognita*, *M. phaseolina* and *M. incognita* + *M. phaseolina*. Each treatment was replicated four times and the experiments were repeated twice. The pots were kept in a rendomized fashion on glass house bench. Pots were watered when needed and the experiment was terminated 90 days after inoculation. Data were recorded on dry shoot 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. The roots were cut into small pieces and mixed, 1 g root was 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 scale ranging from 0 (no disease) to 5 (severe root-rot).

Paecilomyces lilacinus and *B. subtilis* were reisolated from the eggs and females of *M. incognita* to determine the percentage infection on the remaining population. For reisolation, eggs and females were surface sterilized with 0.1 % mercuric chloride for 2 min washed three times in distilled water and placed in potato dextrose agar and nutrient agar medium for fungus and bacterial growth respectively. The plates were incubated at desired temperatures as described earlier. The growth of fungus and bacteria if found were identified. All the data collected were analysed statistically using multifactorial analysis and critical differences (C. D.) were calculated at 5 % level.

Results

Treatments of plants with biocontrol agents without pathogens resulted in increased dry shoot weight and nodulation over to plants inoculated with pathogens and treated with biocontrol agents (Table 1). Dry shoot weight of plants inoculated with *M. incognita* and treated with biocontrol agents was same to plants treated with biocontrol agents and *M. phaseolina*. Moreover, plants inoculated with both pathogens together and treated with biocontrol agents resulted in the greatest reduction in dry shoot weight and nodulation. Nematode multiplication and galling was adversally affected by *M. phaseolina* (Table 1).

Table 1. Biological control of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* by *Paecilomyces lilacinus* and *Bacillus subtilis* on chickpea.

Treatments	Dry shoot weight (g)	No. of nodules per root system	Nematode populations in 1000 s	No. of galls per root system
Control with treatments	7.9	46	–	–
M. incognita (MI) effect	6.5	32	23.0	165
M. phaseolina (MP) effect	6.6	37	–	–
MI + MP effect	5.5	24	14.5	112
C.D. 5 %	0.3	2	0.5	5

Inoculation of plant with biocontrol agents alone resulted in some dry shoot weight and nodulation as control without pathogens. *Paecilomyces lilacinus* treatment alone was found effective in improving dry shoot weight of plants inoculated with *M. incognita* alone or in combination with *M. phaseolina* (Table 2). The treatment of *P. lilacinus* was not effective against *M. phaseolina* alone. *Bacillus subtilis* effectively increase the dry shoot weight and nodulation of plants when inoculated with *M. incognita* or *M. phaseolina* or both. *Paecilomyces lilacinus* and *B. subtilis* inoculated together improved dry shoot weight and nodulation to the greatest extent (Table 2).

Highest reductions in nematode multiplication and galling were caused when *P. lilacinus* and *B. subtilis* were used together followed by *P. lilacinus* treatment alone. *Bacillus subtilis* treatment was least effective in reducing nematode multiplication and galling (Table 2).

Approximately 40 % of the females and 70 % of the eggs of *M. incognita* were found to be infected with *P. lilacinus* when reisolation of the fungus was made from the final nematode population. When reisolation of the bacteria from females and eggs was made the results were statistically negative.

Root-rot indices were found 4 and 5 when *M. phaseolina* was inoculated alone or with *M. incognita* respectively. The index was found 4 when *P. lilacinus* was inoculated with *M. phaseolina* or *M. phaseolina* plus *M. incognita*. The indices were reduce to 3 and 4 when

Table 2. Biological control of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* by *Paecilomyces lilacinus* and *Bacillus subtilis* on chickpea.

Treatments		Dry shoot weight (g)	No. of nodules per root system	Nematode population in 1000 s	No. of galls	Reisolation of *P. lilacinus* in percentage		Reisolation of *B. subtilis* in percentage		Root-rot Index
						Females	Eggs	Females	Eggs	
Control		7.7	44	–	–	–	–	–	–	–
M. incognita (MI)		5.5	28	42.6	286	–	–	–	–	–
M. phaseolina (MP)		5.9	31	–	–	–	–	–	–	4
MI + MP		3.5	14	25.8	204	–	–	–	–	5
Control		7.9	46	–	–	–	–	–	–	–
MI	1 g	6.8	37	18.4	142	43	75	–	–	–
MP	*P. lilacinus*	6.4	36	–	–	–	–	–	–	4
MI + MP		5.7	22	11.6	104	38	67	–	–	4
Control		7.8	47	–	–	–	–	–	–	–
MI	10 ml	6.5	35	20.7	164	–	–	4	2	–
MP	*B. subtilis*	6.9	38	–	–	–	–	–	–	3
MI + MP		5.9	21	14.1	114	–	–	2	3	4
Control		8.0	48	–	–	–	–	–	–	–
MI	*P. lilacinus*	7.1	40	10.2	67	41	73	3	2	–
MP	+	7.3	42	–		–				2
MI + MP	*B. subtilis*	6.7	38	6.3	26	39	65	2	2	4
C.D. 5 %		0.6	4	0.8	10	4	8	4	3	

plants inoculated with *M. phaseolina* or with *M. phaseolina* and *M. incognita* and treated with *B. subtilis*. The treatments of both biocontrol agents with *M. phaseolina* and with both pathogens resulted in 2 and 4 root-rot indices.

Discussion

The parasitism on *M. incognita* eggs and females by *P. lilacinus* resulted in reduced nematode multiplication thereby improving plant growth of nematode infected plants. *P. lilacinus* infected eggs of *M. incognita* more frequently and destroyed the embryo while females were parasitized through anus. The infection process was found to begin with the growth of *P. lilacinus* hyphae in the gelatinous matrix. The similar parasitism of *P. lilacinus* has been reported by others (Jatala *et al.,* 1979; Jatala, 1986). *Bacillus subtilis* inhibited both pathogens either individually or simultaneously. *Bacillus subtilis* is known to have inhibitory effects against several plant pathogens (Broadbent *et al.,* 1971, 1977; Yuen *et al.,* 1985). Its treatment were found effective in increasing yield of carrot by 48 %, oats by 33 % (Merriman *et al.,* 1974) and peanuts upto 37 % (Turner & Backman, 1986). The use of *B. subtilis* may also improve plant growth by suppressing non-parasitic root pathogen or by the production of biologically active sustances or by unavailable mineral and organic compounds into forms available to plants (Broadbent *et al.,* 1977).

It was concluded that *P. lilacinus* is successful biocontrol agent against root-knot nematode *M. incognita* while *B. subtilis* can be used against both *M. incognita* and *M. phaseolina*. This study suggests that combined inoculation of *P. lilacinus* and *B. subtilis* will be best for the control of this root-rot disease complex as inhibitory effect of *B. subtilis* was not effective on the parasitic behavior of *P. lilacinus*. However, we feel that combined application of both organisms as soil drench in field conditions will be very costly. We were not able to use *B. subtilis* as seed treatment to reduce the cost of application because *Bradyrhizobium* was used with the seeds. Further studies are needed on the biological control using both organism as seed treatment without *Bradyrhizobium* and with *Bradyrhizobium*.

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