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Compatibility of *Pasteuria penetrans* with fungal parasite *Paecilomyces lilacinus* against root knot nematode on Chilli (*Capsicum annuum* L.)

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ABSTRACT

Chilli (*Capsicum annuum* L.) crop is highly susceptible for the root knot nematode *Meloidogyne incognita* and every year this nematode causes great loss to the crop. The present study investigated the cumulative effect of two biocontrol agents viz. *Pasteuria penetrans* and *Paecilomyces lilacinus* against *M. incognita*. Two doses of *P. penetrans* i.e. 50g/Kg and 100g/Kg infested soil were applied either alone or in combination with two doses of *P. lilacinus* i.e. 4g spore culture/Kg and 6g spore culture/Kg of soil. Application of *P. penetrans* with *P. lilacinus* resulted into relatively better improvement in various growth attributes of chilli when compared with the individual application. Amongst the various treatments tested combined application with the higher dose of both bioagents (i.e. 100g *P. penetrans* infested soil with 6g of *P. lilacinus*/Kg) showed maximum improvement in fresh and dry weight of shoot and root over the nematode check and it was almost at par with that of the absolute check. The combined application of both the bioagents at higher dose resulted in 139 and 84% increase in dry weight of shoot and root over the nematode check respectively. The combined application of both the bioagents was also observed to cause higher reduction in gall number and nematode population than their individual application except for the treatment having lower dose of the *P. penetrans* with *P. lilacinus* where reduction in both the parameters was observed to be at par with that of the either dose of *P. penetrans* or higher dose of *P. lilacinus*. Combined application with higher dose of *P. penetrans* and *P. lilacinus* showed maximum reduction of 62.6 and 82.2% in gall numbers and nematode population over the nematode check.

1. Introduction

Amongst various vegetable and spice crops, chilli (*Capsicum annuum* L.) has its own importance. This is widely grown vegetable crop in both tropics and subtropics regions. But with this high importance, this crop is highly susceptible for the various types of fungal, bacterial, viral and nematode pathogens. Amongst various nematode pathogens, root-knot nematode *Meloidogyne* species stand out the most dominant group of plant parasitic nematodes and, in almost every vegetable field it causes enormous losses every year in the nursery as well as planted fields. For reducing the multiplication of this nematode several chemical nematicides like methyl bromide, carbofuran, oxamyl, aldicarb, phorate, phenamiphos (fenamiphos), itaconate dimethyl and cadusafos have been recommended by various researchers. However, the management of the *Meloidogyne* species using nematicides is highly expensive and less effective method. Moreover, it is toxic to the beneficial soil microflora and causes the environmental pollution. Public concerns over the toxicity of nematicides and their impact on the environmental damage and public health have necessitated research on alternative eco-friendly and sustainable methods to control plant-parasitic nematodes (Thomason et al., 1987; Carneiro et al., 2007). *Pasteuria penetrans* (Thorne, 1940**)** Sayer & Starr, an obligate hyperparasites of root-knot nematodes (RKN) has for many years shown itself to be a very promising biological control agent. Endospore of *P. penetrans* attach to the cuticle of the motile second stage juvenile (J2) of *Meloidogyne* species when they migrate through the soil in search of the food. The germ tube emerging from these spores penetrates the nematode cuticle and produces dichotomously branched micro-colonies. These micro-colonies subsequently divide and proliferate through the pseudocoelom eventually killing the nematode and producing a cavader filled with spores (Sayre and Starr, 1988; Kariuki et al., 2006). The number of endospores per juvenile is correlated with infection and is considered as the best measurement of biocontrol potential of the bacterium. However, high numbers of endospores attached per juvenile may reduce or impede their penetration into the roots of the host plants and increase final production or yield (Gomathi et al., 2006; Das et al., 2007; Rehiayani, 2007). Although *Pasteuria penetrans* alone itself is a good biocontrol agent but host specificity (Kumari and Sivakumar, 2006; Davies et al., 2008) and infection only on juveniles are some of the limiting factors for this bacterium to be used as biocontrol agent. However, its combination with other microbial agent, organic amendments or chemical nematicides has shown cumulative effect on its performance (Curto, 2006; Kumari and Sivakumar, 2006; Javed et al., 2008; Kumar, 2008).

Like *P. penetrans*, egg parasitic fungus *Paecilomyces lilacinus* also has shown very good biocontrol potential against nematode pest and insects (Bhat et al., 2009; Demirci and Deniahan, 2010). Combination of this fungus with other biocontrol agents and organic amendment also exhibited synergistic effect on the efficiency of this fungus (Pandey et al., 2007; Rao, 2007; Anastasiadis et al., 2008). Although individually both biocontrol agents have excellent biocontrol potential and also combination of these two shows collective effect on nematode penetration and multiplication but very limited information is available about the combined application of these two agents under arid conditions of Rajasthan. The main objective of this investigation was to determine the effective dose of application and evaluate the efficacy of a combination of bacterial parasite, *P. penetrans* and fungal parasite *P. lilacinus* for management of *M. incognita* on chilli under arid zone conditions. In addition, in this study first time we used bacteria-infested soil as inoculums considering the easier application of soil based inoculums on large scale by the farmers.

2. Materials and Methods

2.1. Preparation of microbial inoculum

Pure culture of *P. penetrans* was raised in pot on *M. incognita* infected brinjal plant var. pusa purple long. Bacteria-infested soil of the pots were air dried completely and initial spore attachment per larvae was measured by adding 100 freshly hatched juvenile of *M. incognita* in 10g infested soil for 72h at 28°C. The initial spore attachment was 18.2 Spore/J2. Simultaneously, pure culture of *P. lilacinus* was raised on rice grain. 100 ml capacity Erlenmeyer flasks were filled with 20g of rice grains. The flasks were plugged with cotton plug and autoclaved at 121°C under 1.5046 kg cm^{-1} pressure for 45 min. Fresh culture of *P. lilacinus* was raised on Potato Dextrose Agar medium. After 2 weeks, spores were scrapped from the plates and were inoculated to rice grains. The flasks were kept in an incubator at 28°C. After three weeks, the grains were thoroughly mixed. From this mixture, 500mg of culture was taken and mixed in 30ml of 0.025% sterile agar solution to make a uniform suspension of spore. The number of spore/ml was calculated by haemocytometer. The average number of spore/500 mg of culture was calculated which came out to be 4.66 X 10^{8}.

2.2. Experimental set-up

The study was conducted in 10cm pot containing 500cc of sterilized soil. Chilli seedlings (Var. Haripur Raipur) were grown in pots @ one seedling / pot. The two dosages of *P. lilacinus* i.e. 4 and 6g culture/ Kg of soil and *P. penetrans* i.e. 50 and 100g infested of soil/kg were tasted either alone or in combination. In case of combined application both the bioagents were mixed together and were then applied to the pot soil. Different treatments were imposed when the seedlings were 45 days old, one cm surface soil from the pot were removed. *M. incognita* juveniles were inoculated in the rhizosphere of the plants. After the inoculation the top surface of the pot was again covered with autoclaved soil and plants were lightly irrigated. The experiment was continued until 60 days after nematode inoculation.

2.3. Collection of data and statistical analysis

After 60 days the plants were harvested and the data pertaining to plant growth attributes (shoot and root weight) of chilli and number of galls and *M. incognita* population were recorded. Data were subjected to statistical analysis and critical difference (CD) at 5% was calculated using standard procedures.

3. Results and Discussion

Application of *P. penetrans* either alone or in combination with *P. lilacinus* affected growth characteristics of chilli and multiplication of *M. incognita* at varying degrees. Combining *P. penetrans* with *P. lilacinus* resulted into reverse the adverse effect of nematode on plant growth. Amongst the various treatments evaluated, maximum increase in the weight was recorded in the treatment having combined application of higher dose of both the bio-

Treatments	Shoot Weight (gm)		Root Weight (gm)		No. of galls/ plant	Final Nematode Population
	Fresh	Dry	Fresh	Dry		
1Pp + 1 Pl + N	18.4367	2.4387	6.3339	1.0536	173.6	50713.2 (10.795)
2Pp + 1 Pl + N	19.0541	2.8754	7.5466	1.1343	152.4	30599.6 (10.295)
1Pp + 2 Pl + N	20.0063	2.5294	7.5498	1.0562	166.8	30768.8 (10.328)
2Pp + 2 Pl + N	25.3737	4.2162	9.2665	1.3608	138.2	24629.2 (10.025)
1 Pl + N	15.1956	1.8979	6.1427	0.8447	246	89272.4 (11.392)
2 Pl + N	16.0347	1.9299	6.1897	0.8969	179.6	60618.6 (11.004)
1Pp + N	15.8193	1.9448	6.0856	0.9217	224.4	62347 (11.023)
2Pp + N	16.9457	1.9925	6.1832	0.9481	174.2	61114.6 (11.009)
Nematode Check	14.4743	1.7609	5.2192	0.7376	369.8	139004.6 (11.839)
1 Pl	24.0077	4.6969	11.639	1.4007	0.0	0.0
2 Pl	24.1382	4.6954	11.896	1.4102	0.0	0.0
1Pp	23.3077	4.8827	11.289	1.3989	0.0	0.0
2Pp	25.6895	4.6997	11.698	1.4166	0.0	0.0
Absolute Check	26.3712	4.8831	12.016	1.4388	0.0	0.0
CD 5%	5.59	0.76	1.83	0.34	62.23	0.3157

Table 1: Effect of either individual or combined application of *Pasteuria penetrans* and *Paecilomyces lilacinus* on various growth characters of chilli and multiplication of nematode (Mean of Five replicate)
1 Pl: *Paecilomyces lilacinus* @ 4g/Kg; 2 Pl: *Paecilomyces lilacinus* @ 6g/Kg; 1Pp: *Pasteuria penetrans* @ 50g/Kg; 2Pp: *Pasteuria penetrans* @ 100g/Kg; N: *Meloidogyne incognita*; Figure in parenthesis are log-transformed value.

agents. In case of shoot weight, the effect was more pronounced as this treatment registered complete reversal of the negative effect of nematode and both fresh and dry weight were at par with that of the absolute check. The same treatment was also observed to cause maximum improvement in root weight but the improvement was observed to be less significant than the absolute check. In general, improvement in weight of shoot in combination having lower dose of *P. penetrans* with higher dose of *P. lilacinus* or higher dose of *P. penetrans* with lower dose of *P. lilacinus* was recorded to be at par and these treatments suppress the adverse effect of nematode on shoot weight to a great extent than other combination or their individual application. With regard to root, almost similar trend was also visible in case of root weight. Higher dose of *P. penetrans* along with both the dosages of *P. lilacinus* completely reversed the adverse effect of nematode on dry weight of root and it was at par with that of absolute check. In general, improvement in fresh and dry weight of root in combination having lower dose of *P. penetrans* along with either of the dose of *P. lilacinus* or higher dose of *P. penetrans* with lower dose of *P. lilacinus* were at par to each other. Similarly, individual application of either *P. penetrans* or *P. lilacinus* at both the dosage and combined application of lower dose of *P. penetrans* and *P. lilacinus* were not found to have any significant effect on the improvement of fresh and dry weight of root and it was at par with that of the nematode

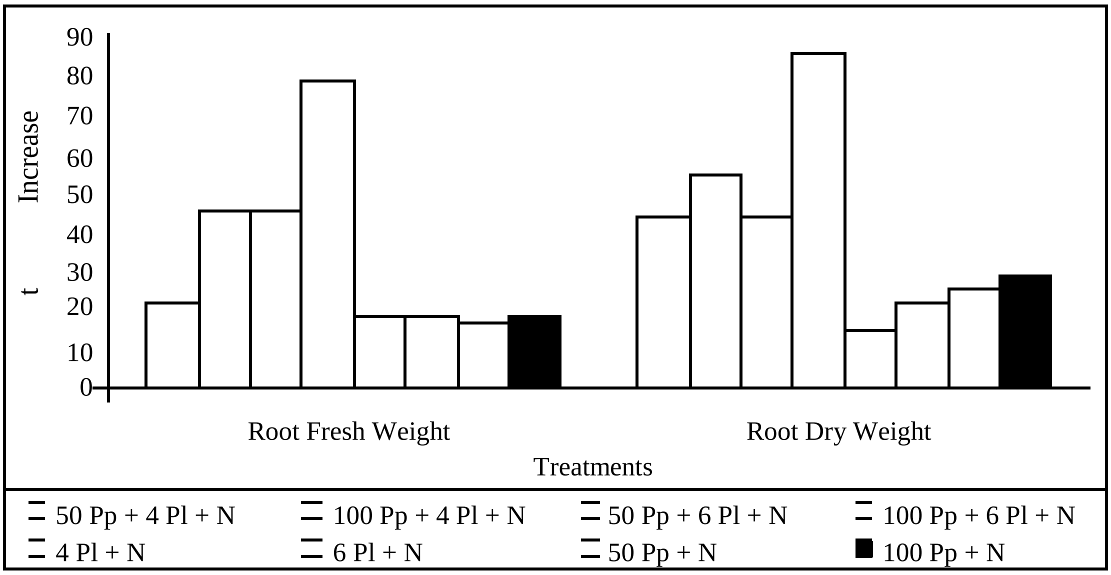

Figure 1: Effect of the individual or combined application on *Pasteuria penetrans* and *Paecilomyces lilacinus* on plant growth characters of chilli (Percentage increase over nematode check)

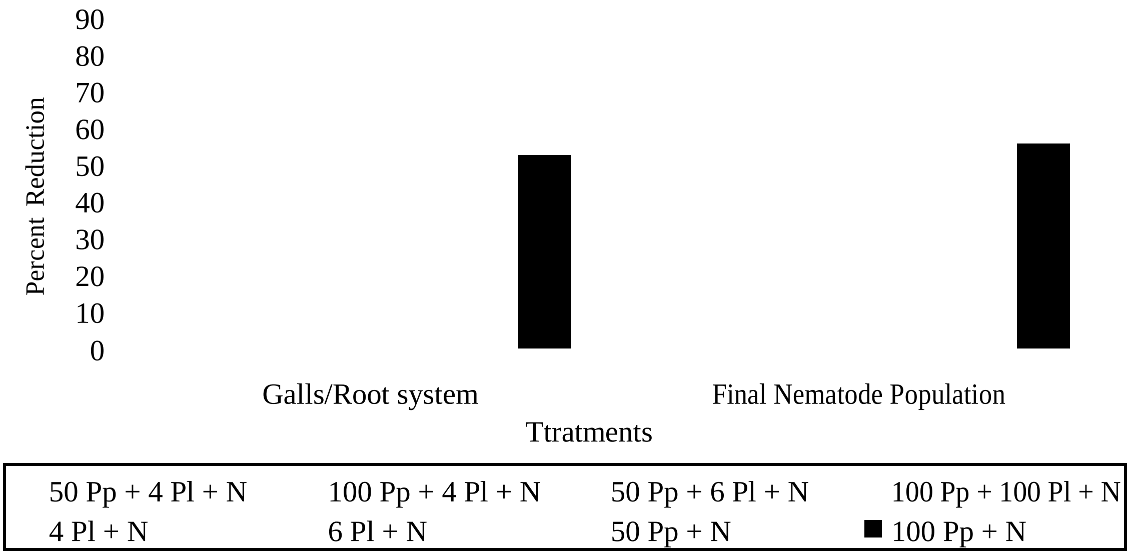

Figure 2: Effect of the individual or combined application on *Pasteuria penetrans* and *Paecilomyces lilacinus* on gall number and *Meloidogyne incognita* multiplication (Percentage reduction over nematode check)

check treatment. The best treatment i.e. higher dose of *P. penetrans* with higher dose of *P. lilacinus* yielded 75 and 77% improvement in fresh weight of shoot and root and 139 and 84% increase in dry weight of shoot and root respectively over the nematode check. These improvements were recorded to maximum amongst all the treatments tested except the absolute and associated control treatments.

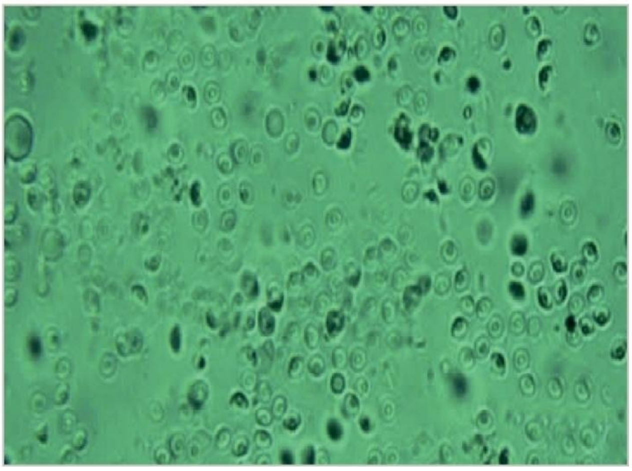

Figure 3: The oil immersion view of *Pasteuria penetrans* spores, (isolated from the infected female).

Application of both the bioagents alone also indicates utmost increase in the weight over nematode check in the treatment receiving higher dose of both bioagents. Amongst all treatment tested, lower dose of *P. lilacinus* proved least effective in the improving the growth characters of chilli. Combined as well as the individual application of both the bioagents was also observed to drastically reduce the average no. of gall/root system as well as the final population of the nematode. In general, combined application of both the bioagents invariably caused higher reduction in gall numbers and final nematode population then their single application except for treatment having combined application of lower dose of *P. penetrans* and *P. lilacinus* where reduction in both the parameters was found to be at par with that of either of the dose of *P. penetrans* or higher dose of *P. lilacinus*. Both the dosages of *P. penetrans* with lower dose of *P. lilacinus* or higher dose of *P. penetrans* were found at par with each other with respect of gall numbers and with higher dosage of individual application of *P. penetrans* or *P. lilacinus* with respect to nematode population. In general, combined application with higher dose of *Pasteuria penetrans* and *Paecilomyces lilacinus* resulted in maximum 62.6% reduction in gall number and 82.2% reduction in nematode population over the nematode check treatment. This was followed by lower application of *P. penetrans* with higher dosage of *P. lilacinus*.

Figure 4: Showing the oil immersion of *P. penetrans* spore attachment on and in the nematode body.

This study clearly indicated that the combined application of both agents showed cumulative effect and was superior to the individual application of both bioagents. Our results are in conformity with that of Koshy et al. (2003), who observed that the black pepper plants inoculated with burrowing nematode (*Radopholus similis*) alone had the minimum vine length, number of leaves, shoot and root weight, maximum root-lesion index and highest population of burrowing nematodes. The biological control agents individually or in combinations were highly effective in enhancing plant growth and suppressing nematode multiplication.

Figure 5: Showing the infection of *Paecilomyces lilacinus* on the nematode eggs and this infection disslove the cutin of the eggs.

Similar findings were reported by the Maheswari and Mani (1988), where the combined application of *Pasteuria penetrans* and *Paecilomyces lilacinus* caused 33.3% increase in the dry weight of tomato shoot and 66.2% reduction in *M. javanica* population. Studies conducted by Sosamma and Koshy (1997) on *M. incognita* infected black pepper also revealed the combination of *P. penetrans* with *P. lilacinus* to be more effective in increasing the fresh weight of shoot and root and reducing the nematode population which corroborates our findings.

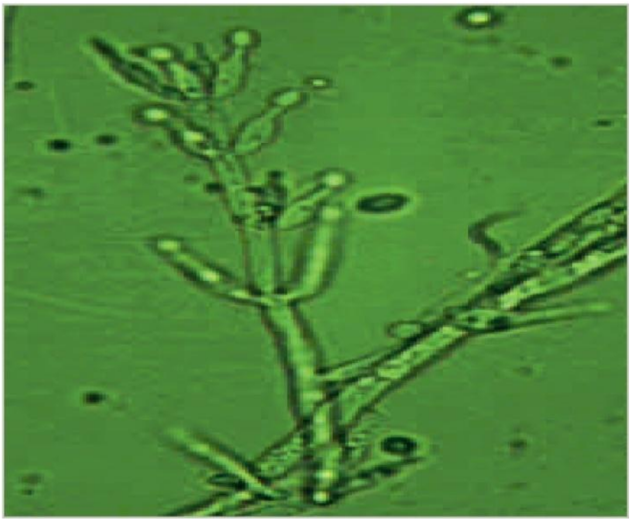

Figure 6: Showing the phialides and conidiophores of the fungus *Paecilomyces lilacinus* isolate from infected egg.

In a micro-plot experiment, also similar type of observation was reported by the Curto (2006) on biocontrol of *Meloidogyne incognita*. Our results slightly differ from those reported by the Zaki and Maqbool (1992), and Dube and Smart (1987), who observed very high improvement in the weight of shoot and root over nematode check at individual as well as combined application level of *P. penetrans* and *P. lilacinus*. Zaki and Maqbool (1992) observed that at individual application of *P. penetrans* caused 94% and *P. lilacinus* caused 170% while in combined application of both of them caused 230% increase in the weight of shoot over nematode check. Similar type of very high improvement was also reported by the Dube and Smart (1987). Result of our study also showing the effectiveness of *P. penetrans* in combination with *P. lilacinus* but in our study, at individual as well as combined application level the improvement in growth characters were less than that reported by these researchers. At individual level both agents proved less significant in increasing the fresh and dry weight of shoot and root and in combination they showed superiority over nematode check but this is less than these two studies. This variation may be result of the variation in the experimental conditions or may be because of the environmental effects on the performance of bioagents. Based on the observations of this study it could be concluded that the application of *P. penetrans* in combination with *P. lilacinus* had cumulative effect on improvement of plant growth and reduction in nematode population.

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References

Anastasiadis IA, Giannakou IO, Prophetou-Athanasiadou DA, Gowen SR (2008) The combined effect of the application of a biocontrol agent *Paecilomyces lilacinus*, with various practices for the control of root-knot nematodes. Crop-Protection 27: 352-361.

Bhat MY, Muddin H, Bhat NA (2009) Histological Interaction of *Paecilomyces lilacinus* and *Meloidogyne incognita* on bitter ground. Journal of American Science 5: 8-12.

Carneiro RMDG, Mesquita LFG, Cirotto PAS, Mota FC, Almeida MRA (2007) Biological control of *Meloidogyne incognita* on coffee plants using the isolates P10 of *Pasteuria penetrans*. Proceeding in the 21st-International-Conference on Coffee Science, Montpellier, France, 2006: pp. 1409-1414.

Curto G (2006) Ecocompatible systems against nematodes. Colture Protette 35: 36-41.

Das M, Gogoi BB, Neog PP (2007) Multiplication of *Pasteuria penetrans* in root-knot nematode in different cropping sequences. Crop Research 34: 246-248.

Davies KG, Rowe JA, Williamson VM (2008) Inter- and intra-specific cuticle variation between amphimictic and parthenogenetic species of root-knot nematode (*Meloidogyne* spp.) as revealed by a bacterial parasite (*Pasteuria penetrans*). International Journal for Parasitology 38: 851-859.

Demirci F, Deniahan E (2010) *Paecilomyces lilacinus,* a potential biocontrol agent on apple rust mite *Aculus schechtendali* and interaction with some fungicide in vitro. Phytoparasitica 38: 125-132.

Dube B, Smart Jr GC (1987) Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. Journal of Nematology 19:222-227.

Gomathi C, Kumar S, Subramanian S (2006) Effective dose and methods of delivery of *Pasteuria penetrans* against root-knot nematode in brinjal. Annals of Plant Protection Sciences 14:452-455.

Javed N, El Hassan S, Gowen S, Pemproke B, Inam ul Haq M (2008) The potential of combining *Pasteuria penetrans* and neem (*Azadirachta indica*) formulations as a management system for root-knot nematodes on tomato. European Journal of Plant Pathology 120:53-60.

Kariuki GM, Brito JA, Dickson DW (2006) Effects of *Pasteuria penetrans* endospore rate of attachment on root penetration and fecundity of *Meloidogyne arenaria* race. Nematropica 36: 261-267.

Koshy PK, Sosamma VK, Samuel R (2003) Bio-control of *Radopholus similis* on black pepper, *Piper nigrum* L. under field conditions. Indian Journal of Nematology 33: 43-46.

Kumar S (2008) Compatibility of *Pasteuria penetrans* with bio-control agents against root knot nematode in tomato. Annals of Plant Protection Sciences 16: 262-263.

Kumari NS, Sivakumar CV (2006) Host range and parasitizing ability of the obligate bacterial parasite, *Pasteuria penetrans* on different populations of the root-knot nematode, *Meloidogyne incognita*. Journal of Ecobiology 18: 225-228.

Kumari NS, Sivakumar CV (2005) Integrated management of root-knot nematode, *Meloidogyne incognita* infestation in tomato and grapevine. Communications in Agricultural and Applied Biological Sciences 70: 909-914.

Maheswari TU, Mani A (1988) Combined efficacy of *Pasteuria penetrans* and *Paecilomyces lilacinus* on the biocontrol of *Meloidogyne javanica* on tomato. International Nematology Network Newsletter 5: 10-11.

Pandey KC, Hasan N, Bhaskar RB (2007) Field evaluation of indigenous Biopesticides and resident bioagents for pest management in sorghum-cowpea intercrop. Range Management and Agroforestry 28: 159-161.

Rao MS (2007) Production of bio-agent colonized vegetable seedlings for sustainable nematode management. Acta Horticulturae 752: 545-548.

Rehiayani AS (2007) Suppression of *Meloidogyne incognita* by soil application of *Pasteuria penetrans* isolate from Al-Qassim area, Saudi Arabia. International Journal of Nematology 17: 130-136.

Sayre RM, Starr MP (1988) *Pasteuria thornei* sp. nov. and *Pasteuria penetrans* sensu stricto emend., mycelial and endospore-forming bacteria parasitic respectively, on plant-parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne*. Annales de l'Institut Pasteur, Microbiology 139: 11-31.

Sosamma VK, Koshy PK (1997) Biological control of *Meloidogyne incognita* on black pepper by *Pasteuria penetrans* and *Paecilomyces lilacinus*. Journal of Plantation Crop 25:72-76.

Thomason IJ (1987) Challenges facing nematology: environmental risk with nematicide and the need for new approaches. In: Veech JA, Dickson DW, (Ed.), Vistas on Nematology, Society of Nematologists, Havattsville. M.D., USA, pp. 469-479.

Thorne G (1940) *Duboseqia penetrans* n.sp. (sporozoa, Microsporidia, Nosematidae), a parasite of the nematode *Pratylenchus pratensis* (de.Man) Filipjev. Proceeding of the Helminthological Society of Washington 7: 51-53.

Zaki MJ, Maqbool MA (1992) Effects of *Pasteuria penetrans* and *Paecilomyces lilacinus* on the control of root-knot nematodes on brinjal and mung. Pakistan Journal of Nematology 10: 75-79.