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REGULAR ARTICLE

EFFECT OF THE TIME OF APPLICATION ON THE BIOCONTROL EFFICACY OF *PASTEURIA PENETRANS* AGAINST ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

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SUMMARY

In the present study, the effect of pre, post and simultaneous applications of *Pasteuria penetrans* to nematode *Meloidogyne incognita* was determined. Time of application of *P. penetrans* to soil was observed to have profound effect on the efficacy of the bacterium and it also affected the plant growth and multiplication of the nematode. The simultaneous application of *P. penetrans* infested soil with *M. incognita* J2 was found to be the most effective in the improving fresh and dry biomass of the chilli crop. This treatment was followed by the seven days prior application of *P. penetrans* to Nematode. The simultaneous application of *P. penetrans* with nematode treatment was found to cause maximum reduction in final population of *M. incognita*. This treatment yielded 45-53% improvement in the various parameters of the fresh and dry biomass of the crop and 71% reduction in final nematode population when compared with the control.

Key words: Biocontrol, *Pasteuria penetrans*, *Meloidogyne incognita*, Time of application

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1. Introduction

Pasteuria penetrans (Thorne, 1940), an obligate, gram positive, mycelial, endospore forming bacterium, is reported to have very good biocontrol potential against the plant parasitic nematode. Amongst the various bioagents tested against root-knot nematode (RKN) *P. penetrans* is considered to be the most promising biocontrol agent against the *Meloidogyne incognita* (Sudheer *et al.,* 2008; Shahid *et al.,* 2007; Brown and Smart, 1984; Mankau and Prasad, 1977). The specialized parasitic natures, species specificity and high degree of virulence are some of the important characters, which make *P. penetrans* a potential biocontrol agent. Biocontrol efficacy of bacterium is directly correlated with the spore attachment on the nematode cuticle (Das *et al.,* 2007; Kariuki *et al.,* 2006). Several biotic and abiotic factors like type of soil, soil pH, soil moisture, soil texture, host nematode, dose of spore inoculums, spore exposure and cropping sequence directly influence efficacy of this bacterium (Carneiro *et al*., 2007; Gomathi *et al*., 2006; Mukhtar *et al*., 2005; Gogoi and Gill, 2003; Nazir *et al*., 2002) against *M. incognita*.

Many researchers reported that the method and type of spore inoculation affect the efficacy of this bacterium but no thorough investigations about the proper timing of application have been carried out so far. Talavera and Mizukubo (2003) reported that the young age juvenile favor more spore attachment than the old age, in this situation information about the proper time of application is must. The Main objective of this study was to ascertain the most suitable timing of application of bacterium for controlling the nematode disease.

2. Materials and Methods

Establishment of nematode culture

The nematode culture was raised from single egg mass of *M. incognita*. The egg mass was picked from already maintained pot culture of the nematode and was kept for hatching in water at 28◦C. The larvae emerging from the egg mass were inoculated to plants of tomato raised in 10 cm diameter pot containing autoclaved soil. This served as mother culture and from this culture bulk multiplication of the nematode was carried out.

Raising of Chilli seedlings

For raising the nursery of chilli (*Capsicum annuum* L. var. Haripur-raipur), healthy seeds were sown in cemented pots (1.5′ X 1′ X 8″) containing autoclaved soil. Ten days after the germination of seed, 40 plants in each pot were maintained. When the seedlings were 45 days old they were transplanted in another pot for establishment of experiment.

Preparation of bacterial inoculums

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 was 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^{O}C. The initial spore attachment was 18.2 Spore/J2. This dried bacterial culture was used for the further study.

Set up of experiment

The experiment was conducted in 10 cm diameter earthen pots under net house conditions. From the developed chilli nursery, healthy plants were selected and were established in the earthen pots @ 1 plant/pot and simultaneously various treatments were imposed. *P. penetrans* infested soil (@100g *P. penetrans* infested soil/kg soil) was either applied simultaneously with nematode or 7 days prior or post to nematode application. Nematode inoculums were applied @4 juvenile *M. incognita*/g of soil. The experiment were set in RBD, various treatment those imposed were (i) first nematode than 7 days later *P. penetrans* infested soil; (ii) first *P. penetrans* infested soil than 7 days later nematode (iii) nematode and *P. penetrans* infested soil simultaneously (iv) nematode check (v) *P. penetrans* check and (vi) Absolute check. The plants were allowed to grow for 60 days after inoculation and after this, observation on various plant growth attributes and nematode multiplication were recorded.

3. Results

Time of bacterial inoculation was found to affect various plants growth character at various degrees (Table 1.). Application of *P. penetrans* prior or post to nematode or simultaneously with the nematode was found to mitigate the adverse effect of the nematode on the various plant growth characters and such an effect was more prominent in treatment receiving simultaneous inoculation of bacterium and nematode. This was followed by the prior application of *P. penetrans*. The adverse effect of nematode on fresh and dry biomass of plant was completely reversed when *P. penetrans* infested soil was applied either prior or along with the nematode and it was at par with that of the absolute check treatments. In case of dry weight of root, simultaneous application treatment was differing than the prior application and it was significantly superior over the nematode check treatments. In general, post application of the *P. penetrans* was found least effective in reducing the adverse effect of nematode and fresh and dry biomass of plant were observed to be at par with that of the nematode check treatment excepting fresh shoot weight where it was significantly higher than the nematode check treatment. Amongst different time of inoculation, simultaneous application of *P. penetrans* with nematode caused maximum improvement in various plant growth characters e.g. 49 and 52% in case of fresh and dry weight of shoot while this improvement was 45 and 53% in case of fresh and dry weight of root over the nematode control treatment (Fig. 1) and this improvement was immediately followed by the prior application of the *P. penetrans*. Similarly, with respect of post inoculation of

bacterium to nematode, this improvement was 38.8% and 30.9% higher in dry weight of shoot and root in simultaneous application of both the agents.

Fig. 1.Effect of different time of inoculation of *Pasteuria penetrans* on plant growth characters of chilli (Percent increase over nematode check)

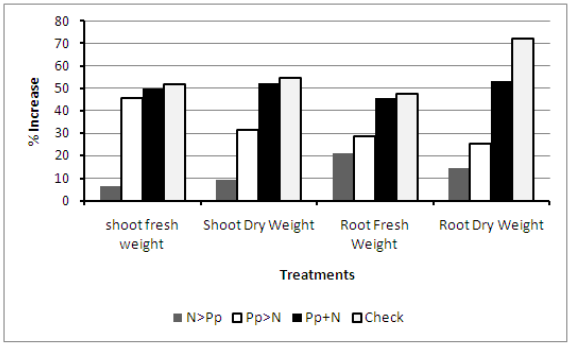
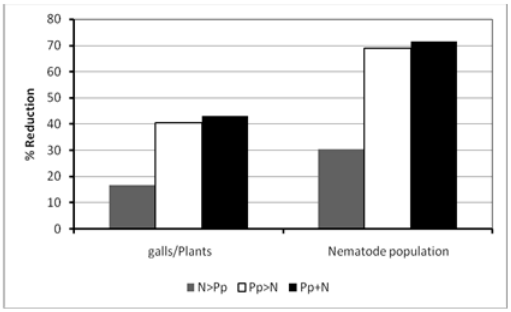


Fig. 2. Effect of different time of inoculation of *Pasteuria penetrans* on *Meloidogyne incognita* multiplication characters (Percent reduction over nematode check).



Data on the effect of sequence of application of bioagent and nematode also revealed maximum reduction in average number of gall/root system and *M. incognita* population in the treatment having simultaneous inoculation of both of them (Table 1). However, this reduction was at par with the treatment where *P. penetrans* infested soil was applied prior to nematode. Post inoculation of *P. penetrans* infested soil to nematode was also observed to significantly reduce the gall numbers as well as nematode population over the nematode control but this reduction was not at par with the two other type of inoculation. Simultaneous application of bacterium and nematode caused 43 and 71.4% reduction in gall number and *M. incognita* population over the nematode check while this reduction was only 16.87 and 30.53% in case of post application of bacteria (Fig. 2).

Table.1. Effect of different time of application of *Pasteuria penetrans* on growth characteristics of chilli and multiplication of *Meloidogyne incognita* (Mean of five replicates)

Treatments	Shoot Weight (g)		Root Weight (g)		No. of gall/ Plant	Final Nematode Population
	Fresh	Dry	Fresh	Dry		
N > Pp	08.026	1.353	3.976	0.711	148.8	38298.6 (10.54)
Pp > N	10.958	1.623	4.228	0.779	106.6	17084.0 (9.726)
Pp + N	11.276	1.879	4.782	0.952	102.0	15761.4 (9.656)
Nematode Check	07.518	1.236	3.284	0.621	179.0	55130.6 (10.913)
Pp Check	11.354	1.917	4.828	1.060	0.00	0.00
Absolute Check	11.424	1.912	4.840	1.069	0.00	0.00
CD 5%	1.50	0.56	0.68	0.12	19.47	0.232

Pp: *Pasteuria penetrans*; N: *M. incognita* J2; Figure in parenthesis are log transformed value; N>Pp: Post application of *P. penetrans*; Pp>N Prior application of *P. penetrans*; N + Pp: Simultaneous application of *P. penetrans* and J2 of *M. incognita*

4. Discussion and Conclusion

Time of application of *P. penetrans* was observed to play an important role in the efficacy of the bacterium. Application of *P. penetrans* simultaneously with nematode was observed to be the most effective in improving fresh and dry weight of plant and reducing gall number and nematode population. This was followed by the prior application of *P. penetrans* to nematode. The application of *P. penetrans* post to nematode inoculation was observed to be least effective in the all tested treatments. Simultaneous application treatment caused 45-53% improvement over the nematode check in various plant growth characters. Similarly, the same treatment caused 71% reduction in the final population of *Meloidogyne incognita* and this reduction was only 69 and 30% recorded in case of prior and post application of *P. penetrans* to nematode. Our results are in conformity with that of Nazir *et al.* (2002); Gogoi and Gill (2003) and Alves *et al.* (2004) who observed that the exposure or agitation period can affect the efficacy of the bacterium. In our study we find out that the prior and post application of *P. penetrans* represent less exposure or agitation with nematode than the simultaneous application and because of this these treatments were less effective than the simultaneous application. When we discuss about the exposure or agitation period of the prior and simultaneous application of the *P. penetrans* with juvenile of the nematode, it is almost same that's why the result of the both treatment are almost at par with each other but after that also pre application of the *P. penetrans* is slightly lesser than the simultaneous application and it is because of the seven day prior inoculation of the bacterium. Freitas *et al.* (1997) reported that 10 days storage of the *P. penetrans* at natural condition (30 -50◦C) can decrease the attachment efficacy of the *P. penetrans*. Our study also corroborated these findings and because of this only pre application of the *P. penetrans* was slightly lesser affective than the simultaneous application. Talavera and Mizukubo (2003) reported that nematode age can affect the spore attachment and biocontrol potential of the bacterium. They reported that 0-6 days old nematodes show higher rate of spore attachment than the 7-30 days old nematodes. In our study, in case of post application of the *P. penetrans,* nematodes were already 7 days old and in that situation the attachment rate of bacterial spore were less than the pre and simultaneous application of the *P. penetrans*. While in the case of pre and simultaneous application of the *P. penetrans* the age of nematode was not the key factor and that's why the efficacy of the bacterium is almost at par with each other. Kumar *et al.* (2005) reported that application of the *P. penetrans* at the time of nursery application can give better results than the after plantation and our study are in conformity with this finding.

The results of our study allow us to suggest that the time of *P. penetrans* inoculation is the major key factor in the strategy of *M. incognita* management on chilli because the efficacy of this bacterium is affected by the age of nematode and exposure or agitation time. Although there is no prior information about the proper time of application but on the behalf of related references of agitation, exposure time, nematode age and our study we can suggest that the application of *P. penetrans* at the time of transplanting or nursery development can give better control of the nematode than its prior or post to the transplanting of the crop.

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