BIO-MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON TUBEROSE USING A FORMULATION OF *POCHONIA CHLAMYDOSPORIA*

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Summary. Field experiments conducted to evaluate the efficacy of a formulation of the bio-control fungus *Pochonia chlamydosporia* Zare *et al.*, for the management of *Meloidogyne incognita* Chitw. on tuberose indicated the potential of the use of this bio-agent. Application of 100 g of the formulation per plot (m 2 x m 2) reduced the population of the nematode significantly and improved the floral characteristics in this crop.

Tuberose, *Polyanthus tuberosa* L., is commercially cultivated in open field conditions for its fragrant flowers. The loose flowers are used in garlands and floral decorations while spikes are used as cut flowers. Natural oil is extracted from the flowers for the use in the perfume industry. It is a half-hardy bulbous perennial plant that perpetuates itself through bulblets. Root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., was reported as one of the important factors affecting commercial cultivation of tuberose (Sunderbabu and Vadivelu, 1988). It was found to reduce the yield of the crop by more than 10% (Khan and Parvatha Reddy, 1992). Further, it was observed to make the plants highly susceptible to attack by *Fusarium oxysporum* f.sp. *dianthi* (Rao *et al.*, 2003). Recent surveys also indicated the widespread nature of this nematode in most of the fields in Southern India where tuberose is cultivated (Rao *et al.*, 2001). The cost of the chemicals required for the effective control of *M. incognita* in the field, and the importance of the sustainability of control methods, indicated a need to develop a reliable bio-management strategy. The material chosen for this was a formulation of the bio-control fungus *Pochonia chlamydosporia* Zare *et al.*, which has been reported as very promising by various researchers (De Leij and Kerry, 1991; Kerry *et al.*, 1993; Reddy *et al.*, 1999; Rao *et al.*, 1997).

MATERIALS AND METHODS

A local isolate of *P. chlamydosporia* (the identification of which was confirmed by Professor Brian Kerry, Rothamsted Research, United Kingdom by a molecular diagnostic test based on the Beta tubulin gene) was mass produced through liquid and solid fermentation processes. In the field experiments, a formulated product of *P. chlamydosporia*, containing 10^6 chlamydospores/gram, was applied at different dosages and a bio-control strategy for the management of *M. incognita* on tuberose under field conditions was evaluated.

The experiment was conducted at the Indian Institute of Horticultural Research farm, in a field that was infested with *M. incognita* at a mean population density of 129 ± 12 J_2 per 100 g of soil. The field was divided into twenty (2 x 2 m) plots. The soil in the experimental field was treated with a formulation of the bio-agent at dosages of 50 g, 100 g or 200 g per plot by mixing the bio-agent in to the top 10 cm of soil of the plot. All the treatments were randomly distributed and replicated five times in a randomized block design.

Ten months after planting tuberose *cv.* IIHR Prajwal, data on fresh plant weight, mean spike number/plot, mean spike length and weight, floral number/spike, root-knot index on a 1-10 scale (Bridge and Page, 1980), nematode population densities in roots and soil, root colonization by the bio-control fungus, propagule density in the soil and number of egg masses and eggs parasitized by the bio-agent were recorded.

To evaluate the colonization by *P. chlamydosporia,* the root system was carefully washed to remove soil, blotted dry, weighed and cut into small pieces of about 1 cm each. One-gram samples of roots were taken at random and root colonization by *P. chlamydosporia* was assessed by using the semi-selective medium developed by Kerry *et al.* (1993). The Petri plates were incubated at 25-27 °C for 15 days. To study egg parasitism, 20 egg masses from each replicate were randomly selected, treated with 0.05% sodium hypochlorite and the number of eggs infected counted. *Pochonia chlamydosporia* was isolated from adult females and eggs of *M. incognita* by using the semi-selective medium mentioned above. The number of nematodes in the roots was estimated by staining 5 g of root samples in acid fuchsin, homogenizing them and counting the nematodes using a microscope. The data were analyzed by ANOVA.

The experiment was repeated three times to confirm

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Table I. Effect of different dosages of a formulation of *Pochonia chlamydosporia* on plant growth parameters and the management of *Meloidogyne incognita* on tuberose under field conditions.

Treatment	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Root-knot index on 1-10 scale	Nematode population in 100 cc soil	Nematode population in 5 g root
P. chlamydosporia 50 g/plot	41.7	35.9	137.9	13.5	6.5	79	33
P. chlamydosporia 100 g/plot	48.8	44.2	150.6	14.8	4.9	55	25
P. chlamydosporia 200 g/plot	49.7	45.7	146.8	14.2	4.2	52	22
Control	35.2	30.2	112.6	11.3	8.3	127	42
C. D. 5%	7.42	6.48	22.76	2.54	1.94	24.32	7.47

Table II. Effect of different dosages of a formulation of *P. chlamydosporia* on the floral characteristics of tuberose under field conditions.

Treatment	No. spikes/plot	No. flowers/ spike	Length of spike (cm)	Weight of spike (g)
P. chlamydosporia 50 g/plot	39	31	64.3	41.2
P. chlamydosporia 100 g/plot	54	35	75.3	45.5
P. chlamydosporia 200 g/plot	56	37	78.5	46.9
Control	27	24	56.4	33.4
C. D. 5%	9.38	6.49	12.34	8.37

Table III. Effect of different dosages of a formulation of *P. chlamydosporia* on the colonization on the roots of tuberose and parasitization of eggs of *M. incognita* under field conditions.

Treatment	Root colonization by bio-agent (CFU/g root*)	Percent eggs parasitized by the bio-agent
P. chlamydosporia 50 g/plot	39,353	42.7
P. chlamydosporia 100 g/plot	47,489	56.2
P. chlamydosporia 200 g/plot	51,986	59.7
Control	0.0	0.0
C. D. 5%	6347.86	9.48

*CFU/g root = Colony forming units per gram root

the results on the efficacy of the formulation of the bio-agent on the management of root-knot nematodes on tuberose under the field conditions. Hence, the results presented in the tables are means of the values from the three experiments.

RESULTS AND DISCUSSION

The results indicated a significant reduction in the root-knot index on tuberose roots and significant increases in shoot length, shoot weight and root length following the incorporation of the formulation of *P. chlamydosporia* (Table I). Application of the bio-agent formulation also significantly improved the floral attributes of tuberose, such as the number of spikes/plot, number of flowers/spike and length of the spike (Table II).

The data on root colonization by the bio-agent and its parasitization of the nematode eggs indicate that the strain of *P. chlamydosporia* is rhizosphere-competent, as there was good colonization of the roots of tuberose and parasitization of nematode eggs 10 months after its application (Table III).

The efficacy of two of the dosages (100 g/plot and

200 g/plot) of the bio-agent formulation was similar in terms of reducing the root-knot index and the nematode population in both the soil and roots, and also in the extent of colonization of the roots (Table I, II and III). However, at these two dosages the formulation was significantly more effective in the management of root-knot nematodes on tuberose than the lower dosage of 50 g/plot (Table I).

Pochonia chlamydosporia was reported to parasitize the eggs and egg masses of root-knot nematodes (De Leij and Kerry, 1991; Kerry *et al.*,1993). However, the role of *P. chlamydosporia* in increasing the growth of tuberose needs to be investigated further, especially its precise role in plant growth promotion, the effect of its colonization on changes in the root physiology, and its possible role in inducing systemic resistance.

The results of these field experiments clearly demonstrate the potential of use of the formulation of *P. chlamydosporia* for the sustainable management of *M. incognita* on tuberose under field conditions.

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