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Research Note

Effect of different methods of application of the plant growth promoting bacterium, *Pseudomonas fluorescens* Migula on the management of *Meloidogyne graminicola* Golden and Birchfield (1965) infecting rice

B. ANITA and G. RAJENDRAN[1]
Horticultural Research Station
Vijayanagaram, Ooty 643 001, The Nilgiris, India
E-mail: anitabellie@yahoo.co.in

ABSTRACT: **Investigations were carried out under glasshouse conditions to study the effect of different methods of application of *Pseudomonas fluorescens* Migula for the management of *Meloidogyne graminicola* Golden and Birchfield infecting rice. The plant growth promoting bacterium *P. fluorescens* was applied as seed treatment, soil application and a combination of both seed and soil application. Dual application of *P. fluorescens* both as seed treatment and soil application proved to be the most effective treatment with highest reduction of 60.18 per cent in nematode population in root and soil, and enhanced growth of rice seedlings. Colonization of *P. fluorescens* was the highest in roots (36.76 x 10^8 cfu/ g) receiving the bacterium through seed treatment and soil application.**

KEY WORDS: *Meloidogyne graminicola, Pseudomonas fluorescens*, rice

The rice root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield is the most widely distributed pest of rice in the sub-tropics and tropics, and has been considered economically important (Panwar and Rao, 1988). Resistance inducing rhizobacteria offer an excellent alternative in providing a natural, effective, safe, persistent and durable protection. Rhizosphere bacteria mainly fluorescent *Pseudomonas* have been reported to be antagonistic to nematodes infecting rice (Spiegel *et al.*, 1991).

Any biocontrol agent having the ability to suppress the disease needs to be applied through a reliable established method for its consistent performance. Hence, this study was carried out with the objective of selecting a suitable method of application of *P. fluorescens* for the management of rice root-knot nematode *M. graminicola*.

Susceptible cultivar CO 43 and the sample of *M. graminicola* obtained from the culture collection were used for the study. *P. fluorescens* strain Pf1 was obtained from the culture collections of the Department of Plant Pathology, TNAU, Coimbatore. The efficacy of different methods of application of *P. fluorescens viz.*, seed treatment, soil application and a combination of both seed and soil treatment

[1] Ex-Professor & Head, Department of Nematology, TNAU, Coimbatore

for the management of *M. graminicola* was compared with a chemical treatment and an untreated control.

Pseudomonas treatment in glasshouse and experimental design

For seed treatment, the rice seeds were surface sterilized with sodium hypochlorite solution (2%) and soaked in a bacterial suspension containing 9×10^8 cfu ml^{-1}. After 24 hours the bacterial suspension was drained and the seeds were shade dried for 30 minutes. For soil application the seeds were allowed to sprout and 25 ml of bacterial suspension (9×10^8 cfu ml^{-1}) was poured per pot before sowing.

Eggs of rice root-knot nematode were extracted from root-knot nematode infected rice roots and allowed to hatch. Infective juveniles were inoculated at the rate of 200 per plant before sowing. All the treatments were replicated thrice in a randomized block design.

The experiment was terminated 30 days after planting and observations on shoot growth, root growth and nematode population in root and soil were recorded. Colonization of *P. fluorescens* in roots was also assessed. The data generated from the glasshouse experiment were subjected to statistical analysis following the standard statistical procedures (Gomez and Gomez, 1984).

Dual application of *P. fluorescens* as seed treatment @ 25 ml bacterial suspension containing 9×10^8 cfu/ ml and as soil treatment @ 25 ml bacterial suspension (9×10^8 cfu/ml) was the most effective method of application (Table 1). There was significant increase in shoot and root growth, compared to untreated control. In general all the treatments recorded significant reduction in the population of adult females and eggs in the root.

Table 1. Effect of different methods of application of *P. fluorescens* on growth of rice CV CO 43 infected with *M. graminicola* under screen house conditions

Treatment	Shoot				Root			
	Length (cm)	Per cent increase	Weight (g)	Per cent increase	Length (cm)	Per cent increase	Weight (g)	Per cent increase
1. *P. fluorescens* - seed treatment @ 25 ml bacterial suspension	22.58^{d}	116.49	0.28^{b}	154.55	9.15^{d}	27.44	0.27^{d}	80.00
2. *P. fluorescens* - soil application @ 25 ml bacterial suspension	24.58^{c}	135.67	0.30^{ab}	172.73	9.55^{c}	33.01	0.30^{c}	100.00
3. *P. fluorescens* - seed treatment + soil application @ 25 ml bacterial suspension	29.18^{a}	179.77	0.34^{a}	209.09	12.55^{a}	74.79	0.41^{a}	173.33
4. Carbofuran 3G @ 1 kg a.i./ ha	27.40^{b}	162.70	0.26^{ab}	136.36	10.93^{b}	53.73	0.36^{b}	140.00
5. Untreated control	10.43^{e}	-	0.11	-	7.18^{e}	-	0.15^{e}	-
CD (P = 0.05)	1.49	-	0.05	-	0.42	-	0.02	-

Figures followed by the same alphabets are not statistically significant.

Table 2. Effect of different methods of application of *P. fluorescens* on the population of *M. graminicola* infecting rice cv. CO 43 under screen house conditions

Treatment	No. of females/g root	Per cent decrease	Nematodes/ 100 cc soil	Per cent decrease	No. of eggs/g root	Per cent decrease	Gall index	*P. fluorescens* (x10⁸cfu /g soil)
1. *P. fluorescens* - seed treatment @ 25 ml bacterial suspension	35.75^{c}	35.29	48.25^{b}	30.82	706.00^{d}	20.56	3	16.00 (4.06)
2. *P. fluorescens* - soil application @ 25 ml bacterial suspension	27.00^{b}	51.13	32.75^{a}	53.05	458.25^{b}	48.47	2	25.75 (5.12)
3. *P. fluorescens* - seed treatment + Soil application @ 25 ml bacterial suspension	22.00^{a}	60.18	33.50^{a}	51.97	380.75^{a}	57.10	1	36.75 (6.10)
4. Carbofuran 3G @ 1 kg a.i./ha	29.25^{b}	47.06	33.75^{a}	51.61	526.00^{c}	40.82	3	0.00 (0.71)
5. Untreated control	55.25^{d}	-	69.75^{c}	-	888.75^{e}	-	5	0.0 (0.71)
CD (P=0.05)	3.93	-	4.12	-	58.00	-	-	-

Figures within parentheses indicate log-transformed values.

The highest reduction of 60.18 per cent and 57.10 per cent was observed in plants treated with *P. fluorescens* applied through seed and soil, respectively. The root gall index ranged between 1 and 3 in plants treated with *P. fluorescens*.

Soil application of *P. fluorescens* at the time of sowing resulted in significant reduction (53.03%) in soil nematode population, which was closely followed by the dual application of *P. fluorescens* of seed and soil treatment (51.97%). Both these treatments were on par with the chemical carbofuron 3G @ 1 kg a. i. ha^{-1} in reducing the nematode population in the soil. Seed treatment with *P. fluorescens* was least effective.

Observation of rice roots treated with *P. fluorescens* for root colonization revealed significantly higher number of colony forming units (36.75 x $10^{8}g^{-1}$) in plant roots applied with *P. fluorescens* as seed treatment and soil application simultaneously (Table 2). Soil application of *P. fluorescens* was also found to increase the root colonization (25.75 x 10^{8} cfu g^{-1}). The least number of colonies (16 x 10^{8}) were observed in roots, which were applied with *P. fluorescens* as seed treatment alone.

Combination of different methods of application could be more effective in disease management than a single method of application (Vidhyasekaran and Muthamilan, 1999). The combined application of *P. fluorescens* as seed treatment and soil application proved to be effective in reducing the nematode severity, though seed treatment alone or soil application alone could manage the nematode and it may be due to increased inoculum potential in inducing resistance. The inoculum level may determine the efficacy of the antagonist in controlling the nematode. Combined application of *P. fluorescens* as seed treatment and nursery soil application was more effective in reducing *H. gracilis* population in rice both in nursery and main field (Ramakrishnan and Sivakumar, 1999; Ramakrishnan *et al.*, 1998) than seed treatment alone. Seed bacterization of tomato with *P. fluorescens* improved germination and reduced *Rotylenchulus reniformis* penetration and

multiplication. However, maximum reduction of the nematode was obtained through seed inoculation with the bacterium followed by soil drench (Nikam and Dhawan, 2001).

Colonization of *P. fluorescens* was highest in the roots of seedlings receiving dual application of the bacterium both through seed treatment and soil application. Combined application of *P. fluorescens* as seed treatment and soil application was found effective in enhancing the growth of black gram. Significant reduction in cyst nematode population was observed. Root colonization by *P. fluorescens* was also significantly more in the combined treatment (Senthamizh and Rajendran, 2003).

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