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Management of root knot nematode (*Meloidogyne incognita*) race 2 in cowpea through bio-agents.

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ABSTRACT

The outcome of the experiment conducted at the Central Research Farm, Gayeshpur during 2016 and 2017 on different bioagents against root knot nematode in cowpea exposed that amendment of soil with bioagents i.e. Pseudomonas fluorescens @ 20 g m^{-2} and Purpureocillium lilacinum @ 20 g m^{-2} along with neemcake @ 100g m^{-2} has given higher cowpea yield (3.69 t ha^{-1} and 3.64 t ha^{-1}) than the solitary application of the bioagents (2.64 t ha^{-1} and 2.50 t ha^{-1}). Application of neemcake in soil restricted the nematode multiplication with higher yield of 2.82 t ha^{-1} that was obtained from solitary application of neemcake @ 100 g m^{-2}. The final soil nematode populations was significantly found less in number (133.5 and145.4 per 200 cc soil) where the bioagents were applied as treatments with neemcake. However, in terms of incremental cost benefit ratio, application of carbofuran 3G was found more effective than the bioagents along with neemcake.

***Keywords*:** Cowpea, neemcake, *Pseudomonas fluorescens*, *Purpureocillium lilacinum*, root knot nematode.

Pulses are the major source of protein in vegetarian diet of the people of India. The production of pulse crops in India unfortunately suffers from several constraints of which pest and disease are the most important ones. Cowpea (*Vigna unguiculata* (L.) Walp) is a plant belonging to the family Fabaceae under pulse group. It is also valued as the cheapest dietary and high quality vegetable protein of about 25-43per cent **(**Nielson *et al*., 1977**).** The crop is also grown for grain, fodder, vegetable and green manure purposes in India. Among pests, phytoparasitic nematodes have been recognized as one of the major constraint in pulse production (Bhatti, 1992). The extent of losses due to nematodes especially in pulse crops is yet to be estimated properly but in cowpea production is estimated to cause annual yield losses of nearly 15per cent worldwide (Sasser, 1987). According to Parvatha, 1981, *Meloidogyne incognita* Chitwood race 2 could cause 28.60 per cent losses in cowpea.

M. incognita is reported to be widely distributed in the pulse growing areas of the country and causes extensive loss in grain yield (Gupta and Verma, 1990). Role of plant parasitic nematodes in agricultural production has remained underestimated due to their soil born nature, microscopic size and hidden mode of life and non-typical visible feeding symptoms on the plants. The enormous economic damage to plants by their root feeding and interactions with other organisms renders the plants for further vulnerable to other biotic and abiotic stresses, microscopic size and hidden mode of life and non-typical visible feeding symptoms on the plants.

There are number of practices for management of plant parasitic nematodes in which chemical nematicides is used against nematodes by farmer because it is effective, easy to apply, and show rapid effects. But it may cause degradation in soil fertility, environmental pollution and also hazardous for animals and human being. That is why biological control are more promising management practice and also economic and ecofriendly. The existing recommendation such as application of granular nematicides *viz.*, carbofuran or phorate are the only short term solution to the nematode problem, as their populations increase a few months later which requiring repeated applications of pesticides with higher dosages. This finally becomes hazardous to the soil health and uneconomical. Considering the long term hazards posed by the indiscriminate use of pesticides to control the root knot nematodes, bio-agents pledge to be the next best alternative for the nematode management and with this aim, the present study was conducted to evaluate the efficacy bioagents such as *Pseudomonas fluorescens* and *Purpureocillium lilacinum* against the root knot nematode.

MATERIALS AND METHODS

The experiment was carried out in the Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur for two consecutive year during the month of May-July 2016 and 2017. The experiment was designed in Randomized Block Design using seven treatments *i.e*. T_1 = *Pseudomonas fluorescens* @ 20 g m^{-2} (CFU 2 x 10^6)**,** T_2 = *Purpureocillium lilacinum* 20 g m^{-2} (CFU 2 x 10^6), T_3 = neem cake @100 g m^{-2}, T_4 = *P. fluorescens* 20 g m^{-2} + neem cake @100 g m^{-2} , T_5 = *P. lilacinum* @ 20 g m^{-2} + Neem cake @100g m^{-2} , T_6 = Carbofuran 3G @ 10 g m^{-2} (Standard check),

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T_7 = Untreated control with three replications. This present study was conducted in a sick plot infested with *M. incognita* race 2. The leading cowpea variety Kashi Kanchan was selected for experimental purpose. Soil from the experimental field was collected before land preparation and Initial nematode population (J_2) of root knot nematode (268/200 cc soil in 2016 and 416/200 cc soil in 2017) was recorded in AICRP Nematodes laboratory. The picking of pod from each plot was done separately at regular interval. At final harvest, plants from each plot were carefully uprooted to observe root galling and indexed using 1-5 scale where 0 = 0% galled roots; 1 = <10%, 2 = 10 - 25 %, 3 = 25 - 50%, 4 = 50 - 75% and 5 = >75% galled roots (Imelda *et al*., 2000). Soil samples were again collected at final harvest from each plot separately and processed by Cobb's sieving and decanting technique (Cobb, 1918) followed by modified Baermann's funnel technique (Christie, 1951) for the estimation of nematode populations in soil. At final harvest soil from bioagents treated plots were also collected to count the CFU g^{-1} of soil. To assess the root colonization of introduced bio-agents, one gram of soil from adjoining root was taken at random from each plant, serial dilutions were made in sterile distilled water and plating was done on Kings B medium (King *et al.*, 1954) for *P. fluorescens*, on potato dextrose agar medium for *P. lilacinum.* After incubation at 27±1°C for 7 days, *P. fluorescens* colonies emitting a pale green fluorescent light under UV at 302 nm, while, *P. lilacinus* colonies changing from white to lilac colour. The collected data were subjected to analysis of variance (ANOVA) and treatment means were separated using standard error of means (SEM) at 5 % level of probability. The suitable data transformation was done as and when necessary.

RESULTS AND DISCUSSION

The results of the field trials conducted during 2016 and 2017 revealed that all the treatments were significantly superior over untreated control to increase the yield. During 2016 mean number of final nematode populations in root after harvest was ranged from 12.03 to 47.40 per 5 g of roots. However, there was no significant difference among the treatments and control. The final soil nematode populations were ranged from 115 to 381.33 mean numbers of nematodes per 200 cc of soil and there was significant difference observed among the treatments and control. The lowest soil nematode populations (115 per 200 cc of soil) were also found in T_4 *i.e*. soil treated with *P. fluorescens* @ 20 g m^{-2} with Neem cake @100 g m^{-2} and was significantly superior over other treatments. However, this treatment was found statistically on par with that of T_5 where *P. lilacinum* 20 g m^{-2} along with Neem cake @100g m^{-2} was applied in soil. The root knot index (RKI) was ranged from 2.2 to 3.8 but there was no significant difference among the treatments. Highest yield of 3.17 t ha^{-1} was obtained from T_4 followed by T_5 but when the incremental cost benefit ratio was calculate the highest return was obtained from T_6 *i.e.* application of Carbofuran 3G @ 10 g m^{-2}.

During 2017, it was recorded that the treatment T_4 was superior over the other treatments and control to reduce the soil nematode populations. However, this treatment was found statistically on par with that of T_5 where *Purpureocillium lilacinum* 20 g m^{-2} along with Neem cake @100g m^{-2} was applied in soil. No significant difference was observed among the treatments and control in relation to root nematode populations. The root knot index was ranged from 2.2 to 3.1. Minimum root knot index was observed in T_4 treated plots. No significant difference was found among the treatments but significant difference was observed with the control. Significantly highest yield was obtained from T_4 treatment and it was statistically on par with T_5 and T_6. Like the previous year highest incremental cost benefit ratio was reported from T_6 (4.18) followed by T_4 (1.20) and T_5 (1.16).

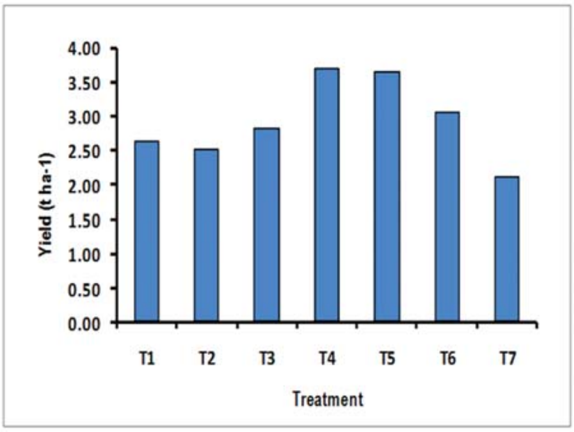

Fig. 1: Effect of different bioagents against root knot nematode (*M. incognita*) on cowpea yield.

The mean data of two years comprehensively revealed that amendment of soil with *P. fluorescens* 20 g m^{-2} along with Neem cake @100 g m^{-2} could effectively control the root knot nematode populations in cowpea. The next best treatment was *P. lilacinum* @ 20 g m^{-2} along with Neem cake @100g m^{-2}. The mean cfu for both the treatments was recorded 3.21 x10^6 and 2.78 x10^5 per g of soil while cfu recorded from sole applied plots of *P. fluorescens* (T_1) and *P. lilacinum* (T_2) were recorded with 2.74 x10^6 and 2.11 x10^5. Highest yield of cowpea was recorded from T_4 (3.69 t ha^{-1}) treatment followed by T_5 and T_6. However, in terms of incremental cost benefit ratio the treatment T6 was found superior to T_4. The result of present study was in conformity with Khan

Table 1: Comparative efficacy of different bioagents against population of root knot nematode (*M. incognita*) in cowpea.

Treatment	FNP 200 cc^{-1} (Soil)			FNP 5 g^{-1} (root)			RKI (0-5 scale)		
	2016	**2017**	**mean**	**2016**	**2017**	**mean**	**2016**	**2017**	**Mean**
T_1	167.0 (12.9)	203.3 (14.6)	185.2 (13.6)	39.0 (6.3)	38.0 (5.4)	38.5 (6.1)	2.6	2.7	2.7
T_2	176.5 (13.3)	237.3 (15.4)	206.9 (14.4)	47.4 (6.8)	36.7 (4.2)	42.1 (6.4)	3.1	2.8	2.9
T_3	220.5 (14.9)	211.7 (14.6)	216.1 (14.8)	13.4 (3.4)	50.7 (6.1)	32.0 (4.9)	3.5	2.9	3.2
T_4	115.0 (10.8)	152.0 (12.4)	133.5 (11.6)	22.3 (4.1)	34.7 (4.1)	28.5 (5.1)	2.2	2.2	2.2
T_5	123.7 (11.2)	167.0 (12.9)	145.4 (12.1)	17.9 (3.8)	36.0 (5.3)	27.0 (5.2)	2.2	2.7	2.4
T_6	165.7 (12.9)	173.3 (13.2)	169.5 (13.0)	12.0 (3.2)	38.7 (4.3)	25.4 (4.2)	3.0	2.5	2.7
T_7	381.3 (19.6)	286.0 (16.9)	333.7 (18.3)	18.2 (4.3)	97.7 (9.3)	57.9 (7.6)	3.8	3.1	3.5
SEm(±)	**0.41**	**0.95**	**0.53**	**1.40**	**3.80**	**1.76**	**0.13**	**0.71**	**0.51**
LSD (0.05)	**0.91**	**2.09**	**1.17**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Note: Figure in parentheses indicate Ö(x+0.5) transformed values, NS= Not significant.

Table 2: Effect of different bioagents against root knot nematode (*M. incognita*) on cowpea.

Treatment	CFU g^{-1} soil at harvest			Yield (t ha^{-1})			ICBR		
	2016	**2017**	**Mean**	**2016**	**2017**	**Mean**	**2016**	**2017**	**Mean**
T_1	3.33×10^{6}	2.15×10^{6}	2.74×10^{6}	2.30	2.97	2.64	0.84	0.95	0.90
T_2	2.66×10^{5}	1.55×10^{5}	2.11×10^{5}	2.17	2.83	2.50	0.63	0.71	0.67
T_3	-	-	-	2.28	3.36	2.82	0.53	1.03	0.78
T_4	2.66×10^{6}	3.75×10^{6}	3.21×10^{6}	3.17	4.21	3.69	0.91	1.20	1.06
T_5	2.33×10^{5}	3.22×10^{5}	2.78×10^{5}	3.13	4.14	3.64	0.89	1.16	1.03
T_6	-	-	-	2.48	3.64	3.06	2.33	4.18	3.26
T_7	-	-	-	1.79	2.40	2.10	-	-	-
SEm(±)				**0.22**	**0.28**				
LSD (0.05)				**0.48**	**0.61**				

Note – ICBR means Incremental Cost Benefit Ratio

and Rathi (2001) and Kumar and Khanna (2008) who has reported that neem cake could effectively reduce populations of *M. incognita* and better plant status in tomato. Rajvanshi and Bishnoi (2012) viewed that the neem based formulations were found effective in reducing the populations of root knot nematodes with increased grain yield and better plant growth response. According to them it may be due to the fact that besides having nematicidal potential, might have increased the tolerance level of plant and develop potential to resist the nematode attack. *Konsam et al.*, 2013 has also reported that application of jatropha cake, neem cake and castor cake could enhance growth and yield of cucumber by reducing nematode population when applied @ 30g pit^{-1} at 10 days prior to sowing. The efficacy of *P. lilacinus* as an egg parasite of *M. incognita* has been reported by Jonathan *et al*. (2000). In confirmity to the present study, Nama and Sharma (2017) also reported that *P. flurescens* could increase the plant growth by reducing nematode reproduction in cowpea.

The experiment of two successive year (2016 and 2017) could be concluded that application of *P. fluorescens* @ 20 g m^{-2} + neem cake @100 g m^{-2} increased the cowpea production by reducing nematode population. It was also viewed that neem cake is having some property that could enhance the bacteria and fungus to act more effectively and these strategies could be included in integrated nematode management practices (INM) of cowpea.

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