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Evaluation of components of IPM for basmati rice against rice root-knot nematode, *Meloidogyne graminicola*

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ABSTRACT

The IPM module for basmati rice, synthesised and validated by ICAR-NCIPM on a large-scale in farmers' participatory mode during 2010-2014 at Bambawad (Gautam Budh Nagar, UP) area, mainly involved green manuring, seed treatment with carbendazim or *Trichoderma harzianum*, seedling root dip in *Pseudomonas fluorescens*, pest monitoring and need-based application of bio-agents/chemical pesticides. During the recent survey, *Meloidogyne graminicola* was identified as a serious pest, however, it was not recorded above threshold levels in villages where IPM was implemented. With this view, repeated *In vitro* and *In vivo* trials were conducted to evaluate the role of individual IPM component against *M. graminicola* infesting rice cultivar PB 1121 during 2016-2018 at NCIPM-Rajpur Khurd campus. Highest mortality of *M. graminicola* juveniles was observed in carbendazim treatment (93%) followed by *T. harzianum* + *P. fluorescens* (75%). However, revival was recorded lowest in the latter. Results of *In vivo* studies indicated carbofuran as the most effective treatment in reducing the multiplication of *M. graminicola* as compared to other treatments. Among the bio-pesticides, *T. harzianum* significantly reduced number of galls per plant as well as soil population. *Sesbania* (chopped plant) extract significantly ($P<0.05$) enhanced biomass of rice plant up to 68% followed by *T. harzianum* (56%). *Sesbania* extract also enabled plant to tolerate higher number of *M. graminicola* galls with a reduction of 37.5% in nematode population as compared to control. Study suggests that incorporation of IPM components over the years not only improve the soil health and fertility but also helps to manage the root-knot nematode, *M. graminicola*.

Key words: Basmati rice, Bio-agents, Integrated Pest Management (IPM), *Meloidogyne graminicola, Sesbania*

Rice root-knot nematode, *Meloidogyne graminicola,* a sedentary endo-parasite of rice roots, is a major species affecting rice production and is an emerging threat to rice cultivation in various rice growing regions of India (Gour and Pankaj 2010). It is a serious problem in the nurseries and from there spread to main fields. During the survey conducted in and around Bambawad (Gautam Budh Nagar) in 2016, rice root-knot nematode, *M. graminicola* was recorded in traces or at low level in fields under IPM practices whereas in fields where IPM practices were not imposed, the pest survived and multiplied well. The population of rice root-knot nematode was recorded 38.3, 35.5 and 37.4 in IPM as against 114.9, 213 and 180 per 200 g soil in non-IPM (farmer's practice) at pre-transplanting, mid-season and harvesting time respectively. It appeared that components of IPM practices especially the antagonistic fungi or bacteria used in IPM module, might have played important role in containing the nematode population. Presently, field application of biological control agents (*Trichoderma harzianum* and *Pseudomonas fluorescens*) is emerging as a promising alternative strategy in the management of root-knot nematodes. Biological control of plant parasitic nematodes with antagonistic micro-organisms is a promising technique which has already been incorporated in integrated nematode management (Singh and Mathur 2010b, Singh and Singh 2012, Singh 2013) infesting agricultural crops.

The objectives of the present investigation is to find out the role of different IPM components, under *In vitro* and *In vivo* conditions, on basmati rice plant health and root-knot nematode, *M. graminicola* population in soil and plant roots.

MATERIALS AND METHODS

The investigations were conducted at the ICAR–National Research Centre for Integrated Pest Management (ICAR-NCIPM), Rajpur Khurd Campus (28º 38′ N; 77º 08′ E), New Delhi in two crop seasons (2016–17 and 2017-18).

Maintenance of nematode culture: Rice knot-knot nematode, *M. graminicola* was obtained from fields surveyed at Bambawad area (Gautam Budh Nagar, UP) and

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maintained on earthen pots (content was autoclave-killed after investigation). Infested soil and roots were processed using Cobb's sieving and decanting method followed by Baermann funnels to obtain nematode inoculum. After 24 h, the juveniles (J2) were collected, counted and exposed for bioassay test against antagonistic fungi (*T. harzianum* and *T. viride*), bacteria (*P. fluorescens*), *Sesbania* crude extract and carbendazim aqueous solution with controls.

Fungal and bacterial culture: Isolates of bio-agents i.e. *T. harzianum*, *T. viride* and *P. fluorescens* at NCIPM were used in the present investigation. *T. harzianum* and *T. viride* were cultured in autoclaved 250 ml flasks containing 100 ml Potato dextrose (PD) broth medium along with streptomycin at 1 ml/l to prevent bacterial growth at room temperature. After two weeks of inoculation the culture broth was filtered to obtain fungal filtrates (standard extract) which were used in experiments. Five days old culture of *P. fluorescens* maintained in King's broth media was used in the experiments.

Experimentation

In vitro: In vitro studies included seven treatments with four replications in randomised block design (RBD). The test was conducted in sterile cavity blocks filled with 2 ml of each testing material i.e. fungal culture filtrate, bacterial suspension, carbendazim solution (2.0g/l (w/v) sterile water), crude *Sesbania* extract and water as control. In each cavity block 1 ml of nematode suspension containing 100 juveniles (J2) of *M. graminicola* was inoculated and the observations were recorded after 24, 48, 72 and 96 h. Toxicity was estimated according to the percentage of paralysed nematodes which was determined after revival test for 24 h.

In vivo: In vivo studies comprised of eight treatments each replicated six times laid out in RBD. *Sesbania* plants attaining a height of approximately one meter were chopped and mixed well with soil 15 days before transplanting. The nematodes were inoculated at 1.3 J2/g soil. The studies were conducted for two crop seasons (2016-17 and 2017-18). The observations were recorded on plant height, bio-mass, root proliferation, number of galls/plant and nematode population in soil. Seeds of rice cv. PB 1121 were soaked overnight in water before sowing. In case of seed treatment carbendazim was added in water for overnight soaking at 2.0 g/kg seed. Twenty five-day old seedlings of rice were transplanted in plastic pots containing 0.6 kg of sterilized soil (sandy loam). All standard agronomic practices were followed to obtained healthy nursery and plants (Scientific cultivation of basmati rice – Agriculture Department Uttar Pradesh).

Statistical analysis: The data for both the experiments were analysed and subjected to Analysis of Variance. A test of homogeneity of variances for *In vitro* trials was conducted prior to pooling the data. The original data was subjected to angular transformation to normalize the distribution. The original *In vivo* trials data was subjected to square root transformation to normalize the distribution. Pooled data analysis was conducted using SPSS 16.0 and the differences between treatments were determined by Duncan's Multiple Range Test at 5% significance level.

RESULTS AND DISCUSSION

Results of *In vitro* studies (Table 1) indicated highest mortality of *M. graminicola* juveniles in carbendazim treatment followed by *T. harzianum* + *P. fluorescens* whereas revival of juveniles was recorded lowest in *T. harzianum* + *P. fluorescens* followed by control. Though the chemical treatment remained most effective but the bio-agents have also proved effective in inducing mortality. The information on effect of carbendazim on mortality of root-knot nematode, *M. graminicola* was scanty in the literature. The mortality of tested nematode with carbendazim may be due to direct exposure to chemical which is a broad spectrum benzimidazole and a metabolite of benzoyl. In the present study toxicity due to carbendazim resulting 93% mortality after 96 h and revived only 1.5% after 24 h when kept in sterile water. In confirmation of present findings, significant paralysis and mortality effect of *P. fluorescens* culture filtrates on *M. graminicola* juveniles has been reported by Seenivasan *et al.* 2012.

Results of *In vivo* studies indicated the higher efficacy of carbofuran in reducing the number of galls/plant and population of *M. graminicola* in soil as compared to other treatments (Table 2). In the treatment where seed of basmati rice cv. PB 1121 was treated with carbendazim has also effectively reduced number of galls but failed to check nematode population in soil. This could be due to the presence of carbendazim on the rootlets at initial stage which prevented the entry of *M. graminicola* juveniles in seedlings as nematode completes its life cycle in 21 days on scented rice (Kumar 2014). Later on, the effect of carbendazim got reduced and thereby failed to stop the penetration and multiplication of *M. graminicola*. The eggs hatched into larvae and emerged well in the soil causing higher number of *M. graminicola* juveniles in the soil. Moser *et al.* 2004 while studying the efficacy of carbendazim on nematodes, indicated that overall abundance of nematode was not affected by the chemical under field validation studies.

Among the bio-pesticides, soil application of *T. harzianum* treatment significantly reduced number of galls/plant and nematode population in soil followed by soil application by *T. viride* and dip of seedlings in suspension of *P. fluorescens*. Soil population of nematodes in extract of *Sesbania* treatment was recorded lower with higher number of galls/plant as comparison to seed treatment with carbendazim where number of galls/plant was recorded lower and soil population was higher. Minimum gall index and reproductive factor was recorded in *M. graminicola* + carbofuran followed by *M. graminicola* + *T. harzianum* (SA). *Trichoderma,* as a potential bio-agent reduced galling severity of *M. graminicola* in rice up to 38% with an economic impact on rice production, has been reported by Houng *et al.* (2009).

Studies conducted on rice plant health in different

Table 1 Mortality and revival of Meloidogyne graminicola (J2) after 24, 48, 72 and 96 h of exposure to various IPM components (*In vitro*)

Treatment	% mortality after various time intervals					% revival after 24 h
	24 h	48 h	72 h	96 h	Av.	
Control (Water) + *M. graminicola*)	2.3* (7.4±2.6)d	8.0 (16.4±0.4)e	11.3 (19.5±1.0)e	12.0 (20.2±0.9)e	8.4	0.0 (1.0±0.0)e
T. harzianum (Th) + *M. graminicola*	25.0 (30.0±1.1)a	42.3 (40.5±0.8)b	55.3 (47.1±0.9)c	64.5 (53.5±2.4)c	46.8	4.0 (11.0±2.1)b
T. viride (Th) + *M. graminicola*	16.8 (24.1±1.0)b	33.3 (35.2±0.5)c	43.3 (41.1±0.5)d	53.3 (46.9±0.5)d	36.6	10.8 (19.1±0.8)a
P. fluorescens (Pf) + *M. graminicola*	24.5 (29.6±1.1)a	43.0 (41.0±0.8)b	61.3 (51.2±1.9)b	64.5 (53.4±1.1)c	48.3	2.8 (9.2±1.5)bc
Th + Pf (Dual appl) + *M. graminicola*	29.5 (32.9±0.6)a	45.3 (42.3±1.3)b	64.8 (53.6±1.1)b	75.5 (60.7±3.5)b	53.8	0.0 (1.0±0.0)e
Sesbania + *M. graminicola*	7.5 (15.9±0.7)c	11.8 (20.0±0.8)d	14.5 (22.3±0.9)e	17.0 (24.3±0.7)e	12.7	0.8 (3.5±2.1)de
Carbendazim + *M. graminicola*	24.5 (29.6±1.2)a	61.5 (51.7±1.2)a	84.0 (66.6±1.9)a	93.0 (75.3±2.7a	65.8	1.5 (6.0±2.2)cd
LSD (P< 0.05)	4.3	2.6	3.7	5.5		3.9

(Mean in each column with different letters differ significantly (P ≥0.05); *original value; **values in parentheses are angular transformed value ± standard Error)

Table 2 Effects of individual components of IPM module on plant health and multiplication on basmati rice (cv. PB 1121) under *In vivo* condition

Treatment	Plant health		Meloidogyne graminicola multiplication			
	Height (cm)	Biomass (g)	Number of galls/ plant	G.I	Soil population/600g soil	R.F (Pf/Pi)
Uninoculated Check	89.8# (9.5±0.1)*a {+74.8}**	34.0(5.9±0.2)b {+58.8}	1.0 (1.0±0.0) {0.0}	0	0.0 (1.0±0.0)g {0.0}	0
Meloidogyne graminicola (Mg) alone	22.7 (4.7±0.5)c {0.0}	14.0 (3.8±0.2)f {0.0}	89.2 (9.5±0.3)e {0.0}	4	875.0 (29.6±0.4)a {0.0}	1.1
Mg + *Trichoderma harzianum* (soil application / SA)	99.3 (10.0±0.1)a {+77.2}	32.0 (5.6±0.2)c {+56.3}	44.3 (6.7±0.3)b {-50.3}	2	378.3 (19.5±0.2)e {-56.7}	0.5
Mg + *Trichoderma viride* (SA)	96.3 (9.9±0.1)a {+76.5}	26.2 (5.1±0.2)d {+46.5}	68.2 (8.5±0.2)d {-23.5}	3	444.0 (21.1±0.2)d {-49.2}	0.6
Mg + *Pseudomonas fluorescens* (seedling dip)	99.7 (10.0±0.0)a {+77.3}	30.5 (5.6±0.2)c {+54.1}	59.0 (7.7±0.4cd {-33.9}	3	473.7 (21.8±0.4)d {-45.8}	0.6
Mg + Carbofuran (SA)	74.2 (8.6±0.6)b {+69.4}	31.5 (5.7±0.2)c {+55.6}	16.8 (4.1±0.4)a {-81.2}	1	204.2 (14.3±0.4)f {-76.7}	0.3
Mg+Sesbania (chopped plant) (SA)	99.5 (10.0±0.1)a {+77.2}	44.3 (6.7±0.2)a {+68.4}	99.0 (10.0±0.2)e {+11.0}	4	547.0 (23.3±0.2)c {-37.5}	0.7
Mg + Carbendazim (seed treatment)	91.8 (9.6±0.1)a {+75.3}	24.3 (5.0±0.2)e {+42.5}	49.0 (7.0±0.3)bc {-45.1}	2	730.0 (27.0±0.2)b {-16.5}	0.9
LSD (P< 0.05)	0.8	0.1	0.3		1.2	

Mean in each column with different letters differ significantly (P ≥0.05); # data presented in Table are pooled data of two consecutive trials; Mg- Meloidogyne graminicola; G.I. – gall index; R.F. - reproductive factor; Figures presented in parentheses ( ) are square root transformed value ± standard error; figures presented in parentheses { } are percentage over respective control; (+) and (-) denotes increase or decrease over their respective control treatments indicated lowest plant height and biomass in *M. graminicola* alone as compared to other treatments (Table 2). Highest biomass was recorded in *Sesbania* extract treated plants followed by un-inoculated check and *M. graminicola* + *T. harzianum* (SA). Comparison of root proliferation among all the eight treatments indicated higher root growth in *M. graminicola* with *Sesbania* (chooped plant) (SA) and bio-agents. Green manure crops not only improve the soil health and fertility but also helps to manage the agriculture pests (Kumar *et al.* 2014) by promoting bio-agents against nematodes (Agbenin 2011). Incorporation of green manuring (*Sesbania*) over the years before transplanting of rice also helps in improving DTPA-extractable micro-nutrient cations of the soil (Nayyar and Chhibba 2000). Organic matter serves as primary source of nutrients for bio-agents and nematode as secondary source. In the present study, although the *Sesbania* was not found very effective in reducing the root galls but caused 37.5% reduction in nematode population in soil, and significantly enhanced the plant height as well as biomass.

It is evident from the present study that among various bio-agents tested, the highest mortality of nematode with no revival (Table 1) and maximum plant growth (Table 2) was observed in combined application of *T. harzianum* + *P. fluorescens*. However, the study conducted by Gangwar *et al.* (2013) on growth and survival of *T. harzianum* and *P. fluorescens* in irrigated rice ecosystem reported lesser population in their combined application than the individual treatment. Keeping in view the anaerobic conditions prevailing in irrigated rice, in the present rice IPM module, only *P. fluorescens* was recommended for seedling root dipping (Tanwar *et al.* 2016). Prior to parasitism and predation, introduced micro-organisms must be able to grow and colonize the soil and/or rhizosphere, which require energy provided by organic matter. In the present basmati rice IPM module, it is assumed that *Sesbania* might have provided necessary nutrients/energy to bio-agent for their survival and proliferation. Organic matter produced by *Sesbania* would be acting as substrate for multiplication of bio-agents. Need for a combination of different management tactics has already been advocated for management of root-knot nematode on a longer term basis on various economic important crops (Singh and Mathur 2010b, Singh and Singh 2012, Singh 2013) and the same has been reflected in ICAR-NCIPM module implemented in basmati rice (Tanwar *et al.* 2016).

It is concluded from the present study that components of IPM for basmati rice has significant antagonistic impact on rice root-knot nematode, *M. graminicola* multiplication and reproduction individually as well as together and helps to proliferate the root system and enhance the plant health as well as biomass. It is also concluded that incorporation of IPM components over the years not only improve the soil health and fertility but also helps to manage the root-knot nematode, *M. graminicola*.

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