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An Eco-friendly Management Option Against Reniform Nematode, *Rotylenchulus reniformis* infecting Castor (*Ricinus communis* L.)

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ABSTRACT: Castor is an important non-edible oilseed crop and plays a vital role in Indian economy. Castor is affected by several biotic and abiotic factors. Among plant parasitic nematode, reniform nematode (*Rotylenchulus reniformis*) is the most widely distributed and the destructive nematode. It is considered as one of the major limiting factors in the production of castor. Owing to adverse impacts of chemicals on human health, there is need to evolve ecofriendly methods of nematode management. Biological nematode control in relation to crop production system is a subject of considerable current interest because of a perceived urgency to develop and adopt safe, economic and efficient method for managing nematode pest of crops. During present investigation, Farm Yard Manure (FYM) enriched with bio-agents *viz., Glomus fasciculatum, Trichoderma harzianum* and *Beauveria bassiana* were tested as soil treatment at 2.5, 5 and 10 g/plant for the management of *R. reniformis* on castor. Results showed that *T. harzianum* at 10 g/plant was found most effective followed by *Glomus fasciculatum* at 10 g/plant and *Beauveria bassiana* at 10 g/plant which significantly enhanced the plant growth parameters of castor and also reduced the nematode multiplication factors. Bioagents *T.harzianum* reduce the density of reniform nematode (31.16-66.18%) and enhance the plant growth (30.23-94.70%).

Keywords: *Glomus fasciculatum,* Bio-agents, Castor, *Rotylenchulus reniformis, Trichoderma harzianum, Beauveria bassiana* and FYM.

INTRODUCTION

Castor (*Ricinus communis* L.) is a widely cultivated crop belongs to family Euphorbiacae is an important oilseed crop. The major countries growing castor are India, China, Brazil and Thailand. India ranks first in the world in respect of area and production (Anonymous, 2021-22). The major castor growing states in the country are Gujarat, Rajasthan Andhra Pradesh, Tamil Nadu, Orissa, Uttar Pradesh, Maharashtra, Madhya Pradesh and Bihar. Castor is an important crop having multifarious industrial applications. Castor seed oil and its derivatives are used in manufacturing of several industrial products including paints, coatings, inks and lubricants (Ogunniyi, 2006). Recently castor is prove good candidate for bio diesel production owing to its ability to grow as annual crop in marginal soils and shorter growing duration compared to other non-edible oil seeds like Jatropha (Shrirame *et al.,* 2011).

In India, castor is cultivated in an area of 0.75 million hectares, which accounts for 65% of the world's castor acreage. India produces 1.2 million tonnes of castor seed per annum which contributes to more than 85% of the world's castor production. Castor is known to be infected by many nematode species among which, reniform nematode (*Rotylenchulus reniformis*) is considered as an economically important pest (Seshadri and Shivakumar 1963; Ram and Baheti 2003; Nagori *et al*., 2023). *Rotylenchulus reniformis* is an obligate, sedentary semi-endo parasite. The pre mature females penetrate the host root system where they establish a feeding site. It has a wide host range affecting more than 300 plant species including important crops like castor, cotton, cowpea, grapes, papaya etc. Symptoms are not specific and in general not very apparent. Dieback, stunting and growth reduction have been reported on agri-horticultural crops heavily infested with reniform nematodes (Verma and Prasad 1969; Ram and Baheti 2003). In addition, leaf shedding, early flowering, malformed and discoloured seeds, decreased yield and inferior quality of oil have also been reported as the consequences of reniform nematode infestation in castor (Sivakumar and Seshadri 1971). The estimated yield loss due to reniform nematode was 13.9% (Jain *et al*., 2007).

MATERIALS AND METHODS

Castor have been affected by several pest and pathogens including phyto-nematodes in India and abroad. Among plant parasitic nematodes, reniform nematode, *R. reniformis* is considered to be the most important nematode pest of castor and causes severe losses in India due to favourable soil and environmental conditions. Therefore, in this experiment eco-friendly management trial was carried out against reniform nematode on Castor, FYM enriched with bio- agents *viz., Glomus fasciculatum*, *Trichoderma harzianum* and *Beauveria bassiana* (5 kg bio-agents in 100 kg FYM) were used at three different doses *i.e.* 2.5g/plant, 5 g/plant and 10 g/plant along with an untreated check. All the treatments were replicated three times in completely randomized design (C.R.D.). Earthen pots were washed, cleaned and disinfected them through 4% formalin solution. Then pots were filled with 1 kg infested soil having an initial inoculum of 540 larvae / 200 cc soil with test nematode. Three Castor seeds (cv.GCH-4) were sown in each pot. After 10 days of germination, one healthy plant in each pot was maintained and others were uprooted carefully.

Plants were harvested after 60 days of germination. Observation on shoot length (cm), shoot weight (g), root length (cm), and root weight (g) were taken at harvest. The root were washed carefully under tap water and stained with 0.1 per cent acid fuschin lactophenol at 80°C and after wash kept in clear lactophenol for 24 hrs. Thereafter, the roots were examined thoroughly under a stereoscopic binocular microscope for counting number of females and egg masses per plant. Soil of each pot was thoroughly mixed and 200 cc soil from each pot were taken and processed by Cobb's Sieving and Decanting Technique (Cobb, 1918) followed by Baermann's funnel technique (Christie and Perry, 1951) for estimation of soil nematode population /200 cc soil. The data were statistically analysed for interpretation of experimental findings.

RESULTS

A. Plant Growth Parameters

1. Shoot length: Results showed that FYM enriched with bio-agents found significantly superior over untreated check. Among treatments, maximum shoot length was recorded 37.06 cm with FYM enriched with *T. harzianum* 10 g per plant followed by *Glomus fasciculatum* (35.10 cm) and *B. bassiana* (33.36 cm) at same dose. *Beauveria bassiana* when applied at 2.5 g per plant (24.03 cm) was found to be least effective but significantly superior over untreated check (20.46 cm).

Experimental findings indicated that FYM enriched with *T. harzianum* 10 g per plant showed maximum increase in shoot length (81.13%) followed by *Glomus fasciculatum* at 10 g per plant (71.55%) and *B. bassiana* at 10 g per plant (63.04%) over untreated check. Minimum increase in shoot length was obtained with *B. bassiana* at 2.5 g per plant (17.44%).

2. Root length: Experimental findings indicated that FYM enriched with *T. harzianum* 10 g per plant showed maximum increase in shoot length (81.13%) followed by *Glomus fasciculatum* at 10 g per plant (71.55%) and *B. bassiana* at 10 g per plant (63.04%) over untreated check. Minimum increase in shoot length was obtained with *B. bassiana* at 2.5 g per plant (17.44%).

Results illustrated that maximum increase in root length was estimated by with FYM enriched with *T. harzianum* 10 g per plant (91.19%) subsequently by *Glomus fasciculatum* at 10 g per plant (71.36%) and *B. bassiana* at 10 g per plant (59.06%). Minimum increase in root length 7.74% was obtained with *B. bassiana* at 2.5 g per plant as compared to untreated check.

3. Shoot weight: Results exhibited that all the treatments of FYM enriched with bio-agents significantly increased shoot weight of castor over untreated check. Among various treatments, maximum shoot weight was observed 18.03 g with FYM enriched with *T. harzianum* 10 g per plant followed by *Glomus fasciculatum* at 10 g per plant(16.50 g) and *B. bassiana* at 10 g per plant (14.53 g). *Beauveria bassiana* at 2.5 g per plant (11.00 g) was found to be least effective and significantly superior over untreated check (9.26 g).

Maximum increase (94.70%) in shoot weight was observed when FYM enriched with *T. harzianum* 10 g per plant followed by *Glomus fasciculatum* at 10 g per plant (78.18%) and *B. bassiana* at 10 g per plant (56.91%). However, minimum shoot weight was obtained (18.79%) with *B. bassiana* at 2.5 g per plant over untreated check (Fig- 1).

4. Root weight: Data presented in Table-1 clearly showed that FYM enriched with bio-agents significantly increased root weight as compared to untreated check. Among various treatments, maximum root weight was recorded with *T. harzianum* 10 g per plant (9.03 g) followed by *Glomus fasciculatum* (8.30 g) and *B. bassiana* at 10 g per plant (7.63 g). These treatments found significantly better over untreated check (4.50 g). *Beauveria bassiana* applied at 2.5 g per plant (4.80 g) was found least effective with regards to root weight.

Experimental findings showed maximum increase in root weight with *T. harzianum* 10 g per plant (100.66) followed by *Glomus fasciculatum* at 10 g per plant (84.44%) and *B. bassiana* at 10 g per plant (69.55%). Minimum increase in root weight (6.66%) was obtained with *B. bassiana* at 2.5 g per plant over untreated check.

A. Nematode Reproduction Parameters

1. Number of females per plant: Results indicated (Table 1) that all the treatments significantly reduced the number of females per plant over control. Among various treatments, lowest numbers of females per plant was found with FYM enriched with *T. harzianum* 10 g per plant (18.33) followed by *Glomus fasciculatum* at 10 g per plant (21.66) and *B. bassiana* at 10 g per plant (26.00) over untreated check (54.00). These treatments significantly changed number of females per plant from each other. Highest numbers of females per plant was obtained with *Beauveria bassiana* applied at 2.5 g per plant (41.00).

Results showed maximum reduction in number of females per plant by FYM enriched with *T. harzianum* 10 g per plant (66.05%) followed by *Glomus fasciculatum* at 10 g per plant (59.88%) and *B. bassiana* at 10 g per plant (51.85%). Minimum reduction was obtained with *B. bassiana* at 2.5 *g* per plant (24.07%)

over the untreated check.

2. Number of egg masses per plant: Result exhibited that all the treatments found significantly superior over untreated check to reduced number of egg masses per plant produced by reniform nematode on castor. Among treatments, FYM enriched with *T. harzianum* 10 g per plant (15.33) proved to be most effective followed by *Glomus fasciculatum* at 10 g per plant (18.66) and *B. bassiana* at 10 g per plant (22.00). These treatments significantly differed from each other. *Beauveria bassiana* at 2.5 g per plant (35.33) was found to be least effective over untreated check (84.66).

Experimental findings showed that FYM enriched with *T. harzianum* 10 g per plant reduced egg masses per plant to the tune of 66.18% followed by *Glomus fasciculatum* at 10 g per plant (58.83%) and *B. bassiana* at 10 g per plant (51.46%). It was observed to be minimum (22.06%) with *B. bassiana* at 2.5 g per plant over untreated check.

3. Final Nematode Population per 200 cc soil: Data illustrated through Fig.1 showed that all the treatments significantly reduced final nematode population over untreated check. It was recorded minimum with FYM enriched with *T. harzianum* 10 g per plant (360.00) followed by *Glomus fasciculatum* (410.66) and *B. bassiana* (439.66) at same dose. All treatments differed significantly from each other. *B. bassiana* was found least effective when applied at 2.5 g per plant (578.66) but found significantly superior over untreated check (785.00).

Experimental results showed highest reduction in final nematode population per 200 cc soil with *Trichoderma harzianum* at 10 g per plant (54.14%). It was determined 47.68% and 43.99% in *Glomus fasciculatum* at 10 g per plant and *Beauveria bassiana* at 10 g per plant, respectively. Minimum reduction in nematode population was noticed in *Beauveria bassiana* at 2.5 g per plant (26.28%) over untreated check.

Table 1: Efficacy of FYM enriched with bio agents on plant growth of castor and multiplication of *Rotylenchulus reniformis.*

Detail of Treatments	Shoot length* (cm)	Shoot weight* (g)	Root length* (cm)	Root weight* (g)	No of females/ Plant**	No of egg masses/ plant**	Final Nema Pop' n / 200 cc soil**
FYM enriched with *G. fasciculatum* 2.5g/plant (T1)	26.50 (29.52)	11.53 (24.51)	10.36 (9.86)	5.23 (16.22)	39.00 (27.77)	33.33 (26.47)	559.66 (28.70)
FYM enriched with *G. fasciculatum* 5g/plant (T2)	30.20 (47.60)	13.00 (40.38)	12.50 (32.55)	6.53 (45.11)	30.00 (44.44)	25.00 (44.84)	475.00 (39.49)
FYM enriched with *G. fasciculatum* 10g/plant (T3)	35.10 (71.55)	16.50 (78.18)	16.16 (71.36)	8.30 (84.44)	21.66 (59.88)	18.66 (58.83)	410.66 (47.68)
FYM enriched with *T. harzianum* 2.5g/plant (T4)	27.13 (32.60)	12.06 (30.23)	11.13 (18.02)	5.50 (22.22)	37.66 (30.25)	31.00 (31.61)	540.33 (31.16)
FYM enriched with *T .harzianum* 5g/ plant (T5)	31.00 (51.51)	13.50 (45.78)	13.20 (39.97)	7.00 (55.55)	28.33 (47.53)	22.33 (50.73)	460.33 (41.35)
FYM enriched with *T. harzianum* 10g/ plant (T6)	37.06 (81.13)	18.03 (94.70)	18.03 (91.19)	9.03 (100.66)	18.33 (66.05)	15.33 (66.18)	360.00 (54.14)
FYM enriched with *B. bassiana* 2.5g/plant (T7)	24.03 (17.44)	11.00 (18.79)	10.16 (7.74)	4.80 (6.66)	41.00 (24.07)	35.33 (22.06)	578.66 (26.28)
FYM enriched with *B. bassiana* 5g/plant (T8)	29.43 (43.84)	12.56 (35.63)	12.06 (27.88)	6.06 (34.66)	31.33 (41.98)	28.00 (38.23)	500.66 (36.22)
FYM enriched with *B. bassiana*10 g/plant (T9)	33.36 (63.04)	14.53 (56.91)	15.00 (59.06)	7.63 (69.55)	26.00 (51.85)	22.00 (51.46)	439.66 (43.99)
Untreatedcheck (T10)	20.46	9.26	9.43	4.50	54	45.33	785.00
SEm±	**0.34**	**0.26**	**0.20**	**0.08**	**1.10**	**0.75**	**3.60**
CD at 5%	**1.01**	**0.78**	**0.61**	**0.25**	**3.26**	**2.22**	**10.62**

Data are the average value of three replications; Figures in parentheses are % increase* or decrease **over untreated check.

Plate 1: Eco-friendly Management of reniform nematode, *R. reniformis* infecting castor.

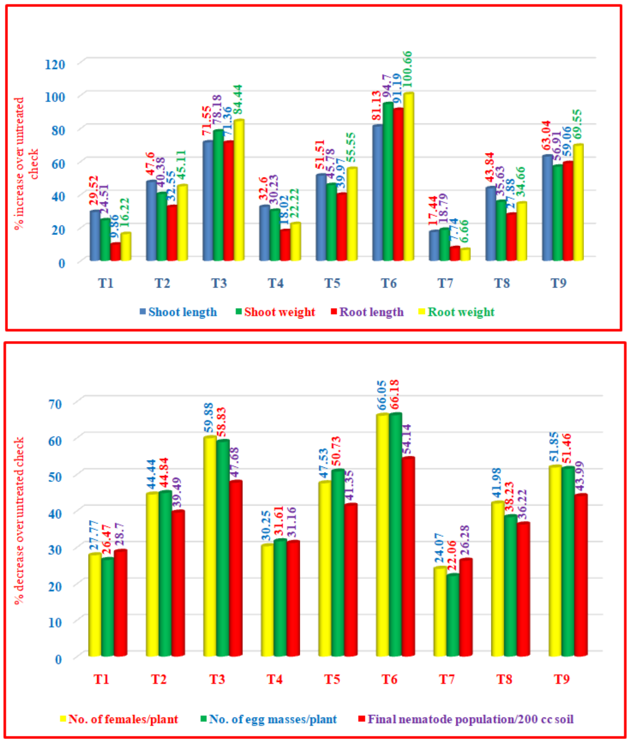

Fig. 1. Effect of FYM enriched with bioagents on growth of castor infected with reniform nematode, *Rotylenchulus reniformis.*

DISCUSSION

Eco-friendly approaches are the need of the hours as these are safe for human beings, domestic animals and environment. The earliest record of fungi as antagonists of plant parasitic nematode was reported by Duddington (1954); Mankau (1980); Jatala (1985). Biological control of plant parasitic nematodes by bacteria was first observed by Cobb in 1906 by Pasteuria sp. and later on by Mankau (1975); Stirling (1991). Organic amendments rapidly decline in nematode population due to decomposition and release of toxic compounds and increase in nematode antagonists. Therefore, in present investigation, efficacy of FYM enriched with bioagents *viz, Glomus fasciculatum*, *Trichoderma harzianum* and *Beauveria bassiana* were tested at 2.5 g, 5g and 10 g per plant as soil application for the management of reniform nematode, *R. reniformis* on castor.

Results exhibited that various combination of bio-agents with FYM were found significantly superior over untreated check. Among the treatments, maximum (37.06 cm) shoot length was recorded with FYM enriched with *T. harzianum* at 10 g per plant followed by *Glomus fasciculatum* (35.10 cm) and *Beauveria bassiana* (33.36 cm) at 10 g per plant. Highest reduction in shoot length was observed with *T. harzianum* at 10g per plant (81.13%) followed by *Glomus fasciculatum* (71.55%) and *Beauveria bassiana* (63.04%) at 10g per plant as compared to untreated check. Minimum reduction (17.44%) was noticed in *B. bassiana* at 2.5 g per plant. Almost similar trend was noticed pertaining to other plant growth characters viz., root length (cm), shoot weight (g) and root weight (g).

Results revealed that all treatments significantly reduced final nematode population as compared to untreated check. It was recorded minimum with FYM enriched with bioagents *Trichoderma harzianum* at 10 g per plant (360.00) followed by *Glomus fasciculatum* (410.66) and *Beauveria bassiana* (439.66) at same dose. *Beauveria bassiana* was found least effective when applied at 2.5 g per plant (578.66) but found significantly superior over untreated check (785.00). Highest reduction in final nematode population per 200 cc soil determined with *Trichoderma harzianum* at 10 g per plant (66.05%) followed by 59.88% and 51.85 % in *Glomus fasciculatum* and *Beauveria bassiana* at 10 g per plant, respectively. Minimum reduction in nematode population/200 cc soil was noticed in *Beauveria bassiana* at 2.5 g per plant (24.07%) over untreated check. Correspondingly, all other nematode reproduction characters (number of females and egg masses per plant) noticed similar effect during present investigation.

The efficacy of various bio-agents (*Paecilomyces lilacinus,Pseudomonas fluorescens, Glomus fasciculatum, Trichoderma harzianum, Beauveria bassiana*) with organic amendments separately as well as in combination (FYM, vermicompost, Poultry manure, decomposite tea waste, oil cakes etc.) were also reported by Sharma *et al.* (1996); Visalakshi *et al*. (2003); Ram and Baheti (2004); Baheti *et al.* (2015); Baheti *et al*. (2019); Kumar and Dwivedi (2012); Bhati *et al.* (2022).

Visalakshi *et al*. (2003) reported the biological control potential of *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Verticillium lecani* [*V.lecanii*], *V. chlamydosporium*, *Trichoderma* *viride*, *Arthrobotrys* spp., *Bacillus subtilis*, *Bacillus cereus* and vesicular arbuscular mycorrhiza (VAM) for the management of reniform nematode (*Rotylenchulus reniformis*) in papaya (cv. CO.3) under glasshouse conditions. They reported that nursery soil application of VAM was effective in reducing reniform nematode population by 80% and improved the growth of the seedlings in terms of length and weight of shoot and root. Ram and Baheti (2004) tested neem, castor and karanj products (leaf and seed kernel) as seed treatment (10% wlw) along with soil applicant (2.5 q/ha) for management of *Rotylenchulus reniformis* on cowpea (Pusa Barsati). Results revealed that plant products were effective in improving plant growth and reducing nematode reproduction over untreated check. However, neem seed kernel was found to be the most effective among all the plant products. Baheti *et al*. (2017) eco-friendly management of maize cyst nematode, *Heterodera zeae* on sweet corn (*Zea mays* L. saccharata). Baheti *et al*. (2019) efficacy of different oil-cakes as soil amendment for the management of root-knot nematode, *Meloidogyne incognita* infecting okra (*Abelmoschus esculentus* L.). Kumar and Dwivedi (2012) conducted a trail for the management of reniform nematode (*Rotylenchulus reniformis*) infecting bitter gourd (*Momordica charantia* L.) through seed treatment with bioagents. All the bioagents used were found effective over untreated control but *Pseudomonas fluorescens* @ 20 g/kg seed was most effective in increasing growth parameters of plant. Final nematode population was reduced 23.0% and yield was increased up to 2.7% in comparison to untreated control. This was followed by *Paecilomyces lilacinus* and *Pochonia chlamydosporia*. Experimental findings exhibited that the seed treatment with *P. lilacinum* at 20g/kg seed was found best followed by *P. fluorescens* at 20g/kg seed and *T. harzianum* at 20g/kg seed in improving plant growth of chickpea and reduced reproduction of reniform nematode, *R. reniformis.* Bhati *et al.* (2022) reported impact of bio-agents against *Meloidogyne incognita* infecting cucumber in poly-house.

The reniform nematode population can be suppressed with application of FYM enriched with different bio-agents, due to the fact that organic amendment (FYM) decomposes in soil, improves physical properties of soil and increases the population of natural or beneficial microbes in soil. These all factors enhance the growth of castor infested with reniform nematode. The results of present investigation clearly showed that combination of bioagents with FYM increased the plant growth and decreased nematode population in castor. The reason may be due to reduction in nematode population by parasitisation of eggs and females of *R. reniformis* and competition for food and space for nematode survival. Due to decomposition of FYM, various substances, biochemicals and metabolites etc. were released in rhizosphere that converted the insoluble nutrients into soluble form which ultimately increased the population of bioagents. The biochemical released from FYM played a toxic effect on nematode physiology. Looking to the short life cycle and fast multiplication *T. harzianum* causes great competition and adverse affected for nematode for space, oxygen, movement etc. It also release trichodermin which directly affected nematode population. It has earlier been reported that *Beauveria bassiana* present in soil released some toxicants like Beauvericin in rhizosphere which negatively impacted the biology and development of *R. reniformis.* This egg parasitising fungus killed the juveniles inside the eggshell of nematode through their fast growing hyphae. Many enzymes (proteases and chitinase) also produced by fungus. These enzymes have nematicidal activity so they degraded the eggshell and inhibited egg hatching. Hence, the reduction in the nematode population resulted by a way of fungal attack on larvae or death of females before egg laying.

Utilization of eco-friendly techniques is a current and for future research practices of nematode management. Due to the wide versatility of this area and synergistic or additive effect with other agricultural inputs, it can be included in eco-friendly management approach. Present investigation focused on the use of bio-agents and organic amendments in sustainable agriculture system and opens up vistas for the use of bionematicides which are promising as well as ecologically sound and safe. Hence, efforts in discovering new non-chemical or eco-friendly strategies for nematode management should be continued to overcome future challenges.

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