

Original Research Article

Effect of Bio-agent as Seed Treatment to Check the Initial Infection of Reniform Nematode, *Rotylenchulus reniformis* on Summer Mungbean (*Vigna radiata* L.)

ABSTRACT

Reniform nematode, *Rotylenchulus reniformis* received greater attention due to its polyphagous nature, cosmopolitan distribution and adaptability to different agro-ecological condition. It causes great losses to pulses including Mungbean (Singh, 2015). Therefore, an experiment was carried out to test the efficacy of botanicals to manage the reniform nematode infecting summer mungbean. Bioagents *i.e.* *Metarhizium anisopliae* and *Beauveria bassiana* were used at 5%, 10% and 15% as seed treatment. *Trichoderma viride* at 10% was kept as standard check. An untreated check was also maintained to compare the experimental findings. The experiment was conducted in completely randomized design with five replications. Results revealed that *Metarhizium anisopliae* at 15 % w/w as seed treatment significantly improve the plant growth parameters (44.81–77.21%) of summer mungbean followed by *Beauveria bassiana* at 15% (38.44-70.88%) and *Metarhizium anisopliae* at 10% (34.13–62.50%) as compared to untreated check. These treatments reduced the infestation of reniform nematode, *R. reniformis* on mungbean to the tune of 36.04-47.43%.

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Key words: *Rotylenchulus reniformis*, Summer Mungbean, *Metarhizium anisopliae*, *Beauveria bassiana*, Seed Treatment

Introduction

Plant parasitic nematodes are considered as one of the major constraint in Kharif pulses *i.e.* mungbean, black gram, cowpea and cluster bean production (Grewal , 1988 ; Baheti & Yadav, 1990 ; Ram & Baheti,2003; Nagar *et al.*,2009 and Yadav *et al*, 2009). Plant parasitic nematodes *viz.* *Rotylenchulus reniformis*, *Meloidogyne incognita*, *M. javanica*, *Heterodera cajani*, *Pratylenchus thornei*, *Helicotylenchus spp.*, *Hoplolaimus spp.* and *Tylenchorhynchus spp.* have been found to be associated with pulses mainly mungbean, black gram, cowpea and clusterbean (Baheti and Yadav,1991; Zareena Beghum and Shivkumar, 2005 and Khoraniya & Baheti,

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2020) . Among these nematodes, *R. reniformis* is considered to be the more serious pest causing not only direct losses but also indirect by interacting with other fungal pathogens.

Linford and Oliveira (1940) detected and described reniform nematode, *Rotylenchulus reniformis* for the first time from the roots of cowpea grown in pineapple field in the Island of Ohio, Hawaii (U.S.A.). In India, it was first reported by Siddiqi and Basir (1959) on the roots of coffee from South India. It causes serious losses in vegetables, fruits, pulses, oilseeds, cereals, millets and other ornamental crops (Seshadri and Shiva Kumar, 1963). Swarup *et al.* (1967) were first to report this important nematode from the state of Rajasthan. Verma and Prasad (1969) while studying pathogenic effect of the reniform nematode on different hosts found castor as highly preferred host for this nematode. Reniform nematode has been reported to cause 13.2 and 8.7 percent loss in yield of cowpea and black gram, respectively (Jonathan, 2001). Singh (2015) reported yield losses to the tune of 10.0 - 28.7 percent in mungbean due to reniform nematode, *R. reniformis* in Rajasthan. Recently, Kumar *et al.* (2020) reported losses to the tune of 29.00% in green gram by *Meloidogyne incognita* with estimated monetary loss of Rs 2001.00 million in India. However, at present very less information is being available on management of reniform nematode, *Rotylenchulus reniformis* - An important nematode pest of mungbean through bioagents. Keeping this view, the present investigation was under taken to find out the low cost and eco-friendly protection technology against reniform nematode, *R. reniformis* on mungbean through bioagents which earlier reported as nematicidal properties against plant parasitic nematodes.

Material and Methods:

An experiment was carried out to test the efficacy of bioagents *viz.*, *Metarhizium anisopliae* (1×10^8 cfu/g) and *Beauveria bassiana* (1×10^8 cfu/g) as seed treatment to check the initial infection of reniform nematode, *R. reniformis* on summer mungbean. Bioagents used at 5%, 10% and 15% w/w as seed treatment. Standard treated (*Trichoderma viride* at 10% w/w 2×10^8 cfu/g) and untreated checks were also taken for comparison and to interpret the experimental findings. Weighed quantity of seeds were taken in a beaker, added few drops of gum and stirred with the help of glass rod and thereafter required quantity of bioagents were added to it and mix thoroughly to provide uniform smooth coating of bioagents over seeds. The chalk powder was used as drying agent. The experiment was laid out in completely randomized

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design and all the treatments were replicated five times. Earthen clay pots were filled with soil infested with test nematode having an initial inoculum of 440 larvae/100 cc soil. After 10 days of mungbean (cv. SML-668) sowing, one healthy plant in each pot was maintained and watered regularly as and when required. Fungicide and insecticide were sprayed to safeguard the crop from insect pest and pathogens. Weeding and hoeing was done as per agronomic practices for better growth of plants. The experimental pots were regularly rotated to avoid sun and shade effect. Observations on plant growth parameters viz., shoot length (cm), shoot weight (g), root length (cm), root weight (g) and nodules per plant as well as nematode reproduction parameters i.e., number of egg masses per plant, number of females per plant and final nematode population per 200cc soil were taken. Statistical analysis was done to interpret and see significance of the treatment effects.

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Results and discussions:

Bio-agents play an important role in nematode management. Therefore, in present investigation efficacy of bio-agents viz. *Metarhizium anisoplae* and *Beauveria bassiana* were tested as seed treatment at 5%, 10% and 15% w/w as seed treatment against reniform nematode, *Rotylenchulus reniformis* on summer mungbean. A standard treated (*Trichoderma viride* at 10% as seed treatment) and untreated checks were also kept to interpret and compare the different treatment effects. Observations on plant growth parameters as well as nematode parameters were recorded to interpretate the experimental findings. Results has been presented in Table-1 and illustrated through Fig: 1-2, Plate-1.

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Plate 1: Effect of bio-agents as seed treatment against *Rotylenchulus reniformis* on summer mungbean.

Results exhibited that all the treatments significantly enhanced shoot length of mungbean over untreated check. Among bio-agents, maximum shoot length (46.34 cm) was recorded with application of *M. anisopliae* at 15% w/w followed by *B. bassiana* at 15% (44.30 cm) and *M. anisopliae* (42.92 cm) at 10% w/w. These treatments significantly enhanced shoot length over rest of the treatments. *B. bassiana* (38.08 cm) was found least effective when applied at 5% but found significantly better over check (32.00 cm). Among all the treatments, maximum shoot length (50.00 cm) was observed with *T. viride* at 10% w/w which was maintained as standard check and it differed significantly from rest of the treatments. Maximum increase in shoot length (44.81%) was recorded with *M. anisopliae* at 15% followed by *B. bassiana* at 15% (38.44%) and *M. anisopliae* at 10% (34.13%). Minimum increase in shoot length was obtained with *B. bassiana* (19.00%) when applied at lower dose *i.e.* 5% w/w over untreated check. However, highest increase in shoot length (56.25%) was observed with *T. viride* at 10% (w/w). Almost similar trend was noticed with regards to other plant growth parameters *i.e.* shoot weight (g), root length (cm), root weight (g) and nodules per plant.

Table 1: Effect of bio-agents as seed treatment against reniform nematode, *Rotylenchulus reniformis* on summer mungbean (*Vigna radiata* L)

Treatments	Shoot length (cm)*	Root length (cm)*	Shoot weight (g)*	Root weight (g)*	No. of nodules /plant*	No. of egg masses /plant**	No. of females/ Plant**	Final nematode population/ 200cc soil**
<i>M. anisopliae</i> 5% (T ₁)	39.84 (24.50)	11.00 (37.50)	16.84 (35.81)	4.50 (18.42)	21.80 (37.97)	11.40 (25.00)	13.00 (24.41)	550.00 (29.48)
<i>M. anisopliae</i> 10% (T ₂)	42.92 (34.13)	13.00 (62.50)	18.70 (50.81)	5.46 (43.76)	24.20 (53.16)	9.40 (38.15)	11.00 (36.04)	480.00 (38.46)
<i>M. anisopliae</i> 15% (T ₃)	46.34 (44.81)	14.00 (75.00)	21.28 (71.61)	6.52 (71.58)	28.00 (77.21)	8.40 (44.73)	9.40 (45.34)	410.00 (47.43)
<i>B. bassiana</i> 5% (T ₄)	38.08 (19.00)	10.30 (28.75)	14.82 (19.52)	4.50 (18.40)	21.00 (32.91)	12.80 (15.78)	14.20 (17.44)	630.00 (19.23)

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<i>B. bassiana</i> 10% (T5)	40.48 (26.50)	12.40 (55.00)	17.94 (44.68)	5.00 (31.57)	23.40 (48.10)	10.20 (32.89)	12.00 (30.23)	585.00 (25.00)	Comment [A17]: subscript
<i>B. bassiana</i> 15% (T6)	44.30 (38.44)	13.30 (66.25)	20.02 (61.45)	5.66 (48.95)	27.00 (70.88)	9.00 (40.78)	10.00 (41.86)	465.00 (40.38)	Comment [A18]: subscript
<i>T. viride</i> 10% (T7)	50.00 (56.25)	15.24 (90.50)	22.40 (80.65)	7.12 (87.39)	28.80 (82.27)	7.20 (52.63)	8.80 (48.83)	375.00 (51.92)	Comment [A19]: subscript
Uncontrol check (T8)	32.00	8.00	12.40	3.80	15.80	15.20	17.20	780.00	Comment [A20]: subscript
SEM+	1.358	0.434	0.500	0.201	0.800	0.439	0.485	22.312	
CD 5%	3.912	1.251	1.44	0.579	2.305	1.264	1.396	64.272	

Initial nematode population: 440 juveniles/200 cc soil

Data are the average value of five replications.

Design of experiment: C.R.D. Crop variety: SML-668 Figures in parentheses are % increase (*) or decrease (**) over check.

Nematode reproduction parameters were also recorded to interpretate the experimental findings.

Data pertaining to number of egg masses per plant produced by *R. reniformis* on summer mungbean reduced significantly with bio-agent treatments as compared to untreated check.

Minimum number of egg masses per plant (8.40) was observed with *Metarhizium anisopliae* at 15% (w/w) followed by *Beauveria bassiana* at 15% (9.00) and *Metarhizium anisopliae* at 10% (9.40). These treatments significantly decreased number of egg masses over check. Maximum number of egg masses per plant (15.20) was obtained in untreated check. *Beauveria bassiana* at 5% (12.80) was found least effective. Among all the treatments, *T. viride* at 10% w/w was found best with respect to reducing number of egg masses per plant (7.20) of *R. reniformis* on summer mungbean. Results showed that maximum reduction in number of egg masses per plant (44.73%) was recorded with the seed treatment of *Metarhizium anisopliae* at 15% (w/w) followed by *Beauveria bassiana* at 15% (40.78%) and *Metarhizium anisopliae* at 10% (38.15%). Minimum reduction was obtained with *Beauveria bassiana* at 5% (15.78%) over check. However, highest reduction (52.63%) was noticed with *T. viride* at 10% w/w. Almost similar trend was observed with respect to other nematode parameters i.e. number of females per plant and final nematode population per 200cc soil. Experimental findings also shows that application of higher dose of *Metarhizium anisopliae* and *Beauveria bassiana* (15%) is more effective as seed treatment to enhanced plant growth and crop yield in nematode infested areas.

Reduced germination percentage has been recorded when dosage of more than 15% w/w were applied to the seeds.

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Results of present investigation are in accordance with the findings of previous workers who reported that application of bio-agent found effective to manage plant parasitic nematodes in different agri-horticultural crops. Kerry (1978) reported that almost all *Heterodera avenae* cysts were parasitized by naturally occurring soil fungi (*V. chlamydosporium* and *N. gynophila*) and controlled the population of nematodes in British soil. Jatala *et al.* (1979) observed the parasitization of eggs and females of *Meloidogyne incognita* by *Paecilomyces lilacinus* on potato. Mankau (1980) carried out an experiment trial to evaluate the fungal antagonist of nematodes consisting of nematode trapping fungi and endo-parasitic fungi. They reported that fungi can effectively be employed as bio-control agents for plant parasitic nematodes and may be a suitable alternative to chemicals specially in integrated management system. David and Zorilla (1990) conducted an experiment on potato field infested with golden cyst nematode and found that *M. anisopliae* gave 67.2% control and was better than *P. lilacinus* (63.2%). Ekanayake and Jayasundara (1994) conducted an experiment to test the efficacy of nematophagous fungi, *Paecilomyces lilacinus* and *Beauveria bassiana* as biocontrol agents against *Meloidogyne incognita* on tomato and compared with the nematicide carbofuran. Carbofuran and *P. lilacinus* significantly control the root-knot nematode and increased the growth of plants; *B. bassiana* was comparatively found less effective. Tribhuvaneshwar *et al.* (2008) conducted an experiment to measure the suppressive effect of different doses of *Metarhizium anisopliae* on *R. reniformis* infesting tomato. The final nematode population in soil significantly decreased with increasing dose of fungus. The maximum population reduction (51 %) was obtained at the higher dose of 12g/ kg soil. Bokhari (2009) tested the efficacy of *Trichoderma species* for control of reniform nematode (*Rotylenchulus reniformis*) and root-knot nematode (*Meloidogyne javanica*). All culture filtrates of the *Trichoderma species* were highly significant in controlling both nematode genera on eggplant. *T.harzianum*, *T. hamatum* and *T. koningii* culture filtrates gave a significant reduction and decreased the female and egg-masses of reniform and root-knot nematodes. Gurjar *et al.* (2012) conducted an experiment to test the efficacy of *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* at 1 and 2 g/kg soil against reniform nematode, *Rotylenchulus reniformis* on soyabean as soil application. Results revealed that *P. lilacinus* was found most effective followed by *P. chlamydosporia* and *T. harzianum* in improving growth characters of soybean and to reduce the nematode reproduction parameters.

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These studies clearly shows that seed treatment with various bio-agents not only reduced nematode population but also increased the plant growth characters may be due to release of substances hazards or toxic for nematodes, competition for oxygen, nutrition, space or due to hyperparasitism . Thus it is suggested that bio-agents viz. *Trichoderma viride* at 10% followed by *Metarhizium anisopliae* at 15% and *Beauveria bassiana* at 15% w/w be used as seed treatment to promote the plant growth and to reduced the infection of reniform nematode, *R. reniformis* on summer mungbean.

Comment [A23]: Result given in abstract is different from your conclusion. Which treatment you are recommending?

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