

Evaluation of Bio-control Agents for the Management of Reniform Nematode (*Rotylenchulus reniformis* Linford and Oliveira, 1960) on Cowpea

ABSTRACT

To evaluate the efficacy of different bio-control agents against reniform nematode, *Rotylenchulus reniformis* on cowpea (var. local) investigations were carried out under net house conditions. Results illustrated that all the treatments were effective in improving the plant growth parameters and reducing the nematode population. Among all the treatments, Biozin-PTB (10% w/w) proved to be best in promoting shoot length, fresh and dry weight of shoot of the plant with the percentages of increase 64.15%, 85.25% and 65.12% respectively. Followed by treatment with Biofor-PF2 (10% w/w) and Biozium (10% w/w) in stimulating shoot length with the percentages of increase 55.90% and 47.38%, fresh weight of shoot with the percentages of increase 68.05% and 55.95% and dry weight of shoot with the percentages of increase 61.12% and 50.00% respectively. Similarly, Biozin-PTB (10% w/w) followed by Biofor-PF2 (10% w/w) and Biozium (10%w/w) proved to be efficient in reducing the nematode population, number of females and egg masses as well as reproduction rate. The number of healthy *Rhizobium* nodules was increased by all the treatments and the highest nodule development was found in treatment with Biozin-PTB (10% w/w). It was observed that the combination product of bioagent performed better than the single bioagent.

Keywords *Rotylenchulus reniformis*, Biocontrol agents, Bioveer, Biozium, Biomonas, Biofor-PF2, Biozin-PTB

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a legume that belongs to the family Fabaceae which is widely cultivated in the tropics and subtropics. The crop is cultivated primarily for seed and fodder production, but also as a vegetable and as a cover crop. The crop can tolerate drought and can fix atmospheric nitrogen. Cowpea seeds are a rich source of protein, vitamins and minerals. Among the plant-parasitic nematodes, reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira, 1940) has been recognized as a major

constraint to cowpea cultivation in India and other parts of the world [1-4]. (fig. 11-15). The crop losses caused to cowpea were estimated to be 10% in India [5].

Nematicides and crop rotation are some of the management tactics of *R. reniformis* [6]. Very few commercial cultivars are available and few alternative crops can be rotated with cowpea. However, the use of chemicals in a fodder or vegetable crop could pose hazards to human health as well as the environment. Biological control of *R. reniformis* may shorten rotations and can be integrated with other control methods to help manage this nematode pest [7]. *Paecilomyces lilacinus*, *Pochonia chlamydospora* and Arkansas fungi have been documented as parasites of the egg stage of *R. reniformis* [8-11]. Furthermore, strains of the bacteria *Pasteuria* spp. and *Pseudomonas fluorescens* have been reported to reduce the number of *R. reniformis* in soil [12-14].

The objective of the study was to evaluate the commercial biocontrol products to determine their potential to reduce *R. reniformis* populations and enhance cowpea plant growth under greenhouse conditions.

2. MATERIALS AND METHODS

The pot experiment was carried out during the kharif season (June 2022 -August 2022) in the net house of the Department of Nematology, Assam Agricultural University, Jorhat. The laboratory investigations were conducted in the Post-Graduate Laboratory of the Department of Nematology, Assam Agricultural University, Jorhat.

Inocula were obtained from a pure culture of *R. reniformis* propagated on castor in greenhouse conditions. Talc based formulation of 5 biopesticides viz., *Trichoderma viride* (Bioveer) 1×10^8 , *Trichoderma harzianum* (Biozium) 1×10^8 , *Pseudomonas fluorescens* (Biomonas) 1×10^8 , *Pseudomonas fluorescens* & *Trichoderma harzianum* (Biofor-PF-2) (1×10^9) & (1×10^8), *Pseudomonas fluorescens* + *Trichoderma harzianum* + *Bacillus brevis* (Biozin-PTB) (1×10^9) & (2×10^9) & (1×10^9) were procured from Department of Plant Pathology, Assam Agricultural University, Jorhat. One kg of each talc formulation of biopesticides was mixed with 10kg of decomposed cow dung for 15 days. After 15 days of incubation, the enriched biopesticides were applied according to the required amount (10% w/w). Surface sterilized seeds of cowpea (var. local) were sown in 15cm diameter pots filled (1 kg/pot) with autoclaved sandy loam soil compost mixture (2:2:1 soil: sand: compost). Inoculation of reniform nematode was done at the pathogenic level (1000 nematodes per kg soil) on the one-week-old seedling. Treatments were applied one week after the inoculation of the nematode. Treatment with Nimbecidine ® @ 5 ml/l as soil drench was included for

comparison. Inoculated seedlings without treatments served as untreated control. Pots of neither treated nematodes nor treatments were also included (uninoculated control). Pots were arranged on the greenhouse bench in a completely randomized block design with three replications.

Observations were taken after 60 days after planting. Potted plants were uprooted with tap water very carefully and slowly remove the adhering soil particles from the root system. Shoot length, root length, fresh and dry weight of shoot and root, number of females and egg masses per root system, larval population (200cc of soil), total nematode population, and reproductive rate were recorded. The tested concentration of fungi (final CFU /g of soil) was determined by the plate count technique [15]. For recording the dry weight of the shoot and root, materials were packed in a paper bag labeled according to the treatment and dried at 30-35°C and weighed. The vermiform life stages of *R. reniformis* were extracted from the soil by Cobb's decanting and sieving technique followed by the sucrose centrifugation flotation technique [16, 17].

Healthy *Rhizobium* nodules were counted with the naked eye and if necessary, with the help of a stereo microscope. Root populations of nematodes, both immobile and vermiform stages, were assessed after differential staining by the NaOCl acid fuchsin method [18]. Estimation of reproductive rate was calculated by:

$$\text{Reproductive rate} = \frac{\text{Final nematode population}}{\text{Initial population}}$$

Data were analyzed and means were compared according to Duncan's Multiple Range Test at the 5% level of probability.

3. RESULTS AND DISCUSSION

The results revealed that all the treatments, *viz.*, Bioveer, Biozium, Biomonas, Biofor-PF2 and Biozin-PTB at 10% w/w improved the cowpea plant growth characters and reduced the number of females, egg masses per root system, and nematode population in comparison to the control (Table 1 and 2) and illustrated in Figure 1-7. Concerning the plant growth parameters of cowpea plants, a high percentage of increase was recorded in concomitant treatments using three biocontrol agents as compared to other treatments. There was a significant increase in plant growth parameters *viz.*, shoot length (64.15 % increase), root length (100 % increase), fresh weight of shoot (85.25 % increase), dry weight of shoot (65.12 % increase), fresh weight of root (100 % increase) and dry weight of root (100 % increase) with the soil treatment with Biozin-PTB @ 10% w/w followed by Biofor-PF2 with shoot

length (55.90 % increase), root length (100 % increase), fresh weight of shoot (68.05 % increase), dry weight of shoot (61.12 % increase), fresh weight of root (100 % increase) and dry weight of root (100 % increase) and Biozium with shoot length (47.38 % increase), root length (96.34 % increase), fresh weight of shoot (55.95 % increase), dry weight of shoot (50.00 % increase), fresh weight of root (100 % increase) and dry weight of root (100 % increase) as compared to untreated control as shown in Table 1 and illustrated in figure 1, 2 and 3 . The number of healthy rhizobium nodules was increased by all the treatments. Treatment with Biozin-PTB @ 10% w/w showed maximum nodule development (100% increase) as compared to the untreated control.

The results also revealed the maximum reduction in the number of females (71.72 % decrease), egg masses per root system (81.81 % decrease), larval population/200cc of soil (85.89 % decrease), total population (85.89 % decrease) and minimum reproductive rate 1.27% in the treatment with soil application of Biozin-PTB @ 10% w/w followed by Biofor-PF2 with reduction in the number of females (65.51 % decrease), egg-masses per root system (72.72 % decrease), larval population/200cc of soil (82.74 % decrease) and total population (82.65 % decrease) as compared to untreated control shown in Table 2 and illustrated in figure 4,5,6 and 7. It was observed that the combination product of bioagent performed better than the single bioagent. This may be the cumulative effect of bioagents as Biozin-PTB contains *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus brevis* that aid in promoting plant growth and suppressing nematode population.

The result of the present investigation is in agreement with Mohanty and Mahapatra [19] who reported that combined application of *P. fluorescens* and *T. viride* @ 5g + 5g/kg seeds of cowpea, registered 57.4% and 45.08% reduction in reniform nematode population in the soil. Jonathan *et al.* [20] reported the consortium application of *P. fluorescens* and *Bacillus subtilis* as seed treatment and soil application improved the plant growth and reduced the number of females, number of nematode populations in root and soil in tomato. Lira *et al.* [21] recorded that the culture filtrates of *T. harzianum* caused a significant reduction in the number of egg masses and the reproductive factor of *Rotylenchulus reniformis* in cowpea and coriander. Bokhari [22] also found that the culture filtrate of *T. harzianum* significantly decreased female and egg masses of *R. reniformis*. Patil and Sharma [23] observed the efficacy of *Trichoderma harzianum* @ 2g/kg soil against *R. reniformis*, in cowpea. *Trichoderma* species led to a direct effect on toxic metabolites and inhibit nematode

penetration and development. Khan *et al.* [24] reported that seed treatment with *Pseudomonas fluorescens* @5ml/kg was found to be the best in advancing plant growth in chickpea as they are found to be produced more Indol acetic acid (IAA). *Pseudomonas* species were recognized by their antagonistic activities towards some plant-parasitic nematodes through the production of secondary active metabolites siderophores, salicylic acid, 2,4-diacetyl phloroglucinol and hydrogen cyanide and extracellular protease.

UNDER PEER REVIEW

Table 1. Effect of different treatments on the plant growth parameters of cowpea infected by *Rotylenchulus reniformis* (Mean of 3 replications)

Treatments	Shoot length (cm)	% increase over untreated control	Fresh weight ofshoot (g)	% increase over untreated control	Dry weight ofshoot (g)	% increase over untreated control	Root length (cm)	% increase over untreated control	Fresh ofroot (g)	% increase over untreated control	Dry ofroot (g)	% increase over untreated control	No. of nodules	% increase over untreated control
T1	79.46 ^e	34.60	29.46 ^d	39.23	12.03 ^d	33.67	18.10 ^e	65.59	9.26 ^d	81.56	2.76 ^c	100.00	14.67	15.78
T2	87.00 ^c	47.38	33.00 ^{bc}	55.95	13.50 ^b	50.00	21.46 ^c	96.34	11.70 ^c	100.00	3.20 ^{bc}	100.00	22.67	78.92
T3	82.67 ^d	40.03	30.10 ^{cd}	42.24	12.43 ^{cd}	38.12	18.50 ^{de}	69.25	9.56 ^d	87.45	2.93 ^c	100.00	18.34	44.75
T4	92.03 ^b	55.90	35.56 ^b	68.05	14.50 ^a	61.12	24.70 ^b	100.00	12.86 ^b	100.00	3.56 ^b	100.00	25.00	97.31
T5	96.90 ^a	64.15	39.20 ^a	85.25	14.86 ^a	65.12	27.50 ^a	100.00	13.86 ^a	100.00	4.13 ^a	100.00	28.34	100.00
T6	86.20 ^c	46.02	32.23 ^{cd}	52.31	13.03 ^{bc}	44.78	21.16 ^{cd}	93.59	10.93 ^c	100.00	3.13 ^{bc}	100.00	17.67	39.46
T7	59.03 ^g	-	21.16 ^f	-	9.00 ^f	-	10.93 ^g	-	5.10 ^f	-	1.06 ^f	-	12.67	-
T8	66.67 ^f	-	26.06 ^e	-	11.23 ^e	-	15.10 ^f	-	8.06 ^e	-	2.10 ^e	-	14.00	-
S.Ed. (±)	1.24	-	1.51	-	0.41	-	1.30	-	0.43	-	0.26	-	1.51	-
C.D (P=0.05)	2.66	-	3.23	-	0.88	-	2.79	-	0.92	-	0.54	-	3.20	-

Mean followed by the same letter in the superscript(s) are statistically *at par*. *T1: Soil treatment with Bioveer (*Trichoderma viride* AAU strain); T2: Soil treatment with Biozium (*Trichoderma harzianum* AAU strain); T3: Soil treatment with Biomonas (*Pseudomonas fluorescens* AAU strain); T4: Soil treatment with Biofor-PF2 (*Trichoderma harzianum* + *Pseudomonas fluorescens* AAU strain); T5: Soil treatment with Biozin-PTB (*Trichoderma harzianum* + *Pseudomonas fluorescens* + *Bacillus brevis* AAU strain); T6: Soil treatment with Nimbecidine; T7: Untreated control; T8: Uninoculated control.

Table 2. Effect of different treatments on number of females, egg masses and nematode population of *Rotylenchulus reniformis* on cowpea (Mean of 3 replications)

Treatments	No. of females/root system	% decrease over untreated control	No. of eggmasses/ root system	% decrease over untreated control	Larval population/ 200 cc of soil	% decrease over untreated control	Total population/pot	% decrease over untreated control	Reproductive rate (%)	cfu/g of soil
T ₁	25.67 (5.16) ^b	46.90	23.67 (4.96) ^b	46.20	440.00 (20.99) ^b	75.51	2225.67 (47.18) ^b	75.35	2.22 ^b	3.5x10 ⁴
T ₂	20.00 (4.57) ^c	58.61	17.00 (4.23) ^d	61.36	376.67 (19.42) ^{bc}	79.03	1903.34 (43.62) ^{bc}	78.92	1.90 ^{bc}	6x10 ⁴
T ₃	23.34 (4.92) ^{bc}	51.72	20.00 (4.54) ^c	54.54	403.34 (20.10) ^{bc}	77.56	2032.67 (45.08) ^{bc}	77.49	2.03 ^b	4x10 ⁶
T ₄	16.67 (4.19) ^d	65.51	12.00 (3.59) ^e	72.72	310.00 (17.61) ^d	82.74	1566.67 (39.55) ^d	82.65	1.56 ^d	7x10 ⁵
T ₅	13.67 (3.82) ^e	71.72	8.00 (2.99) ^f	81.81	253.34 (15.87) ^e	85.89	1280.34 (35.67) ^e	85.82	1.27 ^e	9x10 ⁶
T ₆	21.00 (4.68) ^a	56.56	18.00 (4.35) ^{cd}	59.09	373.34 (19.31) ^c	79.23	1887.67 (43.39) ^c	79.09	1.88 ^{bc}	-
T ₇	48.34 (7.02)	-	44.00 (6.70) ^a	-	1796.67 (42.39) ^a	-	9031.67 (91.96) ^a	-	9.02 ^a	-
T ₈	-	-	-	-	-	-	-	-	-	-
S.Ed. (±)	0.17	-	0.15	-	0.73	-	1.61	-	0.14	-
C.D (P=0.05)	0.35	-	0.31	-	1.55	-	3.44	-	0.29	-

Mean followed by the same letter in the superscript(s) are statistically *at par*. *T₁: Soil treatment with Bioveer (*Trichoderma viride* AAU strain); T₂: Soil treatment with Biozium (*Trichoderma harzianum* AAU strain); T₃: Soil treatment with Biomonas (*Pseudomonas fluorescens* AAU strain); T₄: Soil treatment with Biofor-PF2 (*Trichoderma harzianum* + *Pseudomonas fluorescens* AAU strain); T₅: Soil treatment with Biozin-PTB (*Trichoderma harzianum* + *Pseudomonas fluorescens* + *Bacillus brevis* AAU strain); T₆: Soil treatment with Nimbecidine; T₇: Untreated control; T₈: Uninoculated control

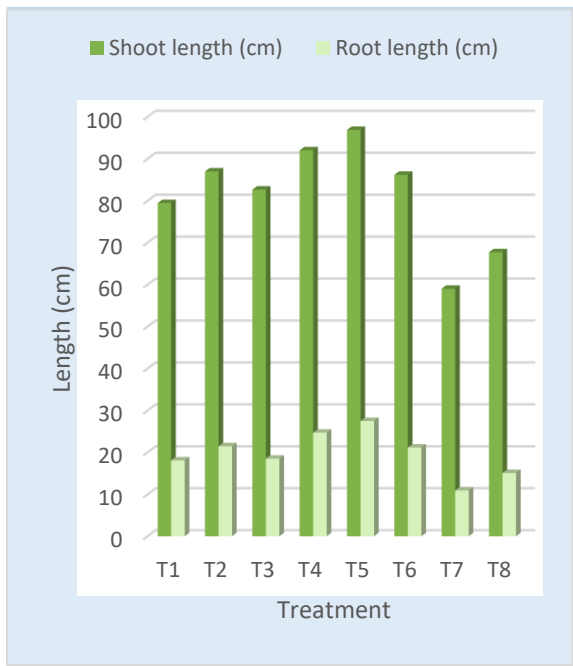


FIGURE 1. EFFECT OF DIFFERENT TREATMENTS ON SHOOT AND ROOT LENGTH OF COWPEA

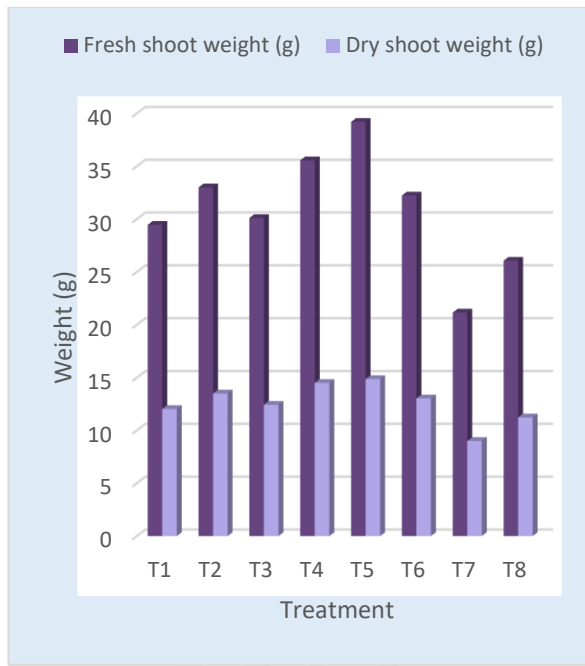


FIGURE 2. EFFECT OF DIFFERENT TREATMENTS ON FRESH AND DRY WEIGHT OF SHOOT OF COWPEA

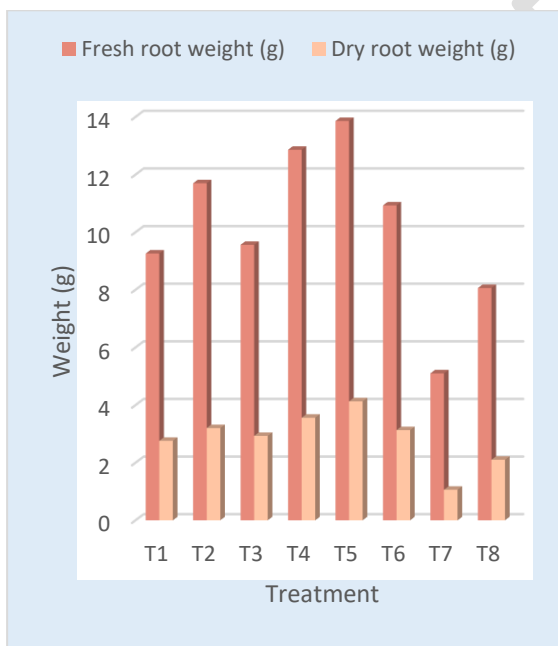


FIGURE 3. EFFECT OF DIFFERENT TREATMENTS ON FRESH AND DRY WEIGHT OF ROOT OF COWPEA

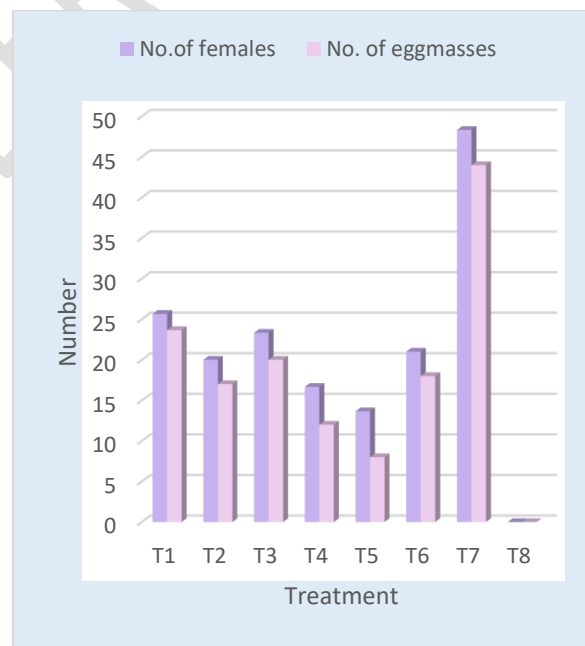


FIGURE 4. EFFECT OF DIFFERENT TREATMENTS ON NUMBER OF FEMALES AND EGG MASSES PER ROOT SYSTEM

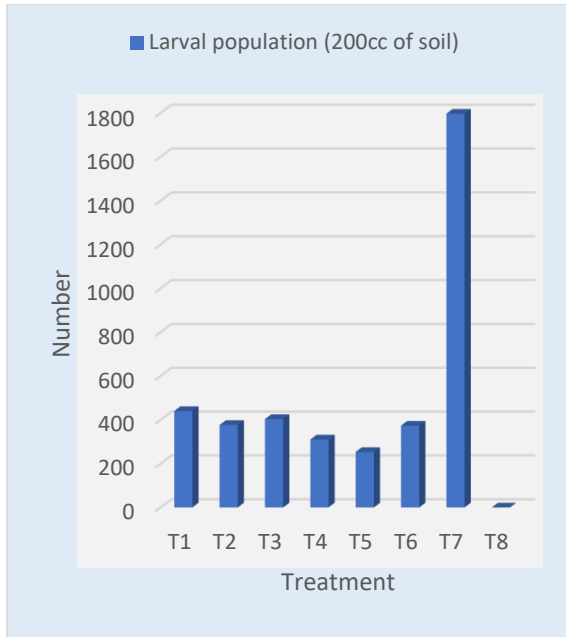


FIGURE 5. EFFECT OF DIFFERENT TREATMENTS ON LARVAL POPULATION

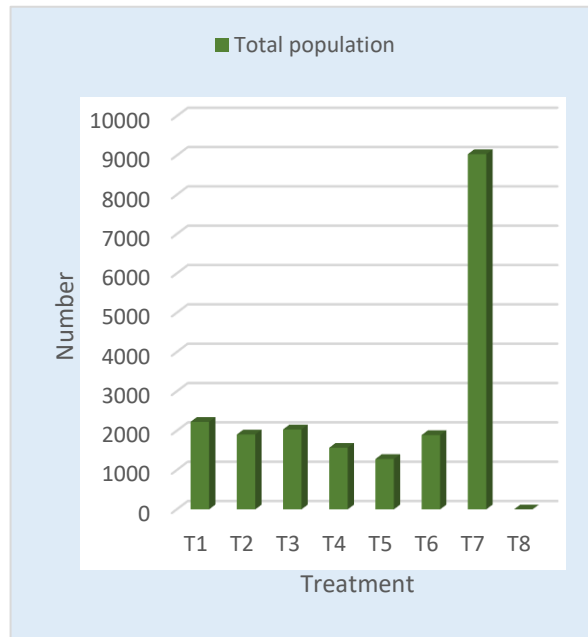


FIGURE 6. EFFECT OF DIFFERENT TREATMENTS ON TOTAL NEMATODE POPULATION

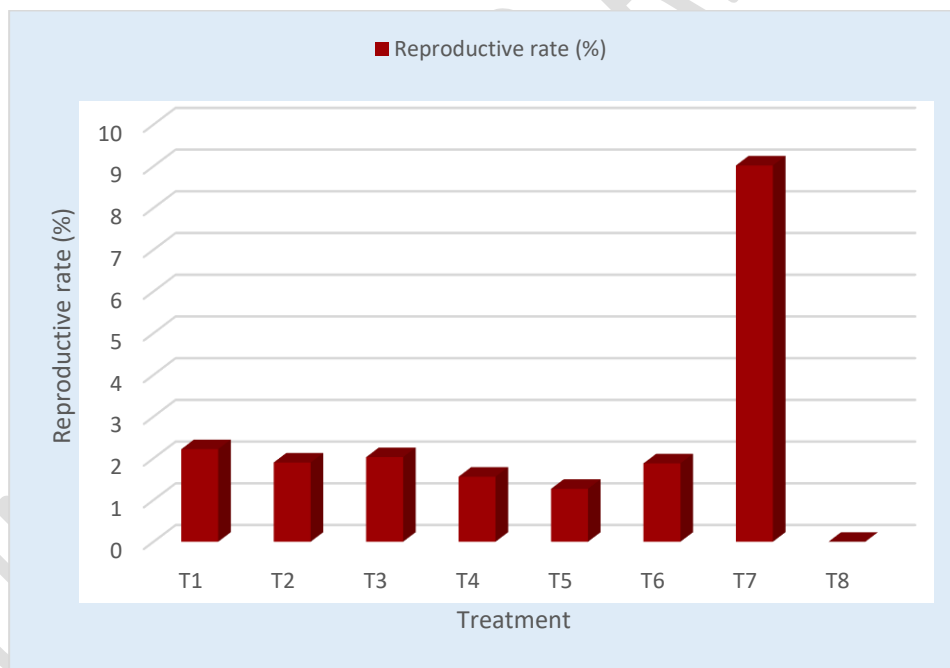


FIGURE 7. EFFECT OF DIFFERENT TREATMENTS ON REPRODUCTIVE RATE (%) OF NEMATODE

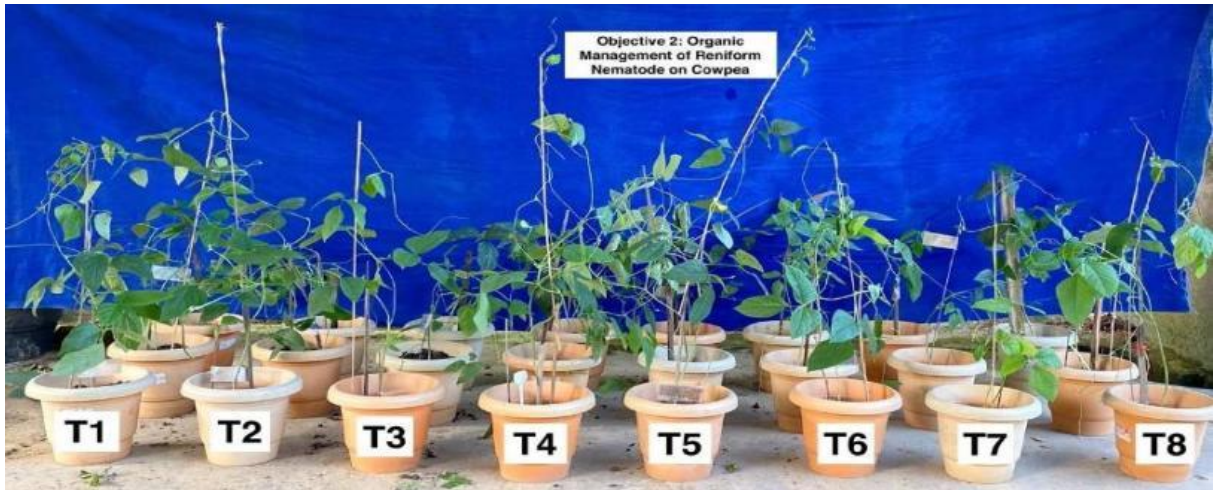


Figure 8. Overview of the pot experiment on cowpea



Figure 9. Effect of different treatments on plant growth of cowpea infected by reniform nematode



Figure 10. Comparison between the best treatment (T5) and untreated control (T7)

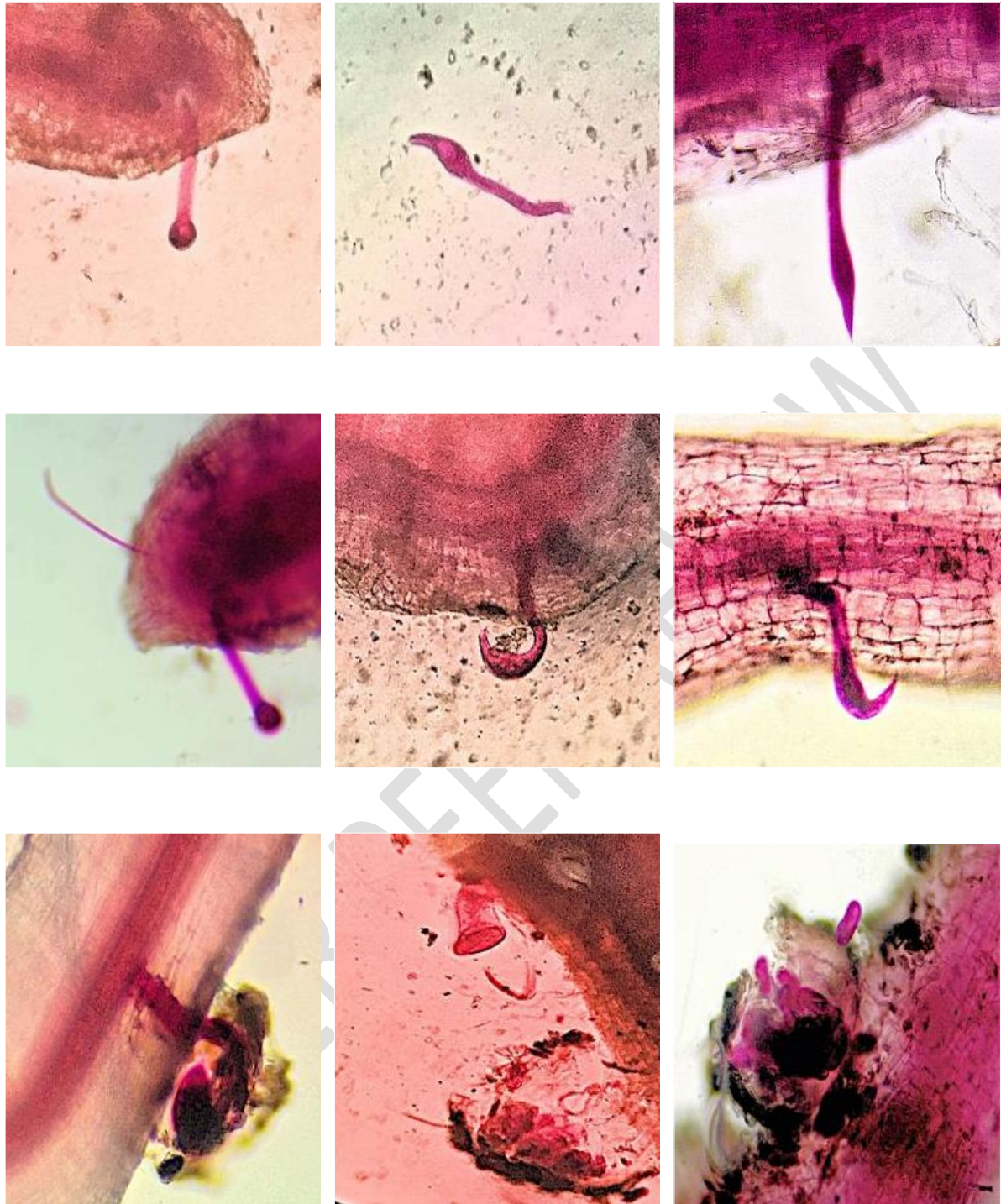


Figure 11. Post penetration development of *Rotylenchulus reniformis* and eggmass in untreated control



Figure 12. Immature female of reniform nematode

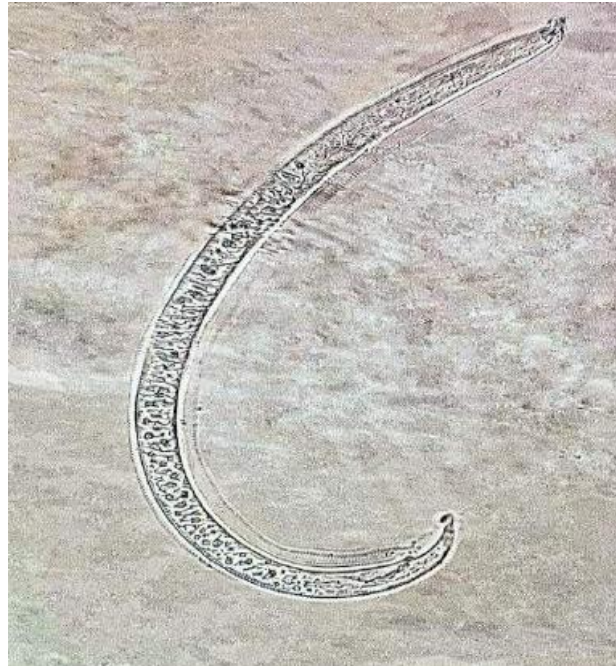


Figure 13. Male of reniform nematode



Figure 14. Mature female of reniform nematode

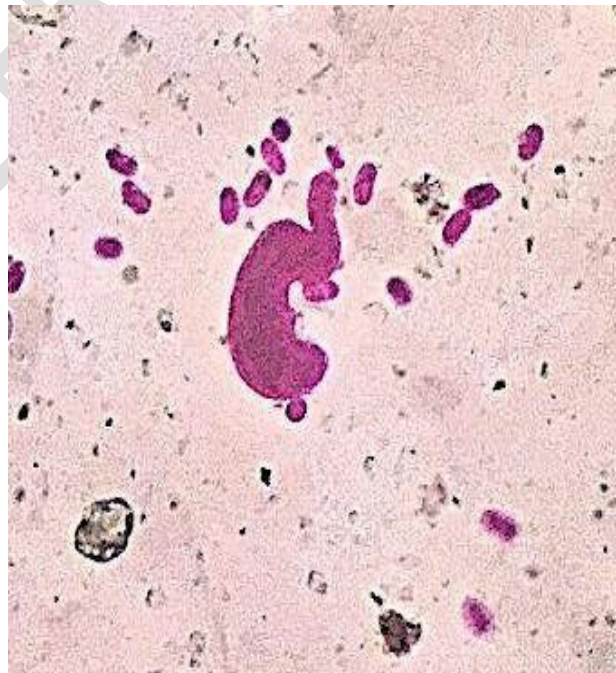


Figure 15. Stained mature female of reniform nematode

4. CONCLUSION

Considering the importance of reniform nematode as a limiting factor in the production of cowpea, the use of biological agents is an alternative to pesticides. More studies with various native biocontrol agents, with different combination, will improve the deployment of biocontrol agents in integrated pest management of *Rotylenchulus reniformis*.

REFERENCES

1. Singh RV, Khera S. Culturing and the life history studies of *Rotylenchulus reniformis* Linford and Oliveira on brinjal. (Nematoda). Bulletin of the Zoological Survey of India 1978;1:115-128.
2. Sikora RA, Greco N, Velosa SJF. Nematode parasites of food legumes in Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, Second Edition (LucM., Sikora R.A. and Bridge J., eds). 2005; pp: 259-318.
3. Robinson AF, Inserra RN, Caswell-Chen EP, Vovlas N, Troccoli A. *Rotylenchulus* species: identification, distribution, host ranges, and crop plant resistance. Nematropica. 1997;27: 127-180.
4. Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol. 2013;14: 946–961
5. Anonymous (2004) Third Annual Report (2003-2004), ICAR Network Project on Reniform Nematodes, *Rotylenchulus reniformis*, Division of Nematology, Indian Agricultural Research Institute, New Delhi, India pp. 67-68.
6. Rathore BS. The effect of neem, mustard and karanj cakes against reniform nematode *Rotylenchulus reniformis* on cowpea. Indian Journal of Mycology and Plant Pathology. 1994; 24:51-52
7. Kerry BR. An assessment of progress toward microbial control of plant- parasitic nematodes. Supplement to the Journal of Nematology. 1990;22:621-631.
8. Reddy P, Khan RM. Evaluation of *Paecilomyces lilacinus* for the biological control of *Rotylenchulus reniformis* infecting tomato, compared with carbofuran. Nematologia Mediterranea. 1988;16:113–115.
9. Waters SA, Barker KR. Efficacy of *Paecilomyces lilacinus* in suppressing *Rotylenchulus reniformis* on tomato. Journal of Nematology. 1994;26:600-605.
10. Wangs K, Riggs RD, Crippen D. Suppression of *Rotylenchulus reniformis* on cotton by the nematophagous fungus ARF. Journal of Nematology. 2004;36:186-191.

11. Zhao DZH, Zhao D, Zhu X, Wang Y, Duan Y. Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. *Journal of Biological Control*. 2018;119:12-19.
12. Hewlett TE, Stetina SR, Schmidt LM, Waters JP, Simmons LJ, Rich JR. Identification of *Pasteuria* spp. that parasitize *Rotylenchulus reniformis*. (Abstr.) *Journal of Nematology*. 2009; 41:338. 17.
13. Jayakumar J, Ramakrishnan S, Rajendran G. Bio-efficacy of *Pseudomonas fluorescent* isolates against reniform nematode, *Rotylenchulus reniformis* infecting cotton. *Indian Journal of Nematology*. 2003;33:13-15.22
14. Schmidt LM, Hewlett TE, Green A, Simmons LJ, Kelley K, Doroh M, Stetina SR. Molecular and morphological characterization and biological control capabilities of a *Pasteuria* spp. parasitizing *Rotylenchulus reniformis*, the reniform nematode. *Journal of Nematology*. 2010;42:207-217
15. Vieira FCS, Nahas E. Comparison of microbial numbers in soil by using various culture media and temperatures. *Microbiological Research*. 2005;160:197-202
16. Christie JR, Perry VG Removing nematodes from soil. *Proceedings of the Helminthological Society of Washington*. 1951;18: 106-108
17. Muraellidharan R, Sivakumar CV. An improved method of recovery of reniform nematode, *Rotylenchulus reniformis* from soil. *Indian Journal of Nematology*. 1975;5:114-115
18. Byrd DW, Kirkpatrick T, Barker KR. An improved technique for clearing and staining plant tissue for detection of nematodes. *Journal of Nematology*. 1983;15: 142-143
19. Mohanty KC., Mahapatra SN. Bio-management of *Rotylenchulus reniformis* in cowpea through seed treatment. *Indian Journal of Nematology*. 2009;39(2):228-252
20. Jonathan EI, Raguchander T, Zareena BM, Sundaramoorthy S. Field efficacy of biocontrol agents for the management of root knot nematode *Meloidogyne incognita* (Kofoid & White) Chitw. and reniform nematode *Rotylenchulus reniformis* (Linford & Oliviera) in tomato. *Journal of Biological Control*. 2009;23(3): 311–316
21. Lira VL, Santos DV, Barbosa RN, Costa AF, Maia LC, Moura RM. Biocontrol potential of fungal filtrates on reniform nematode (*Rotylenchulus reniformis*) in coriander and cowpea. *Nematropica*. 2020;50:86-95
22. Bokhari FM. Efficacy of some Trichoderma species in the control of *Rotylenchulus reniformis* and *Meloidogyne javanica*. *Archives of Phytopathology*. 2009;42(4): 361-369.

23. Patil J, Sharma MK. Management of reniform nematode, *Rotylenchulus reniformis* by soil application with bioagents on cowpea. International Journal of Life Sciences Research. 2016;(1): 2347-8691
24. Khan MR, Khan MM, Anwer MA, Haque Z. Laboratory and field performance of some bacteria used as seed treatment on *Meloidogyne incognita* in chickpea. Nematologia Mediterranea. 2012; 40: 143-151

UNDER PEER REVIEW