Original Research Article

Organic Management of Reniform Nematode (*Rotylenchulusreniformis*Linford and Oliveira, 1960) on Cowpea (*Vigna unguiculata)*

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 followed by Biofor-PF2 (10% w/w) and Biozium (10%w/w) in promoting shoot length,fresh and dry weight of shoot of the plant with the percentages of increase64.15%, 55.90% and 47.38% for shoot length;85.25%, 68.05%, and 55.95% for fresh shoot weight; 65.12%, 61.12% and 50.00% for dry shoot weight 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, cowpea

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 Sing and Khera; Sikora

et al; Robinson et al; Jones et al. [1-4] (fig. 11-15). The crop losses caused to cowpea were estimated to be 10% in India Anon. [5].

Nematicides and crop rotation are some of the management tactics of *R. reniformis*Rathore [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 helpmanage this nematode pest Kerry [7]. *Paecilomyces lilacinus, Pochoniachlamydospora* and Arkansas fungi have been documented as parasites of the egg stage of *R. reniformis*Reddy and Khan; Waters and Barker; Wangs et al; Zhao et al. [8-11]. Furthermore, strains of the bacteria *Pasteuria* spp. and *Pseudomonas fluorescens* have been reported to reduce the number of *R. reniformis* in soil Hewlett et al; Jayakumar et al; Schmidt et al. [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) $1x10^8$, *Trichoderma harzianum* (Biozium) $1x10^8$, *Pseudomonas fluorescens* (Biomonas) $1x10^8$, *Pseudomonas fluorescens* &*Trichoderma harzianum* (Biofor-PF-2) ($1x10^9$) & ($1x10^8$), *Pseudomonas fluorescens* + *Trichoderma harzianum* + *Bacillus brevis* (Biozin-PTB) ($1x10^9$) & ($2x10^9$) & ($1x10^9$) 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 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 Vieira and Nahas [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 Christie and Perry; Muraellidharan[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 Byrd et al. [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 fluorescen*s @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.

Table 1. Effect of different treatments on the plant growth parameters of cowpea infected by *Rotylenchulus reniformis* (Mean of3replications)

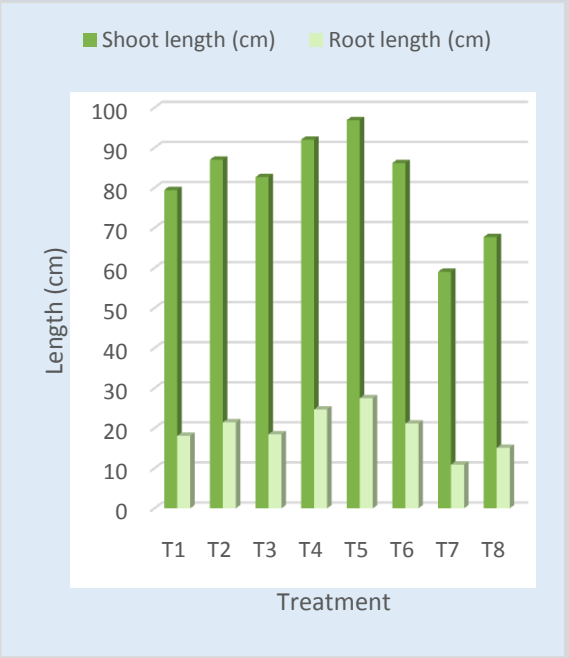
Treatments	Shoot length(cm)	% increase over untreated control	Freshweightofshoot (g)	% increase over untreated control	Dryweightofshoot (g)	% increase over untreated control	Root length(cm)	% increase over untreated control	Freshweightofroot(g)	% increase over untreated control	Dryweightofroot(g)
T_1	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}
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}
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}
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}
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}
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}
T7	59.03^{g}	-	21.16^{f}	-	9.00^{f}	-	10.93^{g}	-	5.10^{f}	-	1.06^{f}
T8	66.67^{f}	-	26.06^{e}	-	11.23^{e}	-	15.10^{f}	-	8.06^{e}	-	2.10^{e}
S.Ed.(±)	1.24	-	1.51	-	0.41	-	1.30	-	0.43	-	0.26
C.D(P=0.05)	2.66	-	3.23	-	0.88	-	2.79	-	0.92	-	0.54

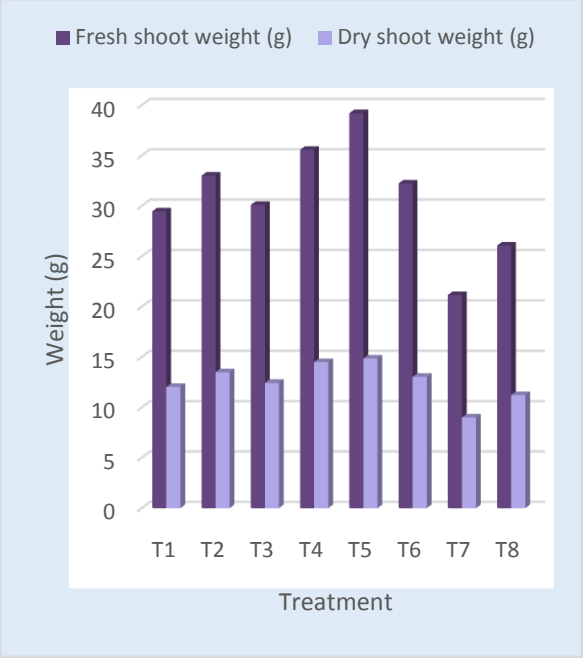
Meanfollowedbythesameletterinthesuperscript(s)arestatistically*atpar*.*T1:SoiltreatmentwithBioveer(*Trichodermaviride*AAUstrain);T2:Soiltreatment with Biozium (*Trichoderma harzianum* AAU strain); T3: Soil treatment with Biomonas (*Pseudomonas fluorescens* AAU strain); T4: Soiltreatmentwith Biofor-PF2 (*Trichoderma harzianum + Pseudomonas fluorescens* AAU strain); T5: Soil treatment with Biozin-PTB (*Trichodermaharzianum + Pseudomonas fluorescens + Bacillus brevis* AAU strain); T6: Soil treatment with Nimbecidine; T7: Untreated control; T8: Uninoculatedcontrol.

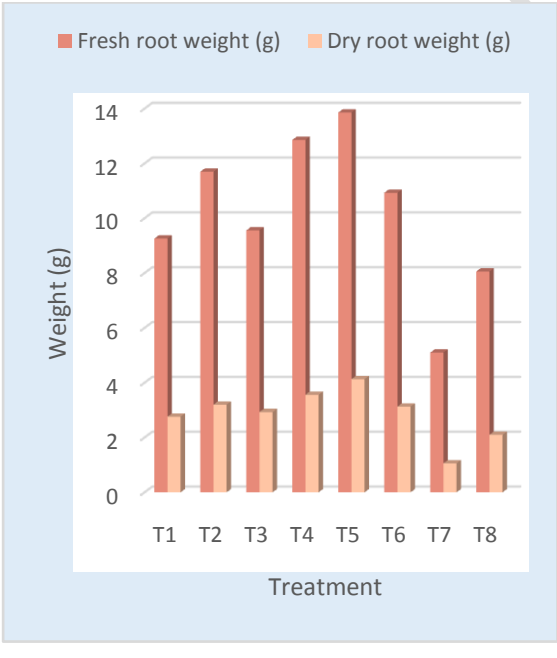
Table 2.Effectofdifferenttreatmentsonnumberoffemales,eggmassesandnematodepopulationof*Rotylenchulus reniformis*oncowpea(Meanof3 replications)

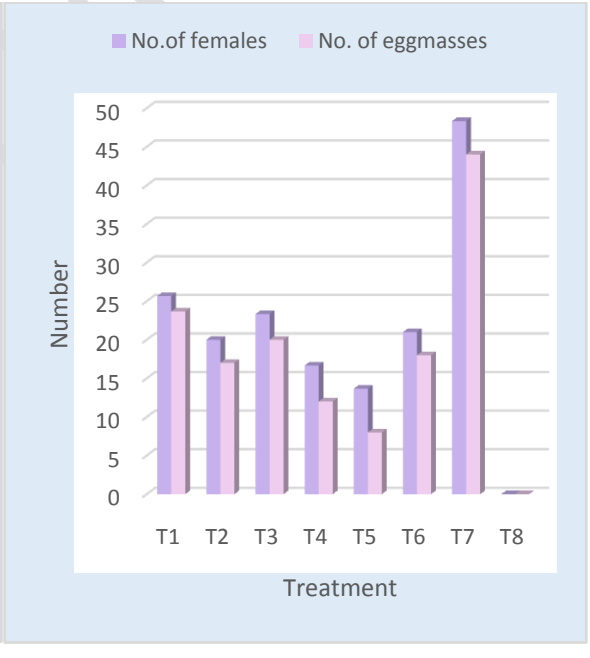
Treatments	No.offemales/ rootsystem	% decrease over untreated control	No.ofeggmasses /rootsystem	% decrease over untreated control	Larvalpopulation/ 200ccof soil	% decrease over untreated control	Total population/pot	% decrease over untreated control	Reproductive rate(%)	cfu/g of soil
T_1	25.67 (5.16)^{b}	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	-

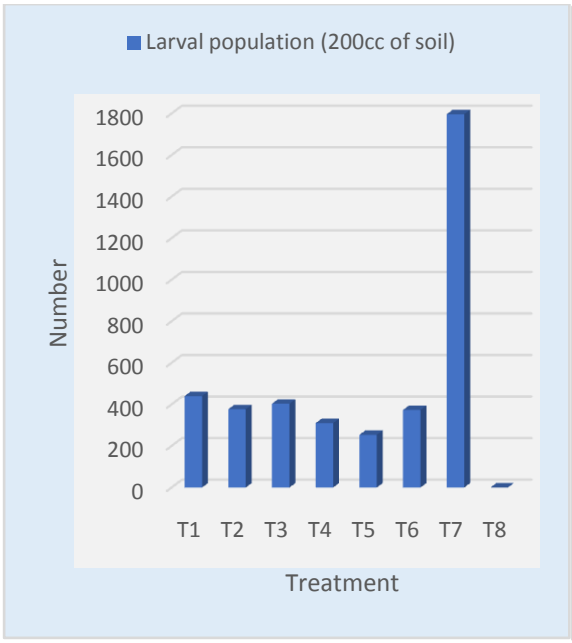
Meanfollowedbythesameletterinthesuperscript(s)arestatistically*atpar*.*T1:SoiltreatmentwithBioveer(*Trichodermaviride*AAUstrain);T2:Soiltreatment with Biozium (*Trichoderma harzianum* AAU strain); T3: Soil treatment with Biomonas (*Pseudomonas fluorescens* AAU strain); T4: Soiltreatmentwith Biofor-PF2 (*Trichoderma harzianum + Pseudomonas fluorescens* AAU strain); T5: Soil treatment with Biozin-PTB (*Trichodermaharzianum + Pseudomonas fluorescens + Bacillus brevis* AAU strain); T6: Soil treatment with Nimbecidine; T7: Untreated control; T8: Uninoculatedcontrol

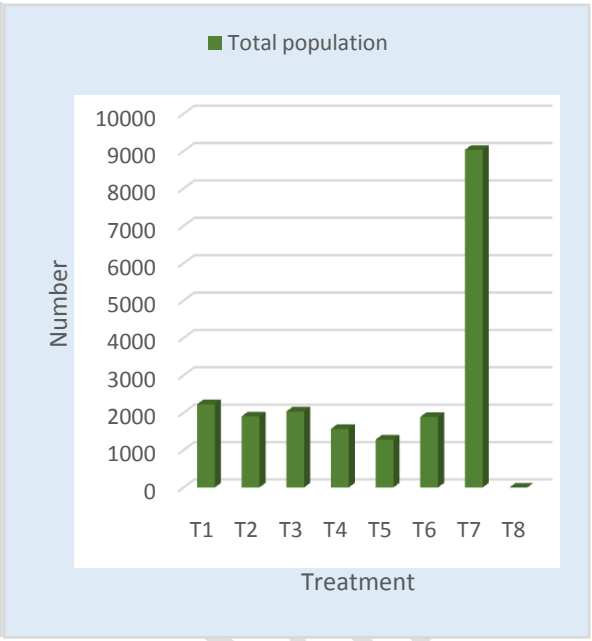

Shoot length (cm)
Root length (cm)
Length (cm)
100
90
80
70
60
50
40
30
20
10
0
T1 T2 T3 T4 T5 T6 T7 T8
Treatment

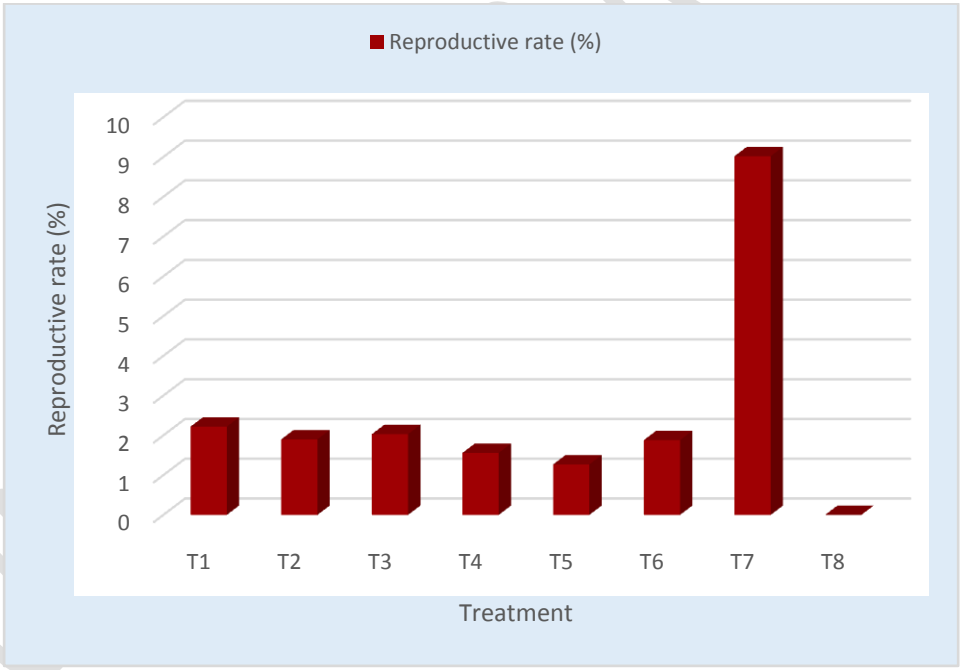

Fresh shoot weight (g)
Dry shoot weight (g)
Weight (g)
40
35
30
25
20
15
10
5
0
T1 T2 T3 T4 T5 T6 T7 T8
Treatment


Fresh root weight (g)
Dry root weight (g)
Weight (g)
14
12
10
8
6
4
2
0
T1 T2 T3 T4 T5 T6 T7 T8
Treatment


No.of females
No. of eggmasses
Number
50
45
40
35
30
25
20
15
10
5
0
T1 T2 T3 T4 T5 T6 T7 T8
Treatment


Larval population (200cc of soil)
Number
1800
1600
1400
1200
1000
800
600
400
200
0
T1 T2 T3 T4 T5 T6 T7 T8
Treatment


Total population
Number
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
T1 T2 T3 T4 T5 T6 T7 T8
Treatment


Reproductive rate (%)
Reproductive rate (%)
10
9
8
7
6
5
4
3
2
1
0
T1 T2 T3 T4 T5 T6 T7 T8
Treatment

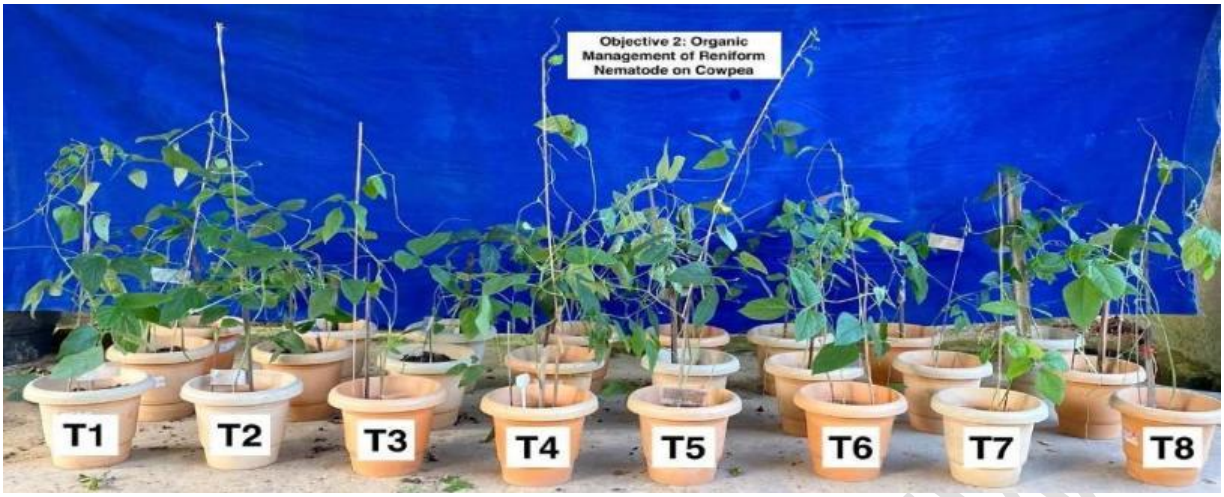


Figure 8. Overview ofthepotexperiment oncowpea

Figure 9.Effectofdifferenttreatmentsonplantgrowthofcowpea infectedby reniform nematode

Figure 10.Comparison betweenthebesttreatment(T5)anduntreatedcontrol(T7)

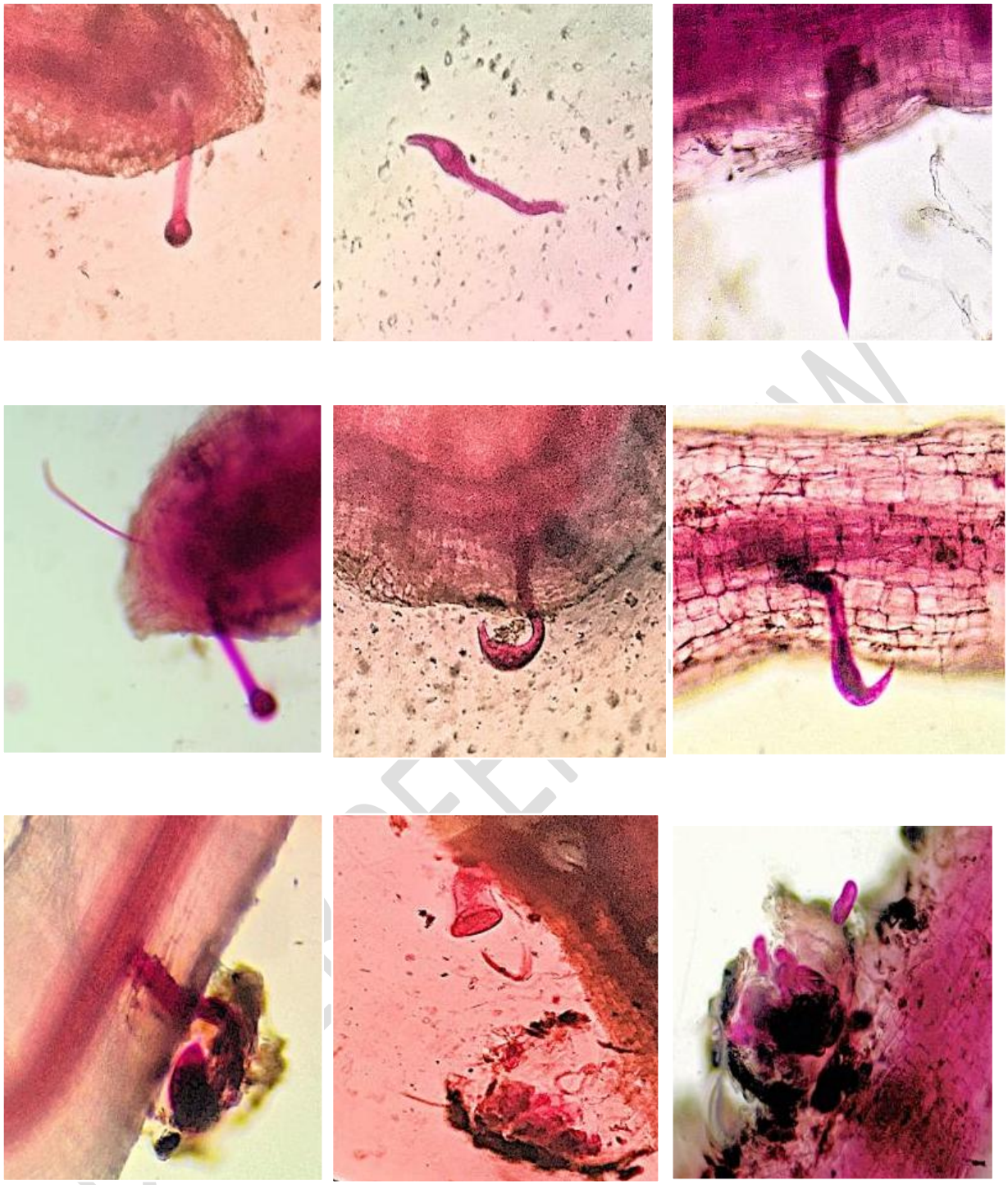


Figure 11. Post penetration development of *Rotylenchulus reniformis*and eggmassin untreated control

Figure 12. Immature female of reniform nematode

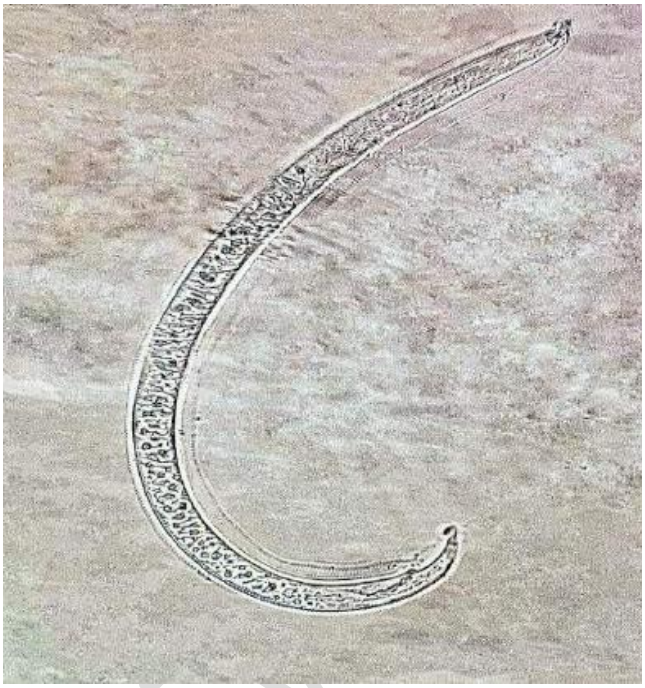


Figure 13. Male of reniform nematode

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*.

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