www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(9): 475-478

www.thepharmajournal.com
Received: 19-07-2021
Accepted: 21-08-2021

Pawan Kumar
Department of Nematology, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Dr. HK Sharma
Department of Nematology, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Corresponding Author:
Pawan Kumar
Department of Nematology, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Bio efficacy of bio-agents against reniform nematode, *Rotylenchulus reniforrmis* infesting chickpea (*Cicer arietinum* L.)

Pawan Kumar and Dr. HK Sharma

Abstract
Reniform nematode, *Rotylenchulus reniformis* is considered to be the most important nematode of pulse crops including chickpea. Farmers experience chronic losses by reniform nematode because of its wide distribution and high frequency in all agro-climatic zones of India. Looking to it's economic importance, several pesticides have been tried for the management of nematode, but due to environmental pollution, health hazards, high cost and lack of easy availability, their adoption at farmer's level has been limited. Therefore, attempts were made to determine alternative methods which may be effective, economical and eco-friendly for the management of reniform nematode, *R. reniformis* on chickpea. 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*.

Keywords: chickpea, reniform nematode, *P. lilacinum*, *P. fluorescens* and *T. harzianum*

1. Introduction
Chickpea is a cool season legume crop and is grown in several countries worldwide as a food source. Chickpea (*Cicer arietinum* L.) is among the most widely consumed legumes in the world, particularly in tropical and subtropical areas (Chhangani). Seed is the main edible part of the plant and is a rich source of protein, carbohydrates and minerals especially for the vegetarian population. As in case of other legume crops, chickpea fix atmospheric nitrogen through its symbiotic association with *Rhizobium spp*. thus helping in enhancing the soil quality for subsequent cereal crop cultivation. Chickpea is basically grown in the dried region of India. The major chickpea producing States of India includes Rajasthan, Madhya Pradesh, Maharashtra, Uttar Pradesh, Andhra Pradesh and Karnataka. Two types of chickpeas are recognized, the white-seeded "Kabuli" and the brown coloured "Desi" types. Chickpea is a good source of carbohydrates and protein and protein quality is considered to be better than other pulses. Chickpea is rich in nutritionally important unsaturated fatty acids such as linoleic and oleic acids. β-Sitosterol, campesterol and stigmasterol are important sterols present in chickpea oil. Ca, Mg, P and especially K are also present in chickpea seeds. Mature chickpeas are cooked to prepare various delectable dishes in number of social functions. The Reniform nematode *Rotylenchulus reniformis* is an obligate sedentary semi-endo devastating nematode parasite attacks over 300 plant species belonging to 46 families grown in the tropical, sub-tropical and warm- temperate regions of the world (Robinson *et al*., 1997) [10]. The maximum number of pathogens has been reported from India alone with the number rising to 89 pathogens in 1995 from 35 in 1978 (Nene *et al*., 1991) [7]. In recent years, there has been tremendous increase in public awareness on environment pollution and climate change associated with pesticides toxicity and residues. This resulted in the shift in pest management strategies from chemical era in the late 1980s. Since then, the search for novel environmentally friendly.

2. Materials and methods
Experiment was performed in the pots to evoluted the management module for Reniform nematode *R. reniformis* on Chickpea. Pots filled with Reniform nematode infested soil taken from the pure culture field of Department of Nematology, RCA, Udaipur. Bio-agents *viz. Purpureocillium lilacinum, Pseudomonas fluorescens* and *Trichoderma harzianum* were used at ten, fifteen and twenty g/kg seed as seed treatment.

A standard check (*Trichoderma viride* at ten g/kg seed) and untreated check was also maintained for comparison with the experimental results. Weighed quantity of seeds (100 g) were taken in a beaker and few drops of gum aerobics were added and continuous stirred with the help of glass rod and after that the required quantity of bio-agents were added and mix thoroughly to spread the uniform coating of bio-agents over seeds. Initial nematode population was calculated before sowing. The experiment was laid out in completely randomized design and all the treatments were replicated four times. At most care was taken from sowing to till harvest of experiment for proper growth and development of plants. After ten days of sowing, one healthy plant in each pot was maintained and watered regularly as and when required. Plants were harvested after 45 days of sowing.

Observations on shoot length, shoot weight, root length, root weight and number of nodules per plant were taken at harvest. Then the root were washed carefully in tap water and stained with 0.1% acid fuchsin in lacto phenol and kept in lacto phenol for 24 hrs. Thereafter, the roots were examined thoroughly under a stereoscopic binocular microscope for counting number of females per plant, number of egg masses per plant and number of eggs and larvae per egg mass and final nematode population/200cc soil. After removing the plant from pots, soil was thoroughly mixed and 200cc from each pot were taken and processed by Cobb's sieving and decanting technique (N.A. Cobb, 1918). followed by Baremann's funnel technique (Christie & Perry, 1951) for estimation of nematode population in soil.

Preparation and maintenance of pure culture of *Rotylenchulus remformis*

Castor plants were infected with *R. reniformis* then uprooted from the difference micro plots of the department of nematology, Rajasthan college of agriculture, udaipur and brought into laboratory. In our department of nematology population or colony of Reniform nematode has been already maintained on castor crop in micro plots therefore, Reniform nematode isolated from castor plants. The roots were first rinsed carefully in tap water to remove the attached soil particle from the root. Egg masses were carefully take out from roots under a stereoscopic binocular microscope while using teasing needle and forcep. For the hatching of eggs using Baermann's funnel method in which the freshly picked eggmasses were kept on it. The larvae were kept in water for about ten days to reach up to pre adult stage. The obtained culture was inoculated on ten days old chickpea plant seedlings raised in sterilized soil in earthen day pots of nine inch size to provide adequate pure population of Reniform nematode, *R. reniformis* on plants and in soil to carry out further studies.

Methodology

Weighed quantity of seeds were taken in a beaker, added few drops of gum and stirred with the help of glass rod and there after required quantity of bio-agents were added to it and mix thoroughly to provide uniform smooth coating of bio-agent over seeds. The chalk powder was used as drying agent. The experiment was laid out in completely randomized design and all the treatments were replicated four times. The soil samples were collected before sowing to determine initial inoculum level. After 10 days of sowing, one healthy plant in each pot was maintained and watered regularly as and when required. Observations on plant growth parameters *viz.*, shoot length (cm), shoot weight (g), root length (cm), root weight (g) and No. of nodules per plant as well as nematode reproduction parameters *i.e.* number of females per plant, number of egg masses per plant, number of eggs & larvae per egg mass and final nematode population per 200cc soil were taken 45 days after sowing. For studying the nematode infection, the roots were stained with 0.1% acid fuchsin lacto-phenol at 80ºC for 2-3 minute (McBeth, Taylor and Smith).

3. Results and Discussion

3.1 Effect of different bio-agents as seed treatment for the management of Reniform nematode, *R. reniformis* on chickpea

The results of experiments showed that the different bio-control agents namely *P. lilacinum*, *P. fluorescens and T. harzianum* had significantly reduced the number of egg masses per plant, number of eggs and larvae per egg mass, number of females per plant and final nematode population per 200cc soil and considerably increase the shoot and root length, shoot and root weight and number of nodules per plant of chickpea. Among all bio-control agents, *Purpureocillium lilacinum* was better as compared to *Pseudomonas fluorescens* for the management of *R. reniformis* on chickpea crop. However all the treatments were significantly better over untreated check. The results for the recorded observations are shown below points which are presented in Table.1.

The result of experiment showed that the seed treatment of *Purpureocillium lilacinum* at 20g/kg seed significantly reduced the number of females /plant, number of eggmasses/ plant, number of eggs & larvae /egg mass and final nematode population/200cc soil and considerably increased the shoot length, shoot weight, root length, root weight and number of nodules per plant as compared to control. Among all bio-agents *Purpureocillium lilacinum* was better as compared to *Pseudomonas fluorescens* and *T. harzianum* against reniform nematode, *R. reniformis* on chickpea, recorded observations are presented in Table 1.

A. Plant Growth Parameters

Data presented in Table-1 revealed that the seed treatment of *Purpureocillium lilacinum* significantly increased shoot length (81.81%), shoot weight (71.98%), root length (81.35%), root weight (109.93%) and number of nodules per plant (29.35%) as compared to control. Among all the treatments, maximum shoot length (90.90%), shoot weight (80.44%), root length (104.38%), root weight (115.12%) and number of nodules per plant (33.02%) were observed with *T. viride* at 10g/kg seed which was maintained as standard check and it differed significantly from rest of the treatments.

B. Nematode Parameters

Data presented in Table-1 revealed that the seed treatment of *Purpureocillium lilacinum* at 20g/kg seed significantly reduced the number of females per plant (44.26%), number of eggmasses/ plant (58.82%), number of eggs & larvae /egg mass (44.38%) and final nematode population/ 200cc soil (49.35%) as compared to control. However, *T. viride* was found superior in terms of reducing infection as compared to other treatment. On the whole, *Purpureocillium lilacinum* 20g/kg as seed treatment proved to be most effective in reducing the infection of reniform nematode, *R. reniformis* and to boost up plant growth characters of chickpea. The present findings are in the line with the findings of Bari *et al*.

(2004) who reported that *T. harzianum* @ 1 g/plant reduced root-knot nematode population and enhancing vegetative growth of lady's finger in the field. Similarly Pandey (2005) [8] reported that *T. harzianum* as soil application significantly enhanced crop yield of menthol mint (*Mentha arvensis*) cv. Kosi and reduced the nematode populations and root-knot indices. While Pathak *et al.* (2005) [9] found that *T. harzianum* @ 4 g/kg soil significantly improved the plant growth characters and suppressed the number of galls, penetrations of *M. graminicola* larvae and final nematode population in soil when compared with control and Joshi *et al.* (2012) [12], reported that among fugal bio-agents, *P. lilacinus* @ 2 g/kg soil was best treatment in increasing plant growth and in reducing nematode reproduction over other fungal bio-agents. Mahdy *et al.* (2006) [5] used powder formulation of *T. harzianum* as soil application for the management of root-knot and root rot disease complex, caused by the root-knot nematode *M. javanica* and the fungus *R. solani*, on soybean. Number of galls, root galling, egg masses and disease severity were reduced sharply with the application of *T. harzianum* over the control.

In the present investigation among dose, 2 g/kg seed for each bio-agent was found most effective in reducing nematode reproduction over 1 g/kg soil. These finding are in agreement with Barua and Bora (2008) [2] who reported significant increased the plant growth and reduction in final nematode population when treated with *T. harzianum* and *P. fluorescens* at higher level.

Table 1: Effect of bio-agents against reniform nematode, *Rotylenchulus reniformis* on chickpea as seed treatment

Treatments	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	No. of Nodules/ plant	No. of egg masses/ plant	No of eggs and larvae/ egg mass	No of females/ plant	Final nematode population
	A	B	C	D	E	F	G	H	I
P. lilacinum 10 g/kg seed (T_1)	25.00 (51.51)	13.79 (37.48)	27.34 (51.04)	5.85 (32.05)	29.75 (9.17)	16.50 (22.35)	64.25 (27.80)	29.00 (4.91)	505.00 (35.25)
P. lilacinum 15 g/kg seed (T_2)	25.70 (55.75)	16.34 (62.91)	30.05 (66.02)	7.53 (69.97)	32.75 (20.18)	12.00 (43.52)	55.00 (38.20)	20.50 (32.78)	440.00 (43.58)
P. lilacinum 20 g/kg seed (T_3)	30.00 (81.81)	18.19 (81.35)	31.13 (71.98)	9.30 (109.93)	35.25 (29.35)	8.75 (58.82)	49.50 (44.38)	17.00 (44.26)	395.00 (49.35)
P. fluorescens 10 g/kg seed (T_4)	23.27 (41.03)	12.16 (21.23)	26.40 (45.85)	5.77 (30.24)	28.75 (5.50)	16.75 (21.17)	69.00 (22.47)	25.75 (15.57)	550.00 (29.48)
P. fluorescens 15 g/kg seed (T_5)	27.24 (65.09)	15.98 (59.32)	29.45 (62.70)	7.44 (67.94)	31.50 (15.59)	13.75 (35.29)	57.75 (35.11)	21.75 (28.68)	465.00 (40.38)
P. fluorescens 20 g/kg seed (T_6)	28.50 (72.72)	17.23 (71.78)	31.01 (71.32)	9.15 (106.54)	34.75 (27.52)	9.50 (55.29)	52.75 (40.73)	18.75 (38.52)	410.00 (47.43)
T. harzianum 10 g/kg seed (T_7)	19.83 (20.18)	11.00 (9.67)	22.10 (22.09)	5.19 (17.15)	23.25 (14.67)	18.00 (15.29)	80.50 (9.55)	27.50 (9.83)	630.00 (19.23)
T. harzianum 15 g/kg seed (T_8)	23.10 (40.00)	11.98 (19.44)	23.80 (31.49)	5.60 (26.41)	24.00 (11.92)	17.00 (20.00)	74.50 (16.29)	26.25 (13.93)	585.00 (25.00)
T. harzianum 20 g/kg seed (T_9)	26.77 (62.24)	14.29 (42.47)	28.75 (58.83)	5.96 (34.53)	30.50 (11.92)	15.25 (28.23)	59.50 (33.14)	23.50 (22.95)	480.00 (38.46)
T. viride 10 g/kg seed (T_{10})	31.50 (90.90)	20.50 (104.38)	32.66 (80.44)	9.53 (115.12)	36.25 (33.02)	7.25 (65.88)	47.50 (46.62)	15.00 (50.81)	375.00 (51.92)
Untreated check (T_{11})	16.50	10.03	18.10	4.43	27.25	21.25	89.00	30.50	780.00
Sem+	0.74	0.28	0.62	0.22	0.97	0.45	1.99	0.72	14.98
CD at 5%	2.12	0.81	1.80	0.65	2.80	1.29	5.74	2.06	43.10

Note: Data are average value of four replications Initial inoculum level: 595 pre adults per 200 cc soil
Data in parantheses are per cent increase*/decrease** over check

5. Conclusion

The present investigation reveals that there is a potential scope for the utilization of different bio-agents for nematode control. However further research should be carried out to testing the different bio-agents under different conditions for managing the plant parasitic nematodes.

6. References

1. Bari MK, Faruk MI, Rahman ML, Ali MR. Management options for root-knot nematode in lady's finger. Bangladesh Journal of Plant pathology 2004;20:49-51.
2. Barua L, Bora BC. Comparative efficacy of Trichoderma harzianum and *Pseudomonas fluorescens* against Meloidogyne incognita and *Ralstonia solanacearum* complex in brinjal. Indian Journal of Nematology. 2008;38:86-89.
3. Chhangani G, Vyas A, Mahla MK, Tali MK. Impact of farmscaping on the bioecology of gram pod borer. Journal of Entomological Research 2018;42:495-498
4. Joshi G, Bhargava S, Sharma MK. Evaluation of fungal bio-agents and plant extracts for the management of Meloidogyne incognita in tomato. Journal of Mycology and Plant pathology 2012;42(04):523-525.
5. Mahdy ME, EI-Shennawy RZ, Khalifa EZ. Biological control of *Meloidogyne javanica* and *Rhizoctonia solani* on soyabean by formulation of *Bacillus thuringiesis* and *Trichoderma harzianum*. Arab Universities Journal of Agriculture Sciences 2006;14:411-423.
6. McBeth CW, Taylor AL, Smith AL. Note on staining nematodes in root tissues. Proceeding of Helminthological Society of Washington 1941;8:26.
7. Nene YL, Reddy MV, Haware MP, Ghaneka AM, Amin KS. Field diagnosis of chickpea diseases and their control. Info Bull No. 28, ICRISAT, Hyderabad, India 1991.
8. Pandey R. Field application of bio-organics in the management of *Meloidogyne incognita* in Mentha arvensis. Nematologia Mediterranea 2005;33:51-54.
9. Pathak KN, Ranjan R, Kumar M, Kumar B. Bio-management of *Meloidogyne graminicola* by *Trichoderma harzianum* and *T. virens* in rice. Annals of plant protection sciences 2005;13:438-440.

10. 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.