Original Research Article

Investigation on management of root-knot nematode, *Meloidogyne incognita* through soil application of biocontrol agents in Field Pea

Abstract: The pot experiment was conducted at AICRP on vegetables, Pusa farm, Dr. Rajendra Prasad Central Agricultural University during 2020-21. The bio-control agents viz. *Glomus fasciculatum* (85-90 spores/g), *Trichoderma harzianum* 1.0% WP (2×106 cfu/g), *Pseudomonas fluorescens* 1.0% WP (1×108 cfu/g), *Purpureocillium lilacinum* 1.0% WP (2×106 cfu/g) either singly or in combined application shown significant improvement in plant growth and development and in declining nematode population. The combined application of *P. fluorescens* 1.0% WP (1×10^{8} $cfug^{-1}$) and *P. lilacinum* 1.0% WP (2×10^{6} $cfug^{-1}$) @ 10g per pot each is efficient in improving plant growth and on other hand, plants treated with Cartap hydrochloride 4G @ 5g per pot had the lowest nematode population, galls per plant, and Reproduction factor (Rf). *P. lilacinum* 1.0% WP (2×10^{6} cfu/g) @ 10g/pot demonstrated promising effects in plants when just single bio-control agent was used. This study discovered that utilizing a mixture of bio-control agents was more effective than using bio-control agents alone in reducing the population of M. incognita. According to the study, bio-control agents had the same effects as Cartap hydrochloride 4G. As a result, bio-control agents can be used instead of nematicides.

Keywords: Biocontrol, *Meloidogyne incognita*, *Purpureocilium lilacinum*, *Pseudomonas fluorescens*, *Trichoderma harzanium*

1. Introduction:

Plant-parasitic nematodes, popularly known as “hidden foes to farmers”, are a key limiting factor in crop productivity. Root-Knot Nematode, *M. incognita* is a polyphagous and detrimental pest of field pea, *Pisum sativum* var. *arvense* and has been observed to be a great obstacle to field pea production i.e., accounts for 40-45% loss in pea [1] Apart from causing direct losses in yields, they also play a significant role in disease-complexes with other pathogens [2]. They establishes a parasitic relationship with host plants and produces transfer cells or metabolic sinks i.e., giant cells. These giant cells transfer the nutrients consumed by the roots to the nematodes for their growth and development. Thus, root development and plant growth is hampered [3]. The life cycle of most of the root knot nematode species takes between 25 and 40 days at temperatures ranging from 25 and 30℃ [4, 5]. The infected plants shown stunting, yellowing of leaves, patchy symptoms and roots were severely galled, poor plant growth and followed by chlorosis [6, 7]. Different treatments, such as nematicides, resistant cultivars, crop rotation, hot water treatment, and various cultural practices are utilized to alleviate the losses evoked by the root knot nematode, Meloidogyne incognita. The continued use of nematicides is limited owing to their skyrocketing cost and it is harmful to human health and the environment by diminishing beneficial soil flora and fauna in soil ecosystems [8] as well as toxicity from lingering effects. The creation of resistant cultivars is a lengthy and difficult procedure. There is also constraint for farmers to procure them. Cultural approaches are widely used, however they do not produce satisfactory results and thus farmers are forced to use other methods. As a result, there is an urgent need for a necessary alternative technique that is both effective and environmentally acceptable, such as organic amendments, bio-pesticides, and so

on. Biological control is seen as an eco-benign and cost-effective alternative to chemical nematicides.

Biological control methods diminish nematode population density and fungi, bacteria, viruses, and other species have exhibited antagonistic action against plant parasitic nematodes [9,10,11]. Of the micro-organisms that parasitize or prey on nematodes, fungi hold an important position and some of them have shown great potential as bio-control agents [12,13] *Trichoderma* spp. are basically mycoparasites [14], but in recent years, their suppressive effects against plant nematodes have also been reported [15,16]. Soils rich in organic matter are generally colonized by some bio-control agents such as *T. harzianum* that improve bio-control activity [17]. *Trichoderma* spp. are also highly rhizosphere component i.e., able to colonize on roots as they develop, thus promote plant growth. They may also exert several other mechanisms such as tolerance to stress through enhanced root and plant development, induced resistance, inactivation of the pathogens enzymes in promoting plant growth and suppressing plant pathogens [18]. VAM fungi, *Glomus fasciculatum* not only increasing plant growth parameters but also confer resistance to major plant pathogens. The VAM fungi provides mechanical barrier i.e., the fungal mantle and also act as antagonistic rhizosphere micro-organisms. *P. lilacinum* is an egg-pathogenic fungus and one of the most widely tested fungus for the control of root knot nematodes [19 , 20]. *Pseudomonas fluorescens* is a plant growth promoting rhizhobacteria (PGPR) suppresses root knot nematodes by modifying the root exudates and thereby prevention of juvenile penetration. They also regulate nematode population by production of siderophore, hydrogen cyanide and through induction of systemic resistance [21]. Bearing in mind the above points, an investigation was setup to test the efficacy of commercially available bio-control agents, *Glomus fasciculatum, Trichoderma harzianum, Pseudomonas fluorescens* and

P. lilacinum as treatments singly or in combination along with Cartap hydrochloride 4G as standard chemical check against root knot nematode, *M. incognita* infestation on field pea.

Material and Methods:

1.1. Nematode inoculum

The root knot infested galled roots were collected from infested soils of field pea plants. The adult females were first collected and distinguished morphologically by perineal patterns, the key identification characteristic of root knot nematode, *Meloidogyne incognita.* To obtain J2s, egg masses retrieved from infected tomato and were incubated in distilled water for three days at 28 ± 2°C in the dark. A nematode stock solution with a final concentration of 100 ± 5 J2 ml^{-1} was prepared. The harvested juveniles were inoculated on the roots of tomato plants cv. Pusa ruby in glasshouse for maintenance of pure culture and were used for further experiments.

1.2.Bio-control agents and Nematicide:

The biocontrol agents *Glomus fasciculatum* (85 to 90 spores/g), *Trichoderma harzianum* 1.0% WP (2×10^6 $cfug^{-1}$), *Pseudomonas fluorescens* 1.0% WP (1×10^8 $cfug^{-1}$), *Purpureocillium lilacinum* 1.0% WP (2×10^6 $cfug^{-1}$) and the nematicide, Cartap hydrochloride 4G were procured from a commercial store and quality test of commercial agents was done *In-vitro* by checking the spore count by haemocytometer and viable colonies formed by them on potato dextrose agar media.

2.3. *In vivo* nematicidal assay with different biocontrol agents

The well pulverized and sterilized pot mixture containing sandy loam soil, sand and FYM in 2:1:1 ratio was filled in the earthen pots. The selected biocontrol agent were mixed with

vermicompost and applied @ 10 g pot^{-1} each (T1-T4), then in different possible combinations of bio-control agents@ 10g pot^{-1} (T5-T10), Treated check with Cartap hydrochloride 4G@ 5 g pot^{-1} and untreated control with only nematodes were applied two weeks after sowing. Field pea variety, HUDP-15 seeds were sown in pots. Each treatment was replicated thrice in Completely Randomized Design (CRD). The treatment schedule applied was: T1- Soil application of *G. fasciculatum* @ 10 g pot-1; T2- Soil application of *T. harzianum* @ 10 g pot-1: T3- Soil application of *P. fluorescens* @ 10 g pot^{-1};T4- Soil application of *P. lilacinum* @ 10 g pot^{-1}; T5- *G. fasciculatum* @ 10 g pot^{-1} + *T. harzianum* @ 10 g pot-1; T6- *G. fasciculatum* @ 10 g pot^{-1} + *P. fluorescens* @ 10 g pot^{-1}; T7- *G. fasciculatum* @ 10 g pot^{-1} + *P. lilacinum* @ 10 g pot^{-1};T8- *T. harzianum* @ 10 g pot^{-1} + *P. fluorescens* @ 10 g pot^{-1}; T9- *T. harzianum* @ 10 g pot^{-1} + *P. lilacinum* @ 10 g pot^{-1}; T10- *P. fluorescens* @ 10 g pot^{-1} + *P. lilacinum* @ 10 g pot^{-1}; T11- Cartap hydrochloride 4G @ 5 g pot^{-1} (Treated check); T12- Untreated check

Plants were grown in conditions at an average temperature of 9.3 to 21.1°C, 12 h: 12 h L: D with 90-95% relative humidity. Every second or fourth day, the plants were watered. After 45 days of inoculation, the plants were uprooted, and roots and aerial parts (stem with leaves) and pods for each plant were separated. The length of main stem and root, fresh weight of shoot and root, dry weight of shoot and root (after 3 days in oven at 55-60°C), number of pods/plant and weight of pods were recorded. For assessing nematode reproduction, the number of root galls per plant, number of eggs per gram of root, initial nematode population, final nematode population and Rf were determined.

2.4.Statistical analysis and data interpretation

The experiment was carried out in Completely Randomized Design (CRD) with twelve treatments, each treatment replicated thrice. The data on number of root galls per root, egg masses

per root and final nematode population in soil and root were analyzed after square root transformation. The Fisher's methods of analysis of variance at 5% level of significance were followed. Further, the comparison of the treatment means was done by calculating standard error of mean S.E. (m) and critical difference (C.D.) in the following manner:

$$\text{S.E. (m) (Standard Error of Mean)} = \sqrt{2 \times \frac{EMS}{r}}$$

$$\text{C.D. at } 0.05 = t_{\text{at } 0.05 \text{ error d.f.}} \times \text{S.E. (m)}$$

Where,

df = Degree of freedom

r = Number of replication

EMS = Error mean sum of square

The difference between the means of two treatments, if greater than the CD value, it indicated the significant difference between the two treatments. In this manner comparison between the two treatments was made.

3. RESULTS AND DISCUSSION:

The results reported were shown significant difference among the treatments at P=0.05% level of significance and observed increase in the plant growth parameters when compared to control. The plant growth promotion parameters i.e., root length (13.52 cm) and plant height (96.00 cm), fresh weight of root (9.7g) and shoot (26.24g), dry weight of root (1.53 g) and shoot (3.50), number of pods (8.02) and pod weight (35.00) was more in plants treated with combination of biocontrol agents, *P. fluorescens* and *P. lilacinum* @ 10 g pot^{-1} each compared to control. The data on plant growth parameters was presented in Table 1, 2 and 3. The application

of *T. harzianum* @ 10 g pot^{-1} and *P. lilacinum* @ 10 g pot^{-1} shown on par results with the effective treatment.

In case of nematode multiplication parameters, the lowest mean number of galls were observed in Cartap hydrochloride 4G @ 5g/pot applied plants (13.40) i.e., 80.15 percent reduction over control. Among the treatments, the combination of bio-control agents *P. fluorescens* and *P. lilacinum* @ 10g/pot each (17.75) has shown effective results i.e., 73.17 percent reduction over control and it was followed by *T. harzianum* and *P. lilacinum* @ 10g/pot each (19.37), and highest mean number of galls per plant was recorded in untreated check (67.55) (Table 4; Fig 1). The observations recorded on mean number of egg masses per plant shown significant reduction over control. The complete formation of egg masses were not observed in the treatments Cartap hydrochloride 4G @ 5g/pot,*T. harzianum* and *P. lilacinus* @ 10g/pot each, *P. fluorescens* and *P. lilacinum* @ 10g/pot each i.e., 100 percent control . The lowest mean number of final nematode population was seen in Cartap hydrochloride 4G @ 5g/pot (310.20) i.e., 85.31 percent reduction over control (Table 4). The highest Rf was observed in untreated check i.e., 2.11 and lowest Rf was seen in Cartap hydrochloride 4G @010g/pot (0.31). However, the effective results were observed with chemical check i.e., Cartap hydrochloride 4G @ 5g/pot (Table 5).

Table 1. Effect of treatments on Plant height and Root length (Mean of 3 replicates)

S. No.	Treatment	Plant height(cm)	% increase over control	Root length (cm)	% increase over control
1	T1	65.46	18.67	10.90	11.03
2	T2	63.33	14.81	10.66	8.55
3	T3	65.96	19.57	11.18	13.88
4	T4	67.33	22.03	11.79	20.12
5	T5	76.30	38.32	10.65	8.48
6	T6	66.00	19.65	10.85	10.52
7	T7	74.60	35.24	11.19	14.01
8	T8	65.46	18.67	11.46	16.70
9	T9	94.00	70.41	12.73	29.60
10	T10	96.00	74.03	13.52	37.60
11	T11	86.30	56.45	12.45	26.70
12	T12 (Untreated check)	55.16		9.82	
	Mean	73.70		11.43	
	S.Em. ±	1.45		0.09	
	CD (P = 0.05)	4.26		0.26	
	CV (%)	3.4		1.4	

Table 2. Effect of treatments on dry weight of root and shoot (Mean of 3 replicates)

S. No.	Treatment	Fresh wt. of root (g)	% increase over control	Fresh wt. of shoot (g)	% increase over control	Dry wt. of root (g)	% increase over control	Dry wt. of shoot (g)	% increase over control
1	T1	6.56	20.93	19.24	15.34	1.22	2.52	2.25	3.20
2	T2	6.16	13.56	20.78	24.62	1.20	0.84	2.21	1.37
3	T3	6.46	19.09	23.11	38.54	1.23	3.36	2.37	8.71
4	T4	6.86	26.45	23.21	39.14	1.26	5.88	2.44	11.92
5	T5	7.03	29.52	19.46	16.70	1.28	7.56	2.45	12.38
6	T6	7.93	46.1	19.72	18.24	1.32	10.92	2.71	24.31
7	T7	7.53	38.73	21.04	26.15	1.35	13.44	2.80	53.29
8	T8	8.50	56.53	21.40	28.29	1.37	15.12	2.86	54.09
9	T9	9.19	69.30	24.52	47.00	1.41	18.48	3.02	54.89
10	T10	9.70	78.63	26.24	57.33	1.53	28.57	3.50	60.07
11	T11	8.05	48.37	24.80	48.68	1.30	9.24	3.01	54.69
12	T12 (Untreated check)	5.43	5.43	16.68		1.19		2.18	
	Mean	7.45		21.68		1.30		2.65	
	S.Em. ±	0.06		0.12		0.03		0.07	
	CD (P = 0.05)	0.08		0.37		0.09		0.21	
	CV (%)	3.5		1.0		4.5		4.8	

Table 3. Effect of treatments on Pod yield (Mean of 3 replicates)

S. No.	Treatment	No. of pods	% increase over control	Weight of pods (g)	% increase over control
1	T1	5.43	8.38	27.28	15.00
2	T2	5.13	2.39	25.59	7.88
3	T3	5.54	10.57	28.67	20.86
4	T4	5.80	15.76	30.34	27.90
5	T5	5.62	12.17	29.28	23.44
6	T6	7.67	53.09	34.23	44.30
7	T7	7.68	53.29	34.33	44.73
8	T8	7.72	54.09	34.31	44.64
9	T9	7.76	54.89	34.34	44.77
10	T10	8.02	60.07	35.00	47.55
11	T11	7.75	54.69	34.41	45.03
12	T12 (Untreated check)	5.01		23.72	
	Mean	6.59		30.95	
	S.Em. ±	0.11		0.78	
	CD (P = 0.05)	0.16		2.30	
	CV (%)	3.0		4.4	

Table 4. Effect of treatments on Galls and Egg masses (Mean of 3 replicates)

S. No.	Treatment	No. of galls	% decrease over control	No. of egg masses	% decrease over control
1	T1	56.40	16.50	25.93	55.39
2	T2	48.50	28.20	27.23	53.15
3	T3	29.24	56.70	17.73	69.49
4	T4	28.87	57.26	7.83	56.53
5	T5	27.33	59.54	9.96	82.86
6	T6	26.53	60.72	7.430	87.21
7	T7	23.65	64.98	5.53	90.48
8	T8	21.40	68.31	3.00	94.83
9	T9	19.37	71.31	0	100.00
10	T10	17.75	73.71	0	100.00
11	T11	13.40	80.15	0	100.00
12	T12 (Untreated check)	67.55		58.13	
	Mean	31.66		13.56	
	S.Em. ±	0.68		0.31	
	CD (P = 0.05)	2.02		0.91	
	CV (%)	3.8		4.0	

Table 5. Effect of treatments on final nematode population (Mean of 3 replicates)

S. No.	Treatment	Initial nematode population (Pi)	Final nematode population (Pf)	% decrease over control	Rf
1	T1	1000	648.20	69.30	0.64
2	T2	1000	515.33	75.60	0.51
3	T3	1000	521.50	75.31	0.52
4	T4	1000	467.93	77.85	0.46
5	T5	1000	447.76	78.8	0.44
6	T6	1000	426.43	79.81	0.42
7	T7	1000	437.66	79.28	0.43
8	T8	1000	453.79	78.52	0.45
9	T9	1000	432.43	79.53	0.43
10	T10	1000	322.83	84.7	0.32
11	T11	1000	310.20	85.31	0.31
12	T12 (Untreated check)	1000	2112.80		2.11
	Mean		591.40		
	S.Em. ±		0.95		
	CD (P = 0.05)		2.80		
	CV (%)		0.28		

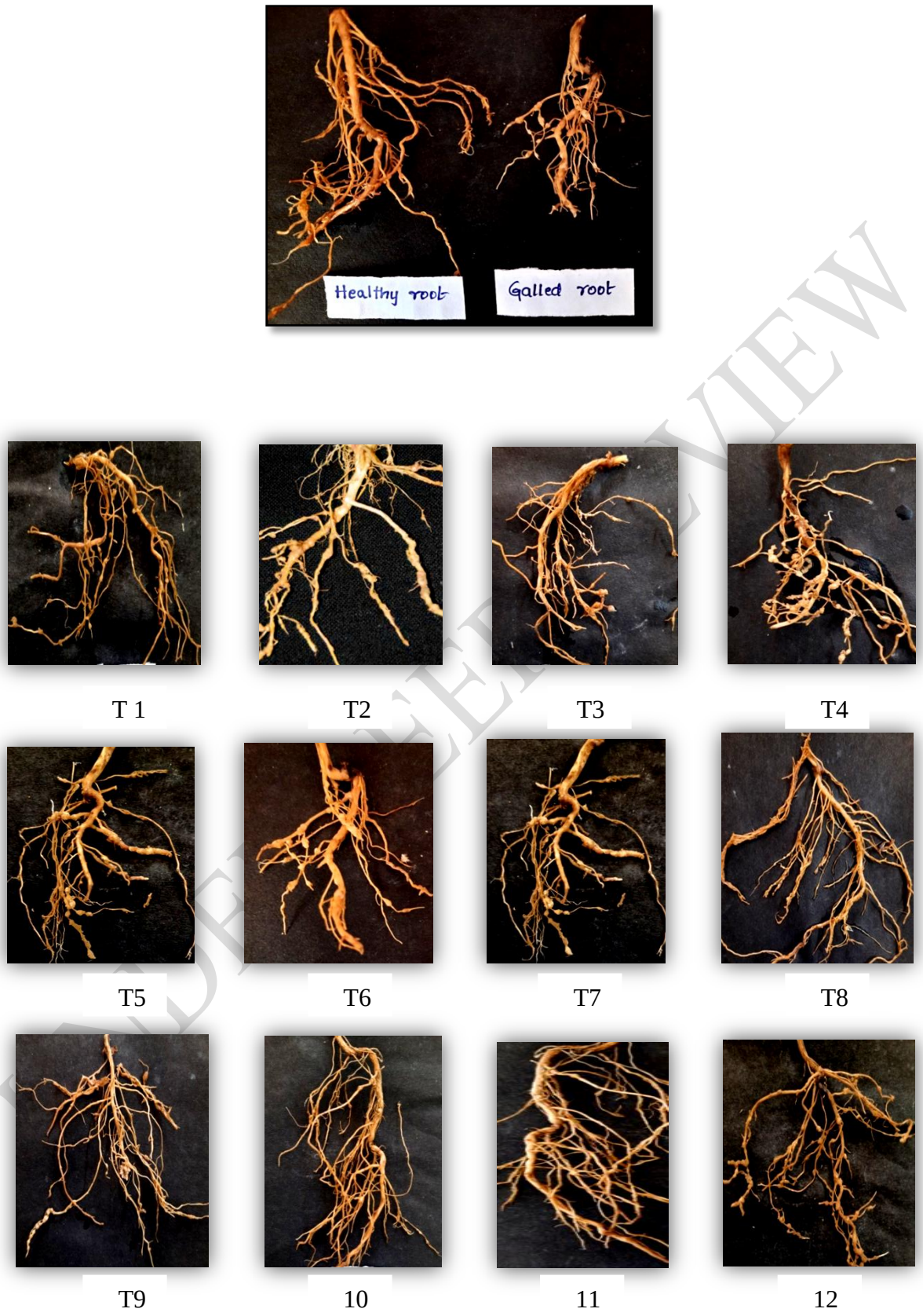

Fig 1. Effect of treatments on field pea plant roots

P. fluorescens, *P. lilacinum*, *T. harzianum* and *G. fasciculatum* were found to be potential bio-control agents and excellent promoters of plant growth in pot studies. It is possible that the lower disease index was due to direct effects of metabolites that cause mortality in J_2, or that it is due to increased host defense mechanisms in roots that resist pathogen invasion and infection. Many investigators have found that *P. fluorescens* and *P. lilacinum* are fatal to *M. incognita* juveniles, and our findings are consistent with their findings [1, 22, 23]. In a study, the treatment of *P. fluorescens* and *P. lilacinum* singly or in combination considerably reduced the nematode population and galling on tomato roots and also substantially boosted the plant growth parameters that corroborated our findings [24]. The better performance of fungal biological control agents may be due to the specific mode of action of *P. fluorescens* and *P. lilacinum*, a well-known egg parasite fungus that attacks nematode eggs in the soil, and *P. fluorescens*, which produces inhibitory allelochemicals and induction of systemic resistance in host plants [25, 26].

It's also worth noting that in the circumstances utilized in these experiments, examined biological control agents, *P. fluorescens* and *P. lilacinum*, were compatible and these are in agreement with findings of existing reports [27]. *P. lilacinum* culture filtrates have been demonstrated to be harmful to nematodes. The worms' cuticles were ruptured, and they died after a few hours of being exposed to the culture filtrates [28]. *P. lilacinum* was found to significantly increase the plant growth parameters by decreasing nematode traits in Bengal gram and tomato respectively [29]. The application of Cartap hydrochloride 4G at different doses declined the severity of nematode infection, root galls and number of egg masses per plant in grapevine and bell pepper [30, 31].

4. CONCLUSION:

The present study shows that the effects of bio-control agents were identical to Cartap hydrochloride 4G, but not as effective as chemical means. The properties of bio-control agents need to be explored such as soluble and volatile metabolites properties, developing the effective formulations, interaction with soil microbiota and their stability under variable environmental conditions as extracting bioresources is an eco friendly and cost effective strategy. Although it is time consuming, but these alternative strategies needed for efficient management of nematodes.

REFERENCES:

1. Siddiqui ZA, Qureshi A, Akhtar MS. Biocontrol of root-knot nematode, *Meloidogyne incognita* by *Pseudomonas* and *Bacillus* isolates on *Pisum sativum*. Archives of Phytopathology and Plant Protection. 2009; 42: 1154-1164.
2. Sasser JN. A world perspective on Nematology: the role of the society. *Vistas on Nematology*. 1987: 7-14.
3. Acharya A, and Padhi NN. Pathogenic relationship of root knot nematode, *Meloidogyne incognita* with betelvine (*Piper betel* L.). *Indian Journal of Nematology*.1987; 17: 127-128.
4. Adesiyan SO, Caveness FE, Adeniji MO, Fawole B. Nematode pests of tropical crops. *Ibadan, Oyo State: Heinemann Educational Books, Nigeria Limited*. 1990; 114.
5. Nwauzor EC, and Fawole, B . The development and life cycle of *Meloidogyne incognita* (race 2) in *Dioscorea rotunda* var. *Okwocha*. In Proceedings: 1st Regional symposium on the Biology and Control of Nematode Pests on Food Crops in Africa. University of Ibadan Press. Nigeria 26th-29th July. 1992: 17-133.

6. Reddy DDR. Analysis of crop losses in tomato due to *Meloidogyne incognita*. *Indian Journal of Nematology*. 1985; 15: 55-59.
7. Singh VK and Satyapriya. New Record of Root knot Nematode, *Meloidogyne incognita* infecting bean and pea in Jammu. *Indian Journal of Nematology*. 2008; 38: 257-258.
8. Cook RJ, Baker KF. The nature and practice of biological control of plant pathogens. American Phytopathological Society. 1983; 539.
9. Siddiqui ZA and Mahmood I. Biological control of plant parasitic nematodes by fungi: a review. *Bioresource Technology*. 1996; 58: 229-239.
10. Dong LQ , Zhang KQ. Microbial control of plant-parasitic nematodes: a five-party interaction. *Plant and Soil*. (2006); 288: 31-45.
11. Tian B, Yang J and Zhang KQ. Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. *Federation of European Microbiological Societies Microbiology Ecology*. (2007); 61: 197-213.
12. Jatala P. Biological control of plant-parasitic nematodes. *Annual review of phytopathology*. 1986; 24: 453-489.
13. Stirling GR. Biological control of plant-parasitic nematodes. Chemical Rubber Company Press.2018: 103-150.
14. Papavizas GC . *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. *Annual review of phytopathology*.1985; 23: 23-54.
15. Siddiqui IA, Shaukat SS . *Trichoderma harzianum* enhances the production of nematicidal compounds *in vitro* and improves biocontrol of *Meloidogyne javanica* by *Pseudomonas fluorescens* in tomato. *Letters in applied microbiology*. 2004; 38: 169-175.

16. Khalili E, Javed MA, Huyop F, Rayatpanah S, Jamshidi S and Wahab RA. Evaluation of *Trichoderma* isolates as potential biological control agent against soybean charcoal rot disease caused by *Macrophomina phaseolina*. *Biotechnology and Biotechnological Equipment*. 2016; 30: 479-488.
17. Murslain, M., Javed, N., Khan, S. A., Khan, H. U., Abbas, H., and Kamran, M. Combined efficacy of *Moringa oleifera* leaves and a fungus, *Trichoderma harzianum* against *Meloidogyne javanica* on eggplant. *Pakistan Journal of Zoology*.2014; 46: 827-832.
18. Weeder CR, Shelton AM, Hoftman MP . Biological Control, A guide to natural enemies in North America.2008: 1-14.
19. Martez JL, Acosta N, Betancourt C, Vicente N, and Rodriguez R. Biological control of *Meloidogyne incognita* in tomato in Puerto Rico. *Nematropica (USA*).1996: 143-152
20. Atkins SD, Clark IM, Pande S, Hirsch PR, Kerry BR. The use of real-time PCR and species-specific primers for the identification and monitoring of *Paecilomyces lilacinus*. *Federatio of European Microbiological Societies Microbiology Ecology*. 2005; 51: 257-264.
21. Subedi P, Gattoni K, Liu W, Lawrence KS, Park SW. Current Utility of Plant Growth-Promoting Rhizobacteria as Biological Control Agents towards Plant-Parasitic Nematodes. *Plants*. 2020; 9: 1167.
22. Khan A, Shaukat SS, Siddiqui IA. A survey of nematodes of pomegranate orchards in Balochistan province, Pakistan. *Nematologia Mediterranea*. 2005; 33: 25-28.
23. Kiewnick S, Sikora RA . Biological control of the Root knot nematode, *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biological control*. 2006; 38: 179-187.

24. Hashem M, Abo-Elyousr KA. Management of the root knot nematode *Meloidogyne incognita* on tomato with combinations of different biocontrol organisms. *Crop Protection*. 2011; 30: 285-292.
25. Singh S. Integrated approach for the management of the root-knot nematode, *Meloidogyne incognita*, on egg plant under field conditions. Nematology.2013; 15: 747-757.
26. Chawla G, Rengasamy S. Seed soaking of okra (*Abelmoschus esculentus* (L.) Moench.) in extracts of neem (*Azadirachta indica* A. Juss) products for the management of plant parasitic nematodes. Indian Journal of Nematology. 2014; 44: 38-43.
27. Shanthi A . Management of root knot nematode, *Meloidogyne incognita* on Ash gourd (*Benincasa hispida*) and Pumpkin (*Cucurbita pepo*) using bio-control agents. Annals of Plant Protection Sciences. 2016; 24: 399-401.
28. Khan MR. Goswami BK. Effect of culture filtrates of *Purpureocillium lilacinum* isolates on hatching of *Meloidogyne incognita* eggs. Annals of Plant Protection Sciences. 2000; 8:62–65.
29. Verma RK, Sharma HK, Bhati SS, Garg S. Investigation on potential of different bio-agents as soil application against root-knot nematode, *Meloidogyne incognita* infecting tomato. International Journal of Chemical Studies. 2020; 8: 2836-2838.
30. Chormule AJ, Mhase, NL, Gurve SS. Management of root-knot nematode, *Meloidogyne incognita* infesting grape under field conditions. Annals of Plant Protection Sciences. 2017; 25:181-185.
31. Narayana R., Thomas S, Sheela MS. Management of root-knot Nematode, *Meloidogyne incognita* infecting black Pepper. Indian Journal of Nematology. 2018; 48: 51-55.