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Investigation on Management of Root-Knot Nematode, *Meloidogyne incognita* through Soil Application of Bio-control Agents in Field Pea

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors contributed to the study conception and design. Author VS performed the statistical analysis, literature searching, protocol for experiment, and written the first draft of the manuscript. Author USS designed the study, managed the analyses of the study and editing of the manuscript. Both authors read and approved the final manuscript.

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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 (2×10^6 cfu/g), *Pseudomonas fluorescens* 1.0% WP (1×10^8 cfu/g), *Purpureocillium lilacinum* 1.0% WP (2×10^6 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}) when applied 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

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nematode population, galls per plant, and Reproduction factor (Rf). *P. lilacinum* 1.0% WP (2×10^6 cfu/g) demonstrated promising effects in plants when just single bio-control agent i.e., 10g/pot 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: Bio-control; Meloidogyne incognita; Purpureocilium lilacinum; Pseudomonas fluorescens; Trichoderma harzianum.

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, *M. 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]. Among the micro-organisms that parasitize or prey on nematodes, fungi and bacteria hold an important position and some of them have shown great potential as bio-control agents [12,13]. Bearing in mind the above points, an investigation was setup to test the efficacy of commercially available bio-control agents, *G. fasciculatum*, *T. harzianum*, *P. 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.

2. MATERIAL AND METHODS

2.1 Host Plant and Test Pathogen

Field pea (*Pisum sativum* var. *arvense*) cv. HUDP-15 (Family-Fabaceae) was selected as host crop. The root-knot nematode, *Meloidogyne incognita* was selected as test pathogen.

2.2 Nematode Inoculums

The population of root-knot nematode, *M. incognita* (Kofoid & White) Chitwood was raised from egg masses, which were collected from infested field pea plants. Identity of the nematode was further confirmed by preparing perenial patterns of 10 adult female nematodes per root system [14]. Large numbers of egg masses were handpicked with the help of sterilized forceps from the galled roots. Egg masses were rinsed with sterile water then placed in 0.5% sodium hypochlorite (NaOCl) solution agitated for 4 minutes and rinsed with sterile water on a 26 µm sieve [15]. The eggs were incubated for 3-5 days at 28 ± 2°C in the dark using a modified

Baermann funnel method [16] to obtain second stage juveniles (J2s). A nematode stock solution with a final concentration of 100 ± 5 second stage juveniles (J2s)/ml 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 [17].

2.3 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. All strains were isolated from their commercial product with a classical microbial insulation protocol (serial dilution technique) on potato dextrose agar (supplemented with 0.01% of tetracycline) and BCAs were conserved under spore forms in glycerol solution and in commercial product aliquots (4°C). The strains were actively grown on PDA at 25°C for 7 days for spore production. The 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 [18].

2.4 *In vivo* Nematicidal Assay with Different Bio-Control Agents on Field Pea

The pot experiment was conducted at AICRP on vegetables, Pusa farm, Dr. Rajendra Prasad Central Agricultural University during 2020-21. 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 bio-control agent i.e., *G. fasciculatum, T. harzianum*, *P. fluorescens, P. lilacinum* were mixed with vermicompost and applied at 10 g pot^{-1}each (T1-T4), then in different possible combinations of bio-control agents i.e., *G. fasciculatum* + *T. harzianum, G. fasciculatum* + *P. fluorescens*, *G. fasciculatum* + *P. lilacinum*, *T. harzianum* + *P. fluorescens*, *T. harzianum* + *P. lilacinum*; *P. fluorescens* + *P. lilacinum* each applied at 10g pot^{-1} (T5-T10), Treated check with Cartap hydrochloride 4G i.e., 5 g pot^{-1} (T11) and untreated control with only nematodes were applied two weeks after sowing (T12). Field pea, *Pisum sativum* var. *arvense* cv. HUDP-15 seeds were sown in pots. One plant per pot was maintained. Sterilized soil was inoculated with 1000 second stage juvemile (J_2)/ kg soil of root-knot nematode by pencil hole method. Each treatment was replicated thrice in Completely Randomized Design (CRD). The treatments involved a single application of each product [19].

Plants were grown in conditions at an average temperature of 9.3 to 21.1℃, 12 h light: 12 h dark 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℃), 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 [20,21]. The roots were cut into small bits and stained with acid- fuchsin to assess the penetration by counting the nematodes inside the root [22].

2.5 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:

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

C.D. at 0.05= $t_{\text{at 0.05 error d.f.}} \times$ 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 [23].

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)(Table 1), number of pods (8.02) and pod weight (35.00) (Table 2) was more in plants treated with combination of bio-control agents, *P. fluorescens* and *P. lilacinum* at 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* and *P. lilacinum* at 10 g pot^{-1} shown on par results with the effective treatment (Fig. 1).

Fig. 1. Effect of promising biocontrol agents on field pea plant growth A. *P. lilacinum* B. *P. fluorescens* + *P. lilacinum* C. Treated check D. Untreated check

Table 1. Effect of treatments on plant growth parameters (mean of 3 replicates)

S. No.	Treatment	Plant height (cm)	% increase over control	Root length (cm)	% increase over control	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	65.46	18.67	10.90	11.03	6.56	20.93	19.24	15.34	1.22	2.52	2.25	3.20
2	T2	63.33	14.81	10.66	8.55	6.16	13.56	20.78	24.62	1.20	0.84	2.21	1.37
3	T3	65.96	19.57	11.18	13.88	6.46	19.09	23.11	38.54	1.23	3.36	2.37	8.71
4	T4	67.33	22.03	11.79	20.12	6.86	26.45	23.21	39.14	1.26	5.88	2.44	11.92
5	T5	76.30	38.32	10.65	8.48	7.03	29.52	19.46	16.70	1.28	7.56	2.45	12.38
6	T6	66.00	19.65	10.85	10.52	7.93	46.1	19.72	18.24	1.32	10.92	2.71	24.31
7	T7	74.60	35.24	11.19	14.01	7.53	38.73	21.04	26.15	1.35	13.44	2.80	53.29
8	T8	65.46	18.67	11.46	16.70	8.50	56.53	21.40	28.29	1.37	15.12	2.86	54.09
9	T9	94.00	70.41	12.73	29.60	9.19	69.30	24.52	47.00	1.41	18.48	3.02	54.89
10	T10	96.00	74.03	13.52	37.60	9.70	78.63	26.24	57.33	1.53	28.57	3.50	60.07
11	T11	86.30	56.45	12.45	26.70	8.05	48.37	24.80	48.68	1.30	9.24	3.01	54.69
12	T12 (Untreated check)	55.16		9.82		5.43	5.43	16.68		1.19		2.18	
	Mean	73.70		11.43		7.45		21.68		1.30		2.65	
	S.Em. ±	1.45		0.09		0.06		0.12		0.03		0.07	
	CD (P = 0.05)	4.26		0.26		0.08		0.37		0.09		0.21	
	CV (%)	3.4		1.4		3.5		1.0		4.5		4.8	

Table 2. 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	

In case of nematode multiplication parameters, the lowest mean number of galls were observed in Cartap hydrochloride 4G when applied at 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* applied at 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* (19.37), and highest mean number of galls per plant was recorded in untreated check (67.55).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, *T. harzianum* and *P. lilacinus*, *P. fluorescens* and *P. lilacinum* i.e., 100 percent control. The lowest mean number of final nematode population was seen in Cartap hydrochloride 4G (310.20) i.e., 85.31 percent reduction over control.The highest Rf was observed in untreated check i.e., 2.11 and lowest Rf was seen in plants treated with Cartap hydrochloride (0.31). However, the effective results were observed with chemical check i.e., Cartap hydrochloride 4G when applied at 5g/pot (Table 3; Fig 2).

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 second stage juveniles (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 [1,24,25]. 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 [26]. 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 [27,28]. 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 [29]. *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 [30]. *P. lilacinum* was found to significantly increase the plant growth parameters by decreasing nematode traits in Bengal gram and tomato respectively [31]. 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 [32,33].

Table 3. 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	Initial nematode population (Pi)	Final nematode population (Pf)	% decrease over control	Rf
1	T1	56.40	16.50	25.93	55.39	1000	648.20	69.30	0.64
2	T2	48.50	28.20	27.23	53.15	1000	515.33	75.60	0.51
3	T3	29.24	56.70	17.73	69.49	1000	521.50	75.31	0.52
4	T4	28.87	57.26	7.83	56.53	1000	467.93	77.85	0.46
5	T5	27.33	59.54	9.96	82.86	1000	447.76	78.8	0.44
6	T6	26.53	60.72	7.430	87.21	1000	426.43	79.81	0.42
7	T7	23.65	64.98	5.53	90.48	1000	437.66	79.28	0.43
8	T8	21.40	68.31	3.00	94.83	1000	453.79	78.52	0.45
9	T9	19.37	71.31	0	100.00	1000	432.43	79.53	0.43
10	T10	17.75	73.71	0	100.00	1000	322.83	84.7	0.32
11	T11	13.40	80.15	0	100.00	1000	310.20	85.31	0.31
12	T12 (Untreated check)	67.55		58.13		1000	2112.80		2.11
	Mean	31.66		13.56			591.40		
	S.Em. ±	0.68		0.31			0.95		
	CD (P = 0.05)	2.02		0.91			2.80		
	CV (%)	3.8		4.0			0.28		

Fig 2. Effect of promising biocontrol agents on field pea plant roots A. *P. lilacinum* B. *P. fluorescens* + *P. lilacinum* C. Treated check D. Untreated check

4. CONCLUSION

The present study shows that the effects of bio-control agents were identical to Cartap hydrochloride 4G in the case of nematode reproduction parameters, whereas the plant growth parameters were increased by the application of bio-control agents either single or combination. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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, 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, 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. 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, 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, 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. Barker KR. Nematode extraction and bioassays In: An Advanced Treatise on *Meloidogyne*. Vol. 2. Methodology. Barker KR, Carter CC, Sasser J.N, eds. North Carolina State University, Raleigh. 1985: 19-35
15. Hussey RS, Barker KR. Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant disease reporter; 1973
16. Southey JF. Laboratory Methods for Work with Plant and Soil Nematodes Her Majesty's Stationery Office. Technical Bulletin, Ministry of Agriculture, Fisheries and Food, London, UK. 1986;202
17. Díaz-Manzano FE, Olmo R, Cabrera J, Barcala M, Escobar C, Fenoll C. Long-term in vitro system for maintenance and amplification of root-knot nematodes in Cucumis sativus roots. Frontiers in Plant Science. 2016;7:124.
18. Waksman SA. A method for counting the number of fungi in the soil. Journal of Bacteriology. 1922;7(3):339-341.
19. Forghani F, Hajihassani A. Recent advances in the development of environmentally benign treatments to control root-knot nematodes. Frontiers in Plant Science. 2020;11:1125.
20. Cobb NA. Estimating the nema population of soil, with special references to the sugarbeet and root-gall nemas, *Heterodera schachtii* Schmidt and *Heterodera radicicola* (Greef) Muller, and with a description of *Tylencholaimus aequalis* n. spp. Agric Tech Circular. 1918;1:48.
21. Schindler A. A simple substitute for a Baermann funnel. Plant Disease Reporter. 1961;45:747-748.
22. Bybd Jr DW, Kirkpatrick T, Barker K. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology. 1983; 15(1):142.
23. Fisher RA. Design of experiments. British Medical Journal. 1936;1(3923):554.
24. Khan A, Shaukat SS, Siddiqui IA. A survey of nematodes of pomegranate orchards in Balochistan province, Pakistan. Nematologia Mediterranea. 2005;33:25-28.
25. Kiewnick S, Sikora RA . Biological control of the Root knot nematode, *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological control. 2006;38:179-187.
26. 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.

27. 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.
28. 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.
29. 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.
30. 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.
31. 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.
32. 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.
33. Narayana R, Thomas S, Sheela MS. Management of root-knot Nematode, *Meloidogyne incognita* infecting black Pepper. Indian Journal of Nematology. 2018;48:51-55.

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