



# Management of Root-Knot Nematode on Okra through Bio-Agents

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/IJECC/2023/v13i92572

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/103645>

**Original Research Article**

**Received: 29/05/2023**

**Accepted: 02/08/2023**

**Published: 09/08/2023**

## ABSTRACT

A pot study was conducted to assess the efficacy of various fungal and bacterial antagonists as seed coating treatments against *Meloidogyne javanica*, a root-knot nematode that infects okra plants. The seeds of okra cv. Pusa Sawani were subjected to treatments with *Trichoderma viride*, *Purpureocillium lilacinum*, and *Pseudomonas fluorescens* at a rate of 2 g/kg seed. As a comparison, Carbosulfan 3G was used as a control, applied at a rate of 3g/kg soil. The treated seeds were then planted in soil infested with two second-stage juveniles of the root-knot nematode per gram of soil. After 45 days of sowing, it was observed that the okra plants exhibited enhanced growth, while the population of root-knot nematodes was significantly reduced in all treatment groups compared to the untreated control. Among the different bio agents tested,

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*Purpureocillium lilacinum* exhibited the best treatment followed by *Trichoderma viride* and *Pseudomonas fluorescens* for increase plant growth characters as well as reduced nematode reproduction.

**Keywords:** Management; *Meloidogyne javanica*; okra; bio-agents.

## 1. INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) is considered one of the oldest and most significant crops worldwide. It is cultivated in regions with Mediterranean, tropical, and subtropical climates. This nutritious vegetable crop plays a vital role in meeting the demand for vegetables in the market. Studies by Pal et al. [1] and Rashwan [2] have shown that okra is a rich source of calcium, vitamins, potassium, carbohydrates, minerals, and fibers. It is particularly renowned for its high iodine content and generous amounts of vitamin C (30 mg/100 g), calcium (90 mg/100 g), and iron (1.5 mg/100 g). Okra also contains crude fiber (6.60% to 10%), protein (14.40% to 18.60%), and ash (8.20% to 9.15%) by weight. Furthermore, it is a rich source of vitamin B6 and folic acid. Dry okra seeds contain approximately 20-30% crude protein [3].

In India, okra is cultivated across an area of approximately 509 thousand hectares, with a production of 6094.9 metric tons. The major okra-producing states are Andhra Pradesh, Bihar, Odisha, Gujarat, West Bengal, Assam, Rajasthan, Madhya Pradesh, Uttar Pradesh, and Karnataka. In Rajasthan specifically, okra is cultivated across an area of 4.15 thousand hectares, yielding 21.39 metric tons [4]. The primary okra-growing districts in Rajasthan include Ajmer, Alwar, Jaipur, Kota, Bundi, Chittorgarh, Bhilwara, Rajsamand, Jhalawar, Jodhpur, Sikar, Nagaur, Dausa, Sriganganagar, Jodhpur, Pali, Sirohi, Banswara, Pratapgarh, and Udaipur.

Root-knot nematodes are a serious pest affecting various vegetable crops, causing stunted growth and nutrient deficiency symptoms. They result in significant economic losses, with severe infestations leading to 10-50% yield reductions [5]. Okra crops are susceptible to various harmful microorganisms such as fungi, bacteria, viruses, nematodes, and insect pests. Among these, the root-knot nematode, *Meloidogyne javanica*, is particularly destructive.

Globally, the estimated yield loss in major crops due to plant parasitic nematodes is reported to

be 12.37% [6], and they significantly reduce okra crop yields [7]. Considering the importance of this crop, an experiment was conducted to manage and determine the minimum population of root-knot nematode (*M. javanica*) infection in okra.

## 2. MATERIALS AND METHODS

An experiment was conducted at the Division of Nematology, Rajasthan Agricultural Research Institute, Durgapura, Jaipur, during 2019-2020 to study the management of root-knot nematode (*Meloidogyne javanica*) on okra using bio-control agents. The experiment utilized 20 cm-sized pots filled with sterilized soil. Soil samples of approximately 200cc were randomly collected from the rhizosphere of okra plants at 4-5 different locations in each field. The samples were obtained at a depth of 15-20cm, homogenized, and stored in labeled polythene bags. These bags were then tied and refrigerated at approximately 10°C. A composite sample of 200cc, along with 5g of roots, was collected from a one-acre field.

To isolate the nematodes from the soil, each soil sample was processed using the Modified Cobb's sieving and decanting technique, followed by the Baermann's funnel technique [8]. Nematode population estimation was performed by adjusting the volume of nematode suspension to 100 ml. The number of *Meloidogyne* species present was counted per 5 ml of suspension using a counting dish, and the average of two counts was recorded as the number of nematodes per ml. The volume of the suspension was multiplied by the average count of nematodes per ml to determine the total nematode population per 200cc of soil.

For counting the nematode population, the roots were carefully uprooted, washed to remove soil, and stained in boiling 0.05% acid fuchsin lactophenol solution for two minutes [9].

The experiment aimed to manage root-knot nematode (*Meloidogyne javanica*) using various bio-control agents and chemicals, including *Purpureocillium lilacinum*, *Trichoderma viride*,

*Pseudomonas fluorescens* at a rate of 2g/kg soil, and carbofuran 3G at a rate of 3g/kg soil. An untreated control group was also included for comparison. Each treatment was replicated four times. The pots were regularly watered as needed. Observations on plant growth and root-knot nematode reproduction were recorded 45 days after sowing.

### 3. RESULTS AND DISCUSSION

An experiment was carried out to assess the effectiveness of various bio agents on the growth characteristics of okra plants and the reproduction of root knot nematodes. The study utilized a talc-based formulation of *Purpureocillium lilacinum* (2g/kg soil), *Trichoderma viride* (2g/kg soil), and *Pseudomonas fluorescens* (2g/kg soil). In addition, an untreated control group and a chemical comparison using carbofuran (3g/kg soil) were included for reference.

#### 3.1 Plant Growth Characters

##### 3.1.1 Shoot length

All of the bio agents demonstrated a significant increase in shoot length compared to the control group (32.12cm). Among the different bio agents tested, *Purpureocillium lilacinum* exhibited the highest shoot length of 42.45cm, followed by *Trichoderma viride* with 38.92cm and *Pseudomonas fluorescens* with 36.07cm. It is worth noting that carbofuran, the chemical comparison, also resulted in the highest shoot length of 48.40cm.

##### 3.1.2 Root length

The plants treated with bio-agents displayed a notable increase in root length compared to the untreated control group (25cm), and the difference was statistically significant. Among the bio-agents tested, *Purpureocillium lilacinum* resulted in the highest root length of 33.35cm, followed by *Trichoderma viride* with 30.40cm and *Pseudomonas fluorescens* with 27.65cm. It is worth mentioning that carbofuran, the chemical comparison, also exhibited the highest root length of 35.07cm.

##### 3.1.3 Shoot weight

*Purpureocillium lilacinum* exhibited the highest shoot weight among the different bio agents, measuring 28.87cm. This was followed by

*Trichoderma viride* with 28.77cm and *Pseudomonas fluorescens* with 27.47cm. However, carbofuran resulted in the maximum shoot weight of 32.25cm. On the other hand, the untreated control group had the lowest shoot weight, measuring 20.60 gm.

##### 3.1.3 Root weight

*Purpureocillium lilacinum* resulted in the highest root weight among the different bio agents, measuring 9.70cm. This was followed by *Trichoderma viride* with 9.40cm and *Pseudomonas fluorescens* with 8.50cm. However, carbofuran also exhibited the maximum root weight of 10.22cm. In contrast, the untreated control group had the lowest root weight, measuring 6.97 gm.

#### 3.2 Nematode Reproduction

##### 3.2.1 Number of galls per plant

The results demonstrated a significant reduction in the number of galls produced by *Meloidogyne javanica* on okra when bio agents were applied, in comparison to the control group. Among the different bio agents, *Purpureocillium lilacinum* exhibited the lowest number of galls (71.25 per plant), followed by *Trichoderma viride* with 78.77 and *Pseudomonas fluorescens* with 95.12. However, carbofuran applied at a rate of 3g/kg soil also resulted in the lowest number of galls (55.30). In contrast, the untreated control group showed the highest number of galls (164.92).

##### 3.2.2 Number of egg masses per plant

The results indicated a significant reduction in the number of egg masses per plant produced by *Meloidogyne javanica* on okra when treated with bio agents, compared to the control group. Among the different bio agents, *Purpureocillium lilacinum* exhibited the lowest number of egg masses (46.35 per plant), followed by *Trichoderma viride* with 51.47 and *Pseudomonas fluorescens* with 65.27. However, carbofuran resulted in the lowest number of egg masses (36.00), while the untreated control group showed the highest number of egg masses (143.90).

##### 3.2.3 No. of eggs/egg masses

The results demonstrate a significant reduction in the number of eggs/egg masses produced by the

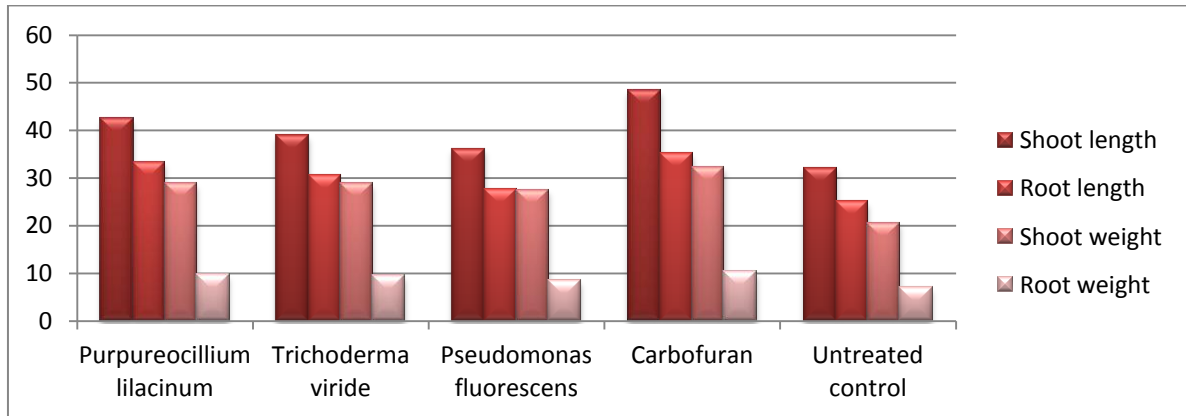
root knot nematode on okra when treated with biocontrol agents compared to the untreated control. Among the different bio agents, *Purpureocillium lilacinum* showed the lowest number of eggs/egg masses (244.00 per plant), followed by *Trichoderma viride* with 254.00 and *Pseudomonas fluorescens* with 260.25. However, carbofuran resulted in the lowest number of galls (229.62), while the untreated control group exhibited the highest number of galls (271.00).

### 3.2.4 Nematode population/200 cc soil

The highest nematode population was observed in the untreated control group (626.25), while the lowest population (298.75) was observed in the treatment using *Purpureocillium lilacinum*. This was followed by *Trichoderma viride* with 308.75 and *Pseudomonas fluorescens* with 330.00. It's worth noting that the nematode population was also minimized in the carbofuran treatment (273.75).

**Table 1. Effect of different bio-agents as seed treatment on plant growth characters of okra**

Treatments	Plant growth characters			
	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
<i>P. lilacinum</i> @2g/kg soil	42.45	33.35	28.87	9.70
<i>T. viride</i> @2g/kg soil	38.92	30.4	28.77	9.40
<i>P. fluorescens</i> @2g/kg soil	36.07	27.65	27.47	8.50
Carbofuran 3G @3g/kg soil	48.4	35.07	32.25	10.22
Untreated control(untreated check)	32.12	25	20.60	6.97
S.Em(±)	0.68	0.49	0.52	0.15
C.D.	2.08	1.51	1.58	0.46
C.V.	3.46	3.28	3.78	3.37



**Fig. 1. Effect of different bio-agents as soil treatment on plant growth characters of okra**

**Table 2. Effect of different bio agents on nematode reproduction**

Treatments	Nematode reproduction				
	No. of galls /plant	No. of egg masses /plant	No. of eggs /egg masses	Nematode population / 200 cc soil	Final / total nematode population/ Pot
<i>Purpureocillium lilacinum</i> @2g/kg soil	71.25	46.35	244.00	298.75	12,802.63
<i>Trichoderma viride</i> @2g/kg soil	78.77	51.47	254.00	308.75	14,618.47
<i>Pseudomonas fluorescens</i> @2g/kg soil	95.12	65.27	260.25	330.00	18,639.60
carbofuran 3G @3g/kg soil	55.30	36.00	229.62	273.75	9,631.60
Untreated control (untreated check)	164.92	143.90	271.00	626.25	42,129.65
S.Em(±)	1.30	0.80	2.77	5.88	343.03
C.D.	3.98	2.43	8.42	17.90	1,043.43
C.V.	2.81	2.33	2.20	3.20	3.50

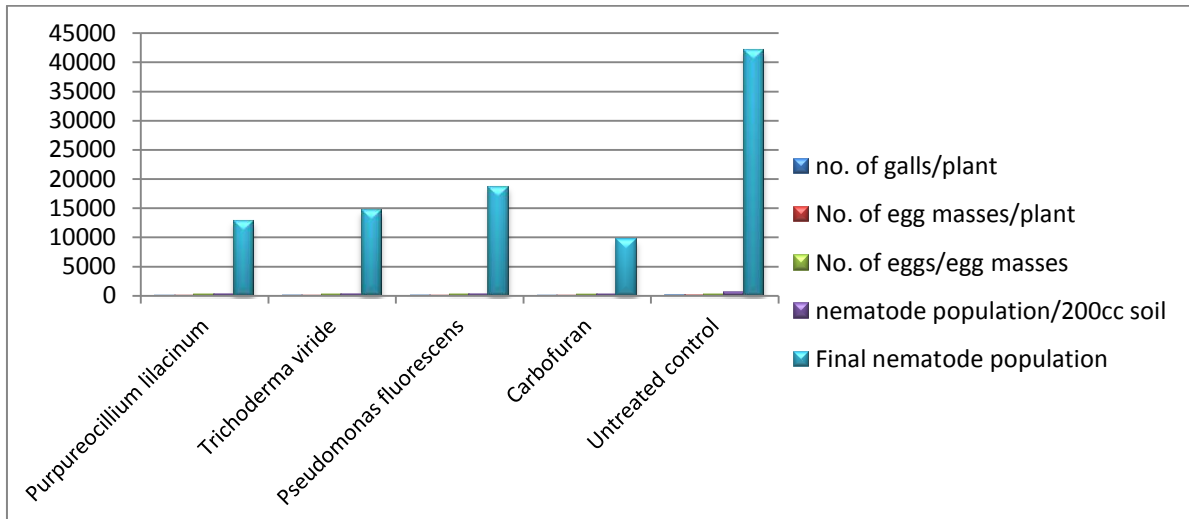


Fig. 2. Effect of different bio-agents on nematode reproduction

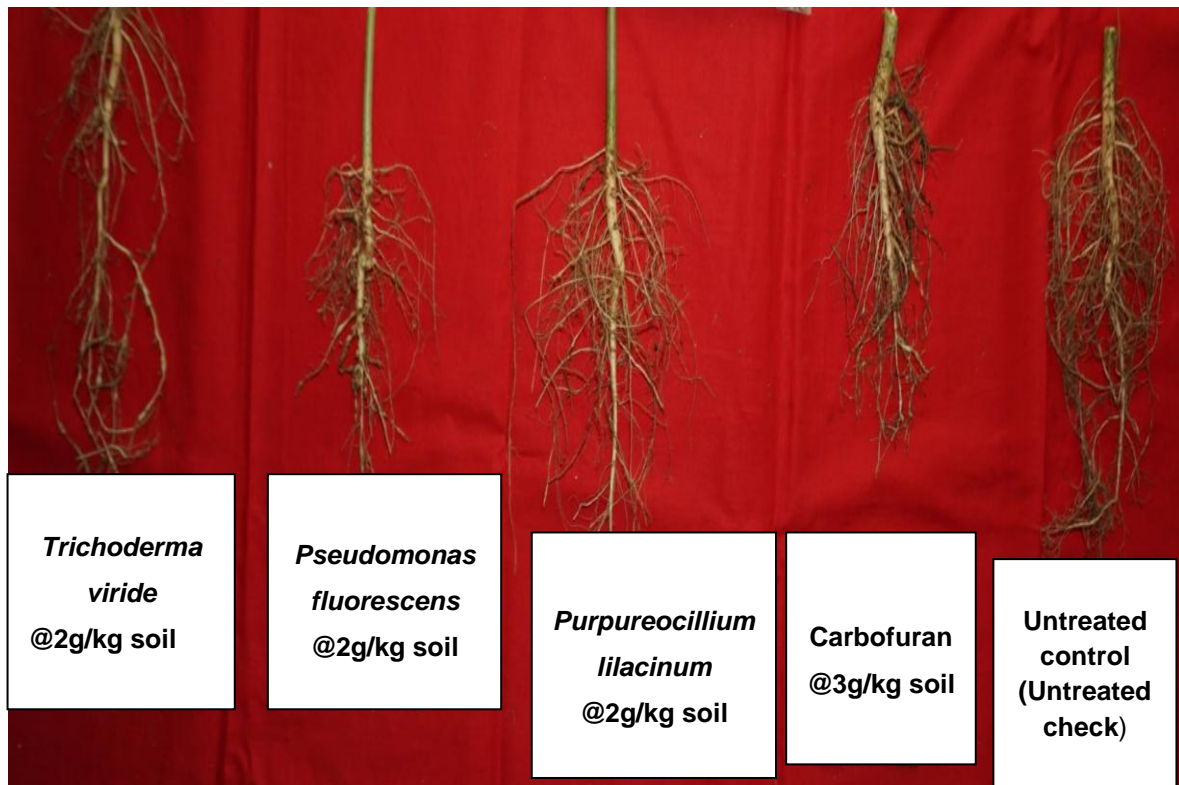


Plate 1. Management of root knot nematode, *Meloidogyne javanica* on okra through bio control agents

### 3.2.5 Final / total nematode population

The untreated control group exhibited the highest final nematode population (42,129.65), while the minimum final nematode population was observed in the treatment using *Purpureocillium lilacinum* (12,802.63). *Trichoderma viride* resulted in a population of 14,618.47, and *Pseudomonas fluorescens* showed a population

of 18,639.60. It's worth mentioning that the carbofuran treatment (3g/kg soil) also resulted in a minimum nematode population of 9,631.60.

### 3.3 Discussion

The results of the experiment indicate that the application of bio-agents, including *Purpureocillium lilacinum* at a rate of 2g/kg soil,

*Trichoderma viridae* at 2g/kg soil, and *Pseudomonas fluorescens* at 2g/kg soil, as well as the chemical control carbofuran at 3g/kg soil, had a positive impact on plant growth and effectively reduced the root-knot index and the populations of both root and soil nematodes. Among the bio-agents, *Purpureocillium lilacinum* showed the maximum improvement in plant growth characteristics, followed by *Trichoderma viridae* and *Pseudomonas fluorescens*. However, the chemical control carbofuran also resulted in high plant growth characteristics.

The treatment with *Purpureocillium lilacinum* exhibited the lowest number of galls, egg masses per plant, eggs per egg mass, nematode population per 200cc soil, and final/total nematode population. It also led to the maximum reduction in root and soil nematode population.

These findings highlight the effectiveness of *Purpureocillium lilacinum* as a bio-agent against *Meloidogyne* spp. in okra. The application of bio-agents, including *Purpureocillium lilacinum*, *Trichoderma viridae*, and *Pseudomonas fluorescens*, can improve plant growth, reduce the root-knot index, and suppress the populations of root and soil nematodes. The results also suggest that chemical control with carbofuran can provide similar benefits in terms of plant growth characteristics and nematode suppression.

Overall, the study supports the use of *Purpureocillium lilacinum* as an effective bio-agent for managing *Meloidogyne* spp. in okra. The results clearly demonstrate the effectiveness of the bio-agent *Purpureocillium lilacinum* at a rate of 2g/kg soil in reducing the number of galls per plant, number of egg masses per plant, number of eggs per egg mass, nematode population per 200cc soil, and the overall total nematode population. Additionally, it significantly increased the shoot length, root length, shoot weight, and root weight of okra compared to the untreated control. Among the biocontrol agents tested, *Purpureocillium lilacinum* showed superior efficacy against the root knot nematode *Meloidogyne javanica* on okra, outperforming *Trichoderma viridae* and *Pseudomonas fluorescens*.

Application of *P. lilacinum* bio-agent increase the fruit yield of okra [10,11] as well as reduced root knot nematode infestation [12]. *Pochonia chlamydosporia* also reported most effective in maize against *Heterodera zea* Kumhar et al.

[13]. Application of *Pseudomonas fluorescens* and *Trichoderma viride* enhance enzymatic activity, improve plant growth and reduction in nematode reproduction in tomato [14] in chilli [15] in cucumber [16]. *Trichoderma viride* and *Paecilomyces lilacinum* reduced nematode population as well as per cent wilt disease incidence in tomato [17]. Bacterial bio-agents also suppress the *Meloidogyne incognita* [18]. Bio-agents also responsible for inhibit nematode hatching and larval mortality [19]. When bio-agents used combination of hot water and bio-waste combination [20].

#### 4. CONCLUSION

In summary, the findings of the study, along with previous research, support the use of *Purpureocillium lilacinum* as a highly effective bio-agents against root knot nematodes in okra. The results highlight its ability to reduce nematode populations and improve plant growth characteristics. Additionally, other studies have emphasized the economic and practical benefits of using biocontrol agents and alternative management strategies for nematode control.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Pal BP, Singh HB, Swarup V. Taxonomic relationship and breeding possibilities of species. *Abelmoschus* related to okra (*Abelmoschus esculentus* (L.) Moench.) *Botanical Gazette*. 1952;113:455-464.
2. Rashwan AMA. Study of genotypic and phenotypic correlation for some agro economics traits in okra (*Abelmoschus esculentus*). *Asian Journal of Crop Sciences*. 2011;3(2):85-91.
3. Anonymous. Horticultural statistics at a glance. Govt of India. 2018:149
4. Anonymous. Horticultural statistics at a glance. Govt of India. 2018:196
5. Berkelaar E. Methods of nematode management ECHO development notes issue 75; 2002.
6. Pankaj, Gaur HS. Nematodes. In. Handbook of agriculture. ICA-R Publications New Delhi, 2009:146.
7. Bhatti DS, Jain RK. Estimation of losses in okra, tomato and brinjal yield due to *Meloidogyne incognita*. *Indian Journal of Nematology*. 1977;7(1):37-41.



8. Christie JR, Perry VG. Removing nematode from soil. Proceedings of Heminthological Society of Washington. 1951;18:106-108.
9. Franklin MT, Goodey JB. A cotton blue lactophenol technique for mounting plant parasitic nematodes. Journal of Helminthology. 1949;23(3):175-178.
10. Kadam V, Khan MR. Biomangement of root knot nematode (*Meloidogyne incognita*) infecting okra in West Bengal, India. Indian Journal of Nematology. 2015; 45(2):178-183.
11. Saha T, Khan MR. Evaluation of bioformulations for management of root knot nematode (*Meloidogyne incognita*) infecting Tuberose. Pakistan Journal of Zoology. 2016;48(3):651-656.
12. Hano P, Khan MR. Evaluation of fungal (*Paecilomyces lilacinus*) formulations against root knot nematode infecting tomato. Journal of Botany. 2016;45(5): 1003-1013.
13. Kumhar RN, Baheti BL, Chandrawat BS, Gurjar Om Prakash. Seed treatment with bio-agents: An environmental suitable approach for management of maize cyst nematode, *Heterodera zea* on maize. Current Nematology. 2018;29(1,2):1-5.
14. Chandrawat BS, Siddiqui AU, Bhati SS, Saharan Vinod. Induction of defence enzymes using bio-agents in tomato infected with root-knot nematode, *Meloidogyne incognita*. Indian Journal of Nematology. 2018;48(2):139-145.
15. Chandrawat BS, Siddiqui AU, Bhati SS, Saharan Vinod. Bio-agents: A source for initiation of defence enzymes in chilli infected with root-knot nematode, *Meloidogyne incognita*. Journal of Entomology and Zoology Studies. 2020;8 (6):1684-1688.
16. Bhati SS, Baheti BL, Singh Ishwar, Chandrawat BS. Impact of bio-agents against *Meloidogyne incognita* infecting cucumber in poly-house. *Biological Forum – An International Journal*. 2022;14(2): 1481-1487.
17. Meena Pinki, Chandrawat BS, Ahir RR. Bio-agents for management of wilt complex in tomato by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*. Annals of Plant Protection Sciences. 2020;28(2):160-162.
18. Kumar N, Bhatt J, Sharma RL, Prahlad. Efficacy of different bio-control agents against *Meloidogyne incognita* and *Fusarium oxysporum* on tomato. International Journal of Current Microbiology and Applied Sciences. 2017; 6(8):1980-1984.
19. Kumari Manju, Sharma MK, Baheti BL, Nama CP, Chandrawat BS. Reaction of locally isolated bio-agents on hatching and mortality of root-knot nematode, *Meloidogyne incognita*. Journal of Entomology and Zoology Studies. 2021; 9(2):1276-1281.
20. Bhati SS, Baheti BL, Chandrawat BS. Influence of eco-friendly management modules for suppression of *Meloidogyne incognita* infecting cucumber in polyhouse. Indian Journal of Nematology. 2021;51(1): 27-33.

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Peer-review history:

The peer review history for this paper can be accessed here:  
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