Management of root-knot nematode on okra through Bio-agents

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

Key- word: Management, *Meloidogyne javanica*, okra, Bio-agents

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*. (1952) and Rashwan (2011) 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 (Anonymous, 2018).

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 (Anonymous, 2018). 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 (Berkerlaar, 2002). 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% (Pankaj and Gaur, 2009), and they significantly reduce okra crop yields (Bhatti and Jain, 1977). 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

Material 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 (Christie and Perry, 1951). 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 (Franklin and Goodey, 1949).

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.

Result & 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.

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)**
P. lilacinum @2g/kg soil	42.45	33.35	28.87	9.70
***T. viride* @2g/kg soil**	38.92	30.4	28.77	9.40
***P.fluorescens* @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

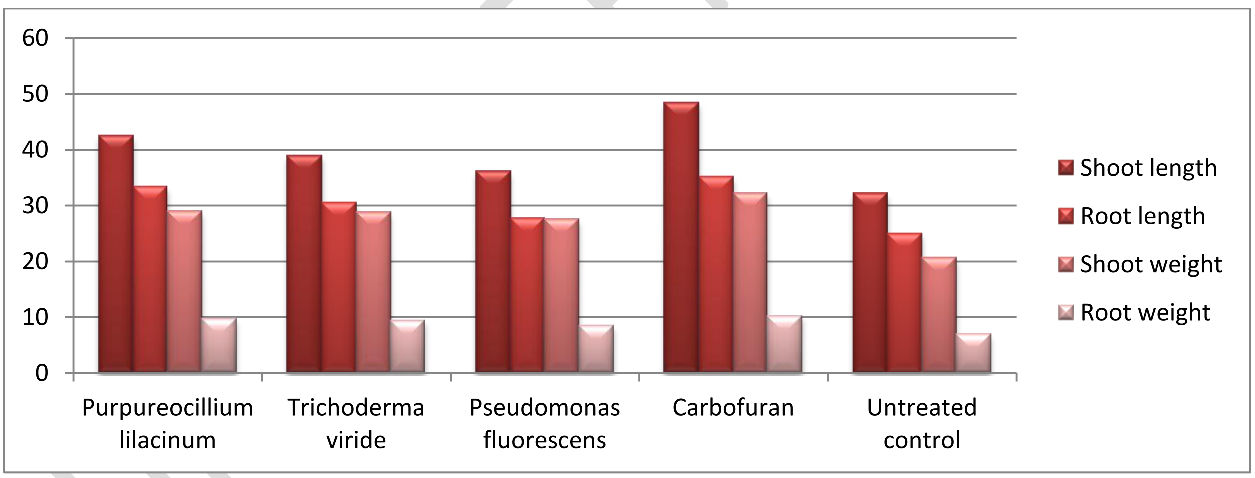

Fig 1: -Effect of different bio-agents as soil treatment on plant growth characters of okra.

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**
***Purpureocillium lilacinum* @2g/kg soil**	71.25	46.35	244.00	298.75	12,802.63
***Trichoderma viride* @2g/kg soil**	78.77	51.47	254.00	308.75	14,618.47
***Pseudomonas fluorescens* @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

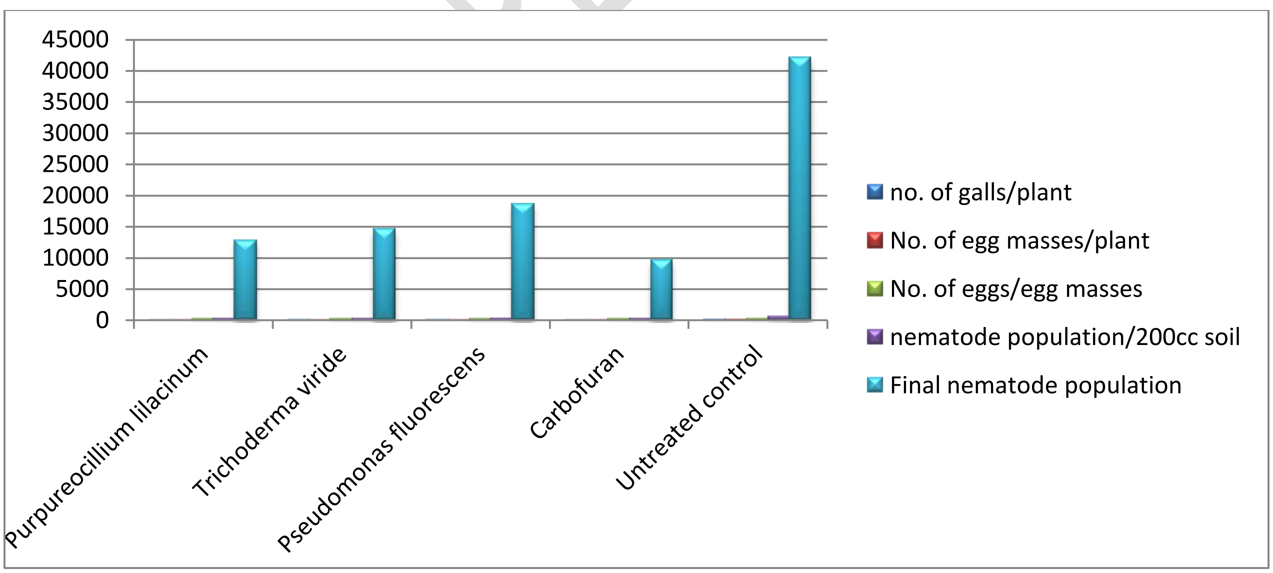

Fig 2:- Effect of different bio-agents on nematode reproduction

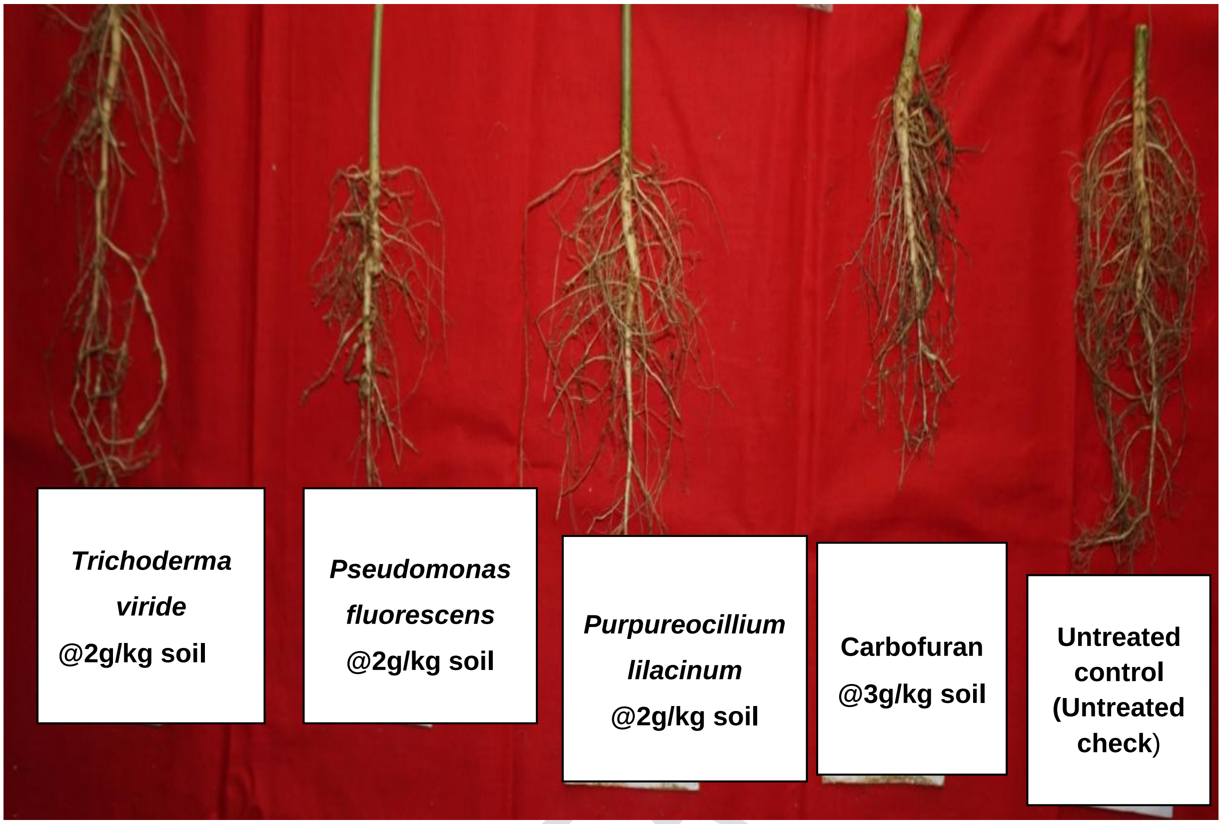

Plate 1: Management of Root knot nematode, *Meloidogyne javanica* on okra through bio control agents.

1. Plant growth characters

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.

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.

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.

1.4 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.

2. Nematode reproduction

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).

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).

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).

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).

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 *Purpureocillum 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.

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-gents, 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 fluorescence.*

Application of *P. lilacinum* bio-agent increase the fruit yield of okra (Kadam and Khan, 2015; Saha and Khan, 2016) as well as reduced root knot nematode infestation (Hano and Khan, 2016). *Pochonia chlamydosporia* also reported most effective in maize against *Heterodera zeae* Kumhar *et al*. (2018). Application of *Pseudomonas fluorescens* and *Trichoderma viride* enhance enzymatic activity, improve plant growth and reduction in nematode reproduction in tomato (Chandrawat *et al*., 2018) in chilli (Chandrawat *et al*., 2020) in cucumber (Bhati *et al*., 2022). *Trichoderma viride* and *Paecilomyces lilacinum* reduced nematode population as well as per cent wilt disease incidence in tomato (Meena *et al*., 2020). Bacterial bio-agents also suppress the *Meloidogyne incognita* (Kumar et al., 2017). Bio-agents also responsible for inhibit nematode hatching and larval mortality (Kumari *et al*., 2021). When bio-agents used combination of hot water and bio-waste combination (Bhati *et al*., 2021)

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.

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