

## Exploitation of Bioagents for Managing Root-Knot Nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 Inciting Black gram (*Vigna mungo* L.)

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**ABSTRACT:** To determine the effect of different bio agents i.e. *Pseudomonas fluorescens*, *Trichoderma viride* on plant growth parameters and the effect of different treatments on root knot nematode multiplication in Black gram (*Vigna mungo* L.). The Completely Randomized Design (CRD) was used to set up a pot culture experiment, which was then replicated three times with ten different treatments. The results showed that all of the treatments improved plant growth parameters while reducing root knot nematode proliferation when compared to the control group. T<sub>8</sub>-(T<sub>2</sub>+T<sub>4</sub>) outperformed the other treatments by increasing plant height, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and decreasing the number of galls, number of egg masses, total nematode population in 200 cc of soil, and total nematode population in soil and root. Followed by T<sub>9</sub> (carbofuran treatment @ 10g/m<sup>2</sup>) and T<sub>5</sub>-(T<sub>1</sub>+T<sub>3</sub>) (Seed and soil treatment of *Trichoderma viride* @ 10g/ka and 20g/m<sup>2</sup> respectively). Application of chemical nematicide, carbofuran @ 10g/m<sup>2</sup> was the 2<sup>nd</sup> best effective way to the management of root knot nematode but losing its popularity due to its cost effectiveness, unfriendly and hazardous environmental consequences. So, based on the findings of this study, seed treatment of Black gram with *Pseudomonas fluorescens* @ 10 g/kg and soil application of *Pseudomonas fluorescens* @20 g/m<sup>2</sup> was most efficient in minimising root knot disease, nematode multiplication, and improving plant growth. However, for meaningful root knot nematode, it is worthwhile to evaluate the efficacy of this treatment in field conditions.

**Keywords:** Root Knot Nematode, Black gram, *Pseudomonas fluorescens*, *Trichoderma viride*.

### INTRODUCTION

Since time immemorial, black gram (*Vigna mungo* (L.) Hepper), often known as poor man's meat, has been planted all over the world and is one of the most significant legume crops cultivated internationally as well as a kharif pulse in India. Urd, Biri, and Mash are all synonyms for black gram. It is a member of the Fabaceae family and is indigenous to India. It is high in phosphoric acid and has 25% protein, 60% carbs, and 1.3 percent fat. As a legume crop, black gram has the ability to fix atmospheric nitrogen by supporting bacteria in the soil, supplementing roughly 42 kg nitrogen per hectare. It is most commonly grown during the kharif (rainy) and summer seasons. It thrives in hot, humid environments with optimal temperatures ranging from 25 to 35 degrees Celsius. In India, black gram was

grown in a total area of 5279 ha, production of 2430 tones and productivity of 1430 kg/ha and occupy second position next to mung (Indian stat). The low yield potential of black gram attributed to various factor like adoption of poor management practices, marginal and poor soil, climate change etc. However, biotic factors including such weeds, insects, fungi, bacteria, and viruses, as well as dangerous nematodes, affected crop output to varying degrees. *Meloidogyne incognita* (Chitwood, 1949) is the main nematode species affecting this crop's growth and productivity. It has a large host range and a wide geographic distribution, ranging from temperate to tropics. *Meloidogyne* species are the most commonly imported nematode pests, which can attack a wide range of crops or, in their absence, weeds (D' Errico *et al.*, 2014). Root knot

nematode illnesses have only been reported in a few cases. *Meloidogyne incognita* Chitwood, (1949) was found on ornamental plants in Turkey, according to Ata, *et al.*, (2021). Following that, mixed populations of *M. arenaria* Chitwood, 1949 and *M. incognita* were reported on rose in Italy by El-Deen & El-Deeb (2018), *M. hapla* Chitw. On rose grown in rockwool and coconut-peat culture in Germany (Sergio *et al.*, (2019), and *M. javanica* (Mukhtar, 2018) Chit (Tzortzakakis, 2004). After nematodes have penetrated the crop, they can reproduce and distribute quickly, causing a rapid devastation of the crop (Kayani *et al.*, (2017). *Trichoderma viride* was found to be effective against *Meloidogyne incognita* on okra (Mukhtar & Hussain 2019) and mulberry (Muthulakshmi *et al.*, 2010). On brinjal, *Pseudomonas fluorescens* was found to be beneficial in reducing *M. incognita* (Dhal, 2001; Barua and Bora, 2008). As a result, the current study uses commercial biocontrol products of *Trichoderma viride* (Tv) and *Pseudomonas fluorescens* (Pf) at various doses, as well as carbofuran as a chemical check, to determine the effectiveness of these microorganisms and their dose on black gram growth Urad kavaya, a sensitive cultivar, and the root-knot nematode, *M. incognita*, proliferation.

## MATERIALS AND METHODS

### Nematode maintenance and multiplication.

*Meloidogyne incognita* egg masses were isolated from infected tomato roots, and the population was replicated on Pusa Ruby, a vulnerable tomato variety planted in 15 cm diameter pots with sterilised soil. This was done some months before the experiment began. Other multicultural operations were carried out when needed.

**Meloidogyne incognita egg masses and juveniles were isolated.** From the cultural pots, galled roots of tomato plants were collected. The roots were gently cleaned out from the dirt using a tab and a mild stream of water. After partial air drying, egg masses were picked up with tweezers and placed on top of the wire gauge tissue paper construction in a petri dish filled with clean tap water, with the bottom of the petri dish likewise covered to prevent evaporation loss. Freshly hatched second stage juveniles (j2) are collected after 24 hours in a petri dish and moved to a beaker, with the

petri dish being replenished with fresh water each time. The juveniles were collected for 7-8 days and then exploited for testing purpose.

### Nematode number standardisation in stock solution.

Newly hatched second stage juveniles (j2) of *Meloidogyne incognita* was identified and externally sterilised by washing them with water Mercurochrome (0.1) solution for 30 minutes to remove any bacterial or fungal contamination. The juveniles also were rinsed in sterile distilled water 4-5 times. In a beaker, the nematode suspension containing second stage juveniles was taken. It was completely mixed before using a 10 ml suspension in a rectangle counting plate to quantify the nematodes three times under a stereoscopic microscope (Doncaster 1962). The maximum number of nematodes contained in the 10 ml stock sample was measured as an average.

**Inoculation of nematodes.** After fifteen days of seeding, infective J2 of the root knot nematode was inoculated at 1000J/Kg soil. In all treatments, three tiny holes of 5cm depth were cut in the soil tightly all around base of the plant, through which a known volume of nematode suspensions (10 ml) was gently placed to release 1000 juveniles each pot, then the pores were immediately closed. Over watering threatened to disrupt the colony formation of biocontrol agents, hence irrigation was limited and only used when absolutely necessary. The experiment finished 45 days after the nematodes were inoculated, during which time the young seedlings were cared for and other intercultural operations were performed on a regular basis.

**Treatments and set-up of the experiment:** The effectiveness of two separate biocontrol agents, *Pseudomonas fluorescens* and *Trichoderma viride*, as well as one nematicide, Carbofuran, was investigated in a pot culture experiment. The two biocontrol agents were pre-incubated in FYM separately in two pot containers for two weeks before being transferred to experimental pots. Bioagents was applied to the different pots according to the treatments fifteen days after the seedlings were sown. The entire experiment was set up in 15cm earthen pots using a Completely Randomised Design (CRD) with ten treatments that were reproduced three times each. The following are the treatments:

- T<sub>1</sub> - Seed treatment with *Trichoderma viride* @ 10 g/kg
- T<sub>2</sub> - Seed treatment with *Pseudomonas fluorescens* @ 10 g/kg
- T<sub>3</sub> - Soil treatment with *Trichoderma viride* @ 20 g/m<sup>2</sup>
- T<sub>4</sub> - Soil treatment with *Pseudomonas fluorescens* @ 20 g/m<sup>2</sup>
- T<sub>5</sub> - T<sub>1</sub>+T<sub>3</sub>
- T<sub>6</sub> - T<sub>1</sub>+T<sub>4</sub>
- T<sub>7</sub> - T<sub>2</sub>+T<sub>3</sub>
- T<sub>8</sub> - T<sub>2</sub>+T<sub>4</sub>
- T<sub>9</sub> - Carbofuran @ 10g/m<sup>2</sup>
- T<sub>10</sub> - Untreated check

**Observations.** Every plant was removed carefully from the pot soil 45 days following inoculation. Roots were rinsed free of soil and other clinging particles with a gentle stream of water, and observations on several plant growth metrics were made.

- Length of shoot
- Weight of fresh shoot
- Weight of dry shoot
- Length of root
- Weight of fresh root
- Weight of dry root
- Numbers of galls in each plant
- Number of egg masses in each plant
- Total nematode population in soil (200 cc) (Cobb, 1918 and Schindler, 1961).
- *Meloidogyne incognita* population in root
- Reproductive factor  $R_f$  value

**Data analysis and interpretation based on statistics.**

In a Completely Randomized Design, numerous observations recorded during the course of the inquiry were statistically analysed (CRD). After square root and log transformations, data on the number of root galls and final nematode population in the soil as well as in the roots were analysed. The analysis of variance was performed using Fisher's method at a 5% level of significance. In addition, the treatment means were compared using the following formulas: S.E.(m).  $2 \text{ EMS} / r$  (standard error of the mean) S.E(m) $\sqrt{0.005}$  at error d.f. L.S.D. at 0.05 = S.E(m) $\sqrt{0.005}$  at error d.f.

Where E.M.S. stands for Error Means Sum of Squares, and

R stands for replication.

S.E.(m) stands for Standard Error Mean.

L.S.D. (0.05) = Least Significant Differences at a 5-percentage-point level

The difference between the two treatments indicates a significant difference between the treatments if it is more than the L.S.D value. In this approach, a comparison of the therapies may be made.

**RESULTS AND DISCUSSION**

Under pot culture conditions, an experiment was done to see how different bio agents, such as soil application and seed treatment, affected the nematode (*Meloidogyne incognita*) on black gram (Ali Moazekho *et al.*, (2020). *Pseudomonas fluorescens* and *Trichoderma viride*, two bio insecticides, were used as seed treatments alone or in combination with soil application (Mostafa *et al.* (2018). Along with an untreated check, a chemical check with carbofuran soil treatment was included. Following the Completely Randomized Design, there were ten treatments, each with three replications.

**Parameters of shoot growth (Table: 1).** Table 1 shows that all treatments increased shoot length, weight of fresh shoot and dry shoot when compare to the untreated control ( $T_{10}$ ). The highest shoot length, weight of fresh shoot and dry shoot (42.3 cm), (20.73 g) and (5.5 g) respectively. It was achieved with a combination of seed and soil treatment with *Pseudomonas fluorescens* ( $T_8$ ), which was 92.27 %, 68.12 % and 63.69 % longer than the untreated control ( $T_{10}$ ).  $T_9$  (69.54 %, 67.06 % and 48.80%),  $T_6$  (64.36 %, 61.33 % and 45.83%),  $T_7$  (55.90 %, 44.4 % and 44.64%),  $T_5$  (45.90 %, 35.46 % and 26.78%),  $T_4$  (40.90 %, 28.8% and 22.91%),  $T_2$  (37.9 %, 20.8 % and 20.83%),  $T_3$  (18.90 %, 15.46 % and 13.09%), and  $T_1$  followed in that order (11.81 %, 8 % and 5.05%).

**Table 1: Impact of bio-control agents on root knot nematode (*Meloidogyne incognita*) on shoot growth characteristics of black gram variety Urad kavva.**

Treatment	Length of shoot(cm)	Increase over control (%)	Wt. of fresh Shoot (g)	Increase Over control (%)	Wt. of dry Shoot (g)	Increase over control (%)
$T_1$ = Seed treatment with <i>T. viride</i> @ 10g/kg	24.6	11.81	14.16	14.84	3.53	5.05
$T_2$ = Seed treatment with <i>P. fluorescens</i> @10g/kg	30.16	37.09	15.83	28.38	4.06	20.83
$T_3$ = Soil treatment with <i>T. viride</i> @20g/m <sup>2</sup>	26.16	18.90	15.5	25.70	3.8	13.09
$T_4$ = Soil treatment with <i>P. fluorescens</i> @ 20g/m <sup>2</sup>	31.00	40.90	17.33	40.55	4.13	22.91
$T_5$ = $T_1 + T_3$	32.1	45.90	17.5	41.93	4.26	26.78
$T_6$ = $T_1 + T_4$	36.16	64.36	17.8	44.36	4.9	45.83
$T_7$ = $T_2 + T_3$	34.30	55.90	17.6	42.74	4.86	44.64
$T_8$ = $T_2 + T_4$	42.3	92.27	20.73	68.12	5.5	63.69
$T_9$ = Carbofuran soil application@10 g/m <sup>2</sup>	37.3	69.54	19.83	60.82	5.0	48.80
$T_{10}$ = Control	22	0.00	12.33	0.00	3.36	0.00
<b>S.E.(m)</b>	<b>0.08</b>	–	<b>0.08</b>	–	<b>0.06</b>	–
<b>LSD (0.05)</b>	<b>0.26</b>	–	<b>0.26</b>	–	<b>0.19</b>	–

(Average of 3 replications)



T<sub>1</sub> - Seed treatment with *Trichoderma viride* @10g/kg, T<sub>2</sub> - Seed treatment with *Pseudomonas fluorescens* @10g/kg, T<sub>3</sub> - Soil treatment with *Trichoderma viride* @20g/m<sup>2</sup>, T<sub>4</sub> - Soil treatment *Pseudomonas fluorescens* @20g/m<sup>2</sup>, T<sub>5</sub> - (T<sub>1</sub>+T<sub>3</sub>), T<sub>6</sub> - (T<sub>1</sub>+T<sub>4</sub>), T<sub>7</sub> - (T<sub>2</sub>+T<sub>3</sub>), T<sub>8</sub> - (T<sub>2</sub>+T<sub>4</sub>), T<sub>9</sub>-Carbofuran@10g/m<sup>2</sup>, T<sub>10</sub> - Untreated check.

**Fig. 1.** Efficacy of bio agents on shoot growth of *Meloidogyne incognita*-infected black gram.

**Parameters of root growth (Table: 2).** The length, weight of fresh and dry root of all black gram plants increased over the check period, according to data on mean root length infected by root knot nematode (T<sub>10</sub>). T<sub>8</sub> topped the table with a length (16.5 cm & 47.72 %), weight of fresh (12.73 g & 69.73%) and dry root (1.21 g & 181.39%) increase in size, followed by T<sub>9</sub> (36.62 %, 67.06% and 172.09%), T<sub>6</sub> (26.92 %, 61.33 and 162.79%), T<sub>7</sub> (25.47 %, 44.4% and 139.53%), T<sub>5</sub> (25.21 %, 35.46% and 109.30%), T<sub>4</sub> (23.75 %, 28.8% and 76.74), T<sub>2</sub> (20.58 %, 20.8% and 39.53%), T<sub>3</sub> (5.74

%, 15.46% and 16.27%), and T<sub>1</sub> (2.91%, 8.0% and 13.95%).

The application of various treatments resulted in an increase in plant growth parameters when compared to the untreated control. In all parameters, the combination of both seed and soil treated with *Pseudomonas fluorescens* produced the best results, accompanied by soil application with carbofuran (chemical check) and seed treatment with *T. viride* and soil application with *P. fluorescens*, all of which had nearly similar effects on plant parameters in the current study (Azam *et al.*, (2018).

**Table 2: The application of bio-control agents reduced root knot nematode (*Meloidogyne incognita*) root development characteristics in the black gram variety Urad kavva.**

Treatments	Length of root (cm)	Increase over control (%)	wt. of fresh root (g)	Increase over control (%)	wt. of dry root (g)	Increase Over Control (%)
T <sub>1</sub> = Seed treatment with <i>T. viride</i> @ 10g/kg	12	2.91	8.1	8.0	0.49	13.95
T <sub>2</sub> = Seed treatment with <i>P. fluorescens</i> @ 10g/kg	14.06	20.58	9.06	20.8	0.6	39.53
T <sub>3</sub> = Soil treatment with <i>T. viride</i> @ 20g/m <sup>2</sup>	12.33	5.74	8.66	15.46	0.50	16.27
T <sub>4</sub> = soil treatment with <i>P. fluorescens</i> @20g/m <sup>2</sup>	14.43	23.75	9.66	28.8	0.76	76.74
T <sub>5</sub> = T <sub>1</sub> + T <sub>3</sub>	14.6	25.21	10.16	35.46	0.9	109.30
T <sub>6</sub> = T <sub>1</sub> + T <sub>4</sub>	14.8	26.92	12.1	61.33	1.13	162.79
T <sub>7</sub> = T <sub>2</sub> + T <sub>3</sub>	14.63	25.47	10.83	44.4	1.03	139.53
T <sub>8</sub> = T <sub>2</sub> + T <sub>4</sub>	16.5	47.72	12.73	69.73	1.21	181.39
T <sub>9</sub> = Carbofuran soil application @ 10g/m <sup>2</sup>	15.93	36.62	12.53	67.06	1.17	172.09
T <sub>10</sub> = Control	11.66	0.00	7.5	0.00	0.43	0.00
<b>S.E.(m)</b>	<b>0.10</b>	—	<b>0.04</b>	—	<b>0.01</b>	—
<b>LSD (0.05)</b>	<b>0.30</b>	—	<b>0.06</b>	—	<b>0.02</b>	—

(Average of 3 replications)





T<sub>1</sub> - Seed treatment with *Trichoderma viride* @10g/kg, T<sub>2</sub> - Seed treatment with *Pseudomonas fluorescens* @10g/kg, T<sub>3</sub> - Soil treatment with *Trichoderma viride* @20g/m<sup>2</sup>, T<sub>4</sub> - Soil treatment *Pseudomonas fluorescens* @20g/m<sup>2</sup>, T<sub>5</sub> - (T<sub>1</sub>+T<sub>3</sub>), T<sub>6</sub> - (T<sub>1</sub>+T<sub>4</sub>), T<sub>7</sub> - (T<sub>2</sub>+T<sub>3</sub>), T<sub>8</sub> - (T<sub>2</sub>+T<sub>4</sub>), T<sub>9</sub> -Carbofuran@10g/m<sup>2</sup>, T<sub>10</sub> - Untreated check.

**Fig. 2.** The effect of bio agents on the root growth of *Meloidogyne incognita*-infected black gram.

**Nematode multiplication (Table 3-4).** A quick examination of the data revealed that all treatments had a considerable reduction in the nematode population i.e. no. of galls, no. of egg masses, nematode population in 200 cc of soil, and nematode population in soil & root when compared to the control (T<sub>10</sub>). T<sub>8</sub> (no. of galls - 84.8 %, no. of egg masses - 79.81%, nematode population in 200 cc of soil - 82.97% and nematode population in soil & root - 79.10%) had the greatest reduction in nematode population, followed by T<sub>9</sub> (84.37 %, 74.18%, 81.17% and 78.12%), T<sub>6</sub> (77.3 %, 66.19%, 78.01% and 75.07%), T<sub>7</sub> (73.33%, 71.36%, 77.29% and 73.16%), T<sub>5</sub> (69.17 %, 72.81%, 75.76% and 71.95%), T<sub>4</sub> (56.25 %, 59.63%, 75.31% and 68.94%), T<sub>2</sub> (26.25 %, 43.66%, 72.97% and 68.25%), T<sub>3</sub> (20.83%, 27.70%, 63.24% and 43.90%), and T<sub>1</sub>(18.75%, 14.56%, 45.04% and 40.63%).

**Reproductive factor (Table: 4).** According to the findings, T<sub>8</sub> had the greatest reduction of nematodes in

soil and roots, followed by T<sub>9</sub> and T<sub>6</sub>. T<sub>9</sub> and T<sub>6</sub> were both in the same ballpark as T<sub>8</sub>. All of the therapies outperformed the untreated control group.

The confluence of both soil & seed application with *Pseudomonas fluorescens* exhibited an increase in length of shoot (92.27%), length of root (47.72%), weight of fresh shoot (68.12%), weight of dry shoot (63.69%), weight of fresh root (69.73%), & weight of dry root (181.39%) respectively and also reduction in root galls (84.8%), total number of egg masses (79.81%), total nematode population in 200 cc of soil (82.97%), & total nematode population (79.10%) over untreated check. This is in agreement with the finding of Osman *et al.*, (2020) in comparison to the untreated control, using *Pseudomonas fluorescens* as a seed treatment + soil application resulted in a considerable increase in plant growth characteristics and groundnut pod yield.

**Table 3: Galls and egg masses are affected by the root knot nematode (*Meloidogyne incognita*).**

Treatments	Total nematode galls in each plant	% Decrease over control	Total number of egg masses in each plant	% Decrease over control
T <sub>1</sub> = Seed treatment with <i>T. viride</i> @ 10g/kg	65	18.75	60.66	14.56
T <sub>2</sub> = Seed treatment with <i>P. fluorescens</i> @ 10 g/kg	59	26.25	40	43.66
T <sub>3</sub> = Soil treatment with <i>T. viride</i> @ 20 g/m <sup>2</sup>	63.33	20.83	51.33	27.70
T <sub>4</sub> = Soil treatment with <i>P. fluorescens</i> @ 20 g/m <sup>2</sup>	35	56.25	28.66	59.63
T <sub>5</sub> = T <sub>1</sub> + T <sub>3</sub>	24.66	69.17	19.3	72.81
T <sub>6</sub> = T <sub>1</sub> + T <sub>4</sub>	18.16	77.3	24	66.19
T <sub>7</sub> = T <sub>2</sub> + T <sub>3</sub>	21.33	73.33	20.33	71.36
T <sub>8</sub> = T <sub>2</sub> + T <sub>4</sub>	12.16	84.8	14.33	79.81
T <sub>9</sub> = Carbofuran soil application @ 10 g/m <sup>2</sup>	12.5	84.37	18.33	74.18
T <sub>10</sub> = Control	80	0.00	71	0.00
<b>S. E.(m)</b>	<b>0.17</b>	—	<b>0.14</b>	—
<b>LSD (0.05)</b>	<b>0.53</b>	—	<b>0.43</b>	—

(Average of 3 replications)

**Table 4: Effect of various bio-agents on root knot nematode (*Meloidogyne incognita*) multiplication in black gram variety Urad kavya.**

Treatment	Root knot nematode population in soil (200cc)	Increase or decrease over control (%)	Root knot nematode population (soil and root)	Increase or decrease over control (%)	Rf= pf/pi
T <sub>1</sub> = Seed treatment with <i>T. viride</i> @ 10 g/kg	203.33	45.04	1026.66	40.63	1.02
T <sub>2</sub> = Seed treatment with <i>P. fluorescens</i> @ 10 g/kg	100	72.97	549	68.25	0.54
T <sub>3</sub> = Soil treatment with <i>T. viride</i> @ 20 g/m <sup>2</sup>	136	63.24	970	43.90	0.97
T <sub>4</sub> = Soil treatment with <i>P. fluorescens</i> @ 20 g/m <sup>2</sup>	91.33	75.31	537	68.94	0.53
T <sub>5</sub> = T <sub>1</sub> + T <sub>3</sub>	89.66	75.76	485	71.95	0.48
T <sub>6</sub> = T <sub>1</sub> + T <sub>4</sub>	81.33	78.01	431	75.07	0.43
T <sub>7</sub> = T <sub>2</sub> + T <sub>3</sub>	84	77.29	464	73.16	0.46
T <sub>8</sub> = T <sub>2</sub> + T <sub>4</sub>	63	82.97	361.33	79.10	0.36
T <sub>9</sub> = Carbofuran soil application @ 10 g/m <sup>2</sup>	69.66	81.17	378.33	78.12	0.37
T <sub>10</sub> = Control	370	0.00	1729.33	0.00	1.72
<b>S.E.(m)</b>	<b>0.20</b>	-	<b>0.60</b>	-	-
<b>LSD (0.05)</b>	<b>0.60</b>	-	<b>1.78</b>	-	-

(Average of 3 replications)

The number of galls/plant, egg mass/plant, eggs/egg-mass, and soil population /200 g soil were all lowest in groundnut plants treated with *P. fluorescens* as both seed treatment and soil application. Hassan *et al.*, (2019) found that treating seeds with *Pseudomonas fluorescens* and soil with both *Pseudomonas fluorescens* and *Trichoderma harzianum* was more effective in reducing root galls index and *Meloidogyne incognita* root and soil population. This is also in conformity with the finding of El-Deriny *et al.*, (2020) also documented that seed treatment with *Pseudomonas fluorescens* increased the height of seedlings significantly. In this regard Sowley *et al.*, (2018), Sreenayana *et al.*, (2021); Navazollah and Gholamrezaee (2021) reported that antagonistic activity of tested bioagents which induced decrease in nematode multiplication and resulted in enhanced plant health and yield. Root knot nematode hatching and penetration were hampered by *Pseudomonas* species (Mohapatra *et al.*, 2020 and Mahfouz *et al.*, 2019). This likely lowered root knot nematode replication and served to mitigate the inverse effects.

So the next best treatment as per the observations was the soil application with carbofuran after that seed treatment with *Trichoderma viride* and soil treatment with *Pseudomonas fluorescens* that has almost same impact factor as T<sub>8</sub> treatment but is associated with the disadvantages as possessed by the later, From the observation . It can be studied that soil application with carbofuran and seed treatment with *Trichoderma viride* + soil application with *Pseudomonas fluorescens* had increase the shoot length (69.54% and 64.36% ), weight of fresh shoot ( 60.82% & 44.36% ), weight of dry shoot ( 48.80% and 45.83%), root length (36.62% and 26.92% ), fresh root weight (67.06% and 61.33%), dry root weight (172.09% and 162.79% ), nematode galling (84.37% and 77.3% ), egg mass per plant (74.18% and 66.19% ), nematode population in soil cc

(81.17% and 78.01%), total nematode in plant and soil (78.12% and 75.07%) over untreated check.

Chen *et al.* (2020) who reported that treatment with chemical nematicide (carbofuran ) is most effective treatment in increasing growth and reduction of galling & root knot nematode reproduction in okra. Another finding of Desaegeer & Watson (2019) and confirmed that length of shoot, weight of shoot dry, reduction in galling of root system and root knot nematode reproduction was studied highest in chemical treatments as compared to bio agents. Bioagents that boost plant growth have also been discovered and inhibit multiplication of *Meloidogyne incognita*. Several earlier studies have also proved the efficacy of carbosulfan and fungal and bacterial antagonists as seed treatment in suppressing *M. incognita* in many field crops like cowpea (Patra *et al.*, 2019; Mohanapriya *et al.*, 2020). Ramadan M El-Ashry *et al.*, (2020) also had reported that maximum reduction in nematode population with application of carbosulfan @ 1kg a.i./ha in cowpea.

Although chemical nematicides are effective for worm control, their high toxicity and persistent effects on the environment, particularly on non-target organisms, necessitated the development of alternate nematode control strategies. It can also be used as a chemical check, although bio pesticides are pest specific, nontoxic to humans, less expensive, and environmentally friendly. In comparison to chemicals, the use of bio agents such as *Pseudomonas fluorescens* and *Trichoderma viride* is considered one of the alternative approaches for nematode management because it is safe for the environment, poses no health risks to humans, is cost-effective, and is readily available to farmers (Ahmad *et al.*, (2021).

In case of reduction in egg mass number of *Meloidogyne incognita* by bio inoculants the application of *Pseudomonas fluorescens* as seed and soil treatment showed best results i.e. (79.81%) increase

over check followed by application of chemical nematicide carbofuran with (74.18%), *Trichoderma viride* is used as a seed and soil treatment (72.81%). The control of egg mass by *Trichoderma viride* may be due to parasitization of *Trichoderma* on egg mass of root knot nematodes. This was in agreement with Meena *et al.*, (2020) who stated could be probably due to direct parasitism of eggs through the increase of extra cellulase chitinase activity. The findings backed with the findings of Jothi *et al.*, (2019); David *et al.*, (2018); Sankari Meena Meena *et al.*, (2019), who found that *T. harzianum* boosted tomato plant growth substantially.

Increased colonisation, competition for nutrients, change in host response, production of toxic compounds, and other factors could all contribute to *Pseudomonas fluorescens* reducing root galling and increasing plant growth metrics. Root colonisation is an important criterion for assembling bio agents and assessing the bio efficacy of any formulated product, according to Tae Gyu Choi *et al.*, 2020, who also stated that the use of *Pseudomonas fluorescens* and *Trichoderma harzianum* did not affect each other in root colonisation. Root colonisation by *Pseudomonas* sp frequently enhances root growth, development, crop productivity. Sidhu (2018) published another study that showed that nematode damage can be decreased by using PGPR.

The harmful allelochemical directly produced by *Pseudomonas fluorescens* may also boost or improve the reduction in worm population in cowpea as quoted by Kanokporn Siengchin *et al.*, (2020); Doaa Khairy *et al.*, (2021); Metwally (2019); Rostami *et al.*, (2021) and Ansari *et al.*, (2020). *Pseudomonas* may boost plant growth (Sohrabi *et al.*, 2020) by producing biologically active substances (Forghani & Hajihassani 2020) or converting unavailable minerals and organic compounds into forms that are available to plants (Forghani & Hajihassani 2020); (Samal *et al.*, 2018). *Pseudomonas* sp. can produce an enzyme that regulates plant hormone levels, limit accessible iron via siderophore synthesis, and use antibiotics to destroy the pathogen (El-Nagadi and Abd-El-Khair, 2017). Furthermore, *Pseudomonas* spp.-induced systemic resistance is suggested to represent a biocontrol strategy against plant diseases.

## CONCLUSION

Based on the findings of this study, seed treatment of black gram with *P. fluorescens* at 10 g/kg and soil application of 20 g/m<sup>2</sup> were beneficial in minimising root knot disease, nematode multiplication, and improving plant growth. However, for a meaningful outcome in managing root knot nematode, it is worthwhile to study the efficacy of this treatment in field conditions.

## FUTURE SCOPE

Because of the rising nematode pest resistance problem and the high demand for safe and high-quality food items, biocontrol will continue to expand in the future. By lowering the burden of poisonous nematicides and associated adverse effects, bioagents can aid in the formation of population regulating processes for major nematode pests. It frequently involves biocontrol chemicals that can interact with either a plant or plant nematodes to inhibit nematode growth and restrict the nematode's harmful impact on the host plant. Plant development and yield are aided by bioagents. Finally, studies focused solely on the bioagents would yield useful knowledge for better nematode disease control while avoiding injury to other biosystems. However, there will be a number of obstacles to overcome.

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