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RESEARCH ARTICLE

In-vitro and *in-vivo* evaluation of different plant-based by-products against *Meloidogyne incognita* and *Sclerotinia sclerotiorum* in French bean

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Abstract

The present study includes *in-vitro* evaluation of plant-based products against *Meloidogyne incognita* and *Sclerotinia sclerotiorum* under and further integration of the promising treatments with *Trichoderma harzianum* and fungicide, Carbendazim 50WP, to manage the nematode and fungal disease complex in French bean. During *in-vitro* studies, for root knot nematode, *M. incognita,* four doses, *i.e.*, 2.5, 5.0, 10.0 and 15.0% suspension of each plant-based by-products *viz.*, neem cake, mustard cake, garlic extract, and neem leaf extract was tested. In comparison, for *S. sclerotiorum* three doses, 5.0%, 10.0%, 15.0% suspension of each plant-based by-product was tested. Maximum egg hatch inhibition was observed in neem leaf extract at 15.0% concentration with 83.65% inhibition over the untreated control after 72 hours, followed by mustard cake at 15% after 72 hours (82.52%). Further, maximum juvenile mortality was recorded in mustard cake 15% recording 90.0% mortality, followed by neem cake 15% with 89.67% mortality over control after 72 hours. The LC50 of neem leaf extract for egg hatch inhibition was 743.32 ppm, while for mustard cake it was 854.38 ppm after 72 hours. While for juvenile mortality mustard cake showed LC50 at the concentration of 343.22 ppm after 72 hours. For *S. sclerotiorum,* maximum percent mycelial inhibition was recorded in neem leaf extract with 70.0% inhibition at 15.0% concentration over control, followed by neem cake with 69.89% inhibition at 15.0%. Among the total 12 treatments tested under *in-vivo* conditions, the results revealed soil application of mustard cake 2.5 g/kg soil before sowing +foliar spray of carbendazim 50 WP (0.25%) gave maximum reduction in sclerotinia rot disease (58.55%) along with 36.70% reduction in root knot nematode infestation while soil application of MC 2.5 g/kg soil before sowing + *T. harzianum* 20 g/kg soil 15 DAS recorded minimum root nematode infestation along with 50.97% reduction in sclerotinia rot disease. The results indicate that integration of soil amendments with bio-agent or fungicide can be utilised to develop cost-effective safer alternative to manage root knot nematode and sclerotinia rot disease complex in French bean.

Keywords: Root knot nematode, Sclerotinia rot, French bean, Mustard cake, Neem cake.

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Introduction

French bean (*Phaseolus vulgaris* L.) is an important and widely cultivated legume crop in tropics, sub-tropics and temperate regions; and known to be a rich source of dietary protein, vitamins, minerals and various polyphenols (Singh & Singh, 2015; Datt et al., 2013). The French bean crop is affected by various biotic and abiotic stresses which hinder its successful cultivation; affecting its yield along with the quality of the produce. Amongst these, soil borne pathogens pose a major problem, especially under protected cultivation. Major soil-borne pathogens affecting French bean crop are root knot nematodes and fungus *Sclerotinia sclerotiorum*. Root knot nematodes (RKNs) of the genus *Meloidogyne* have been considered as the most widespread and serious plant parasitic nematodes causing diseases to a wide range of legume crops including French beans in the tropics and subtropical regions (Shree & Schwartz, 2011). These nematodes are malignant curse to vegetables which undermine the production and very few farmers aware of

them and the damage they cause (Coyne et al., 2018). They produce characteristic galls or knots on the roots of the host plants and disrupt water and nutrient uptake ability of the plants. Due to damage caused to the roots the above ground symptoms exhibited by the nematode affected plats involves yellowing, stunting and day time wilting. In addition to the direct damage caused to the host plants these nematodes predispose the roots to other soil bore fungal and bacterial pathogens and thus play very important role in disease complexes (Coyne et al., 2018).

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic soil-borne fungus with a very wide host range as it is known to attack more than 500 species of higher plants. This pathogen can lead to significant and unpredictable yield losses, reaching up to 100% under severe conditions (Willets & Wong 1980). Several researchers have reported this fungus as highly destructive to many crops including French beans (Danielson et al., 2004). It affects all stages of the crop's development, including the seedling, vegetative, flowering, and harvesting stages. This fungus can affect all the above ground plant parts. The fungus produces lesions that are initially small, round, dark green and water-soaked. As the disease progresses, white fungal mycelium develops on the affected plant tissues which finally lead to the development of hard black sclerotia, causing wilting and ultimately death of the host plant. The sclerotia of this fungus can survive in the soil for 5-8 years and may germinate to produce mycelium (myceliogenic germination) or ascoscpores (carpogenic germination). When the soils are moist, cool and shady, sclerotia of top two inches soil profile produces ascospores (Steadman & Boland, 2005).

The root knot nematodes and *S. sclerotiorum* both share the common rhizospheric space due to their soil borne nature. The disease caused by *S. sclerotiorum* is often associated with root knot nematode and the association of both the pathogens is reported to aggravate the disease intensity in different crops (Kalaivanan et al., 2017; Dutta et al., 2020). Management of these soil borne pathogens is thus very important for sustaining crop production. Several management strategies viz., cultural, biological and application of chemicals are being used for the management of root knot nematode and sclerotinia rot. The management of root knot nematodes is difficult due to their soil borne nature, high reproductive potential and extensive host range. The use of nematicides is undesirable due to associated problems of residual toxicity, environmental pollution and public health hazards (Mukhtar et al., 2013). Similarly, due to wide host range, longevity of the soil borne sclerotia and necrotrophic nature, sclerotinia rot is very difficult to manage with fungicides. Moreover, higher usage of fungicides is not a sustainable and/or ecologically viable option. In response to these challenges, alternative methods are gaining prominence for managing postharvest fungal infections while simultaneously enhancing product quality. These methods include the use of natural plant-based products, organic amendments, disinfecting agents, biocontrol agents, and combinations thereof (Romanazzi et al., 2016). Therefore, the present study was undertaken to evaluate the efficacy of plant based organic extracts and amendments against Root knot nematode and *S. sclerotiorum* under both *in-vitro* and *in-vivo* conditions. Further, the promising amendments neem cake and mustard cake were evaluated in pots alone as well as in combination with *Trichoderma harzianum* and Carbendazim50 WP to manage nematode and fungal disease complex.

Materials and Methods

The evaluation of different plant-based extracts viz. (garlic extract, neem leaf extract, neem cake and mustard cake) was done under *in-vitro* and *in-vivo* conditions against *Meloidogyne incognita* and *Sclerotinia sclerotiorum*. The pot trials were conducted at experimental area of Department of Vegetable Science, Punjab Agricultural University, Ludhiana.

In vitro evaluation of different plant-based products against *Meloidogyne incognita*

For *in vitro* evaluation, the efficacy of the plant-based products was tested against the egg hatching and juvenile mortality of *Meloidogyne incognita*.

Nematode inoculum

The pure culture of root knot nematode, *Melidogyne incognita* was multiplied on susceptible brinjal cultivar 'Punjab Sadabahar'. The brinjal plants were raised in protrays filled with sterilized media (cocopeat & perlite) and one month old plants were transferred to 6 inches diameter pots filled with sterilized soil. One week after transplanting, the plants were inoculated with freshly hatched juveniles harvested from the pure culture raised on brinjal plants. After 60 days the plants were removed from the soil and washed under running tap water after that by using forceps, the egg masses were manually collected using forceps and transferred to sterilized water for subsequent use.

Evaluation of different plant-based products against egg hatching of *Meloidogyne incognita*

In all seventeen treatments comprising of four plant-based products viz., garlic extract, neem leaf extract, neem cake and mustard cake at four different concentrations viz., @ 2.5%, 5.0%, 10.0%, and 15.0% along with untreated control (distilled water) were evaluated. Five millilitres of solution from each treatment was dispensed into separate 10 ml Petri dishes, and four freshly extracted egg masses of *M. incognita* were placed in each dish. Five replicates of each treatment were maintained and the Petri dishes were incubated at a temperature of 25±2°C. Observations on hatching of juveniles were recorded under a stereo zoom binocular

microscope. To count the number of juveniles hatched, one millilitre of suspension was transferred to a counting dish, and the juveniles were counted at regular intervals of after 24, 48, and 72 hours. Percent egg hatch inhibition was calculated using the formula as below;

$$\text{Percent egg hatch inhibition} = \frac{\text{Number of } J_2 \text{ in untreated control} - \text{Number of } J_2 \text{ in treatment}}{\text{Number of } J_2 \text{ in untreated control}} \times 100$$

Evaluation of different plant-based products against juvenile mortality of Meloidogyne incognita

For the examination of juvenile mortality, freshly hand-picked egg masses of *M. incognita* were placed on double-layer tissue paper resting on a coarse aluminium wire gauge support, which was then positioned over a 10-centimeter diameter Petri dish containing sufficient amount of water to keep the tissue paper moist to facilitate emergence of juveniles. After 24 hours freshly hatched juveniles were collected in a beaker and adjusted to make standard suspension to achieve a count of 50 juveniles per millilitre of suspension. Prior to inoculation, the count of juveniles per millilitre of suspension was confirmed by averaging at least three counts. Five millilitre suspension of each treatment was taken in the 10 cm Petri dish and was inoculated with a total of 100 juveniles per treatment. The untreated distilled water was taken as control. Each treatment was replicated five times. Observations were recorded on the mortality of J_2 by counting dead and live juveniles at regular intervals after 24, 48 and 72 hours under the stereo zoom binocular microscope by taking 1 ml of suspension in a counting dish and repeating these three times. The average of three observations was taken as the final reading. Percent mortality was calculated by using formula as below;

$$\text{Percent mortality} = \frac{\text{Number of } J_2 \text{ in untreated control} - \text{Number of live } J_2 \text{ in treatment}}{\text{Number of } J_2 \text{ in untreated control}} \times 100$$

In-vitro evaluation of different plant-based products against Sclerotinia sclerotiorum

The same plant-based products were evaluated against *S. sclerotiorum* at concentrations of 5.0%, 10.0%, and 15.0% along with untreated control. Petri dishes containing potato dextrose agar (PDA) supplemented with plant-based products (as per the specified treatments) were inoculated with a 5 mm diameter culture disc. The various extracts were prepared by combining 100g of the plant product with 100ml of sterilised distilled water to create a standard solution (S). This standard solution was then used to prepare PDA amended with 5.0%, 10.0%, and 15.0% concentrations. From the standard solution, 5.0 ml, 10.0 ml, and 15 ml extracts were measured into conical flasks, and corresponding volumes of PDA (95 ml, 90 ml, and 85 ml, respectively) were added to achieve the desired concentrations. The flasks containing the media were autoclaved (121°C for 15 minutes) and extracts were added before pouring the media in Petri plates. The Petri dishes were inoculated with 5 mm discs of three-day-old *S. sclerotiorum* culture maintained on PDA. Five replicates were maintained for each treatment. The Petri dishes were then incubated at 25 ±1°C alongside the control. The radial growth of the fungus was measured in millimetres (mm) every 24 hours until the Petri dishes were completely covered. The percent inhibition over control was calculated by using the bliss formula as given below;

$$\text{Percent growth inhibition over control} = \frac{DC - CT}{DC} \times 100$$

DC: colony diameter in control
CT: colony diameter in treatment.

Management of M. incognita and S. sclerotiorum disease complex in French bean

After *in-vitro* evaluation of plant-based products the mustard cake and neem cake were selected for pot trials to manage root knot nematode and sclerotinia rot disease complex in French bean. The trial was conducted at Vegetable Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana. Total thirteen treatments were composed including application of mustard and neem cake as soil amendments alone or as an integration with *T. harzianum* and chemical Carbendazim 50WP. The pots were filled with sterilized soil and inoculated with freshly hatched second stage juvenile of *M. incognita* with 2 juvenile per gram of soil and *S. sclerotiorum* culture (as sorghum grains covered with mycelium of *S. sclerotiorum*) @ 250g per kg of soil. The amendments (neem cake, mustard cake) were added (as per details given below) into the pots one week before sowing of the French bean and after application soil was mixed properly followed by light watering so as to allow the amendments to decompose in the soil. After one week, the French bean genotype 'FBPVAR-16' susceptible to root knot nematode and Sclerotinia rot was sown in the pots. The treatments were added to the pots as per the details mentioned below:

T1- Soil application of MC (mustard cake) 5g/kg soil before sowing.
T2- Soil application of NC (neem cake) 5g/kg soil before sowing.
T3- *T. harzianum* 20g/kg soil before sowing.
T4- Soil application of NC 2.5g/kg soil before sowing + *T. harzianum* 20g/kg soil 15 DAS.
T5- Soil application of MC 2.5g/kg soil before sowing + *T. harzianum* 20g/kg soil 15 DAS.
T6- Soil application of NC 2.5g/kg soil before sowing + foliar spray of Carbendazim (0.2%) 15 DAS
T7- Soil application of NC 2.5g/kg soil before sowing + foliar spray of *T. harzianum* (6 g/l water) 15 DAS.
T8- Soil application of MC 2.5g/kg soil before sowing +foliar spray of *T. harzianum* (6 g/l water) 15 DAS.

T9- Soil application of MC 2.5g/kg soil before sowing+foliar spray of Carbendazim 50 WP (0.25%) 15 DAS.
T10- Soil application of *T. harzianum* 20 g/kg soil + foliar spray of Carbendazim 50 WP (0.25%) 15 DAS.
T11- Seed treatment with Carbendazim 50WP (0.2%).
T12- Foliar spray of Carbendazim 50WP (0.25%) 15 DAS.
T13- Untreated control

The observations were recorded on fifty days-old plants on plant growth parameters (shoot weight (g) and shoot length (cm), soil nematode population, root gall index, number of egg masses per root system and percent disease index. The plant growth parameters were calculated by using Centimetre scale. For recording root galling index (RGI), after uprooting and gentle washing, the number of galls per plant was counted and on the basis of the number of galls present on the individual root system of the plant, it was graded using 0-5 scale (Taylor & Sesser, 1978).

The root galling index was calculated using the formulae:

$$RGI = \frac{\text{Sum total of grades of all the plants observed}}{\text{Total number of plants observed}}$$

For Sclerotinia rot, scale (0–9) described by Teran et al., (2006) was used to score the plants and percent disease index was calculated using the following formula (Wheeler, 1969):

$$PDI = \frac{\text{Total sum of numerical ratings}}{\text{Total number of plants} \times \text{Maximum disease rating}} \times 100$$

Statistical analysis

The replicated data recorded for *in-vitro* and *in-vivo* studies was subjected to statistical analysis for the computation of the critical difference (CD) at 0.5% using R software. The data recorded for *in-vitro* efficacy of different treatments against root knot nematode was also subjected to Probit analysis to compute the lethal dose expressed in terms of LC50 and LC90 *i.e.* median lethal dose at which 50% and 90% of the nematodes fail to hatch and became dead for egg hatch inhibition and juvenile mortality experiments, respectively.

Results and Discussion

In-vitro evaluation of different plant-based products against juvenile mortality and hatching of Meloidogyne incognita

The data recorded on the effect of plant-based products on egg hatching of root-knot nematode under *in-vitro* conditions revealed that different extracts showed varying degrees of egg hatch inhibition over the untreated control (Table 1). Maximum egg hatch inhibition was observed in neem leaf extract 15% concentration with 66.55, 73.74, 83.65% inhibition over untreated control followed by mustard cake at 15% showing 61.72, 72.63 and 82.52% inhibition over untreated control after 24, 48 and 72 hours. All the treatments exhibited maximum egg hatching inhibition at higher concentrations *i.e.*15% and minimum at 2.5% concentration. Overall maximum egg hatch inhibition (74.27%) was recorded at the highest concentration in neem leaves i.e. at 15% followed by mustard cake 15% conc. (72.15 %) (Table 2). Overall minimum percent egg hatch inhibition (45.60) was recorded at the lowest concentration of neem cake i.e. 2.5% followed by neem leaf extract at 2.5% (48.38%). There was a steady decline in egg hatch inhibition with dilution of extracts from 15% to 2.5%. Thus, the inhibition in egg hatching of *M. incognita* was found to be concentration-dependent.

When the lethal dose expressed in terms of LC50, LC90 *i.e.* median lethal dose at which 50% and 90% of the nematodes fail to hatch was computed using probit analysis method it was observed that, for egg hatching inhibition of *M. incognita*, the neem leaves showed the least LC50 at the concentration of 3243.93 (24 hours), 2332.00 (48 hours) and 743.22 ppm (72 hours) while for mustard cake it was recorded as 3789.27 (24 hours), 3454.55 (48 hours) and 854.38ppm (72 hours). Similarly, neem leaf extract exhibited LC90 at the concentration (in ppm) of 35456.61 (at 24 hours), 23432.38 (48 hours), 8765.24 (72 hours), while for mustard cake, LC90 was observed at 37654 (24 hours), 34353.43 (48 hours) and 9454.82 at 72 hours. Therefore, the treatment (neem leaves) was confirmed to have maximum activity against root-knot nematode egg hatch inhibition, followed by mustard cake and neem cake (Table 3). Kumar et al. (2019) studied the *in-vitro* effect of neem leaves, cauliflower leaves, and cabbage leaves against rice root-knot nematode *M. graminicola*. They found that maximum egg-hatching inhibition was recorded by neem leaves, followed by cauliflower and cabbage. Njenga et al. (2019) studied that maximum egg hatching inhibition was recorded in neem extract and Tithonia against RKN in French bean.

In-vitro evaluation of different plant-based products against juvenile mortality of root-knot nematode, Meloidogyne incognita

As per the observations recorded in Table 4, maximum juvenile mortality was also recorded in mustard cake at 15% with 72.65, 76.67, 90.00% mortality over control after 24, 48 and 72 hours, respectively. This was followed by neem cake at 15% concentration with 70.00, 73.33, 89.67% juvenile mortality after 24, 48 and 72 hours, respectively. All the treatments exhibited maximum juvenile mortality at higher concentrations *i.e.*15% and minimum at 2.5% concentration. Overall maximum juvenile mortality (79.70%) was recorded at the highest concentration of mustard cake at 15% concentration, followed by neem cake at 15% conc. (77.60%) Overall, minimum juvenile mortality (35.5%) was recorded at the lowest concentration of garlic extract, i.e., 2.5%, followed by neem cake extract at 2.5% (42.3%). A steady decline in juvenile mortality was observed as the extracts were diluted from 15 to 2.5%. Thus, juvenile mortality of *M. incognita* was found to be concentration dependent (Table 5).

Table 1: *In-vitro* evaluation of different plant-based products against egg hatching of root-knot nematode, *M. incognita*

Treatment	*Concentration (%)*	*Egg hatching inhibition (%)*		
		24h	*48h*	*72h*
Garlic extract	2.5	38.46	61.40	64.41
	5	40.87	63.46	67.53
	10	51.44	71.23	72.79
	15	57.21	72.63	75.74
Mustard cake	2.5	38.38	47.66	68.77
	5	48.94	53.81	70.55
	10	51.58	59.67	75.19
	15	61.72	71.08	82.52
Neem cake	2.5	33.10	42.16	61.54
	5	41.90	49.32	66.78
	10	47.62	55.86	74.21
	15	59.51	66.51	80.21
Neem Leaves	2.5	34.86	44.44	65.85
	5	48.94	55.48	67.53
	10	60.39	59.67	72.09
	15	66.55	73.74	83.65
CD (0.5%)		Treatments (A)=0.848	Treatments (A)=0.965	Treatments (A)=1.238
		Concentrations (B)=0.548	Concentrations (B)=0.863	Concentrations (B)=1.107
		(A) × (B)=1.454	(A) × (B)=1.765	(A) × (B)=1.989

Table 2: Overall efficacy of different treatments on percent egg hatch inhibition of *Meloidogyne incognita* at different concentrations

Treatments	*Percent egg hatch inhibition at different concentrations*			
	2.5%	*5%*	*10%*	*15%*
Garlic extract	55.11±14.45	56.6±13.96	65.10±11.90	68.5±9.92
Mustard cake	51.60±15.57	57.76±11.34	62.14±12.00	72.15±11.01
Neem cake	45.60±14.53	52.66±12.77	59.23±13.61	68.74±10.53
Neem leaves	48.38±15.87	57.31±9.43	64.05±6.97	74.27±8.00

Table 3: Probit analysis for lethal dose of different treatments against root-knot nematode egg hatching inhibition

Treatments	*Lethal concentration (ppm) (egg hatching inhibition) (LC50)*			*Lethal concentration (ppm) (egg hatching inhibition) (LC90)*		
	24 hours	*48 hours*	*72 hours*	*24 hours*	*48 hours*	*72 hours*
Garlic	5676.92	6765.94	1071.21	63243.93	54323.45	10343.07
Mustard cake	3789.27	3454.55	854.38	37654.43	34353.43	9454.82
Neem cake	4321.93	4578.10	987.19	47876.43	45654.72	9786.67
Neem leaves	3243.93	2332.00	743.32	35456.61	23432.38	8765.24

As per the observation recorded in the Table 6, the computation of lethal dose LC50, LC90 median lethal dose at which 50 and 90% of the nematodes become dead and immobile using probit method revealed that mustard cake showed the LC50 for juvenile mortality at the concentration of 567.34 (after 24 hours), 456.93 (48hours) and 343.22 (72 hours), while for neem cake LC 50 was recorded as 621.92 (after 24 hours), 503.35 (48 hours) and 456.45 (72 hours).

Table 4: *In vitro* evaluation of plant-based by-products against juvenile mortality of root knot nematode, *Meloidogyne incognita*

Treatment	*Concentration (%)*	*Juvenile mortality (%)*		
		24h	*48h*	*72h*
Garlic extract	2.5	20	36.67	50
	5	26.67	45.00	57.33
	10	33.33	48.33	75.00
	15	45.00	53.33	80.00
Mustard cake	2.5	41.33	55.67	68.33
	5	43.33	56.67	78.33
	10	70.33	74.00	79.67
	15	72.65	76.67	90.00
Neem cake	2.5	20.00	41.67	65.00
	5	36.67	46.67	72.33
	10	40.00	63.33	86.67
	15	70.00	73.33	89.67
Neem Leaves	2.5	26.67	47.67	66.67
	5	43.33	57.00	71.67
	10	46.67	60.00	78.33
	15	45.00	65.67	85.00
CD (0.5%)		Treatments (A) = 0.765 Concentrations (B) = 0.456 (A) × (B) = 1.654	Treatments (A) = 0.765 Concentrations (B) = 0.565 (A) × (B) = 1.787	Treatments (A) = 0.987 Concentrations (B) = 0.786 (A) × (B) = 1.987

Table 5: Overall efficacy of treatments on root knot nematode juvenile mortality at different concentrations

Treatments	*Percent juvenile mortality at different concentrations*			
	2.5%	*5%*	*10%*	*15%*
Garlic extract	35.5±15.03	43.00±15.43	52.22±21.11	59.40±18.28
Mustard cake	55.11±13.51	59.40±17.66	74.6±4.71	79.70±9.08
Neem cake	42.3±22.51	51.89±18.39	63.4±23.34	77.60±10.53
Neem leaves	47.0±20.01	57.4±14.17	61.7±15.90	65.30±20.00

Table 6: Probit analysis for lethal dose of different treatments against root knot nematode juvenile mortality

Treatments	*Lethal concentration (ppm) (percent mortality) (LC 50)*			*Lethal concentration (ppm) (Percent mortality) (LC 90)*		
	24 hours	*48 hours*	*72 hours*	*24 hours*	*48 hours*	*72 hours*
Garlic extract	1000.64	987.62	879.93	6765.72	6321.16	5876.82
Mustard cake	567.34	456.93	343.22	3754.28	3432.85	2876.78
Neem cake	621.92	503.35	456.45	4365.34	4021.23	3234.23
Neem leaves	654.68	524.80	586.18	4476.59	4345.73	3432.32

Similarly, mustard cake recorded LC90 at the concentration of 3754.288 (after 24 hours), 3432.85 (48 hours) and 2876.78 (72 hours), neem cake recorded LC90 at 4365.34 9 (24 hours), 4021.23 (48 hours) and 3234.23 (72 hours). Therefore, the treatment (mustard cake) was found to have maximum activity against root knot nematode juvenile mortality, followed by neem cake, neem leaves, and garlic extract. Javed et al. (2021) studied the efficacy of the neem leaves against the mortality of root knot nematode at 48-hour intervals at different concentrations, i.e., 25%, 50%, 100%.

The maximum mortality percentage against the root knot nematode was observed in 100% concentration at 48-hour intervals. The highest larval mortality was observed at 100% concentration as compared to 25 and 50% in the case of neem leaves. Das et al. (2021) studied the concentration of different organic amendments and botanical extracts on the mortality of *M. javanica*. The two organic amendments and two botanical extracts, *i.e.,* vermicompost and biogas digestate, marigold and cabbage, were used. The results showed that maximum mortality was observed in botanical extracts as compared to organic amendments.

In- vitro evaluation of different plant-based products against *S. sclerotiorum*

As per Figure 1, it was observed that the maximum percent inhibition was recorded in neem leaves at different concentrations showing 43.21, 65.23 & 70.0% inhibition at 5, 10, 15% concentration over control followed by neem cake with 41.82, 50.91, 69.89% inhibition at 5, 10 and 15%, respectively. The minimum percent inhibition was recorded in garlic extract, showing 55.0, 45.30, & 20.63% inhibition over control at 15, 10 and 5%, respectively. In another study, Meena et al. (2021) found that Mancozeb + carbendazim and garlic were found to be most effective in the inhibition of mycelial growth under *in-vitro* conditions, as well as in reducing disease intensity under *in-vivo* conditions. Khamari et al. (2018) studied that neem cake registered maximum growth inhibition of 60, 76.57 and 93.73%, followed by Mustard cake (58.20, 68.89, 93.73%) and Mahua cake (51.53, 68.49, 87.13%) at 3, 4, and 5% concentrations, respectively. Kumawat et al. (2021) studied that garlic (*Allium sativum*) clove extract gave maximum inhibition (40.40, 62.60, and 76.30%) of mycelial growth at 5, 10, and 15% concentration.

Management of M. incognita and S. sclerotiorum disease complex in French bean

Data from Table 7 reveals that the minimum average root gall index (2.25) was observed in treatment with T5 (Soil application of MC 2.5 g/kg soil before sowing + *T. harzianum* 20 g/kg soil 15 DAS) with 40.16% reduction over control. It was found to be statistically at par with treatment T4-, *i.e.*, soil application of NC 2.5 g/kg soil before sowing + *T. harzianum* 20 g/kg soil 15 DAS (39.10% reduction over control). Similarly, the minimum average number of egg masses per root system (8.67) was observed in T5, followed by T4 with 9.33 egg masses per root system. The treatments T4 and T5 also showed significant reduction in sclerotinia rot of French bean with 51.17 and 50.97% disease reduction over the control. For *Sclerotinia,* minimum disease severity index 22.26 (58.55% reduction over control) was observed in T9 i.e. soil application of MC 2.5 g/kg soil before sowing + foliar spray of carbendazim (0.25%) 15 DAS which is at par with T6- soil application of NC 2.5 g/kg soil before sowing + foliar spray of carbendazim 50WP (0.2%) 15 DAS with disease severity index of 24.30 (54.75% reduction over control). The treatment T9 and T6 also gave 36.70 and 37.50% reduction in root knot nematode infestation and were statistically at par with the T4 (soil application of NC 2.5 g/kg soil before sowing +*T. harzianum* 20 g/kg soil 15 DAS) and T5 (Soil application of MC 2.5 g/kg soil before sowing + *T. harzianum* 20 g/kg soil 15 DAS) treatments those gave maximum reduction in root knot nematode infestation.

The observations recorded on plant growth parameters showed that the maximum plant height (73.67 cm) and plant weight (12.90 g) was observed in treatment number T9 i.e. soil application of MC 2.5 g/kg soil before sowing + foliar spray of carbendazim 50 WP (0.25%) 15 DAS followed by T6, *i.e.*, Soil application of NC @1 t/ha before sowing +

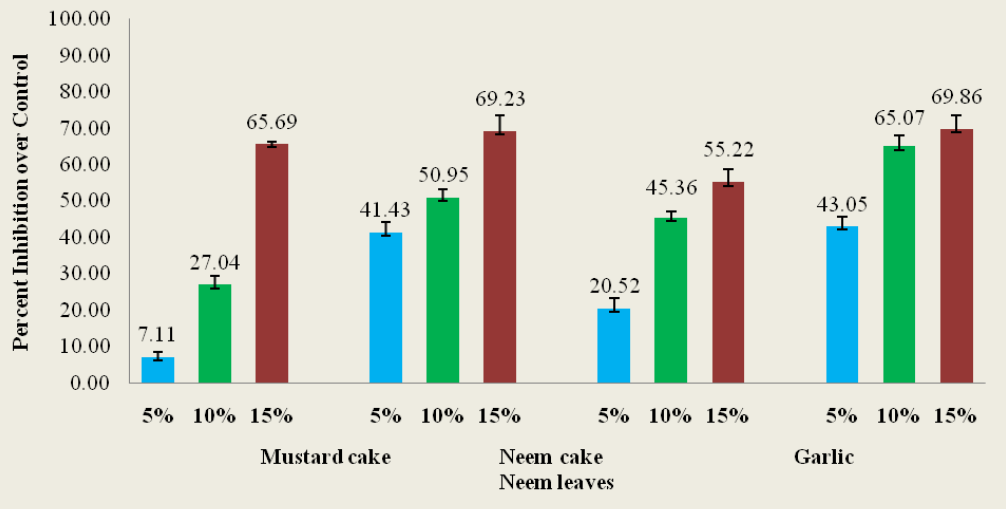

Figure 1: *In-vitro* evaluation of different plant-based products against *Sclerotinia sclerotiorum*

Foliar spray of carbendazim 50 WP (0.2%) 15 DAS exhibiting plant height as 72.67 cm and weight as 12.53 g (Table 7). However, these treatments were statistically at par with treatments T4 and T5. The addition of organic amendments like neem cake and mustard cake is known to release some antimicrobial and antifungal toxins on decomposition (Tayyab et al., 2019), enhance the activity of natural enemies or antagonistic microflora and improve the levels of plant resistance to pathogens (Tayyab et al., 2019). In addition to this, azadirachtin, the main active compound in neem and glucosinolates and isothiocyanates produced by Brassica plants are well known for their pesticidal activity against soil-borne pathogens and nematodes (Syndia et al., 2015). Different studies have documented the antifungal and anti-nematicidal effects of these organic amendments in different crops. Jena et al. (2021) reported that soil application of mustard cake or neem cake @ 50 g/m^2 along with AM fungus (*Glomus fasciculatum*) 5g/m^2 plus seed treatment with *Rhizobium* @ 25 g/kg seed significantly reduced root knot nematode population in soil as well as roots of green gram, along with improvement in plant growth. Similarly, Meena et al. (2020) evaluated the effect of neem cake, mustard cake, mahua cake, vermicompost, and karanj for the management of root knot nematode and *Fusarium oxysporum* disease complexes in the tomato crop. It was observed that neem cake was most effective, followed by castor cake and mahua cake, for the management of RKN population as well as Fusarium growth, with a considerable increase in plant growth parameters. The application of neem cake, caster cake, mustard cake, wheat straw and

Table 7: Integrated management of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* disease complex in French bean

Treatments	*Root knot nematode disease*			*Sclerotinia white rot*			
	RGI (0-5) scale	*Percent decrease over control*	*Number of egg masses/ root system*	*Percent disease index (PDI)*	*Reduction in over control (%)*	*Plant height (cm)*	*Plant weight (g)*
T1- Soil application of MC 5g/kg soil before sowing	2.41	35.90	10.00	29.74	44.62	64.00	10.00
T2- Soil application of NC 5g/kg soil before sowing	2.47	34.31	12.00	30.22	43.72	62.42	9.73
T3-*T. harzianum* 20g/kg soil before sowing	3.29	12.50	14.0	42.59	20.69	55.89	9.30
T4- Soil application of NC 2.5g/kg soil before sowing + *T. harzianum* 20g/kg soil 15 DAS	2.29	39.10	9.33	26.22	51.17	69.22	10.87
T5- Soil application of MC 2.5g/kg soil before sowing + *T. harzianum*20g/kg soil 15 DAS	2.25	40.16	8.67	26.33	50.97	68.78	10.81
T6- Soil application of NC 2.5g/kg soil before sowing + Foliar spray of carbendazim50 WP (0.2%) 15 DAS	2.35	37.50	11.67	24.30	54.75	72.67	12.53
T7- Soil application of NC 2.5g/kg soil before sowing + Foliar spray of *T. harzianum* (6 g/ l water) 15 DAS	2.40	36.17	14.33	27.22	49.31	65.00	10.76
T8- Soil application of MC 2.5g/kg soil before sowing +Foliar spray of *T.harzianum* 6 g/ l of water 15 DAS	2.71	27.93	12.6 7	26.96	49.80	65.56	10.72
T9- Soil application of MC 2.5g/kg soil before sowing +Foliar spray of carbendazim 50 WP (0.25%) 15 DAS	2.38	36.70	12.33	22.26	58.55	73.67	12.90
T10- Soil application of *T. harzianum* 20 g / kg soil + Foliar spray of Carbendazim (0.25%) 15 DAS	3.28	12.77	17.67	25.11	53.24	67.67	12.27
T11-Seed treatment with Carbendazim 50WP (0.2%)	3.67	2.39	18.00	31.11	42.07	66.89	10.73
T12- Foliar spray of Carbendazim 50WP (0.25%) 15 DAS	3.70	1.60	19.00	26.67	50.34	67.20	10.75
T13-Untreated control	3.76	0.00	22.00	53.70	0.00	48.00	8.50
CD (0.05)	0.47	-	4.35	0.35		6.34	2.11

*DAS=Days after sowing

paddy straw significantly reduced the Sclerotinia stem rot of chick pea (Singh et al., 2016). Chandrawat et al. (2020) also proved the effect of oil cakes on the enhancement of plant growth parameters and the reduction in nematode population in tomato. Dhillon et al. (2023) also reported that soil application of neem cake 1t/ ha and mustard cake 1t/ ha, along with Farm Yard Manure (FYM) 2.5 t/ha before sowing, significantly reduced the root knot nematode infestation and enhanced the yield of cucumber under greenhouse conditions.

Conclusion

The present study concludes that for root knot nematode, neem leaf extract and mustard cake (MC) and neem cake were found effective against root knot nematode, *M. incognita* and *S. sclerotiorum* under *in-vitro* conditions. Further, integration of neem cake and mustard cake under *in - vivo* studies alone as well in integration with *Trichoderma* and carbendazim, revealed that soil application of MC 2.5 g/kg soil before sowing + *T. harzianum* 20 g/kg soil 15 DAS gave 40.16% reduction in root knot nematode infestation with 50.97% reduction in sclerotinia rot disease while soil application of MC 2.5g/kg soil before sowing + foliar spray of carbendazim (0.25%) 15 DAS gave 58.55% reduction in screotinia rot with 36.70% reduction in root knot nematode infestation. Thus, integration of plant-based amendments with biocontrol agent *T. harzianum* or carbendazim can be used to effectively manage root knot nematode and sclerotinia disease in French bean.

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सारांश

वर्तमान अध्ययन में, चार अलग-अलग पौध-आधारित उत्पाद (नीम केक, सरसों केक, लहसुन का अर्क, नीम पत्ती अर्क) का मूल्यांकन इन-विट्रो स्थितियों के अंतर्गत सूत्रकृमि मेलॉयडोगाइन इन्कॉग्निटा एवं स्क्लेरोटिनिया स्क्लेरोशियोरम के विरुद्ध किया गया तथा आगे इनके उपयोग को फ्रेंच बीन में नेमाटोड एवं फंगल रोग परिसर के प्रबंधन हेतु ट्राइकोडर्मा हार्ज़ियानम एवं कार्बेन्डाजिम 50 WP के साथ एकीकृत किया गया। इन-विट्रो अध्ययन के दौरान, रूट नॉट नेमाटोड के लिए प्रत्येक उत्पाद की चार सांद्रताएँ (2.5%, 5.0%, 10.0% एवं 15.0%) पर परीक्षण किया गया, जबकि S. sclerotiorum के लिए तीन सांद्रताएँ (5.0%, 10.0% एवं 15.0%) का परीक्षण किया गया। रूट नॉट नेमाटोड के मामले में, अंडों के हैचिंग का अधिकतम अवरोध नीम पत्ती अर्क द्वारा (83.65%) तथा इसके बाद सरसों के केक द्वारा (82.52%) 15% सांद्रता पर 72 घंटे में दर्ज किया गया। किशोर मृत्यु दर में अधिकतम प्रभाव सरसों के केक 15% (90.00%) तथा नीम केक 15% (89.67%) द्वारा 72 घंटे के बाद देखा गया। S. sclerotiorum के लिए, नीम पत्ती अर्क में माइसीलियल वृद्धि का अधिकतम अवरोध 15.0% सांद्रता पर 70.0% दर्ज किया गया। परिणाम दर्शाते हैं कि नीम या सरसों के केक को जैव-कारक या कवकनाशी के साथ मिलाकर फ्रेंच बीन में जड़ गाँठ सूत्रकृमि एवं स्क्लेरोटिनिया सड़न रोग के प्रबंधन हेतु एक लागत-प्रभावी, सुरक्षित एवं टिकाऊ रणनीति विकसित की जा सकती है।