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Integrated application of amendments and chemicals for the management of root-knot nematode (*Meloidogyne incognita*) in cucumber (*Cucumis sativus*)

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ABSTRACT

Root-knot nematode is one of the significant plant-parasitic nematodes in cucumber (*Cucumis sativus* L.). Considering its destructive potential, studies were carried out on effect of organic amendments and their integration with chemicals upon root-knot nematode in cucumber in pots (2016–17) and in infested field (2017–18), at research farm of Punjab Agricultural University, Ludhiana, Punjab. Among the organic amendments, mustard cake treatment (5 t/ha) was found to be most effective with 65.16% nematode infestation reduction in pot experiment and 53.57% nematode infestation reduction in field experiment over control. Integrated treatments were found to be more effective than individual applications. Individually, the maximum reduction in soil nematode population was observed in applying mustard cake (5 t/ha) followed by neem cake (5t/ha). The efficacy of mustard cake was observed to increase in integrated treatments. In integrated treatments, application of mustard cake (2.5 t/ha) + Basamid (20 g/m) was observed to be most effective in reducing nematode infestations in soil and roots of cucumber. Among all the chemicals and amendments evaluated against root-knot nematode, Basamid among chemicals and mustard cake as an amendment in integration were found most effective against root-knot nematode and can be explored further.

Keywords: Basamid, Carbofuran, Mustard Cake, Neem cake

Agricultural productivity is hampered by a slew of issues throughout the world. Plant-parasitic nematodes (PPNs) are a significant constraint in many countries, especially in the tropics and subtropics regions, where they cause considerable production losses in various crops. Among the root knot nematodes, *Meloidogyne incognita* is one of the most significant because of its aggression, broad host range and widespread distribution for various commercially important agriculture and greenhouse crops (Hussain *et al.* 2011a). *Meloidogyne incognita* enters the roots and establishes itself in its feeding site, converting the cells around the stylet into giant cells and, as a result, causing galls on the roots, obstructing the plant's ability to absorb water and nutrients (Karssen *et al.* 2013).

Cucumber (*Cucumis sativus* L.) is a globally significant vegetable documented as a root-knot nematode-prone crop. More and more farmers have recently opted for its cultivation in poly houses/net houses in addition to open cultivation. Under protected structures, the losses might be multiplied because of congenial conditions of higher temperature, humidity, continuous monoculture, maintenance of stable microclimate and recycling of nematode-infected growing medium and planting materials by unaware growers that led to maximum multiplication rate of root knot nematode on Cucurbitaceous crops (Sorribas *et al.* 2020) and caused around 88% yield losses (Gine *et al.* 2014). In a protected cultivation system, plant parasitic nematodes (*Meloidogyne* spp.) are the most damaging and toughest to manage (Sharma *et al.* 2007), which has become a major global concern (Silva *et al.* 2019, Barros *et al.* 2021).

Cucumber being directly consumable, chemicals may prove to be very hazardous to health. With more awareness of health concerns and environmental risks, the consumer gives preference to food grown organically. So there is more focus on the sustainable methods to control pathogens in agroecosystems. However, the farmers have to achieve efficient control of the pests in their crops because these negatively affect the yield and subsequently their income. In such conditions, there is a great need to prospect for the optimal approach in the management of pests, in order to ensure both efficient and sustainable control. Therefore, the efficient management of root-knot nematode becomes vital for sustaining cucumber production. Hence, alternative

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approaches individually or in integration need to be explored to avoid losses and increase yields.

MATERIALS AND METHODS

Nematode Inoculation: Pure culture of root knot nematode was raised by single egg mass technique and maintained in earthen pots. Infested plants from a pure culture maintained in pot and research field at Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab were uprooted and their roots were gently washed with water. After washing the roots, egg masses were removed and placed in Petri plate having water for hatching second stage juvenile. After 24 h, the hatched larvae were used for further inoculations. Before inoculations, the juvenile's number per ml of suspension was counted by taking an average of at least 3 counts. The juvenile levels were adjusted with water to add an equal volume of nematode suspension in each experiment for further experimentation whenever necessary.

Evaluation of amendments and their integration with chemicals against root-knot nematode: Experiments were conducted in earthen pots (2016–17) as well as in infested soil beds (2017–18) under protected conditions at the Department of Plant Pathology, PAU, Ludhiana to ascertain the effect of different integrated approaches for the management of root knot nematode in Cucumber. For the pot experiment, earthen pots of 30 cm diameter were filled with known initial population of *M. incognita* and for the infested bed experiment; seedbeds of 2 sqm with known initial population of *M. incognita* were made. The treatments given in Table 1 were done in pots and seedbeds 15 days before transplanting. Basamid and formalin applications were given simultaneously with other treatments when integrated, and these pots were covered with a sheet for 7 days. After removing a sheet, the soil was upturned twice for 2 days, and then after 6 days, seedlings were transplanted. The seedlings used in the study were raised in plastic trays having cocopeat. This experiment was conducted in a completely randomized design under protected conditions and each treatment was replicated thrice.

For the infested soil bed experiment 14 treatments, viz. T_1, T_2, T_3, T_4, T_5, T_7, T_8, T_9, T_{11}, T_{12}, T_{13}, T_{15}, T_{16}, and T_{17} were selected (Table 1). Observations were recorded after 60 days on soil nematode population/250 cc soil as per modified Cobb's sieving and decanting method (Cobb 1918 and Schnidler 1961). After uprooting and washing, the roots of each plant was spread on paper and graded for root gall index (RGI) based on the per cent root system galled by using a 0-10 scale (Bridge and Page 1980). The reproduction factor (Rf) was calculated by dividing the final population (Pf) by the initial one (Pi). Observations on growth parameters were recorded immediately after uprooting the plants. To remove the soil and adhering inert material, the roots were washed. Excess water from the root surface was removed by placing the root between the blotting papers, and then fresh root weight and shoot weight were taken with the help of an electronic balance. Root portion from the shoot portion was separated by cut with a blade, then the length of the main root was measured up to the growing point, and shoot length was measured from the soil surface to the growing bud with the help of scale. The per cent increases and reductions in these variables were calculated over control as:

Per cent reduction/increase = (Treatment/Control-1) × 100

Table 1 Description of treatments applied for integrated management of *M. incognita* in Cucumber

Treatment	Description (dose)
T_1	Soil application with mustard cake (5 t/ha)
T_2	Soil application with neem cake (5 t/ha)
T_3	Soil application with FYM (5 t/ha)
T_4	Carbofuran (2.0 kg a.i./ha) + FYM (2.5 t/ha)
T_5	Formalin (3%) + FYM (2.5 t/ha)
T_6	Triazophos (0.1%) + FYM (2.5 t/ha)
T_7	Basamid (20 g/sqm) + FYM (2.5 t/ha)
T_8	Carbofuran (3 kg a.i./ha) + mustard cake (2.5 t/ha)
T_9	Formalin (3%) + mustard cake (2.5 t/ha)
T_{10}	Triazophos (0.1%) + mustard cake (2.5 t/ha)
T_{11}	Basamid (20 g/sqm) + mustard cake (2.5 t/ha)
T_{12}	Carbofuran (2.0 kg a.i /ha) + neem cake (2.5 t/ha)
T_{13}	Formalin (3%) + neem cake (2.5 t/ha)
T_{14}	Triazophos (0.1%) + neem cake (2.5 t/ha)
T_{15}	Basamid (20 g/sqm) + neem cake (2.5 t/ha)
T_{16}	Carbofuran (3 kg a.i./ha)
T_{17}	Control

Statistical analysis: All the data were subjected to Analysis of Variance (ANOVA), and means were compared by Duncan's Multiple Range Test (DMRT) at a 5% level of significance and standard errors of means were calculated using R-studio (RStudio Team 2021).

RESULTS AND DISCUSSION

Earthen pot experiment: Results (Table 2) shows that the population of soil nematodes decreased in different ways under different treatments. When compared to control, mustard cake treatment (5 t/ha) was shown to be the most efficient in reducing nematode infestation in soil (65.16%), followed by neem cake application (5 t/ha). In terms of statistics, both of these were at par with each other. Formalin (3%) and carbofuran (2 kg a.i./ha) similarly reduced nematode population, however the reduction was considerably and comparably less than individual mustard cake treatment. In soil treated with mustard cake (5 t/ha), the root galling index on infected cucumber roots was also observed to be lowest (RGI= 4.33), with a reduction of 46.18% over control. It was statistically at par with neem cake (5 t/ha) (RGI=4.67). Root gall index was observed to be higher in the application of FYM (5 t/ha) than mustard cake and neem cake treatments.

When used in integration, the maximum reduction in soil nematode population was recorded when the soil was treated with mustard cake (2.5 t/ha) + basamid (20 g/sqm) (79.07% decrease). In this treatment, the soil population was meager (149.99 nem/250 cc soil) compared to 716.66 J_2/250 cc soil in control (Table 2). Root galling index was also observed to be least amongst all the treatments given (RGI -2.33). The efficiency of T_9 treatment was found to be statistically at par with T_{11} treatment. Integration of formalin (3%) + mustard cake (2.5 t/ha) also gives 76.74% reduction in soil nematode population over control and 65% reduction in root gall index (RGI=3.00). Integration of Basamid (20 g/sqm) + neem cake (2.5 t/ha) was also found to reduce the soil nematode population effectively. The reproduction factor was observed to be less than 1 in this treatment. Reduction in nematode population was (40–55%) also observed in T_4, T_5 where formalin was integrated with carbofuran and FYM. However, the decrease was significantly less than basamid integrated treatments. Treatments with triazophos individually and with integration were found to be the least effective in all the treatments.

Growth parameters were significantly affected by the different treatments. Shoot length and shoot weight was observed to the maximum in T_{11} treatment where integration of basamid (20 g/sqm) with mustard cake (2.5 t/ha) was done (Table 2). Among the treatments, minimum shoot length was observed in treatment T_6. Integration of formalin with mustard cake and neem cake also increase shoot length and shoot weight. Shoot weight was also observed to be maximum in treatment T_9. Shoot length and shoot weight were observed higher in integrated treatments as compared to the individual application. Shoot weight was observed to be more in integrated treatments of mustard cake with basamid and formalin than mustard cake alone. Similar trends were observed in the root length of cucumber, where the primary root was healthier and more prolonged in T_9 and T_{11} treatments than in the control. Due to more galling on the roots in treatments T_6 and $T_{14,}$ root weight was higher in these treatments and control.

Infested soil bed experiment: Trails were conducted in root knot nematode infested soil beds with selected treatments from the earthen pot study. Since triazophos was not found to be effective, it was not used in this trail. This study revealed that the soil nematode population was reduced effectively when the mustard cake was integrated with basamid or formalin chemicals. More than 70%

Table 2 Effect of organic amendments and their integration with chemicals on root-knot nematode population in soil, root gall index (RGI) and on growth parameters of cucumber grown in earthen pots

Treatment	*Earthen pot experiment*								
	Soil nematode population/ 250 cc	Percent (%) decrease over control	Rf	RGI	Percent (%) decrease over control	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T_1	249.66 (16.29*)	65.16	0.91	4.33	46.18	186.67^{c}	86.90^{bc}	14.36^{abc}	2.14^{i}
T_2	349.98 (18.24*)	51.16	1.28	4.67	42.26	156.00^{d}	66.86^{de}	14.30^{abc}	2.39^{h}
T_3	449.98 (20.81*)	37.21	1.64	6.67	30.71	126.38^{efg}	44.00^{fg}	11.89^{bcde}	3.04^{ef}
T_4	366.67 (18.36*)	48.83	1.34	6.33	26.98	111.35^{gh}	48.38^{fg}	9.29^{ef}	3.24^{d}
T_5	396.66 (19.84*)	44.65	1.52	5.67	34.64	141.42^{de}	56.43^{ef}	12.18^{bcde}	2.95^{f}
T_6	583.33 (24.45*)	18.60	2.13	7.33	19.16	107.02^{h}	42.21^{g}	9.22^{ef}	3.85^{b}
T_7	383.33 (19.13*)	46.51	1.40	5.33	38.45	157.25^{d}	71.23^{d}	13.63^{abcd}	2.67^{g}
T_8	333.33 (18.24*)	53.48	1.22	5.67	34.60	129.95^{ef}	45.34^{fg}	10.15^{de}	3.05^{ef}
T_9	166.66 (12.80*)	76.74	0.61	3.00	65.81	206.14^{ab}	95.40^{ab}	16.40^{a}	1.70^{j}
T_{10}	483.33 (21.59*)	32.55	1.77	6.33	26.90	115.98^{fgh}	46.79^{fg}	10.69^{cde}	3.28^{d}
T_{11}	149.99 (11.42*)	79.07	0.54	2.33	69.28	218.31^{a}	107.50^{a}	17.03^{a}	1.49^{k}
T_{12}	350.00 (18.68*)	51.16	1.28	6.00	30.79	124.55^{fg}	45.57^{fg}	9.03^{ef}	3.16^{de}
T_{13}	233.33 (15.23*)	67.44	0.85	4.33	50.00	187.81^{c}	86.10^{bc}	14.47^{abc}	2.28^{hi}
T_{14}	516.66 (22.30*)	27.90	1.89	6.67	23.09	114.43^{fgh}	44.21^{fg}	9.60^{e}	3.63^{c}
T_{15}	183.33 (14.17*)	74.41	0.67	3.67	61.54	198.05^{bc}	76.98^{cd}	15.53^{ab}	1.85^{j}
T_{16}	383.33 (19.91*)	46.51	1.40	6.67	23.06	101.15^{h}	44.04^{fg}	9.55^{ef}	3.56^{c}
T_{17}	716.66 (27.08*)		2.62	8.67		45.63^{i}	21.55^{h}	5.65^{f}	4.84^{a}
CD (P=0.05)	3.55			0.89					

*Figures in parentheses are square root transformed values of respective data Initial population- 273.00 J_2/250 cc soil.

Note: The mean values shown with same superscript are not significant among each other whereas the mean values shown with different superscripts differ significantly. Treatment details are given in Table 1.

Table 3 Effect of organic amendments and their integration with chemicals on root-knot nematode population in soil, root gall index (RGI) and on growth parameters of cucumber grown in infested soil beds

Treatment	*Infested soil bed experiment*								
	Soil nematode population/ 250cc	Percent (%) decrease over control	Rf	RGI	Percent (%) decrease over control	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T_1	433.33 (20.81*)	53.57	1.62	5.67	37.00	204.17^{c}	97.71^{bc}	18.57^{ab}	3.04^{g}
T_2	516.67 (22.30*)	44.64	1.94	6.00	33.33	172.60^{d}	78.18^{de}	17.84^{abc}	3.19^{fg}
T_3	616.67 (25.06*)	33.92	2.31	7.00	22.22	144.50^{f}	56.32^{fg}	15.76^{bcde}	4.05^{cd}
T_4	450.00 (21.00*)	51.78	1.69	7.33	18.55	128.80^{g}	59.36^{fg}	13.63^{de}	4.13^{c}
T_5	583.33 (24.45*)	37.50	2.19	6.67	25.88	160.63^{de}	68.64^{ef}	16.54^{bcd}	3.89^{d}
T_7	533.33 (23.09*)	42.85	2.00	6.00	33.33	175.12^{d}	81.79^{de}	18.66^{ab}	3.51^{e}
T_8	383.33 (20.81*)	58.92	1.44	6.33	29.66	147.08^{ef}	56.77^{fg}	14.82^{cde}	4.19^{c}
T_9	333.33 (18.24*)	64.28	1.25	4.00	55.55	223.12^{ab}	106.05^{ab}	21.25^{a}	2.59^{h}
T_{11}	266.67 (16.29*)	71.42	1.00	2.67	70.33	237.07^{a}	119.28^{a}	20.81^{a}	2.43^{h}
T_{12}	416.67 (19.13*)	55.35	1.56	6.67	25.88	143.97^{f}	56.30^{fg}	12.68^{e}	3.94^{d}
T_{13}	400.00 (19.91*)	57.14	1.50	5.33	40.77	205.37^{c}	97.53^{bc}	19.03^{ab}	3.23^{f}
T_{15}	366.67 (17.96*)	60.71	1.37	4.67	48.11	216.47^{bc}	89.52^{cd}	20.68^{a}	2.60^{h}
T_{16}	466.67 (20.81*)	49.99	1.75	7.67	14.77	119.05^{g}	54.38^{g}	13.23^{de}	4.77^{b}
T_{17}	933.33 (30.55*)		3.50	9.00		61.75^{h}	31.11^{h}	8.90^{f}	6.07^{a}
CD (P=0.05)	4.11			1.18					

*Figures in parentheses are square root transformed values of respective data. Initial population-266.66 nem/250cc soil.

Note: The mean values shown with same superscript are not significant among each other whereas the mean values shown with different superscripts differ significantly. Treatment details are given in Table 1.

reduction in soil nematode population was observed in the integrated treatment of basamid (20 g/sqm) + mustard cake (2.5 t/ha) (Table 3). The application of mustard cake individually was also effective in reducing the nematode population, as observed in the earthen pot experiment. Growth parameters were also observed to be higher in all treatments as compared to control.

Both experiments revealed that in individual applications, organic amendments, mustard and neem cakes were effective in reducing nematode populations. Mustard cake has been effective against root-knot nematode as reported by Charles *et al.* (2015). He studied the nematicidal effects of Brassica cultivars (rape, radish, mustard and cabbage) and their formulations against root-knot nematodes (*Meloidogyne javanica*) in tomatoes and among these, the mustard cake was found to be most effective in reducing soil nematode population and root gall index. Brassicaceae have been reported to produce glucosinolates which are β-D-thioglucosides, which degrade via enzymatic hydrolysis. As a result of tissue damage, the relatively non-reactive glucosinolates react with myrosinase to yield nitriles, epithionitriles, thiocyanates and isothiocyanates (ITCs) (Salem *et al.* 2012), which are nematicidal in nature. Neem cake has also been observed to be effective. Different parts of the neem tree contain over 40 bitter principles belonging to the diterpenoid, triterpenoid, limuloid and flavonoid groups of natural products (Thakur *et al.* 1981). These properties have been effectively used against nematodes and soil borne pathogens.

The present studies also revealed that integrated treatment was more effective than individual applications of chemicals or organic amendments. Integration of Mustard cake and basamid was observed to be most effective in pots and field studies. Growth parameters were also observed maximum in these treatments. Parihar *et al.* (2015) reported that neem cake was effective against *Meloidogyne javanica* infesting tomato when used individually and integrated with *Pochonia chlamydosporia*. Mansoor (2006) also reported the highest reductions in the nematode infections, and improvement in plant growth parameters was noted in pots treated with oilcake combined with carbofuran. Dhilon and Kaur (2016) recommended application of basamid @40 g/sq m for the management of root knot nematode in nursery beds of brinjal and tomato. Different studies have shown that, by mixing soil or furrow applications, dazomet fumigation can suppress many soilborne diseases effectively and thus, increase crop yield (Ma *et al.* 2006, Fu *et al.* 2012). Kella *et al.* (2012) had also reported maximum protection of cucumber plant against root knot nematode and highest yield by application of basamid, metam El-sodium and licorice powder treatments in field trials. Conclusively, integration of organic amendments with chemicals, i.e. mustard cake with basamid, neem cake with basamid, musatard cake with formalin and neem cake with farmalin was found more effective to reduce the soil nematode population and root gall index in cucumber and further experiments need to be conducted for more alternate options for integrated management of root-knot nematode in cucumber, which is becoming a predominant problem in protected cultivation.

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