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Management of Root-Knot Nematode with Non-Chemical Methods for Sustainable Production of Cucumber under Protected Cultivation

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Abstract: The multi-faceted benefits of growing crops under protected cultivation are gradually establishing it as a promising technology for vegetable cultivation in India. However, the adoption of successive cropping patterns in soil-based closed structures is leading to the buildup of soil-borne pathogens and pests, which are becoming a major hindrance to the sustainable production of these crops, particularly in the northern plains. Root-knot nematodes (RKNs) are a critical threat to protected cultivation and farmers are required to contribute a significant amount of time and money for their management. To reduce the overdependence of chemicals, the present study explored the potential of plant-based by-products as amendments for the management of RKN in cucumbers grown under a plastic greenhouse. A pot trial was conducted to assess the efficacy of different plant-based amendments against nematodes in cucumber plants. The pot trial results revealed that the application of mustard cake (MC) and neem cake (NC) at 1 t ha^{-1} either alone or as a combined application with farmyard manure (FYM) of 2.5 t ha^{-1} was effective against RKN infestation, reflecting the improved plant growth parameters of cucumber. Based on the results of the pot trials, treatments with plant-based amendments and FYM i.e., T1: MC 1 t ha^{-1}; T2: NC 1 t ha^{-1}; T3: FYM 2.5 t ha^{-1}; T4: MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}, along with the treated check carbofuran 3 G 2 kg (a.i.) ha^{-1} (T5) and untreated check (T6), were evaluated in multi-locational field trials. The results revealed that the combined application of MC 1 t ha^{-1} + NC 1 tha^{-1} + FYM 2.5 t ha^{-1} exhibited promising results in decreasing RKN infestation (56–58%) in all of the three RKN-infested polyhouses, with significantly enhanced yields at all of the three locations. Soil organic carbon also increased significantly in the amended plots, indicating improved soil health. The results of the present work hold good promise for the management of RKN in the protected cultivation of cucumber with an environment friendly approach, along with the additional incentives of improved soil health.

Keywords: *Cucumis sativus*; *Meloidogyne*; mustard cake; neem cake; greenhouse

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1. Introduction

Protected cultivation is a new-age farming technique that is highly productive, conserves water and land, and also protects the environment [1]. The growing of crops in polyhouses (plastic greenhouses) has reinvigorated farmers' interests to grow off-season or year-round crops for increased economic gains [2]. However, the ecological conditions created in polyhouses are propitious to certain pests and diseases, which are not frequently confronted by crops grown in out-field cultivation conditions [3]. Amongst the various soil-borne pests, plant parasitic nematodes are becoming a major limiting factor in the sustainable production of crops in protected structures in different parts of India. More than 60% of the polyhouses surveyed in the northern state Punjab were infested with root-knot nematodes [4]. Incidentally, cucumber, capsicum and tomato are the main crops cultivated in polyhouses in Punjab, all of which are susceptible to root-knot nematodes. The favourable conditions provided by protected cultivation systems and the year-round availability of the host promote the build-up of high population densities of parasitic nematodes. The species that belong to genus *Meloidogyne* are becoming the most damaging pathogens that pose challenges to the successful cultivation of crops in polyhouses [2,5,6]. Increased nematode infestations due to the monoculture of crops may decrease the yield by 45–50% [7].

To sustain profitability in protected cultivation, there is a need to address the declining factor of crop yields, especially nematodes [3]. In recent years, rising health and environmental concerns caused by the application of synthetic chemicals make these chemicals unacceptable for continued use by farmers [8]. The year-round production of crops, coupled with the indiscriminate use of chemicals, often leads to high levels of pesticide residues, which is not in the line with Good Agricultural Practices protocols [9]. To avoid the detrimental effects of chemical use, there is a need to focus more on the use of plant-based rural strategies to control pests and pathogens with minimum toxic levels [10,11]. A number of botanicals have been reported for their antagonistic and allelopathic nature. The phytochemicals released from these plants or their developed bioproducts when incorporated into the soil have been found to be nematicidal [12,13].

The use of organic amendments is gaining importance in light of the hazardous nature of the chemicals used for the management of nematode diseases. These amendments are known for their biocidal and antimicrobial activity, as well as their ability to improve soil structure and fertility [14,15]. Amongst the various plant groups that produce anti-pest compounds, the Brassica group is known for its characteristic release of glucosinolates such as β-D-thioglucosides, which degrade via enzymatic hydrolysis. Following damage to plant tissue, the relatively non-reactive glucosinolates react with the enzyme myrosinase to yield compounds such as nitriles, epithionitriles, thiocyanates, and isothiocyanates (ITCs), which are inhibitory to pathogens [16,17]. The *Brassica* spp. has been explored for its biofumigant properties as oil cakes or as green manures. Their practical use for bio-fumigation is currently being debated, due to the extensive application of biomass in the field [18]. Brassica by-products such as oil cakes release biocidal compounds [19,20], in addition to providing important benefits to soil health improvement. Apart from the Brassica species, *Azadirachta indica*—commonly known as neem—is well documented for its antimicrobial properties [21]. Neem trees contain over forty bitter principle compounds that belong to various groups, such as diterpenoids, triterpenoids, limuloids and flavonoids [22], which have been effectively used against microbes, soil-borne pests and pathogens. The neem cake, a by-product of neem, upon decomposition produces ammonia and certain other toxic compounds, which have been reported to exhibit anti-pathogenic properties [23]. Furthermore, farmers in their cultivation practices since ancient times have been using farmyard manure (FYM) as an amendment for the improvement of soil nutrition status, plant growth and improving beneficial microflora [24,25].

Cucumber is an economically important crop grown under protective structures, the production of which is limited by the number of biotic factors, including nematodes. Farmers use pesticides for sustaining the yield of the crop. However, as cucumber is a directly consumable crop and with the increased issues associated with health and the environment, the application of pesticides needs to be restricted. In view of this, it is vital to explore the wealth of antagonistic plant species or their products for their anti-pathogenic properties. The present studies were conducted to explore if mustard and neem cake amendments were effective in the management of RKN-infested cucumber. Although both these botanicals have been previously explored for their allelochemical properties against these pathogens, [26] we characterize their relevant use under field conditions.

2. Materials and Methods

2.1. Pot Experiment

The effect of amendments on the cucumber plants in the pot experiment was studied in root-knot nematode (RKN)-infested, as well as non-infested, soil. A total of ten treatments were used, including mustard cake (MC 0.5 t ha^{-1}, 1.0 t ha^{-1}; 2.0 t ha^{-1}), neem cake (NC 0.5 t ha^{-1}, 1.0 t ha^{-1}; 2.0 t ha^{-1}), farmyard manure (FYM 2.5 t ha^{-1}) and the combined application of MC (1 t ha^{-1}) + NC (1 t ha^{-1}) + FYM (2.5 t ha^{-1}), a pesticide-treated control of carbofuran 3 G (2 kg (a.i.) ha^{-1}) and a non-treated control. Each treatment was replicated four times. Two sets of pots (12 inches in diameter) i.e., set-I and set-II, were used, with forty pots in each set. Pots of set-I were filled with root-knot nematode-infested soil (initial soil population = 266 J_2 250 g^{-1} soil) and set-II was filled with autoclaved soil one day prior to the application of treatments. For the estimation of the initial soil nematode population, the soil was thoroughly mixed and three composite samples were drawn. Each composite sample was made by mixing five sub-samples. Nematode extraction was achieved by washing the soil using the modified Cobb's sieving and decanting method [27]. The washed samples were placed over a paper towel supported by a 2 mm wire-mesh sieve placed over a petri dish that contained water. The nematode suspension obtained after washing was collected in a 200 mL beaker after 24 h. The volume of this nematode suspension was brought up to 50 mL for each soil sample washed. About 5 mL of suspension was transferred to a counting dish and the number of nematodes was counted under a stereo zoom binocular microscope. For accurate reading, the nematodes were counted three times from each suspension and an average of these was taken as the final reading.

The treatments of amendments and chemicals (treated check) as described above were applied to the pots in both the sets, followed by light watering, so as to allow the amendments to decompose. Ten days after the application of treatments, three seeds of the cucumber variety 'Punjab Kheera-1' were directly sown in the pots of both the sets. After germination, the seedlings were thinned to two seedlings per pot. When the plants were sixty days old, observations were recorded regarding the soil nematode population, the number of egg masses per plant, root gall index (RGI), plant height and plant weight. For the soil nematode population, nematodes were extracted using the modified Cobb's sieving and decanting method as mentioned above. The number of eggs per plant was also counted under the microscope. The roots of individual plants were uprooted and rated on a 0–5 scale [28] to work out the RGI for each treatment. The plant height was taken with the help of a cm scale and fresh plant weight was taken using an electronic weighing balance. For observing the effect of amendments on plant growth in the infested and non-infested soil, a percent increase in plant height over the untreated control was calculated.

2.2. Identification of RKN Species in the Polyhouses

The study was conducted in polyhouses located at three different locations (as given in the table under Section 2.3). For identification of root-knot nematode species, infested roots were collected from the experimental locations. The RKN species were identified on the basis of perineal pattern morphology of adult females [29] and the species was confirmed using a species-specific SCAR marker (Minc F—CTCTGCCCAATGAGCTGTCC; Minc R—CTCTGCCTCACATTAAG) [30]. For the molecular studies, egg masses were collected from infected cucumber roots and were incubated at 28 ± 1 °C to allow the juveniles to hatch out from the eggs. Total DNA was extracted from the freshly hatched juveniles using DNeasy Tissue and Blood Extraction Kits (Qiagen, Hilden, Germany). The total DNA was subjected to PCR amplification in an Eppendorf PCR system (Mastercycler pro, Eppendorf, Hamburg, Germany) with 25 µL of the reaction mixture, which consisted of 2 µL of template DNA (55 ng/µL), 1.5 µL (25 mM) of $MgCl_2$, 0.5µL (2 mM) of dNTPs, 0.25 µL (500 units) of Taq polymerase (Promega, USA), 1.0 µL (20 picomol/µL)) of each forward and reverse primer in 5 µL of reaction buffer (5×) (Green GoTaq, Promega, Madison, WI, USA). The total volume of 25 µL was reached by adding nuclease-free water. The amplification program comprised of an initial cycle of denaturation at 94 °C for 2 min, thirty-five cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 2 min and extension at 67 °C for 1 min, followed by a final extension for 10 min at 72 °C. The amplified PCR product was visualized by staining 1.0% agarose gel with ethidium bromide and viewing the product under UV trans-illumination (Alpha Innotech AlphaImager Hp System, San Francisco, CA, USA). The results were verified against a DNA marker (100 bp).

2.3. Field Trials

The field trials were conducted at three different locations for two years. Naturally infested polyhouses with a previous history of RKN infestation were selected for the trials. These locations were as follows: (i) Research Farm, Department of Plant Pathology, PAU, Ludhiana; (ii) Research Farm, Department of Vegetable Science, PAU, Ludhiana; (iii) commercial nematode-infested polyhouse farm in Village Sandhuan, Ropar district. In Punjab, the cucumber crop can be grown in any of the two seasons (season-I: February to April and season-II: September to December) under a protected cultivation system. The research trials at location-I and II were performed during season-I (February–April), and the following year, a trial at location-III was conducted in season-II (September–December). The details of the experimental trials at different locations are as follows (Table 1).

Table 1. The details of the experimental trials at different locations.

Trial Location	Geographical Location	Year	Crop Season	Variety	Initial Nematode Population 250 g^{-1} Soil
Polyhouse-I Research Farm, Plant Pathology	N-27°20′38.0″ E-77°50′11.0″	2018	February–April	Punjab Kheera No. 1	466.6 J_2
Polyhouse-II Research Farm, Vegetable Crops	N-30°54′01.5″ E-75°47′33.9″	2018	February–April	Punjab Kheera No. 1	350.3 J_2
Polyhouse-III Commercial polyhouse of farmer	N-30°52′08.9″ E-76°23′30.0″	2019	September–December	KUK-9	473.0 J_2

The trials were carried out using a randomized block design and each treatment was replicated four times at all locations. The treatments comprised of mustard cake (MC, 1.5 t ha^{-1}), neem cake (NC, 1.5 t ha^{-1}), farmyard manure (FYM, 5.0 t ha^{-1}) individually, as well as in combination (MC 1 t ha^{-1} + NC 1 ha^{-1} + FYM 2.5 ha^{-1}), a pesticide-treated control (carbofuran 3 G 2 kg (a.i.) ha^{-1}) and untreated control. The mustard cake was sourced from the local mustard variety grown in the fields of the Department of Plant Pathology as a by-product after the extraction of oil. Neem cake was purchased from Mittal Pesticides, Ludhiana, India and FYM was procured from the Department of Dairy Science, Guru Angad Dev Veterinary University, Ludhiana. Carbofuran 3 G was purchased from Bharat Plant Protectors and Engineers, Ludhiana. At the PAU Research Farms, the trials were carried out using a randomized block design with six treatments and four replications (with 8 plants in each) in twenty-four plots. Individual plots (2.5 m × 1.0 m) were separated by a buffer space of 0.5 m. Each treatment included a total of 32 plants. At the farmer's field, all six treatments were applied in a randomized block design with four replications of each treatment. The individual plots of 4.0 m × 1.0 m size were separated by a buffer space of 0.5 m. Furthermore, each replication included 16 plants, which resulted in a total of 64 plants per treatment. The mean temperature during the trials at all of the three locations ranged from 27 ± 1 °C to 39 ± 1 °C.

Seeds of the commercial cucumber variety ('Punjab Kheera -1' at location-I and II and KUK-9 at location-II) were directly sown with a seed-to-seed spacing of 30 cm on one side of the bed. These are popular cucumber cultivars grown by a large number of farmers in the state. The amendments were added ten days before the sowing of the crop, after which light irrigation was performed. Hoeing of the beds was performed twice for the proper mixing of the amendments over these ten days. Carbofuran 3 G was added at the time of the sowing of the crop. Weeding was performed manually using a hoe.

Conventional irrigation and fertilization were provided according to the requirements of the crop. Proper sanitation practices were followed to prevent the entry of insects inside the structure. At the end of the crop season, observations were recorded on the soil nematode population, root gall index and yield. Analysis of the physical and chemical properties of the soil was also performed in the location-I trial. To evaluate the effect of the treatments on yield, cucumbers were harvested regularly. The harvested cucumbers after each picking were weighed and recorded from each plot. To assess the soil nematode population, five samples were taken randomly using a soil sampling auger (45 $\times$ 2.5 cm, Passey) up to the depth of 20 cm from each plot and mixed thoroughly to obtain a working sample of 250 g [31]. The soil samples were transported to the University Nematology laboratory for nematode extraction. Nematodes were extracted using the modified Cobb's sieving technique as described above. The build-up of the nematode population was estimated as the reproduction factor (Rf = Pf/Pi), where Pf is the final nematode population recorded at end of the crop cycle and Pi is the initial nematode population at the start of the experiment. Rf > 1 indicated the multiplication of RKN in the treatment. Root gall infestation was determined at the end of the crop season as root gall index by randomly digging the roots of eight plants and rating the observations on a 0–10 scale [32].

2.4. Analysis of Soil Properties

The change in soil properties, i.e., soil pH, electrical conductivity (EC), macro-nutrient (phosphorus and potassium) and micro-nutrient (Zn, Fe, Mn and Cu) levels with addition of different amendments were analyzed for location-I in 50-day-old crop. A composite sample that consisted of three sub-samples (taken with the help of a soil auger) from each replication of the respective treatment, were used for the analysis of soil properties. Organic carbon was taken as the representation of soil health and nitrogen levels of the soil. Soil samples were drawn from 0 to 15 cm and taken to the soil laboratory for analysis of physicochemical properties and were air-dried and ground to pass through a 2 mm sieve. These samples were analyzed for pH and EC (1:2: soil:water), organic carbon [33], 0.5 M $NaHCO_3$-extractable P [34], 1 N ammonium acetate (AmOAc)-extractable K [35] and DTPA-extractable micronutrients [36]. The data on temperature during the study period were obtained from the Agro-Meteorological Department, Punjab Agricultural University, Ludhiana.

2.5. Statistical Analysis

The data were subjected to statistical analysis to compute completely randomized design ANOVA for pot experiments and randomized block design ANOVA for field trials using the Statistical Tool for Agricultural Research (STAR 2.0.1) software package. Treatment means for the effect of different amendments on the micronutrient composition of soil were compared using least significant difference (LSD) at a 5% level of probability.

3. Results

3.1. Pot Experiment

The results of the pot experiment confirmed that infestation by nematodes decreased the height and weight of cucumber plants in infested soil (untreated control in infested soil = 70.7 cm, as compared to non-infested soil (untreated control in non-infested soil = 77.2 (Table 2)). The application of soil amendments increased the plant growth parameters both in nematode-infested, as well as non-infested, soils. The mustard and neem cake application at 1 t ha^{-1} and above significantly increased the plant height and weight over the untreated control, counteracting the negative effect of nematodes. However, the maximum plant height (84.7 cm; 85.3 cm) and weight (33.8 g; 35.3 g) were observed in the combined application of MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1} both in nematode-infested and non-infested soil (Table 2).

Table 2. Effects of different soil amendments on root-knot nematode infestations and plant growth parameters of cucumbers grown in pots.

Treatments	Soil Nematode Population		Egg Masses/ Root System	Root Gall Index (RGI)		Nematode-Infested Soil		Non-Nematode-Infested Soil	
	Nematode Population 250 g^{-1} Soil	Reduction over Control (%)		RGI (0–5) Scale	Reduction over Control (%)	Plant Height (cm)	Plant FW (g)	Plant Height (cm)	Plant FW (g)
MC 0.5 t ha^{-1}	282 + 6.6 (16.80) ^{g}	6.2	23.9 ± 1.0 ^{e}	3.5 ± 0.3 ^{f}	10.3	73.3 ± 0.7 ^{g}	28.3 ± 1.5 ^{bdef}	80. 7 ± 0.7 ^{cdef}	32.4 ± 0.71 ^{bcd}
MC 1.0 t ha^{-1}	130 + 7.0 (11.44) ^{bcd}	56.7	11.3 ± 1.5 ^{abc}	1.8 ± 0.4 ^{abc}	53.8	80.6 ± 2.2 ^{bcd}	31.7 ± 3.0 ^{abc}	82.1 ± 2.8 ^{bcd}	33.8 ± 0.9 ^{abc}
MC 2.0 t ha^{-1}	125± 5.0 (11.24) ^{b}	58.3	10.7 ± 1.2 ^{ab}	1.7 ± 1.1 ^{ab}	55.6	81.6 ± 3.5 ^{bc}	32.33± 1.1 ^{ab}	83.8 ± 0.9 ^{ab}	34.4 ± 1.2 ^{ab}
NC 0.5 t ha^{-1}	2845± 8.9 (16.89) ^{g}	5.2	24.3 ± 1.5 ^{e}	3.6 ± 0.3 ^{f}	8.4	72.1 ± 0.8 ^{gh}	27.3 ± 2.1 ^{def}	79.2 ± 0.7 ^{ef}	31.6 ± 1.4 ^{d}
NC 1.0 t ha^{-1}	134 ± 6.1 (11.61) ^{bcde}	55.4	13.7 ± 1.2 ^{cd}	2.2 ± 0.2 ^{cde}	42.7	78.7 ± 3.3 ^{def}	29.7 ± 1.5 ^{bcd}	81.6 ± 0.6 ^{bcde}	33.0 ± 0.71 ^{abcd}
NC 2.0 t ha^{-1}	128 ± 4.0 (11.37) ^{bc}	57.3	13.0 ± 1.0 ^{bcd}	2.0 ± 0.2 ^{abcd}	49.6	82.7 ± 2.4 ^{ab}	31.7 ± 1.3 ^{abc}	83.4 ± 0.6 ^{ac}	32.8 ± 2.3 ^{bcd}
FYM 2.5 t ha^{-1}	287 ± 5.5 (16.96) ^{g}	4.4	25.3 ± 1.3 ^{e}	3.9 ± 0.2 ^{f}	1.0	72.7 ± 1.1 ^{gh}	28.3 ± 2.1 ^{bcdef}	81.8 ± 0.6 ^{bcde}	32.3 ± 0.2 ^{bcd}
MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}	107 ± 3.6 (10.39) ^{a}	64.4	9.7 ± 1.2 ^{a}	1.5 ± 0.2 ^{a}	60.7	84.7 ± 1.3 ^{a}	33.8 ± 1.2 ^{a}	85.3 ± 0.8 ^{a}	35.3 ± 2.0 ^{a}
Carbofuran 3 G 2.0 kg (a.i.) ha^{-1}	135 ± 8.1 (11.67) ^{bcdef}	55.1	11.3 ± 0.6 ^{abc}	1.9 ± 0.2 ^{abcd}	52.1	79.7 ± 2.5 ^{cde}	29.4 ± 2.2 ^{bcde}	82.0 ± 1.4 ^{bcde}	32.3 ± 1.2 ^{bcd}
Untreated control	300 ± 6.6 (17.35) ^{g}	0.0	26.3 ± 1.5 ^{e}	3.9 ± 0.2 ^{f}	-	70.7 ± 1.7 ^{h}	25.8 ± 2.2 ^{f}	77.2 ± 1.8 ^{g}	29.1 ± 2.0 ^{d}
LSD ($p \leq 0.05$)	0.58		2.67	0.46		2.29	3.43	2.96	2.33

Each value is a mean ± S.D. of four replicates; MC—mustard cake; NC—neem cake; FYM—farmyard manure; FW—fresh weight. Means that share common letters within columns do not differ significantly at $p \leq 0.05$%.

In addition, the application of mustard and neem cake amendments (1 t ha^{-1}) was found to significantly reduce the soil root-knot nematode population, the number of egg masses/plant and the root gall index, clearly demonstrating their nematicidal effect. The maximum decrease in soil nematode population (64.4%), as well as in the root gall index (60.7%), was observed in the combined application of MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}, as compared to the untreated control. This effect was even larger than that observed for the carbofuran 3 G 2 kg (a.i.) ha^{-1} application (55.1% and 52.1% reduction in RKN population in soil and roots over control). In contrast to the protective effects of applying mustard and neem cake amendments alone at 1 t ha^{-1} and 2 t ha^{-1}, the applications of 0.5 t ha^{-1} were not effective at reducing nematode infestation.

3.2. Identification of RKN Species in the Polyhouses

The perineal patterns of adult female(s) isolated from polyhouse soils showed a high squarish dorsal arch, and as such, were identified as *M. incognita*. In addition, some of the perineal patterns had distinct lateral lines and these were identified as *M. javanica* (Figure 1a,b). The species-specific markers were used for the confirmation of the dominant species, *M. incognita* (Figure 1c).

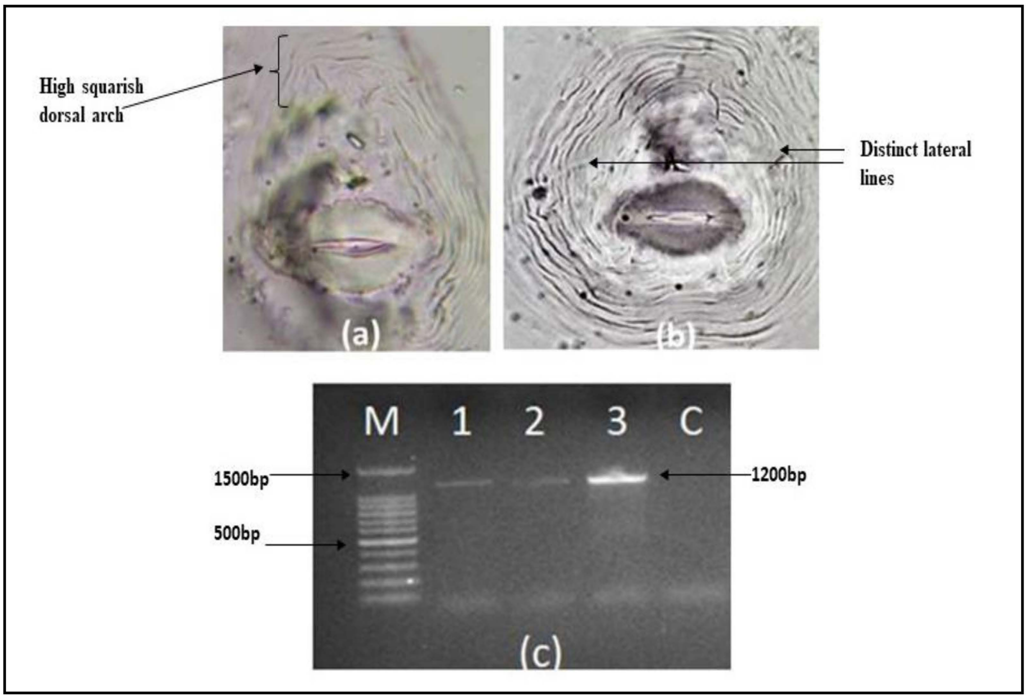

Figure 1. Perineal pattern of adult root-knot nematode females: (**a**) *Meloidogyne incognita* (showing high squarish dorsal arch with no lateral lines); (**b**) *Meloidogyne javanica* (perineal pattern with distinct lateral lines); (**c**) agarose gel (1%), showing amplification of 1200 bp that corresponds to *Meloidogyne incognita* sp. of root-knot nematode, using the SCAR Finc/Rinc primer from all of the three polyhouses; 1 = location-I; 2 = location-II; 3 = location-III; C—negative control; M—DNA marker (100 bp).

3.3. Field Trials

The observations recorded regarding the nematode infestations in the soil and roots of cucumber plants in polyhouse-I (Research Farm, Plant Pathology) revealed that the combined application of amendments, including MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}, significantly decreased the number of root galls (RGI 3.06), in comparison to all the other treatments and the untreated control (Table 3). This amendment also was the most effective at suppressing the soil nematode population to 350 J_2 250 g^{-1} soil, compared to the untreated plots (676 J_2 250 g^{-1} soil). Still effective, although less so, was the individual application of MC at 1.5 t ha^{-1} (451.7 J_2 250 g^{-1} soil). The combined application of amendments gave a >58% reduction in the soil nematode population and the reproduction factor was observed to be <1, indicating less nematode multiplication in this treatment. Heavy galling (RGI 7.3) was observed in the roots of cucumber plants grown in non-amended soil (control). In contrast, the root gall index was observed to be very low in the amended plots. The reproduction factor was also observed to be >1 in the unamended soil. The treatments with the individual application of NC 1.5 t ha^{-1} or MC 1.5 t ha^{-1} also showed a significantly lower RGI (4.5 and 4.6), as compared to the chemical control and untreated control. The influence of farmyard manure as the suppressive amendment was observed to be significantly less than the neem and mustard cake amendments. The application of carbofuran 3 G significantly decreased nematode infestation in the roots by 22.6% and in the soil by 26.8%, as compared to the control, but was significantly lower than the treatments with the application of NC and MC amendments individually or in combination. Compared to the untreated controls (35.29 t ha^{-1}), the total yield of cucumber treated with the combined application of amendments was higher (47.5 t ha^{-1}), although not significantly different from the yield of the chemical control (45.4 t ha^{-1}).

Table 3. Effects of different amendments on root-knot nematode infestation and yield of cucumber (var. Punjab Kheera 1) under protected conditions (polyhouse–I, Department of Plant Pathology).

Treatments	Nematode Population 250 g^{-1} Soil	Reduction over Control (%)	RGI (0–10) Scale	Reduction over Control (%)	Rf = Pf/Pi	Yield (t ha^{-1})	Increase over Control (%)
MC 1.5 t ha^{-1}	452 ± 27.5 (21.26) ^{b}	33.2	4.5 ± 0.4 ^{b}	37.8	1.0	41.9 ± 1.8 ^{bc}	18.8
NC 1.5 t ha^{-1}	481. 7 ± 35.5 (21.96) ^{bc}	28.8	4.6 ± 0.1 ^{bc}	36.8	1.1	40.7 ± 2.1 ^{cd}	15.3
FYM 5.0 t ha^{-1}	633 ± 28.8 (25.18) ^{e}	6.4	6.8 ± 0.4 ^{e}	7.0	1.4	39.8 ± 2.4 ^{cd}	12.7
MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}	350 ± 25.0 (18.72) ^{a}	48.3	3.1 ± 0.3 ^{a}	58.1	0.8	47.5 ± 1.2 ^{a}	34.7
Carbofuran 3 G 2.0 kg (a.i.) ha^{-1}	495 ± 57.6 (22.24) ^{bcd}	26.8	5.6 ± 0.2 ^{d}	22.6	1.1	45.4 ± 2.2 ^{ab}	28.7
Control	677 ± 60.3 (26.01) ^{e}	0.00	7.3 ± 0.5 ^{e}	0.0	1.5	35.3 ± 0.8 ^{e}	-
LSD ($p \leq 0.05$)	1.68		0.64			3.6	

Each value is a mean ± S.D. of four replicates; MC—mustard cake; NC—neem cake; FYM—farmyard manure; scale used for scoring RKN in roots (0–10) described by Bridge and Page, 1980; Rf = reproduction factor; Pi = initial nematode population; Pf = final nematode population; RGI—root gall index; values in () are square-root transformed values; means that share common letters within columns do not differ significantly at $p \leq 0.05\%$.

Similarly, the data recorded from the crops grown in polyhouse-II (Research Farm, Vegetable Science Department) revealed that a maximum reduction in RKN infestation (56.0%) in the roots, as well as in the soil (47.5%), was observed following the application of MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1} (Table 4). The RGI (RGI 2.7) and soil nematode population (287. J_2 250 g^{-1} soil) for this treatment were significantly lower than all the other treatments and the untreated control. The individual application of amendments also reduced the nematode severity, although the reduction was comparatively less than the combined application. The reduction in mustard, as well as neem cake, was observed to be 32.2 and 25.6% in roots and 29.5 and 25.5% in soil, respectively. The application of farmyard manure at 2.5 t ha^{-1} was observed to be less effective than the neem and mustard cake amendments. In the case of the untreated control, the soil nematode population (546/250 g^{-1}) and root gall index (6.2) were at their maximum and the reproduction factor was observed to be 1.6. The combined application of amendments gave a 33.7% higher yield, as compared to the untreated control.

The trends of the results of polyhouse-I and polyhouse–II were followed in a commercial farmer's fields, where the lowest nematode severity was observed in the treatment with the combined application (MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}) of amendments and the highest severity was recorded in the single treatment of FYM 2.5 t ha^{-1}. Treatments with the individual application of mustard cake and neem cake also exhibited reduced infestation of RKN, although it was comparatively less than the treatment with the combined application of amendments. The average cucumber yield was observed to be 38.8% higher in the combined application of amendments, as compared to the control (Table 5). The yield obtained in the commercial farm polyhouse was higher in the amended plots, as compared to the untreated control and chemical control (carbofuran 3 G).

Table 4. Effects of different amendments on root-knot nematode infestation and yield of cucumber (var. Punjab Kheera 1) under polyhouse conditions (polyhouse-II, Department of Vegetable Science).

Treatments	Nematode Population 250 g^{-1} Soil	Reduction over Control (%)	RGI (0–10) Scale	Reduction over Control (%)	Rf = Pf/Pi	Yield (t ha^{-1})	Increase over Control (%)
MC 1.5 t ha^{-1}	385 ± 13.2 (19.64) ^{b}	29.5	4.2 ±+ 0.4 ^{b}	32.2	1.1	64.8 ± 3.5 ^{b}	21.8
NC 1.5 t ha^{-1}	407 ± 18.9 (20.18) ^{bcd}	25.5	4.6 ± 0.1 ^{bc}	25.6	1.2	58.6 ± 4.1 ^{cd}	10.0
FYM 5.0 t ha^{-1}	493 ± 11.5 (22.23) ^{e}	9.6	5.7 ± 0.1 ^{e}	8.5	1.4	56.2 ± 3.5 ^{de}	5.5
MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}	287 ± 15.3 (16.95) ^{a}	47.5	2.7 ± 0.1 ^{a}	56.0	0.8	71.2 ± 3.6 ^{a}	33.7
Carbofuran 3 G 2.0 kg (a.i.) ha^{-1}	388 ± 19.9 (19.73) ^{bc}	28.8	4.7 ± 0.2 ^{bcd}	24.3	1.1	58.8 ± 4.1 ^{c}	10.5
Control	546 ± 14.3 (23.38) ^{f}		6.2 ± 0.7 ^{e}		1.6	53.2 ± 2.9 ^{f}	-
LSD ($p \leq 0.05$)	0.75		0.70			2.53	

Each value is a mean ± S.D. of four replicates; MC—mustard cake; NC—neem cake; FYM—farmyard manure; scale used for scoring RKN in roots (0–10) described by Bridge and Page, 1980; Rf = reproduction factor; Pi = initial nematode population; Pf = final nematode population; RGI—root gall index; values in () are square-root transformed values; means that share common letters within columns do not differ significantly at $p \leq 0.05$%.

Table 5. Effects of different amendments on root-knot nematode infestation and yield of cucumber (var. KUK-9) in a commercial polyhouse (polyhouse-III, farmer's field).

Treatments	Nematode Population 250 g^{-1} Soil	Reduction over Control (%)	RGI (0–10) Scale	Reduction over Control (%)	RF = Pf/Pi	Yield (t ha^{-1})	Increase over Control (%)
MC 1.5 t ha^{-1}	467 ± 57.7 (21.6) ^{b}	34.9	4.7 ± 0.4 ^{b}	36.9	1.0	35.7 ± 2.2 ^{b}	7.3
NC 1.5 t ha^{-1}	500 ± 50.0 (22.3) ^{bc}	30.3	4.9 ± 0.7 ^{bc}	34.2	1.1	35.4 ± 4.0 ^{b}	6.5
FYM 5.0 t ha^{-1}	633 ± 28.8 (26.1) ^{e}	11. 7	6.8 ± 0.2 ^{e}	9.8	1.3	33.3 ± 2.4 ^{b}	0.3
MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}	327 ± 46.2 (18.4) ^{a}	54.4	3.3 ± 0.2 ^{a}	55.6	0.7	46.1 ± 2.0 ^{a}	38.8
Carbofuran 3 G 2.0 kg (a.i.) ha^{-1}	517 ± 76.3 (22.7) ^{bcd}	27.9	5.2 ± 0.2 ^{bcd}	30.2	1.1	37.9 ± 5.2 ^{b}	14.0
Control	717 ± 57.7 (26.7) ^{e}	-	7.5 ± 0.6 ^{e}	36.9	1.5	33.2 ± 1.1 ^{b}	-
LSD ($p \leq 0.05$)	2.59		0.92			4.74	

Each value is a mean ±S.D. of four replicates; MC—mustard cake; NC—neem cake; FYM—farmyard manure; scale used for scoring RKN in roots (0–10) described by Bridge and Page, 1980; Rf = reproduction factor; Pi = initial nematode population; Pf = final nematode population; RGI—root gall index; values in () are square-root transformed values; means that share common letters within columns do not differ significantly at $p \leq 0.05$%.

3.4. Analysis of Soil Properties

As per the observations recorded in Table 6, an increase in organic carbon, phosphorus and potassium availability in the soil was observed in different treatments. Organic carbon was significantly higher in treatments with the application of MC 1 t ha^{-1} (1.02 g kg^{-1} soil) and the combined application of amendments (MC 1 t ha^{-1}+ NC 1 t ha^{-1} + FYM 2.5 t ha^{-1})

(0.96 g kg^{-1} soil), as compared to the untreated control (0.36 g kg^{-1} soil). The available phosphorus content in the soil was also at its maximum with the application of mustard cake (99.0 mg kg^{-1} soil) and the combined application of amendments (96.25 mg kg^{-1} soil). The potassium content was significantly higher in the combined application of amendments (397.5 mg kg^{-1} soil). The soil pH ranged from 7.5 to 7.75. In addition, the availability of micronutrients such as zinc, iron, manganese and copper was at its maximum in the soil treated with the combined application of amendments (Figure 2).

Table 6. Effect of the application of amendments on soil properties and nutrient availability (polyhouse-I, Department of Plant Pathology).

Treatments/ Nutrient Availability	MC 1 t ha^{-1}	NC 1 t ha^{-1}	FYM 2.5 t ha^{-1}	MC + NC + FYM (1.0 + 1.0 + 2.5) t ha^{-1}	Carbofuran 3 G 2 kg (a.i.) ha^{-1}	Untreated Control	LSD ($p \leq 0.05$)
Organic carbon (g kg^{-1} soil)	1.02 ± 0.04 ^{a}	0.42 ± 0.03 ^{c}	0.39 ± 0.01 ^{d}	0.96 ± 0.05 ^{b}	0.32 ± 0.02 ^{d}	0.36 ± 0.05 ^{d}	0.28
Available phosphorus (mg kg^{-1} soil)	99.0 ± 6.16 ^{a}	81.5 + 2.6 ^{c}	76.25 + 3.04 ^{d}	96.25 ± 3.04 ^{b}	67.5 ± 3.60 ^{e}	67.0 ± 2.41 ^{e}	1.87
Available potassium (mg kg^{-1} soil)	277.5 ± 4.58 ^{b}	277.5 ± 4.35 ^{b}	255.0 ± 5.29 ^{c}	397.5 ± 6.08 ^{a}	210.0 ± 4.58 ^{d}	210.0 ± 3.60 ^{d}	3.95
Soil pH	7.52 ± 0.00 ^{ab}	7.50 ± 0.01 ^{ab}	7.70 ± 0.00 ^{a}	7.60 ± 0.02 ^{a}	7.70 ± 0.00 ^{a}	7.75 ± 0.01 ^{a}	0.10

Each value is a mean ±S.D. of four replicates; MC—mustard cake; NC—neem cake; FYM—farmyard manure; means that share common letters within columns do not differ significantly at $p \leq 0.05$.

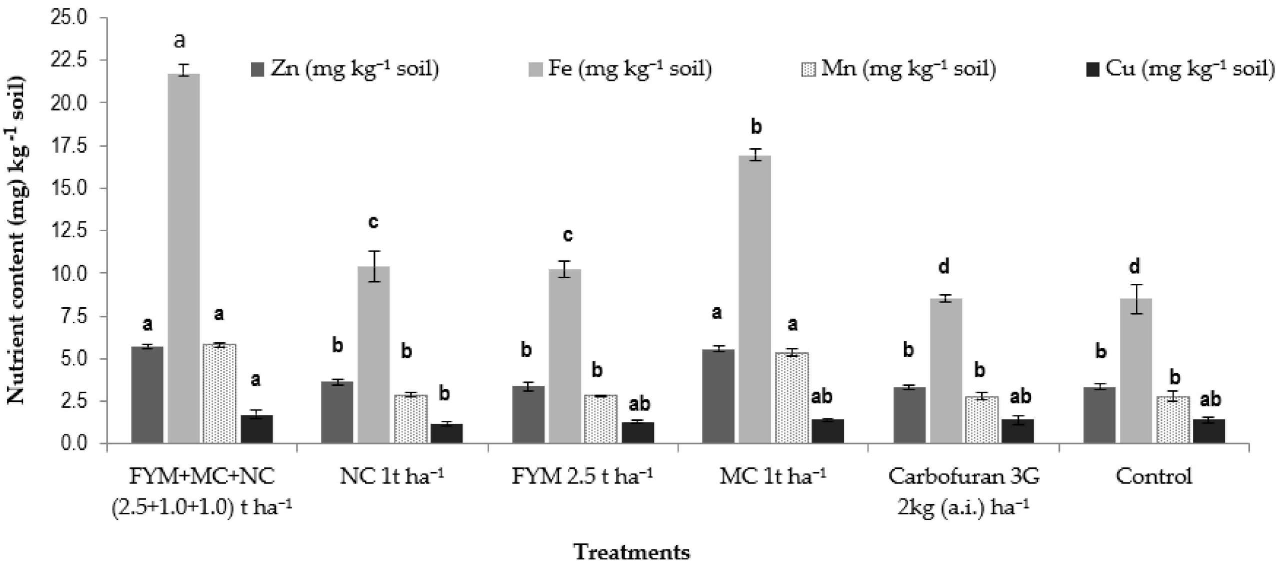

Figure 2. Effect of different amendments on micronutrient composition of the soil (polyhouse-I). The columns show the mean value of three replicates and the bars show the standard error of means. The columns labelled with different letters are significantly different according to the LSD test at 5% probability. MC = mustard cake; NC = neem cake; FYM = farmyard manure.

In summary, this study demonstrates that the combined application of amendments, including MC 1 t ha^{-1}+ NC 1 t ha^{-1} + FYM 2.5 t ha^{-1}, exhibited nematicidal activities against *M. incognita*. There was also a significant increase in the cucumber yield in all three trials conducted in polyhouses located at different places. The physical and chemical characteristics and nutrient contents of the soil were improved with the application of amendments.

4. Discussion

The present study revealed a significant decrease in the final soil nematode populations and root gall index in the integrated application of MC 1 t ha^{-1} + NC 1 t ha^{-1} + FYM 2.5 t ha^{-1} in comparison to all other treatments. The decrease in the population of nematodes could be due to the presence of nematicidal compounds released from the organic cakes applied throughout the crop season. Multiple modes of action have been proposed for the activity of these amendments against plant parasitic nematodes, along with the improvement of soil structure and fertility [2,14]. Azadirachtin is the major active compound released by neem that is reported to be very effective and target-specific in controlling insects and nematode pests of various crops [21,37]. The breakdown of neem cake in the soil also produces certain compounds such as ammonia, fatty acids, formaldehyde and phenols [38], which are toxic to soil-borne pathogens and nematodes. The suppressive effect of the isothiocyanates generated from glucosinolates released by Brassica plants possess wide pesticidal activity against soil-borne pathogens and nematodes [39,40]. All these factors together help to decrease nematode development and reproduction [41]. The application of oil cakes has been reported to suppress plant parasitic nematodes in various crops. The soil application of neem, castor, mustard and duan was found to be effective in decreasing infestations of *M. incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae* and *Helicotylenchus indicus,* as well as pathogenic fungi, in chickpea crops [42]. Soil amendments with neem, linseed, mustard and castor cakes decreased the nematode population (*M. incognita*) and improved the plant growth parameters of okra [43]. The application of mustard and neem cake, in addition to exhibiting allelopathic properties, also revealed nutritional properties.

The organic carbon content of the soil, as well as the availability of phosphorus and potash, was also found to be enhanced with the application of the amendments used in the present study. The incorporation of organic amendments into the soil, in addition to suppressing the nematode population density, also promotes antagonistic microbial activity and improves the fertility and organic matter status of the soil [44]. The use of organic manure either alone or in combination with fertilizer enriched the NH_4 OAc-extractable K forms in Alfisols and Aridisols in India [45]. The increase in the levels of the carbon values in crop production systems helps to retain the nitrogen in the soil [46]. Application of FYM with nitrogen increases the available potassium (K) content of the soil [47]. The increase in NH_4OAc-extractable K with MC + NC and FYM application in the present study may be due to the additional amount of K added through these sources. The increased root biomass doubly increased the levels of NH_4OAc-extractable K. The nematode-suppressive effects of the amendments may have been also increased due to an increase in the phosphorus and potash contents of the soil. Higher levels of potash have shown a nematicidal effect on the activity of *M. javanica* and reduced RKN galls on the roots of tomato plants [48]. A higher K concentration (8 mM) led to the increased enzyme activity of the total phenol and flavonoid contents, enhancing the resistance of the cucumber plant. Phosphate treatments with fertilizers reduced the egg mass contents, resulting in increased plant growth and reduced nematode infestations [49]. The low residence time of fewer than 4 days following the incorporation of the isothiocynates in the soil has additional advantages [50], in addition to the suppression of nematode pathogens in the soil.

5. Conclusions

The present study concludes that the integrated application of mustard and neem cake, along with farmyard manure, is highly effective in managing RKN infestation in cucumbers grown in polyhouses. The additional incentive of soil nutrient enrichment, in addition to its suppressive effect on nematodes, offers a double advantage to the farmers by increasing crop yield. For effective management strategies, calculating the site- and soil-specific dosages of these amendments could prove them to be a sustainable asset for eco-compatible *M. incognita* management in cucumbers.

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