Nematicidal Efficacy of *Cymbopogon nardus*, *Pelargonium graveolens* Essential Oils and *Annona squamosa* Extract against *Meloidogyne incognita* and *M. graminicola*

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A crude hexane extract of seed kernel was processed into fatty acid methyl esters (FAME) using methanolic-sulfuric acid. Herbal mixtures (seed extracts of *A. squamosa* and essential oils of *Cymbopogon nardus* (citronella) and *Pelargonium graveolens* (geranium)) were evaluated against root knot nematode (*Meloidogyne* spp). The mortality of J2s of *M. incognita* in hexane and methanol extracts (AHE+AME) of *A. squamosa* with geranium oil (GO) ranged from 50.75-89.75% to 71.50-99.00% at 31.25 to 1000 μgmL^{-1} in 24 to 96 hours. The number of galls after CO+AHE+AME treatment was 4.0 ± 0.0 and 5.67 ± 0.58 galls/seedling and were recorded at 1000 and 500 μgmL^{-1} treatments on 30 days after inoculation, compared to carbofuran (3.0 ± 0.0 and 4.3 ± 0.6 galls/seedling) at 1000 and 500 μgmL^{-1} and velum prime (3.6 ± 0.6 and 4.6 ± 0.6 galls/seedling) at 1000 and 500 μgmL^{-1}. The maximum shoot length of brinjal seedlings was recorded in CO+AHE+AME treatment compared to carbofuran (47.3 ± 0.6cm) and velum prime (48.7 ± 0.6 cm) at 1000 μg mL^{-1} in soil drenching application. Methanol and hexane extracts (AHE+AME) obtained from *Annona squamosa* seeds were investigated for their nematicidal properties. *M. graminicola* in direct-seeded rice at the nursery level and *M. incognita* in brinjal (eggplant) were controlled for up to sixty days when treated with these mixtures.

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INTRODUCTION

Plant-parasitic nematodes (PPNs) responsible for major damage to important crops worldwide. A recent report showed that PPNs lead to an average yield reduction of 13.5% in 40 key food and export crops, which corresponds to USD 358.24 billion annually (Abd-Elgawad *et al.* 2024). Nematodes are known to infest the roots of vegetables, cereals, and pulses, causing a substantial loss in yield. Rice is a staple food for about four billion people. It is primarily grown in Asia. India, the second-largest rice producer, is facing an emerging problem due to nematode infestation in rice cultivation (Pathak *et al.* 2022). Approximately 4,100 species of plant-parasitic nematodes are described, causing up to US$80 billion in

crop losses annually (Decraemer and Geraert 2006). Species of *Meloidogyne* can parasitize almost all vascular plants, leading to complete crop loss (El-Nagdi *et al*. 2014). Crop losses are often higher in developing countries due to a lack of awareness about nematode infestations (Moens *et al.* 2009; Jain *et al.* 2012; Yadav *et al.* 2021a).

Meloidogyne graminicola, a phytopathogenic nematode, significantly impacts rice cultivation, causing over 20% of total economic loss (Patil *et al.* 2020). These nematodes form hook-shaped galls at rice root tips. *M. incognita*, found in tropical and subtropical regions, cause substantial damage to vegetable crops, including brinjal, causing significant yield losses due to disrupted nutrient uptake and plant growth (Jones *et al.* 2013; Ghosh 2021; Yadav *et al.* 2022).

Synthetic nematicides are highly effective against nematodes. However, their use has declined in recent decades due to their toxicity to non-target organisms and the environment (Yadav *et al.* 2021b). Integrated pest management (IPM) strategies offer promising alternatives to mitigate nematode populations in soil (Ntalli *et al.* 2010; Ajith *et al.* 2020). This study highlights various IPM techniques, including crop rotation, mulching with *Tagetes* and *Crotalaria* species, soil amendment, and the utilization of biocontrol agents such as *Trichoderma spp.*, *Paecilomyces lilacinus*, and *Pseudomonas fluorescens*. Additionally, non-host crops and nematode-resistant varieties are discussed as environmentally benign management strategies.

Botanical pesticides (such as neem oil, pyrethrins, clove essential oil, and karanj oil) offer an alternative to synthetic ones, being less harmful to the environment and human health (Akhtar *et al.* 2000; Acheuk *et al*. 2022). Their biodegradability and compatibility with biocontrol agents make them useful (Kumar *et al.* 2022). Plant-derived secondary metabolites (estragole, γ-eudesmol, *trans*-anethole/geraniol) are gaining attention for effectively targeting root-knot nematodes while sparing non-target organisms (Jirovetz *et al.* 2006).

The use of seed extract and essential oils from plant origins in the management of nematodes in agricultural crops has not been much explored at pot levels except under *in vitro* conditions (Jirovetz *et al.* 2006). That is the reason why up to the present, no nematicidal products of plant origin, either in the form of seed extracts, essential oils, or herbal mixture are available to end users (Ntalli *et al.* 2011a,b). Essential oils, a complex of volatile chemical compounds with multiple modes of action, are reported to possess antifungal, antibacterial, and insecticidal activities (Mossa *et al.* 2016). Citronella essential oil and its isolated compounds were screened *in vitro* for antifungal potential against *F. verticillioides* and *D. maydis* using poisoned food technique. Citronella essential oil (ED_{50} 0.095 mg/ml) was most effective against *F. verticillioides* (Kaur *et al*. 2021). Botanicals were toxic to *S. frugiperda* larvae at a single concentration (1%), and the maximum mortality was observed after 72 h. Geranium oil resulted in the highest mean larval mortality after 24 h of treatment (40%) (Keerthi *et al*. 2023).

However, recent studies highlight the potential efficacy of essential oils in managing nematodes in rice, brinjal, in field and greenhouse environments (Choi *et al.* 2007; Andres *et al.* 2012; Faria *et al.* 2013; Julio *et al.* 2017; Rajasekharan *et al.* 2020). A gap in research could involve doubts about how well mixing seed extracts (*Annona squamosa*) and essential oils from certain plants *Cymbopogon nardus* and *Pelargonium graveolens* can control root-knot nematodes (*M. incognita* and *M. graminicola*). While separate studies have shown good results, nobody has studied how effective they are when used together (Jardim *et al.* 2018).

In vitro studies showed that mixture of essential oil (*Cymbopogon nardus*, and *Pelargonium graveolens*) and seed extracts (*Annona squamosal*) could be an important source of nematicidal agents, especially for the management of *M. graminicola* and *M. incognita* in rice and brinjal.

There are very few studies on the nematicidal activity of essential oil (Shakil et al. 2004) against *Meloidogyne incognita* but in the present work first time we have evaluated the mixture of seed extracts from *Annona squamosa* and essential oils from *Cymbopogon nardus* and *Pelargonium graveolens* against *M. incognita*. Exploring the untapped potential of these botanical products as nematicides could pave the way for new alternatives.

In this study, the essential oils from *Pelargonium graveolens* (geranium) and *Cymbopogon nardus* (citronella) and seed extract from the *Annona squamosa* were isolated, and their chemical profiling was done by gas chromatography with flame-ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS). The mixtures of essential oils *(P. graveolens* and *C. nardus*) and seed extract (*A. squamosa*) were evaluated for nematicidal activity against *M. graminicola* and *M. incognita* both under *in vitro* and pot conditions.

EXPERIMENTAL

Preparation of Hexane Extract and Essential Oils Extraction

Fresh seeds of *Annona squamosa* were procured from the Khari Bably market in New Delhi in September 2021. The seeds were ground into a fine powder using a grinder. Crude fatty oil was then extracted from the powdered seeds using hexane as a solvent in a Soxhlet apparatus (Borosil®, India) over a period of 48 hours. The resulting extract was concentrated using a rotary evaporator.

The essential oils were isolated from shed dried *C. nardus* (citronella) leaves (400 g) and *P. graveolens* (geranium) aerial parts (500 g, leaves and inflorescence) by hydrodistillation method using Clevenger-type apparatus for 4 h and repeated thrice. The distillate was extracted with diethyl ether and the ethereal layer was dried over anhydrous sodium sulphate. The ether was distilled off on a gently heated water bath and stored in amber vials in a refrigerator until analysis and stored at 4 °C. The yields of the oils in *C. nardus* and *P. graveolens* were found to be 0.97% and 0.84%, respectively.

Preparation of Fatty Acid Methyl Esters

The crude hexane extract containing mainly total oil was converted into fatty acid methyl ester using an acidic esterification method (Saxena *et al.* 2005). Briefly, 200 mg of crude total oil was dissolved in 20 mL of sulfuric acid-methanol (1.0%) (0.20 mL H_2SO_4 in 19.80 mL methanol) and was refluxed in a boiling water bath for 2 h. The reaction mixture was cooled and dried under reduced pressure (50 mbar and 45 °C). The dried mixture was dissolved in 20 mL of distilled water and neutralized with a saturated solution of sodium carbonate followed by extraction of methyl esters with diethyl ether (Rana *et al.* 2008). The ethereal mixture of methyl esters was dried over anhydrous sodium sulphate. The ether was removed from the mixture and stored at 4 °C.

GC-FID and GC/MS Analysis

The fatty acid methyl esters were analyzed by GC-FID (Shimadzu GC 2010 Plus) fitted with a capillary column, SH Rtx-5 (30 m × 0.25 mm × 0.25 µm film thickness). GC conditions were: N_2 as carrier gas (1.0 mL/min); injector and flame ionization detector temperatures were 250 ℃ and 260 ℃, injected volume (0.3 µL, 1000 µg mL^{-1} in hexane); and injection mode (split ratio 1:20). The column temperature was held at 60 ℃, then increased to 250 ℃ at 3 ℃/min. The relative percentage composition of the oils were determined by the normalization method from the GC peak areas and then calculated as the mean value of two injections, without using correction factors.

A GC/MS (Focus-Polaris Q; Thermo, USA) bench top quadrupole ion trap mass spectrometer outfitted with a ZB-5 capillary column (30 m × 0.25 mm internal diameter (id) film thickness 0.25 mm) was used to analyze the fatty acid methyl esters. The oven was preheated to 60 °C and programmed to rise to 250 °C in 3 min. The injection volume was 0.2 µL (1000 µg mL^{-1} in hexane), the split flow was 20 mL min^{-1}, and helium was employed as the carrier gas at a flow rate of 1 mL min^{-1} (split mode, 1:20). The temperatures of the injector and transfer line were 220 and 240 ºC, respectively. The mass spectrometer's source temperature was 220 ºC. Analysis was done using the mass range of 40 to 400 atomic mass unit (a.m.u.) at 70 eV in electron ionization mode. Identification of individual compounds was carried out by comparing the retention time and mass spectra with certified reference materials (Sigma-Aldrich), as well as mass spectra in the NIST Mass Spectral Library Version 2.0 d (2005).

Nematicidal Activity

Preparation of test samples

The test solution (1.0%) of mixture of *A. squamosa* hexane extract (AHE) and *A. squamosa* methanol extract from *A. squamosa* seeds, when mixed in equal proportion with citronella oil or geranium oil, was prepared in distilled water with polysorbate 80 (Tween-80) (4%) followed by mixing the ingredients using a lab stirrer for 1 h (Ibrahim *et al.* 2006). Different concentrations (1000, 500, 250, 125, 62.5, and 31.25 µg mL^{-1}) of the test solutions were prepared by serial dilution for their evaluation against *M. graminicola* and *M. incognita*. The stock solution (1.0%) of carbofuran and velum prime prepared in water and applied *via* root dipping and soil drenching using two commercial nematicides, carbofuran 3G (Furadon by Crystal crop) and velum prime (Fluopyram 34.48% SC by Bayer) and desired concentration (62.5 and 31.25 µg mL^{-1}) were prepared by serial dilution from stock the solution.

Isolation of J2s of M. graminicola and M. incognita

Heavily infected rice (cv. PB-1121) plants infested with *M. graminicola* were uprooted from infested plot maintained at the Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi. The roots were thoroughly washed and cleaned under tap water and infected roots bearing galls were cut into small pieces. The infected root pieces were kept on muslin cloth, placed on wire mesh (0.5 mm) and kept for a period of 48 h on a petri plate filled with water. The freshly hatched second-stage juveniles (J2s) of *M. graminicola* emerged from the galls into the water and were collected in a beaker. J2s were counted under the binocular microscope and their numbers were found to be 100+1 mL^{-1} as an average of 1.0 mL aliquots in triplicates.

Tomato (*Solanum lycopersicum*) plants of cultivar Pusa Ruby infested with M. incognita and maintained by the Nematology Division, ICAR-Indian Agricultural

Research Institute, New Delhi were uprooted and washed. Egg masses were hand-picked using sterilized forceps and transferred to a vial containing sodium hypochlorite (NaOCl - 0.5% (v/v)). The egg mass culture was passed through a series of filters (pore size 74, 45, and 25 µm), and eggs were collected in sterile distilled water. The eggs were allowed to hatch in modified Baermann funnels at 28 °C to get J2s for the bioassay. The number of J2s per mL was counted under the binocular microscope and their numbers adjusted to 100+1 per mL, as an average of 1.0 mL aliquot in triplicates.

In-vitro nematicidal bioassay

The nematode suspension (100+1 J2smL^{-1}) was poured into 24-well culture plates and 1.0 mL of different concentrations of test solutions was added, mixed, and incubated at 28 °C. All bioassays were done in triplicate. Polysorbate 80 (Tween-80) (4.0%) in distilled water and sterile distilled water were taken as negative control, while carbofuran 3G and velum prime (Fluopyram 34.48% SC) were used as positive control. The mortality of J2s was recorded after 24, 48, 72, and 96 h of treatment. All dead and alive J2s were counted with the aid of a counting dish under a stereoscopic binocular microscope. The mortality of nematodes was determined by keeping immobile nematodes in fresh distilled water for 24 h and again observed under a stereoscopic binocular microscope. Mortality rates were calculated using Abbott's formula (Abbott 1925; Rana *et al*. 2015).

Pot Experiments

Preparation of soil

A soil-sand mixture (3:1) was autoclaved at 1.06 kg per cm^3 pressure for 4 h in polythene bags and then cooled and dried in sunlight followed by filling of the soil into formalin (4.0%)-treated earthen-ware pots.

Seedlings

Approximately 500 rice Pusa Basmati (PB-1121) seeds were washed with potassium permanganate solution (1.0%) followed by distilled water and were spread on wetted filter papers in a plastic tray and kept up to 10 days to germinate. Similarly, the seedlings of *Solanum melongena* cv. 'Pusa Purple Long' were raised on sterilized oil from seeds in nursery beds for carrying out pot experiments in rice and brinjal seedlings using root dip and soil drenching methods.

Root dip method

The roots of rice (PB-1121,10 days old) and brinjal (cv. Pusa Purple Long, 30 days old) seedlings were dipped in 50 mL volume of concentrations (1000 and 500 µgmL^{-1}) of mixture of *A. squamosa* hexane extract (AHE) and *A. squamosa* methanol extract (AME) from A. squamosa seeds when mixed in equal proportion with citronella oil (CO) or geranium oil (GO) for 30 min. The rice (5 seedlings/pot) and brinjal (2 seedlings pot^{-1}) seedlings were transplanted to the earthen pots containing sterilized soil (1.150 kg pot^{-1}) and kept for 48 h. Freshly hatched J2s (about 2000 J2s pot^{-1}) of *M. graminicola* and *M. incognita* were inoculated directly in the root zones of rice and brinjal seedlings after 48 h of transplantation. Total 9 treatments with triplicates were taken in this study.

Soil drenching method

Sterilized soil (1.150 kg) in earthen-ware pots was drenched (50 mL pot^{-1}) separately with two concentrations (1000 and 500 µg mL^{-1}) of herbal mixture of *A.*

squamosa hexane extract (AHE) and *A. squamosa* methanol extract (AME) from *A. squamosa* seeds when mixed in equal proportion with citronella oil (CO) or geranium oil (GO) and covered with polythene sheets for 2 days. The ten-day-old rice (5 seedlings/pot) and thirty days old brinjal (2 seedling spot^{-1}) seedlings were transplanted to the earthen pots. The freshly hatched J2s (about 2000 J2s pot^{-1}) of *M. graminicola* and *M. incognita* were inoculated directly in the root zones of rice and brinjal seedlings after 48 h of transplantation. A total of 9 treatments with triplicates were taken in this study.

Seedling care and nutrition

Care of seedlings such as watering and application of recommended doses of nitrogenous and phosphoric fertilizers for proper growth of rice and brinjal seedlings together with the random rotation of pots to eliminate the effect of sun and shade were carried out. Also, rice and brinjal seedlings were provided Hoagland's nutrient solution once a fortnight at the rate of 250 mL pot^{-1} (Hoagland and Arnon 1950). The observations for rice seedlings' growth parameters such as root and shoot lengths, fresh root and shoot weights, and the number of galls per seedling in both experiments were recorded on 7, 14, and 30 days after inoculation (DAI). Similarly, root length, shoot length, fresh root weight, shoot weight and number of galls per seedling in both experiments and that of brinjal were recorded in 30 and 60 DAI.

Statistical Analysis

All experiments were performed in triplicate, and the data were subjected to statistical analysis using SPSS (version 22.0) software using one-way analysis of variance (significance level of $p < 0.05$). The results were presented as mean ± standard deviations.

RESULTS AND DISCUSSION

Chemical Composition of Crude Hexane Extract

Dried and powdered seeds were extracted using hexane. The yield of the hexane extract of *A. squamosa* was 22.2%, on a dry-weight basis. The crude hexane extract contained mainly total oil and was thus converted into fatty acid methyl esters using methanolic-sulfuric acid (1.0%) to determine its chemical composition. Fatty acids were identified by GC-MS and quantified by GC-FID using its fatty acid methyl esters (Table 1). Eleven fatty acids, accounted for 99.8% of the oil; the most predominant were oleic acid (50.1%), linoleic acid (23.9%), palmitic acid (14.2%), and stearic acid (11.8%) (Fig. 1). GC-MS analysis showed the presence of heneicosanoic acid (2.3%), eicosanoic acid (0.9%), margaric acid (0.2%), 11-eicosanoic acid (0.2%), palmitoleic acid (0.01%), and 17-methyloctadecanoic acid (0.1%).

Table 1. Major Fatty Acid Composition of the Oil of *Annona squamosa* Seeds

Fatty acid	Amount (%)
Oleic acid	50.10
Palmitic acid	14.24
Linoleic acid	23.85
Stearic acid	11.81

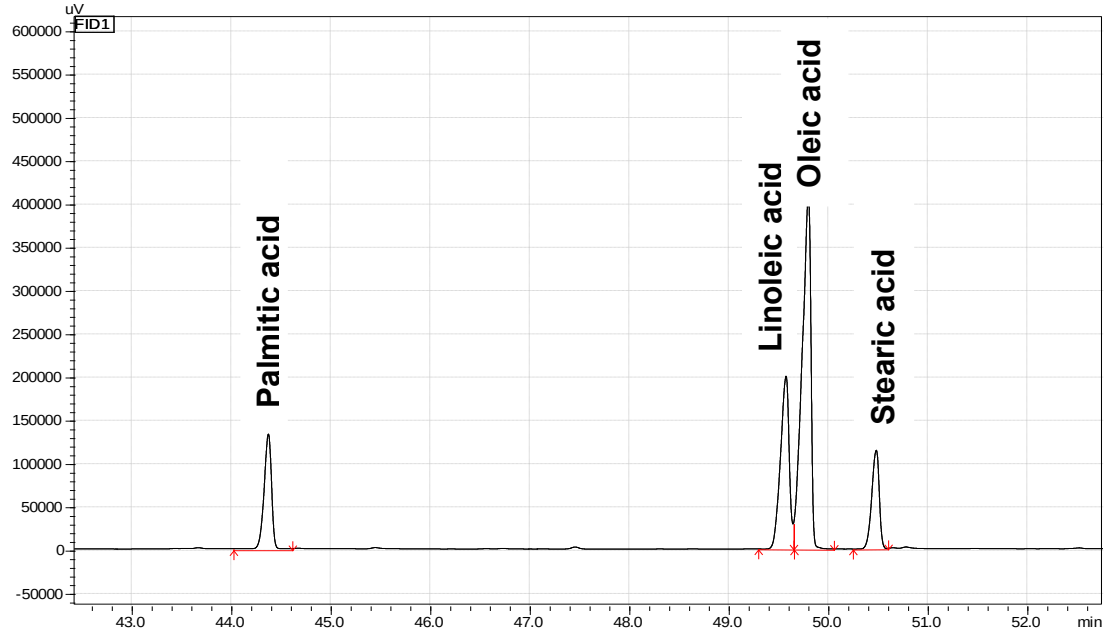

Fig. 1. Gas chromatogram of methyl esters of the fatty oil of *Annona squamosa* seeds

Nematicidal Activity

In-vitro nematicidal activity

The results of in-vitro nematicidal activity of mixture of *A. squamosa* hexane extract (AHE) and *A. squamosa* methanol extract (AME) from *A. squamosa* seeds when mixed in equal proportion with citronella oil (CO) or geranium oil (GO) against J2S of *M. graminicola* and *M. incognita* at a range of concentrations from 31.2 to 1000 µg·mL^{-1} and period of exposures from 24 h to 96 h showed concentration-dependent mortality of J2s. Mortality of J2s increased with concentration and duration of exposure. The evaluation of nematicidal activity of citronella oil plus *A. squamosa* methanol plus *A. squamosa* hexane extract (CO+AHE+AME) mixture against *M. graminicola* and *M. incognita* showed that the oil caused mortality of J2 of both nematode species. The results ranged from 33.5 ± 0.6-91.3 ± 1.0 and 66.5 ± 1.3-99.2 ± 1.0% against *M. graminicola* and 48.5 ± 1.3-88.5 ± 1.3 and 69.7 ± 1.0-99.00 ± 0.8% mortality of *M. incognita* at 31.2 to 1000 µg mL^{-1} from 24 h to 96 h of treatment, as listed in Table 2. Lethal concentration (LC50) values were found to be 13.7 µg mL^{-1} for *M. graminicola* and 11.6 µg mL^{-1} for *M. incognita* after a period of 96 h.

The evaluation of nematicidal activity of geranium oil plus *A. squamosa* methanol plus *A. squamosa* hexane extract (GO+ AME+AHE) mixture against *M. graminicola* and *M. incognita* showed that the oil caused impressive mortality of J2 of both nematodes, at 31.2 to 1000 µg mL^{-1} from 24 h to 96 h of treatment (Table 3). LC50 values were found to be 21.8 µg mL^{-1} for *M. graminicola* and 8.1 µg mL^{-1} for *M. incognita* after a period of 96 h of treatment.

Pot Experiment against *M. graminicola*

Root dip application

In the pot experiments, a single root dip application of a mixture (CO+AHE+AME) increased rice seedling growth parameters and inhibited formation of galls on roots (Table 4). The mean fresh rice shoot length (32.0 ± 1.0 cm), root length (10.5 ± 0.5 cm), shoot weight (3.2 ± 0.1 g), root weight (1.9 ± 0.1 g), and number of root gall (4.0 ± 0.0) resulted

from application of 1000 µg mL^{-1}. Application of 500 µg mL^{-1} CO+AHE+AME resulted in a decrease in shoot length (27.7 ± 0.6cm), root length (9.0 ± 0.5cm), shoot weight (3.20 ± 0.10g), root weight (1.60 ± 0.10 g), and number of root gall (5.6 ± 0.5) recorded on 30 days after inoculation.

The maximum fresh rice shoot length (30.7 ± 1.5cm), root length (09.7 ± 2.1cm), shoot weight (3.3 ± 0.1 g), and root weight (1.7 ± 0.2 g) were found at 1000 µg mL^{-1} compared to 500 µg mL^{-1}, in which rice shoot length (30.7 ± 1.5cm), root length (09.0 ± 0.8cm), shoot weight (2.4 ± 0.1 g), and root weight (1.3 ± 0.1g) were recorded on 30 days after inoculation in the GO+AHE+AME mixture. The number of galls after CO+AHE+AME treatment was 4.0 ± 0.0 and 5.67 ± 0.58 galls/seedling, whereas with treatment GO+AHE+AME it was 2.43 ± 0.1 and 3.26 ± 0.1 galls/seedling that were recorded for the 1000 and 500 µg mL^{-1} treatments, respectively, on 30 days after inoculation, compared to carbofuran (3.0 ± 0.0 and 4.3 ± 0.6 galls/seedling) at concentration of 1000 and 500 µg·$mL^{-1}$and velum prime (3.6 ± 0.6 and 4.6 ± 0.6 galls/seedling) 1000 µg·mL-1and 500 µg·mL-1. The negative control showed a higher number of galls (14.7 ± 0.6 galls/seedling) during the same period of 30 days. The mixture significantly improved and increased the shoot length and inhibited root galling in the roots of rice seedling (Fig. 2).

However, the use of these mixtures of essential oils (*P. graveolens and C. nardus*) and hexane extract of seed *(A. squamosa)* in the management of nematodes in field crops has not been much explored under field conditions or even in greenhouse agriculture and nematicidal products from the mixtures of essential oils and seed extract are not yet developed and available to the farming community.The most effective tested samples have the potential to be used as bionematicides, however, multi-location field trials are suggested to further establish the efficacy under different geographic conditions against *M. graminicola* and *M.incognita* in rice and brinjal.

Soil drenching application

Rice seedlings were planted in the soil drenched with mixture of *A. squamosa* hexane extract (AHE) and *A. squamosa* methanol extract (AME) mixed in equal proportion with citronella oil (CO) or geranium oil (GO) in pots. In the case of *M. graminicola*, the results (Table 5) showed a similar effect on seedling growth parameters and root galling but better inhibition of gall formation in the roots of rice than the root dipping method. The number of galls in the CO+AHE+AME treatment was 3.3 ± 0.6 and 5.0 ± 1.0 galls/seedling, and the number of galls in the GO+AHE+AME treatment was 3.7 ± 0.6 and 4.7 ± 0.6 galls/seedling) at 1000 and 500 µg mL^{-1} treatments *vs.* carbofuran (2.60 ± 0.6 and 4.0 ± 0.5 galls/seedling) and velum prime (3.0 ± 0.0 and 4.3 ± 0.5 galls/seedling) at 1000 and 500 µgmL^{-1}, which were lesser than the negative control (14.66 ± 0.58 galls/seedling) 30 days after inoculation. The rice seedling growth parameters were found similar in both mixture extract and positive control at 1000 µg mL^{-1} and were better than the negative control (Fig. 3).

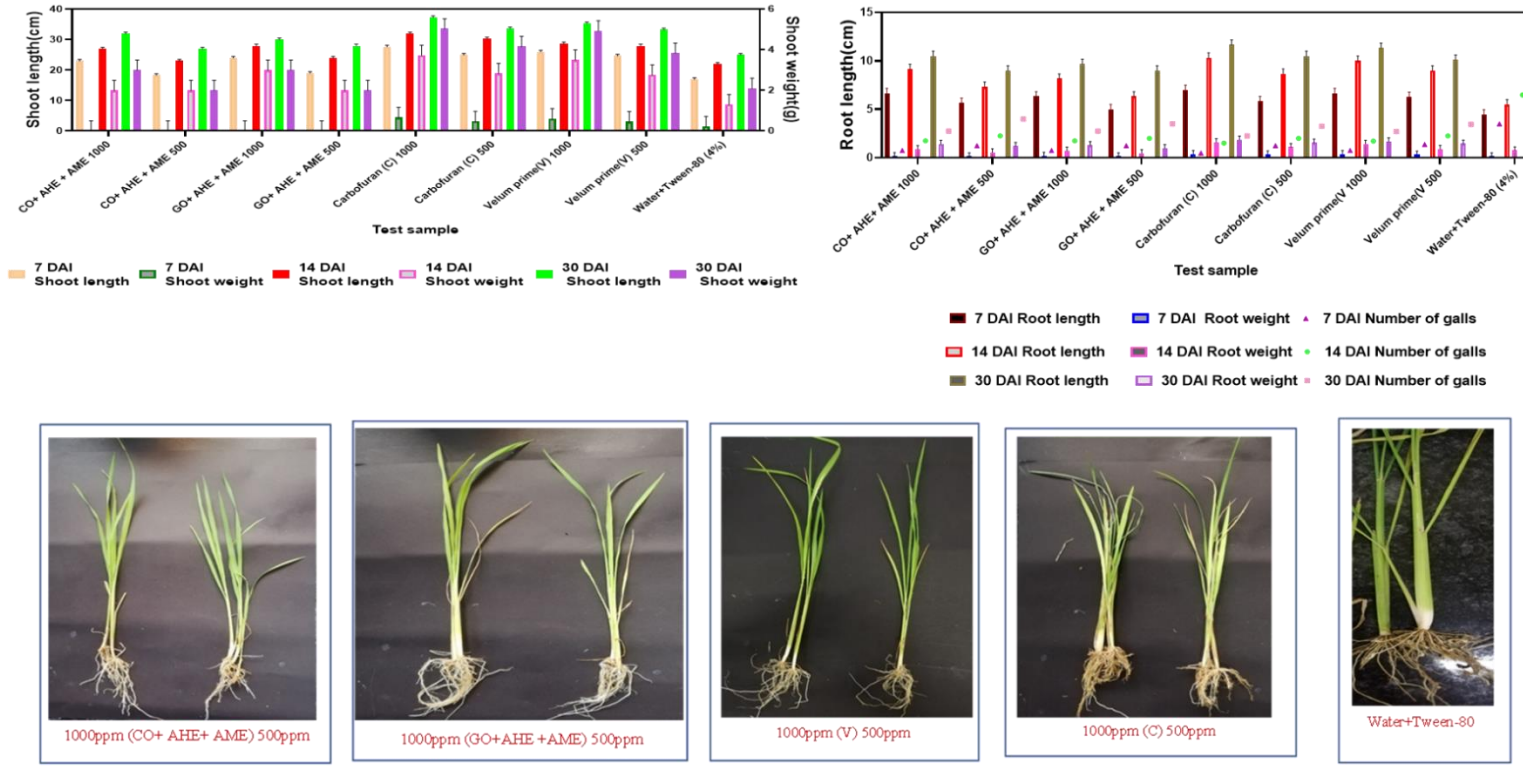

Fig. 2. PB-1121 Root Dipping: Effect of (CO+AHE+AME) and (GO+AHE+AME) on growth parameters and number of galls of *Meloidogyne graminicola* in roots of rice seedlings in root dip application in pot experiment. *CO-Citronella oil, AHE-*A. squamosa* hexane extract, AME-*A. squamosa methanol* extract, GO-Geranium oil

Table 2. *In-vitro* Nematicidal Activity of Citronella Oil +*A. squamosa* Hexane Extract (AHE)+ *A. squamosa* Methanol Extract (AME) on Mortality (%) of J2s of *Meloidogyne graminicola* and *M. incognita*

Conc. (µg mL^{-1})	*Meloidogyne graminicola*				*Meloidogyne incognita*			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
CO+AHE+AME -1000	91.25 ± 0.96	95.50 ± 0.58	97.50 ± 0.58	99.25 ± 0.96	88.50 ± 1.29	93.50 ± 1.29	97.50 ± 1.29	99.00 ± 0.82
CO+AHE+AME -500	83.25 ± 0.50	87.00 ± 1.41	93.00 ± 1.41	95.25 ± 1.26	80.75 ± 0.96	86.00 ± 0.82	93.25 ± 0.96	96.00 ± 0.82
CO+AHE+AME -250	71.25 ± 0.96	77.50 ± 1.29	83.50 ± 1.29	90.00 ± 0.82	72.75 ± 1.26	76.50 ± 2.38	85.25 ± 0.96	90.50 ± 0.58
CO+AHE+AME -125	58.75 ± 0.96	63.50 ± 1.29	71.00 ± 1.41	84.00 ± 0.82	64.00 ± 1.82	71.00 ± 0.82	77.00 ± 0.82	82.75 ± 1.71
CO+AHE+AME -62.5	47.25 ± 0.96	52.50 ± 1.29	62.00 ± 0.82	77.00 ± 0.82	56.50 ± 1.29	62.00 ± 2.16	68.75 ± 1.70	77.75 ± 1.71
CO+AHE+AME -31.25	33.5 ± 0.58	39.00 ± 0.82	46.75 ± 1.70	66.50 ± 1.29	48.50 ± 1.29	53.00 ± 1.82	61.25 ± 0.96	69.75 ± 0.96
C-62.5	76.0 ± 1.00	78.67 ± 0.57	82.67 ± 0.57	87.67 ± 0.57	66.00 ± 1.82	69.00 ± 0.82	72.50 ± 1.29	76.75 ± 0.96
C-31.25	70.67 ± 0.57	74.67 ± 0.57	79.33 ± 0.57	83.33 ± 1.52	61.50 ± 1.29	64.75 ± 0.96	66.50 ± 1.29	71.75 ± 1.26
V-62.5	59.75 ± 1.26	64.75 ± 1.89	70.50 ± 0.58	75.00 ± 0.82	64.50 ± 1.29	69.50 ± 1.29	75.00 ± 0.82	79.75 ± 1.26
V-31.25	55.50 ± 0.58	60.50 ± 0.58	65.25 ± 0.50	70.25 ± 0.50	59.25 ± 0.96	64.25 ± 0.96	70.25 ± 0.50	74.50 ± 1.29
WT (1%)	3.33 ± 1.5	6.75 ± 0.96	9.75 ± 0.50	11.75 ± 2.22	2.50 ± 1.29	4.00 ± 0.82	5.75 ± 0.96	10.75 ± 0.96

* CO+AHE+AME =Citronella oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), GO+AHE+AME =Geranium oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), C=Carbofuran, V=Velum Prime, WT-80= Water+Tween-80. The values given are the mean ± standard deviation of three replicates.

Table 3. *In-vitro* Nematicidal Activity of Geranium Oil +*A. squamosa* Hexane Extract (AHE)+ *A. squamosa* Methanol Extract (AME) on Mortality of J_2s of *Meloidogyne graminicola* and *M. incognita*

Conc. (µg mL^{-1})	*Meloidogyne graminicola*				*Meloidogyne incognita*			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
GO+AHE+AME -1000	87.75 ± 0.96	90.25 ± 1.50	94.75 ± 0.96	99.75 ± 0.50	89.75 ± 1.50	94.25 ± 0.96	97.75 ± 1.26	99.00 ± 0.81
GO+AHE+AME -500	76.50 ± 0.58	82.00 ± 1.41	88.50 ± 0.58	95.00 ± 0.82	82.00 ± 0.82	85.75 ± 1.70	92.25 ± 1.70	94.25 ± 0.95
GO+AHE+AME -250	62.75 ± 1.26	69.75 ± 1.70	75.75 ± 0.96	86.75 ± 1.89	75.75 ± 0.96	81.50 ± 1.29	86.50 ± 1.29	90.25 ± 0.50
GO+AHE+AME -125	50.75 ± 0.96	58.75 ± 1.50	69.00 ± 0.82	80.75 ± 0.96	65.25 ± 1.70	71.25 ± 1.70	78.00 ± 0.82	85.75 ± 1.70
GO+AHE+AME -62.5	40.50 ± 0.58	46.00 ± 0.82	60.50 ± 1.29	70.25 ± 0.96	59.00 ± 0.82	64.75 ± 0.96	69.50 ± 1.29	80.50 ± 0.57
GO+AHE+AME -31.25	31.25 ± 0.50	36.25 ± 1.26	46.25 ± 1.50	60.50 ± 1.29	50.75 ± 0.96	57.00 ± 0.82	61.50 ± 1.29	71.50 ± 1.29
C-62.5	76.0 ± 1.00	78.67 ± 0.57	82.67 ± 0.57	87.67 ± 0.57	66.00 ± 1.82	69.00 ± 0.82	72.50 ± 1.29	76.75 ± 0.96
C-31.25	70.67 ± 0.57	74.67 ± 0.57	79.33 ± 0.57	83.33 ± 1.52	61.50 ± 1.29	64.75 ± 0.96	66.50 ± 1.29	71.75 ± 1.26
V-62.5	59.75 ± 1.26	64.75 ± 1.89	70.50 ± 0.58	75.00 ± 0.82	64.50 ± 1.29	69.50 ± 1.29	75.00 ± 0.82	79.75 ± 1.26
V-31.25	55.50 ± 0.58	60.50 ± 0.58	65.25 ± 0.50	70.25 ± 0.50	59.25 ± 0.96	64.25 ± 0.96	70.25 ± 0.50	74.50 ± 1.29
WT (1%)	3.33 ± 1.5	6.75 ± 0.96	9.75 ± 0.50	11.75 ± 2.22	2.50 ± 1.29	4.00 ± 0.82	5.75 ± 0.96	10.75 ± 0.96

* GO+AHE+AME =Graminicola oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), GO+AHE+AME =Geranium oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), C=Carbofuran, V=Velum Prime, WT-80= Water+Tween-80. The values given are the mean ± standard deviation of three replicates

Table 4. Effect of CO+AHE+AME and GO+AHE+AME on Growth Parameters and Number of Galls of *Meloidogyne graminicola* in Roots of Rice Seedlings in Root Dip Application in Pot Experiment

Sample	Conc. (µg mL^{-1})	Fresh shoot length(cm) and weight(g)/rice seedling						Fresh root length(cm), root weight(g) and number of galls per rice seedling								
		7DAI		14DAI		30DAI		7DAI			14DAI			30DAI		
		SL	SW	SL	SW	SL	SW	RL	RW	NGP	RL	RW	NGP	RL	RW	NGP
CO + AHE + AME	1000	23.00 ± 1.00	0.56 ± 0.005	27.33 ± 0.58	2.80 ± 0.10	32.00 ± 1.00	3.20 ± 0.10	7.00 ± 0.51	0.32 ± 0.05	1.33 ± 0.05	10.00 ± 2.00	1.71 ± 0.19	2.33 ± 0.58	12.16 ± 0.76	1.90 ± 0.05	4.00 ± 0.00
	500	18.33 ± 0.58	0.38 ± 0.005	23.16 ± 0.76	2.10 ± 0.10	27.67 ± 0.58	2.90 ± 0.10	6.00 ± 0.57	0.27 ± 0.03	1.66 ± 0.05	7.67 ± 0.28	0.63 ± 0.05	3.00 ± 0.50	10.23 ± 0.25	1.30 ± 0.10	5.67 ± 0.58
GO + AHE + AME	1000	23.33 ± 1.15	0.60 ± 0.10	28.00 ± 1.00	1.83 ± 0.05	33.33 ± 0.58	3.80 ± 0.10	6.66 ± 0.58	0.60 ± 0.10	1.00 ± 0.05	9.16 ± 0.76	1.18 ± 0.12	3.80 ± 0.10	23.33 ± 1.15	0.60 ± 0.10	2.43 ± .0.12
	500	17.00 ± 1.00	0.38 ± 0.02	21.00 ± 1.00	1.50 ± 0.10	25.50 ± 0.50	1.90 ± 0.05	5.66 ± 0.58	0.38 ± 0.02	1.66 ± 0.05	7.33 ± 0.76	0.73 ± 0.15	1.90 ± 0.05	17.00 ± 1.00	0.38 ± 0.02	3.26 ± 0.12
C	1000	27.66 ± 0.58	0.67 ± 0.06	32.00 ± 1.00	3.73 ± 0.94	37.33 ± 0.58	5.03 ± 1.00	7.00 ± 0.28	0.50 ± 0.06	0.66 ± 0.05	10.33 ± 0.58	2.13 ± 0.23	2.00 ± 0.00	11.66 ± 0.58	2.48 ± 0.02	3.00 ± 0.00
	500	25.00 ± 1.00	0.47 ± 0.06	30.33 ± 0.58	2.83 ± 1.04	33.67 ± 1.52	4.16 ± 0.28	5.83 ± 0.28	0.45 ± 0.05	1.66 ± 0.05	8.67 ± 0.58	1.46 ± 0.50	2.67 ± 0.58	10.50 ± 0.50	2.06 ± 0.20	4.33 ± 0.58
V	1000	26.00 ± 2.00	0.60 ± 0.005	28.67 ± 0.58	3.50 ± 0.36	35.33 ± 0.58	4.93 ± 0.11	6.67 ± 0.28	0.49 ± 0.001	1.00 ± 0.058	10.00 ± 1.00	1.90 ± 0.10	2.30 ± 0.58	11.33 ± 0.58	2.23 ± 47	3.60 ± 0.58
	500	24.67 ± 0.58	0.46 ± 0.002	28.00 ± 0.58	2.76 ± 0.40	33.33 ± 0.58	3.83 ± 0.76	6.26 ± 0.25	0.43 ± 0.005	1.83 ± 0.058	9.00 ± 1.00	1.20 ± 0.20	3.00 ± 1.00	10.11 ± 0.12	1.93 ± 0.11	4.60 ± 0.58
WT-80	4.0%	17.00 ± 1.00	0.22 ± 0.004	22.00 ± 1.00	1.30 ± 0.10	25.00 ± 1.00	2.10 ± 0.26	4.46 ± 0.45	0.19 ± 0.001	4.66 ± 0.58	5.50 ± 0.50	1.00 ± 0.18	8.64 ± 0.58	6.76 ± 0.68	1.36 ± 0.12	14.66 ± 0.58

* DAI= days after inoculation, CO+AHE+AME =Citronella oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), GO+AHE+AME =Geranium oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), C=Carbofuran, V=Velum Prime, WT-80= Water+Tween-80. The values given are the mean ± standard deviation of three replicates.

Pot Experiments against *M. incognita*

Root dip application

A single application of mixture of CO+AHE+AME and GO+AHE+AME using the root dipping method showed nematicidal activity against *M. incognita* in brinjal cv. Pusa Purple Long by inhibiting root galls and improved growth parameters GO+AHE+AME were shown to be better ($P<0.05$) than the positive control treatments (Table 6). The minimum number of galls per seedling were found in GO+AHE+AME (4.7 ± 0.6 galls) followed by carbofuran (3.7 ± 0.6 galls) and velum prime (5.3 ± 0.6 galls) at 1000µg mL^{-1}, but higher galls were recorded in negative control (27.3 ± 1.5 galls) 60 days after inoculation. The mean of maximum shoot length (38.7 ± 0.6 cm) was recorded in GO+AHE+AME, and shoot length (38.0 ± 0.0 cm) was recorded in CO+AHE+AME compared to carbofuran (40.0 ± 1.0 cm) and velum prime (39.0 ± 1.0 cm) at the same concentration and duration, but highly reduced shoot length (30.0 ± 2.1 cm) was found in the negative control (WT-80) due to infestation of *M. incogntia* (Fig. 4).

Soil drench application

The application of the herbal mixture in soil using the drenching method was effective on *M. incognita* in the roots of brinjal by inhibiting gall formation (Table 7). The herbal mixture significantly increased shoot length and weight of brinjal seedlings, comparable to the positive control and better than the negative control. The number of galls/seedling in GO+AHE+AME was 6.0 ± 1.0 galls, and with CO+AHE+AME was 4.7 ± 1.1 galls, more compared to carbofuran (2.0 ± 0.6 galls) and velum prime (2.3 ± 0.6 galls) but higher than the negative control (8.6 ± 1.5galls) at 1000 µg mL^{-1}on plants 60 days after inoculation. Similarly maximum shoot length of 57.3 ± 2.5 cm was recorded in CO+AHE+AME treatment compared to carbofuran (47.3 ± 0.6cm) and velum prime (48.7 ± 0.6cm) at same concentration and duration, but reduced shoot length (32.3 ± 2.1 cm) was found in the negative control (Fig. 5). A similar pattern was found in brinjal biomass and root length parameters in both root dip and soil drench applications.

Chemical Composition of Crude Hexane Extract

The seeds of *A. squamosa* were found to contain hexane extract rich in oleic acid (50.1%) and linoleic acid (23.85%), totaling 73.95% of the oil content. This oil, similar in composition to *J. curcas* used for biodiesel production, is suitable for edible purposes after purification (Shakil *et al*. 2004; Jardim *et al.* 2018; Ajith *et al.* 2020; Ajith *et al*. 2022).

Nematicidal Activity

A mixture, including *A. squamosa* seeds, was evaluated for nematicidal activity against *M. graminicola* and *M. incognita* under in vitro and pot conditions. The bioassay showed activity against J2s of both nematode species. The mortality of nematodes was found to be concentration-dependent and increased with concentration of the mixture and duration of exposure in all the experimental conditions (Ansari *et al.* 1985). *In-vitro* assays of the CO+AHE+AME and GO+AHE+AME mixtures showed the highest mortality of J2s of *M. graminicola* (99.2 ± 1.0%) followed by *M. incognita* (99.2 ± 1.0%) at 1000 µg mL^{-1} at 96 h after treatments and were similar to carbofuran and velum prime at 62.5 µg mL^{-1} (Tables 2, 3). Some mortality of the J2s was also recorded in the negative control (Ibrahim *et al.* 2006; Saxena *et al.* 2005).

In a pot experiment, mixtures CO+AHE+AME and GO+AHE+AME applied to rice seedlings showed better control of *M. graminicola* infestation when applied *via* soil drenching compared to root dipping (Kamatchi *et al.* 2019). Soil application resulted in fewer galls and better growth (Laquale *et al.* 2015). Treatment was comparable to the positive control and superior to the negative control in terms of reducing root galling and improving growth parameters.

Table 5. Effect of (CO+AHE+AME) and (GO+AHE+AME) on Growth Parameters and Number of Galls of *Meloidogyne graminicola* in Roots of Rice Seedlings in Soil Drenching in Pot Experiment

Sample	Conc. (µg mL^{-1})	Fresh shoot length (cm) and weight (g) per rice seedling						Fresh root length (cm), root weight (g) and number of galls per rice seedling								
		7 DAI		14 DAI		30 DAI		7 DAI			14 DAI			30 DAI		
		SL	SW	SL	SW	SL	SW	RL	RW	NGP	RL	RW	NGP	RL	RW	NGP
CO+AHE+AME	1000	23.67 ± 0.58	0.52 ± 0.04	28.67 ± 0.58	1.46 ± 0.05	34.00 ± 1.00	3.60 ± 0.87	10.67 ± 0.68	0.49 ± 0.01	0.33 ± 0.58	12.16 ± 0.76	1.20 ± 0.10	1.00 ± 0.00	13.66 ± 0.28	1.73 ± 0.15	3.33 ± 0.58
	500	17.33 ± 1.15	0.30 ± 0.07	23.00 ± 1.00	1.17 ± 0.05	28.33 ± 0.58	2.60 ± 0.05	6.00 ± 1.00	0.37 ± 0.05	0.67 ± 1.15	8.16 ± 0.28	0.99 ± 0.005	1.67 ± 0.58	11.50 ± 0.50	1.26 ± 0.20	5.00 ± 1.00
GO+AHE+AME	1000	23.33 ± 1.15	0.60 ± 0.10	28.00 ± 1.00	1.83 ± 0.05	33.33 ± 0.58	3.80 ± 0.10	8.66 ± 0.69	0.39 ± 0.01	0.33 ± 0.58	10.50 ± 0.50	1.23 ± 0.05	1.67 ± 0.58	14.83 ± 0.28	1.53 ± 0.30	3.67 ± 0.58
	500	17.00 ± 1.00	0.38 ± 0.02	21.00 ± 1.00	1.50 ± 0.10	25.50 ± 0.50	1.90 ± 0.05	5.33 ± 0.58	0.29 ± 0.01	0.67 ± 0.58	8.50 ± 0.50	0.99 ± 0.10	2.67 ± 0.58	11.83 ± 0.28	1.40 ± 0.10	4.67 ± 0.58
C	1000	23.66 ± 0.58	0.76 ± 0.06	32.00 ± 0.00	3.00 ± 0.62	35.33 ± 0.58	5.03 ± 0.05	9.33 ± 0.58	0.46 ± 0.08	0.33 ± 0.58	12.33 ± 0.58	2.06 ± 0.20	1.33 ± 0.58	14.66 ± 0.58	2.43 ± 0.11	3.60 ± 0.58
	500	20.00 ± 1.00	0.67 ± 0.12	26.33 ± 0.58	2.10 ± 0.17	28.67 ± 0.58	4.16 ± 0.12	7.66 ± 0.58	0.36 ± 0.04	1.00 ± 0.10	11.00 ± 1.00	1.70 ± 0.72	2.00 ± 1.00	12.33 ± 0.58	1.43 ± 0.25	4.00 ± 0.50
V	1000	25.00 ± 1.00	0.80 ± 0.10	32.00 ± 1.00	2.75 ± 0.32	34.67 ± 1.52	4.93 ± 0.15	8.67 ± 1.15	0.51 ± 0.08	0.67 ± 0.58	12.33 ± 0.58	1.80 ± 0.10	1.60 ± 0.58	14.00 ± 0.00	3.00 ± 0.10	3.00 ± 0.00
	500	20.00 ± 1.00	0.67 ± 0.05	25.66 ± 1.52	2.16 ± 0.70	29.33 ± 0.58	3.83 ± 0.10	7.33 ± 0.76	0.43 ± 0.02	1.33 ± 0.58	10.67 ± 0.58	1.50 ± 0.10	2.60 ± 0.58	12.33 ± 0.58	2.23 ± 0.30	4.33 ± 0.58
WT-80	4.0%	18.00 ± 1.73	0.43 ± 0.15	23.00 ± 0.00	1.30 ± 0.20	28.33 ± 0.58	2.10 ± 0.26	4.46 ± 0.76	0.33 ± 0.06	4.33 ± 0.58	7.33 ± 0.58	0.88 ± 0.10	8.00 ± 1.00	9.50 ± 0.50	1.07 ± 0.25	14.66 ± 0.58

* DAI= days after inoculation, CO+AHE+AME = Citronella oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), GO+AHE+AME =Geranium oil + *A. squamosa* hexane extract (AHE)+ *A. squamosa* methanol extract (AME), C=Carbofuran, V=Velum Prime, WT-80= Water+Tween-80, SL= shoot length, SW= shoot weight, RL= root length, RW= root weight, NGP= number of galls. The values given are the mean ± standard deviation of three replicates.

Table 6. Effect of (CO+AHE+AME) and (GO+AHE+AME) on Brinjal Seedling Growth and Number of Galls of *Meloidogyne incognita* in the Roots in Root Dip Application in Pot

Sample	Conc. (µg mL^{-1})	Growth parameters (in cm) and number of galls per brinjal seedling									
		30 DAI		60 DAI		30 DAI			60 DAI		
		SL	SW	SL	SW	RL	RW	NGS	RL	RW	NGS
CO+AHE+AME	1000	27.00 ± 1.00	12.33 ± 2.08	38.00 ± 0.00	18.00 ± 1.00	20.83 ± 0.70	2.00 ± 0.00	2.00 ± 0.00	28.33 ± 0.00	8.00 ± 1.00	5.67 ± 0.58
	500	21.00 ± 1.00	8.33 ± 0.58	35.00 ± 1.00	14.67 ± 1.52	14.67 ± 0.70	1.00 ± 0.00	3.67 ± 0.58	20.33 ± 1.41	5.00 ± 1.00	7.33 ± 0.58
GO+AHE+AME	1000	30.67 ± 1.15	9.67 ± 0.58	38.67 ± 0.58	14.67 ± 0.58	13.00 ± 1.06	1.67 ± 0.58	1.67 ± 0.58	27.33 ± 0.70	7.33 ± 1.15	4.67 ± 0.58
	500	20.83 ± 0.28	7.33 ± 0.58	31.67 ± 1.52	11.67 ± 0.58	9.50 ± 1.41	1.00 ± 0.00	2.67 ± 0.58	20.67 ± 3.50	5.00 ± 1.00	8.00 ± 0.00
C	1000	29.33 ± 0.58	9.67 ± 0.58	40.00 ± 1.00	20.00 ± 1.00	25.00 ± 1.40	2.67 ± 0.00	1.33 ± 0.58	36.00 ± 1.41	7.67 ± 0.58	3.67 ± 0.58
	500	26.33 ± 1.00	8.00 ± 1.00	36.00 ± 1.00	18.67 ± 0.58	22.33 ± 0.70	1.67 ± 0.00	2.67 ± 0.58	28.00 ± 1.42	5.67 ± 1.15	5.67 ± 0.58
V	1000	28.16 ± 0.58	9.67 ± 0.58	39.00 ± 1.00	19.33 ± 0.58	24.33 ± 0.70	2.33 ± 0.58	2.00 ± 0.00	35.33 ± 1.41	8.66 ± 0.58	5.33 ± 0.58
	500	24.66 ± 0.58	8.33 ± 0.58	35.16 ± 0.76	17.67 ± 0.58	21.67 ± 0.70	1.67 ± 0.00	2.67 ± 0.58	29.33 ± 0.70	6.66 ± 0.58	6.33 ± 0.58
WT-80	4.0%	18.50 ± 0.58	3.33 ± 0.58	30.00 ± 2.08	10.67 ± 0.58	11.50 ± 1.76	1.00 ± 0.00	11.33 ± 1.52	21.00 ± 1.41	3.33 ± 0.58	27.33 ± 1.52

DAI= days after inoculation, SL=shoot length, SW shoot weight, RL=root length, RW=root weight, NGS=number of galls per seedling, AHE= Annona squamosa hexane extract, V=velum prime, C=carbofuran, WT-80=water with tween-80, The values given are the mean ± standard deviation of three replicates.

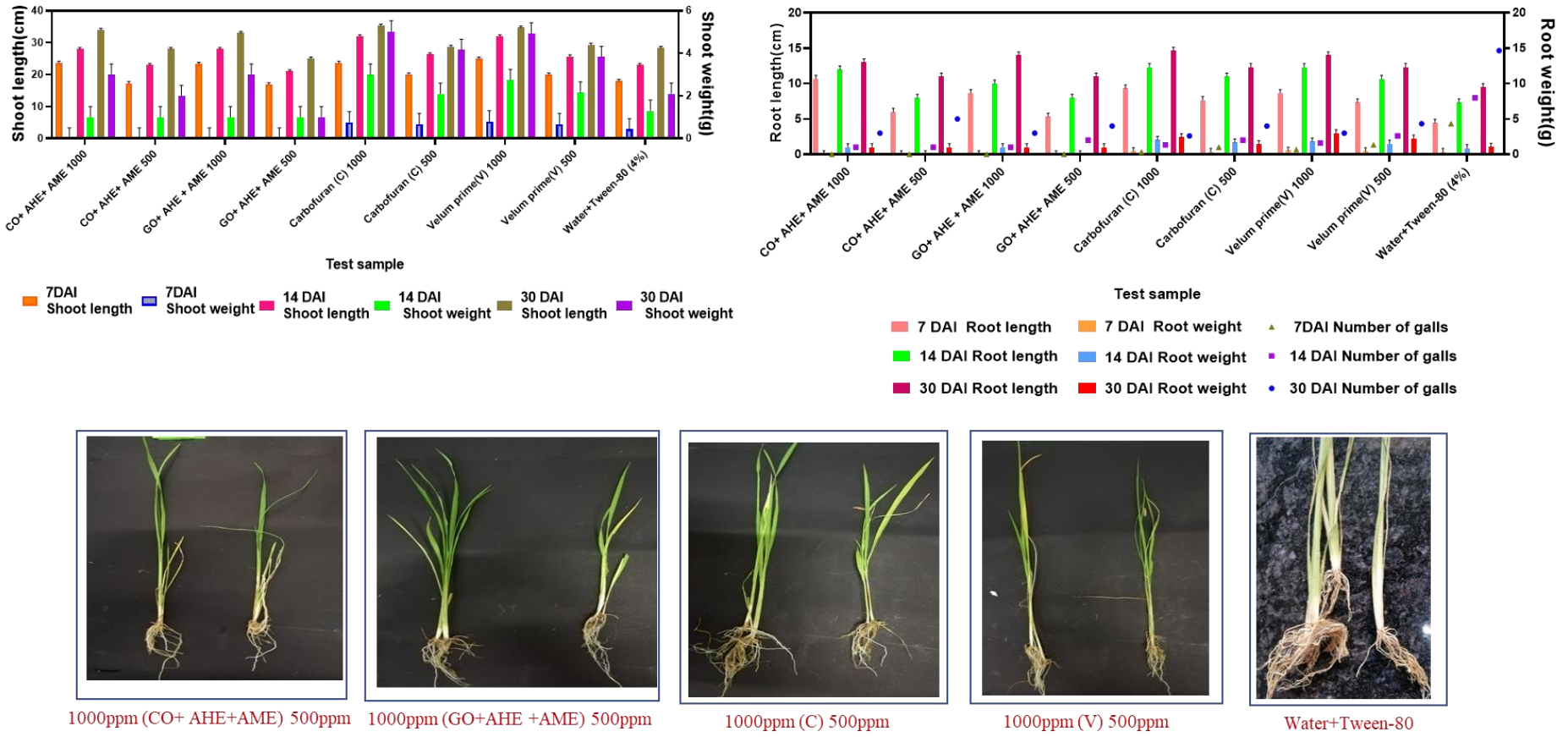

Fig. 3. PB-1121 Soil drenching: Effect of CO+AHE+AME and GO+AHE+AME on growth parameters and number of galls of *Meloidogyne graminicola* in roots of rice seedlings in soil drenching in pot experiment. *CO-Citronella oil, AHE-*A. squamosa* hexane extract, AME-*A. squamosa methanol* extract, GO-Geranium oil

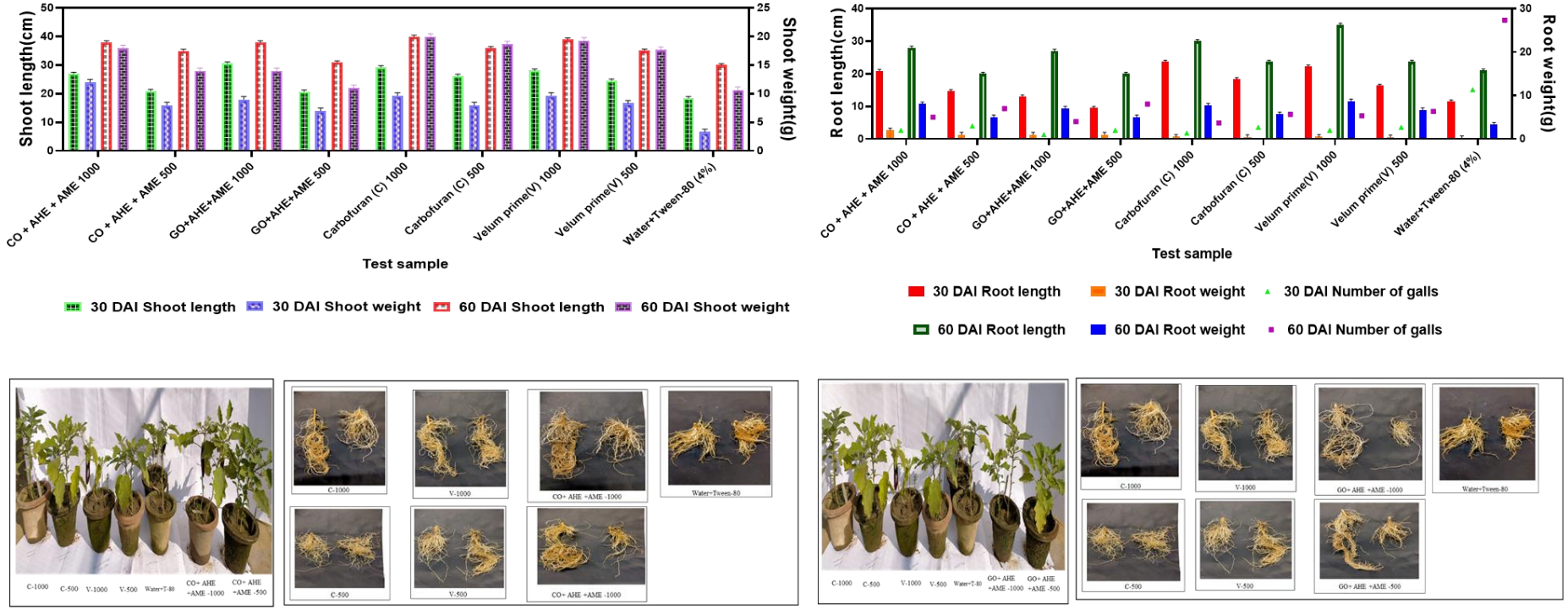

Fig. 4. PPL Root Dipping: Effect of CO+AHE+AME and GO+AHE+AME on brinjal seedling growth and number of galls of Meloidogyne incognita in the roots in root dip application in pot. *CO-Citronella oil, AHE-*A. squamosa* hexane extract, AME-*A. squamosa methanol* extract, GO-Geranium oil

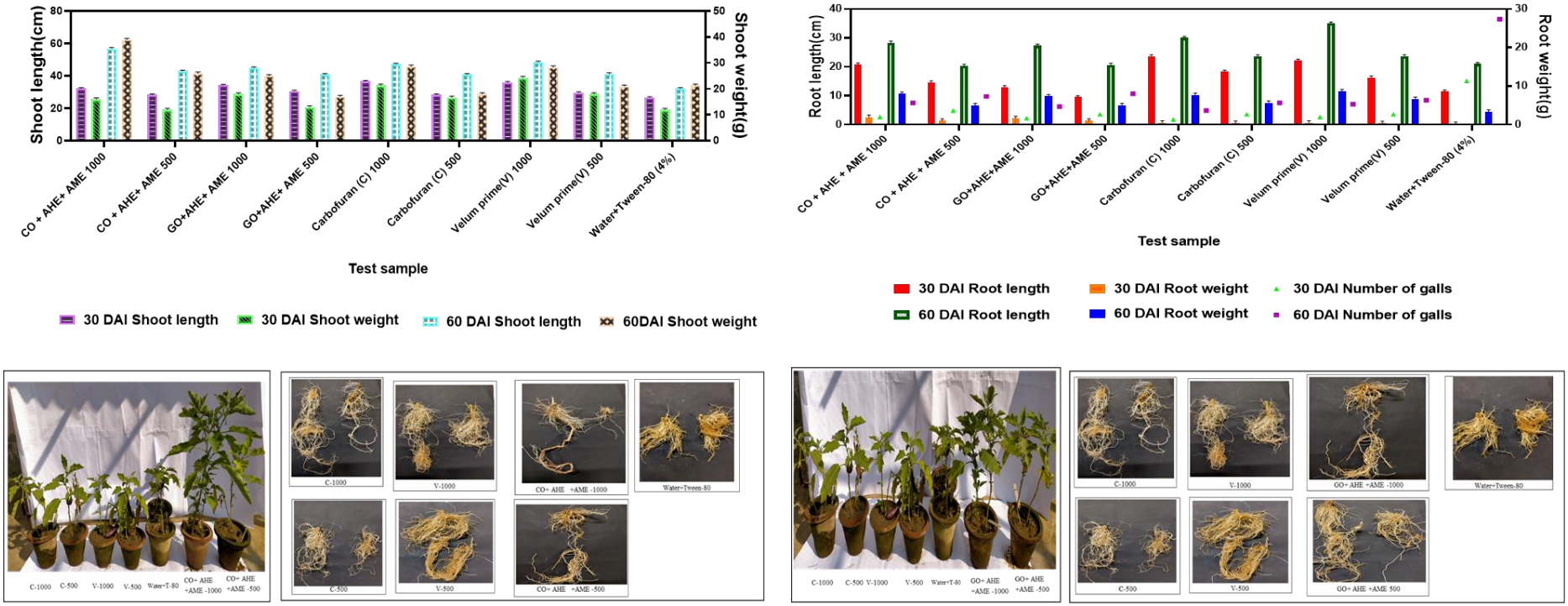

Fig. 5. PPL Soil drenching: Effect of CO+AHE+AME and GO+AHE+AME on brinjal seedling growth and number of galls of *Meloidogyne incognita* in the roots in soil drenching in pot. *CO-Citronella oil, AHE-*A. squamosa* hexane extract, AME-*A. squamosa methanol* extract, GO-Geranium oil

Table 7. Effect of (CO+AHE+AME) and (GO+AHE+AME) on Brinjal Seedling Growth and Number of Galls of *Meloidogyne incognita* in the Roots in Soil Drenching in Pot

Test sample	Conc. (µg mL^{-1})	Growth parameters (in cm) and number of galls per brinjal seedling									
		30 DAI		60 DAI		30 DAI			60 DAI		
		SL	SW	SL	SW	RL	RW	NGS	RL	RW	NGS
CO+AHE+AME	1000	32.33 ± 3.21	16.33 ± 1.15	57.33 ± 2.51	39.00 ± 1.73	24.33 ± 1.15	3.33 ± 0.05	2.00 ± 0.05	41.33 ± 1.52	9.33 ± 0.57	4.67 ± 1.15
	500	28.33 ± 0.58	12.33 ± 1.52	43.33 ± 2.08	26.67 ± 8.96	21.33 ± 0.58	2.00 ± 0.00	3.67 ± 0.05	32.33 ± 2.51	8.00 ± 1.00	7.33 ± 0.58
GO+AHE+AME	1000	34.33 ± 1.52	18.67 ± 0.58	45.33 ± 0.58	25.00 ± 0.00	25.00 ± 1.00	3.33 ± 0.05	2.67 ± 0.05	30.33 ± 1.16	10.67 ± 0.57	6.00 ± 1.00
	500	30.67 ± 1.15	13.00 ± 2.64	41.67 ± 1.52	17.33 ± 2.08	19.00 ± 1.00	2.00 ± 0.00	3.33 ± 0.05	28.00 ± 1.73	7.00 ± 1.00	8.33 ± 0.58
C	1000	36.67 ± 0.58	21.33 ± 0.58	47.33 ± 0.58	28.67 ± 0.58	25.16 ± 1.75	4.33 ± 0.58	0.66 ± 0.05	41.16 ± 1.25	14.33 ± 0.58	2.00 ± 0.05
	500	28.33 ± 2.08	16.67 ± 2.08	41.00 ± 1.00	18.00 ± 1.00	18.00 ± 1.00	3.00 ± 0.00	1.66 ± 0.08	30.66 ± 2.08	12.00 ± 1.00	2.67 ± 0.05
V	1000	36.00 ± 2.08	24.33 ± 2.08	48.66 ± 0.58	28.33 ± 0.58	27.67 ± 0.58	4.33 ± 0.58	1.00 ± 0.08	36.33 ± 0.58	13.67 ± 0.58	2.30 ± 0.05
	500	29.67 ± 1.00	18.00 ± 1.00	41.33 ± 1.52	21.00 ± 1.00	25.00 ± 1.00	3.33 ± 0.58	1.83 ± 0.05	32.33 ± 2.08	11.00 ± 1.00	3.00 ± 0.08
WT-80	4.0%	26.67 ± 2.00	12.00 ± 2.00	32.33 ± 2.08	20.33 ± 1.52	14.33 ± 0.58	1.67 ± 0.58	4.66 ± 1.73	21.00 ± 1.00	7.00 ± 1.00	8.64 ± 1.52

DAI= days after inoculation, SL=shoot length, SW shoot weight, RL=root length, RW=root weight, NGS=number of galls per seedling, AHE= *Annona squamosa* hexane extract, V=velum prime, C=carbofuran, WT-80=water with tween-80, The values given are the mean ± standard deviation of three replicates

Mixtures demonstrated effective nematode control against *M. incognita* in brinjal through root dip and soil drenching. The extracts-controlled gall formation and improved growth parameters in a concentration-dependent manner. Soil drenching was more effective than root application up to 60-day post-inoculation (Knothe *et al.* 2005; Akbar *et al.* 2009).

This study found that herbal mixtures of *A. squamosa* with essential oils effectively controlled *M. graminicola* in rice and *M. incognita* in brinjal for up to 30 and 60 days respectively. A similar result was reported in (Dang *et al.* 2011), where the MeOH extract of *A. squamosa* seeds demonstrated significant nematicidal activity against *M. incognita*, with mortality values of 93%. The EtOAc layer, which was the most active against *M. incognita* among the four layers, was followed by the aqueous, BuOH, and Hex layers (Chowdhury *et al.* 2007). The present findings are also in line with Kamatchi *et al.* (2019), who described that the effect of water extracts of *Chromolaena odorata* and *A. squamosa* on egghatch of *M. incognita* by day four, the percent mortality of the water extracts of *C. odorata* (leaf and root), *A. squamosa* (leaf and bark) at 5,000 mg/kg, 10,000, and 15,000 mg/kg increased from day five today seven; there was 100% mortality at different exposure times and water extract concentrations (Saxena *et al.* 2005). The efficacy was concentration dependent. These non-edible seeds could be used for crop protection against these nematodes, offering a potential alternative to synthetic nematicides. Further field trials are needed to confirm utility in managing root knot nematodes in the field.

CONCLUSIONS

1. This study revealed that a combination of *A. squamosa* herbal mixtures with essential oils, which are rich in polyunsaturated fatty acids, gave promising results in controlling nematodes *in vitro* and in pots.
2. The J_2 stage of both *M. graminicola* in direct-seeded rice at the nursery level and *M. incognita* in brinjal were found to be controlled for up to 30 and 60 days, respectively, with better growth of plants.
3. The efficacy of the extract was concentration dependent. These *A. squamosa seed* is inedible and unutilized. It can be utilised for crop protection products against *M. graminicola* and *M. incognita* and could be an alternative to synthetic nematicides or used in combination with them to slow resistance development.
4. Field trials of its seed oil need to be carried out to confirm larger-scale effectiveness in the management of root-knot nematodes.

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