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Assessment of Neem (*Azadirachta Indica*) and Lemongrass (*Cymbopogon citratus*) Extracts on *Meloidogyne incognita*

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***Abstract*:** Several Phyto-nematicides are available but none of them is capable to control *Meloidogyne* species infestation efficiently. Thus, there is a great need for an eco-friendly, highly effective sustainable control measure for this pest. In this study, to manage the *Meloidogyne* infestation, leaf extract of *Azadirachta indica* and *Cymbopogon citratus* were tested. Organic compounds present in extracts were characterized by GCMS. In vitro bioassays were conducted by applying three concentrations over 100 individuals of the 2nd juvenile stage. The mortality status of the nematode population by counting the dead and live individuals after applying 100 µl of the extract was recorded at different time durations i.e., 24, 48 and 72 hr. Results indicated significant variability toward different concentrations and durations for both extracts. GC-MS profiles of *C. citratus* and *A. indica* revealed the presence of diverse types of compounds in varying quantities. In such a way active compounds present in *A. indica* and *C. citratus* will be screened out. These compound/s-based formulations will be available for Integrated pest management programs, especially in horticultural crops to minimize infestation of *Meloidogyne incognita*.

***Keywords*:** Plant-parasitic nematodes, *Meloidogyne, Azadirachta indica, Cymbopogon citratus*

Nematode pest cause approximately 21.3% downfall in global food production annually (Kumar et al 2020). Approximately 157 billion US dollars in a global loss was reported due to Phyto-parasitic nematodes (Hassan et al 2013). More than 4100 species of plant-parasitic nematodes (PPNs) are reported, out of this *Meloidogyne* species is prominent and worldwide in distribution (Jones et al 2013). *Meloidogyne* species are obligate parasites that parasitize thousands of different plant species including monocotyledons, dicotyledons, herbaceous and woody plants. Various species of genus *Meloidogyne* such as *Meloidogyne incognita* (Kofoid & White), *M. Javanica* (Treub), *M. arenaria* (Neal), *M. acronea* Coetzee are major PPNs species of economically valuable crops that grow in tropical, subtropical, and temperate climates. Several plant products based on nematicides exist in the market but none of them can manage the PPNs population successfully at the commercial level. There is very fewer information available regarding the detailed structure of active chemical compounds, proper stability testing data, and shelf life of these plant products based on nematicides. Thus, this study was conducted to observe the effect of the leaf extract of *A. indica* (A. Juss) and *C. citratus* (DC.) Stapf on the 2nd juveniles' stage of *Meloidogyne* infestation at three-time durations i.e., 24, 48 and 72 hr (hours). Chemical profiling of compounds present in *A. indica* and *C. citratus* extract were also conducted to know the actual active compound responsible for nematicidal activity through GCMS.

MATERIAL AND METHODS

Establishment of *M. Incognita* culture and nematode extraction: Brinjal variety (Hybrid BS6-793) was grown on a nematode-free soil bed specially prepared in an isolated area in a poly house by following standard agronomical practice for pure culture establishment. The study was carried out during the brinjal crop cultivation season in the winter of 2021-22 (September – March at 28° 32' 7.8612" N, 77° 23' 27.7044" E). The nematode-infected plants were collected from the Amity Institute of Organic Agriculture, Amity University Noida Uttar Pradesh India. Females of root-knot nematodes were segregated on the basis of microscopic morphology, from the root-knot of affected plants. Later selected females were transferred to the host brinjal for obtaining pure nematode culture. The purified culture of *M. incognita* was verified, established and mass multiplied. Nematodes were extracted from soil by the modified Baermann funnel method (Cesarz et al 2019). For this, a rubber tube 10 cm in length and 3.3 cm in diameter was connected to the glass funnel stem. The distal end of the rubber tube was tightened with a clamp to regulate the water flow. This assembly was mounted on a funnel stand. Two-third length of the funnel was filled with water. A wire-mesh basket was placed over the funnel in such a manner that the lower end of the mesh was touching to water layer present in the funnel. On the top of the wire-mesh basket double layer of tissue paper was placed. such assembly was used for *M.*

incognita extraction. For *M. incognita* extraction from the brinjal plant, the nematode population containing nodes and associated soil mass of the targeted sample were spread out evenly on the tissue paper layer of the above-mentioned assembly. Adequate quantity of water was added to maintain the water level around 2-3 cm above from the wire mesh. Care was taken for the maintenance of water and soil contact throughout the extraction period. After 24hr clamp was removed and elute was collected from the rubber tube. Elute was filtered from the 400µm sieve for nematode harvesting collected nematode population were diluted in 20 ml amount of water. Nematode density was measured by counting the number of *M. incognita*. The stages of *M. incognita* were identified by microscopic examination and 2nd juvenile stages were utilized for the bioassay process.

Plant extract preparation: From each selected plant, leaves were collected, washed with water, and air dried and 30g leaves were soaked in 300ml HPLC (High-Performance Liquid Chromatography) grade distilled hexane for 24 hr then it was filtered through Whatman no. 1 filter paper. Anhydrous sodium sulfate was added to the filtrate @1/10 gm leaf samples. This was kept for 2 hr for dehydration. After dehydration, it was filtered through Whatman no. 1 filter paper for the removal of sodium sulfate (Singh et al 2019). The filtrate was run through a silica gel column (60-120 mesh size). The extract distillation was conducted at 60-70ºC in a round bottom flask. The residues left over at the bottom of the flask were collected in a glass vial by rinsing with a small quantity of HPLC grade distilled hexane. The solvent was evaporated completely, the residues were collected and diluted with 25ml HPLC grade hexane for preparing different concentrations (100, 50 and 25%) for use in bioassay and Gas Chromatography-Mass Spectrometry (GCMS) studies.

Gas chromatography-mass spectrometry analysis: GCMS analysis for the identification of compounds present in plant extracts was conducted by employing SHIMADZU-GCMSQP2010ULTRA. It was equipped with an Rtx-5 MS column measuring 30 m×0.25 mm I.d. × 0.25 µm film thickness. An electron ionization system was utilized and operated in electron impact form which produced an ionization energy of 70eV. Helium gas (99.9% pure) was adopted as carrier gas. The flow rate of carrier gas was 1 ml/min. The injection volume of the extract was 1 µl (a split ratio of 10:1). The Gas Chromatography column oven's initial temperature was 60°C. The injector temperature was 260°C and the source ion temperature was 230°C. The total run time provided for each sample was 60 min. The mass-to-charge ratio (m/z) taken was 40.00. Illumination on the mass spectrum was carried out using the database of the National Institute of Standards and Technology (NIST). Host plant Brinjal (Hybrid BS6-793) was established for pure culturing *M. incognita*. The purified culture of *M. incognita* was established and mass multiplied.

Impact assessment of plant extract on *M. incognita* population: The tissue culture plate of polystyrene consisting of 24 Wells (each of 2.5 ml capacity) was selected as a platform for in vitro assessment of three concentrations i.e.,100, 50, and 25% of lemongrass and neem on the 2nd juvenile stage of *M. incognita*. For this 500 µl of nematode-rich pure culture and a 100 µl selected concentration of the targeted extract were mixed. For each concentration of targeted extract 3 sets (each containing 10 replicas) were maintained. Along with this for both the extracts control were also maintained (each containing 10 replicas) in a similar kind of Tissue Culture Plate. In one set of control plate 500 µl nematode culture and 100 µl hexane were applied. While in another by using 500 µl nematode culture and 100 µl water was utilised. The mortality status of the nematode population was estimated for each sample by counting the number of live as well as dead individuals in the entire volume of each well of the designated set at different time durations i.e., 24, 48 and 72 hr.

Statistical analysis: All data were analysed by using Windostat software (version 8.5) developed by Indostat Services, Hyderabad, India.

RESULTS AND DISCUSSION

Effect of 100, 50 and 25% dilutions of volatile cues present in lemongrass hexane leaf extract were observed by measuring the mean value of no. of live and dead individuals of root-knot nematodes *M. incognita*. Mortality status was noticed at different time durations (24, 48 and 72 hr). In Lemongrass, mortality status after 72 hr was observed to be 31.40% in 50% concentration and 30.76% in 100 % concentration. Maximum mortality (37.01%) was observed for lemongrass extract after 48 hr at an application of 25% concentration. For Neem extract the highest mortality (29.46%) was recorded after 24 hr on use of 50% concentration followed by 27.80% (24 hr duration and 25% concentration). Least mortality was observed for neem extract.

Chemical profiling of lemongrass shows 66 fractions whereas from neem 36 fractions were obtained. The analysis of various fractions of neem and lemongrass signified the presence of alkane, alkene, alcohol, aldehydes, carboxylic, triene, terpene and ketones. Out of these all compounds having a similarity index of more than or equal to 95% were targeted. Dodecane and Neophytediene exist in both extracts. Pentadecane, heneicosane, pentacosane, tetracontane, hexacosane and hexatriacontane were

prominent alkanes present in neem extract whereas Decane, dodecane, tetradecane, octadecane, dodecylcylohexane, octacosane eicosane tetratetracontane were recorded from lemongrass extract. Izuogu et al (2015) observed that lemongrass at 75% aqueous extract gives the best results due to the presence of tannin, crude alkaloids, saponnins and crude extracts against. *Meloidogyne* species*, Pratylenchus*, *Helicotylenchus*, *Radopholus*, *Rotylenchus* and *Xiphinema*.

Table 1. Effect of lemongrass and neem leaf extracts on mortality of root-knot nematode

Concentration	24hr		48 hr		72 hr	
	Lemongrass	Neem	Lemongrass	Neem	Lemongrass	Neem
100%	18.92	25.59	19.02	26.94	30.76	19.07
50%	31.72	29.46	27.40	18.32	31.40	18.89
25%	22.98	27.80	37.01	18.45	28.20	19.42
Control	7.06	07.06	05.85	05.85	06.60	06.06
Hexane	16.23	16.23	16.88	16.88	25.25	25.25

Table 2. Chemical profile of lemongrass and neem

Name of compound ≥ 95%	Lemon grass		Neem	
	Percent area	S.I.	Percent area	S.I.
Decane	0.23	96	-	-
Dodecane	0.83	97	0.83	97
Cyclohexane, Hexyl-	0.31	97	-	-
1-Tetradecene	1.09	96	-	-
Cyclohexane, 1-Ethenyl-1-Methyl-2,4-Bis(1-M)	0.55	95	-	-
Tetradecane	2.15	97	-	-
Cyclohexane, Octyl-	0.51	96	-	-
1,6-Cyclodecadiene, 1-Methyl-5-Methylene	0.81	95	-	-
E-14-Hexadecenal	2.30	97	-	-
Hexadecane	2.28	97	-	-
Cyclohexane, Decyl-	0.45	96	-	-
Eicosene, (E)	1.86	96	-	-
Octadecane	1.75	97	-	-
Neophytadiene	0.56	95	0.19	96
Dodecylcyclohexane	0.39	97	-	-
1-Nonadecene	1.00	96	-	-
Octacosane	0.46	96	-	-
N-Heptadecylcyclohexane	0.10	95	-	-
Eicosane	0.19	95	-	-
Tetratetracontane	0.51	95	-	-
Pentadecane	-	-	0.22	97
Gamma. -Elemene	-	-	0.17	96
Germacrene B	-	-	0.17	96
Heneicosane	-	-	0.07	97
Pentacosane	-	-	0.74	98
Squalene	-	-	0.24	96
Tetracontane	-	-	15.01	97
Hexacosane	-	-	1.02	97
Hexatriacontane	-	-	1.77	96

Nile et al (2018) studied the nematotoxic potential of neem plants using in vitro and in-planta trials against *M. incognita*. Neem extracts were lethal to second-stage juvenile (J2) and egg hatching. Chavan et al (2021) observed lemongrass, basil and peppermint oil showed the greatest mortality at 96 hr. Shakya and Yadav (2020) observed that neem was the most effective against *M. javanica* in vitro. Divya et al (2021) observed *Purpureocillium lilacinum* in combination with neem cake gave the best results. In accordance with these studies, the elevated mortality rate of *M. incognita* could be ascribed due to the variation of compounds present in lemongrass, especially straight-chain alkanes.

CONCLUSION

Treatment of the host plant with a 25% concentration of lemongrass extract was most effective against *Meloidogyne* species. In further study effective root-knot nematodes repellent compound/s present in the extract may be identified by testing the efficacy of individual compounds to validate their activity. After validation, these compounds may be formulated in an inert base as a commercial eco-friendly nematicide for minimizing infestation of *Meloidogyne* species.

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