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Nematicidal potential of *Azadirachta indica* A. Juss. and *Cymbopogon citratus* DC. to control plant-parasitic nematodes

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ABSTRACT

Plant-parasitic nematodes (PPNs) such as *Meloidogyne incognita* are a serious threat to many crops and causes substantial reduction in productivity. We evaluated the nematotoxic effects of aqueous leachates prepared from *Azadirachta indica* A. Juss. and *Cymbopogon citratus* DC. at 6-concentrations (10,20,40,60,80 and 100 %) against second stage Juveniles (J2) of *M. incognita*. Laboratory bioassays were done to estimate the impact of leachate concentrations against J2 mortality at 24, 48 and 72 h. Results demonstrated concentration and time dependent mortality responses. The 100 % neem leachate exhibited the highest nematicidal activity after 72 h, resulting in complete mortality (100 ± 9.1), similarly, 80 % lemongrass leachate achieved 99 ± 0.9 % mortality at 24 h. In addition to this, GC-MS profiling of both the leachates were performed to identify bioactive compounds potentially responsible for the nematicidal activity. These leachates showed the presence of 2-propyl-1-pentanol, phytol acetate, phytol and squalene at > 90 % similarity index. Overall, neem and lemongrass leachates exhibited 90 % nematicidal activity at higher concentrations and longer exposures, underscoring their potential as biorational alternatives to synthetic nematicides. Given their wide availability, low cost and environmental safety, these botanicals can be effectively integrated into sustainable PPNs management strategies to combat *M. incognita* in agricultural systems.

Keywords: Aqueous leachates, *Azadirachta indica*, *Cymbopogon citratus*, GCMS, Lab. bioassay, *Meloidogyne incognita*, nematicide, *Solanum melongena*

INTRODUCTION

Vegetables, fruits, cereals, grains, flowers, etc. are significant sources of these essential components, but plant-parasitic nematodes (PPNs), greatly reduced their productivity (7). PPNs causes an estimated yield loss of approximately 1.58 billion USD, resulting in 21.3 % reduction in crop production (18). Due to their capacity to parasitize a variety of crops in varied climatic conditions, more than 4100 species of PPNs are widely distributed around the world (8). Among these, *Meloidogyne* species are quite prominent and causes approximately 90 % damage (12). *Meloidogyne incognita* (Kofoid and White) enters the rhizosphere of host plants at the second-juvenile stage (J2) and moves up into the root stele. They establish permanent feeding sites in root stele cells and convert these cells into giant cells, which leads to gall or root-knot formation. This process disrupts normal root physiology and reduces the plant's ability to absorb water and nutrients efficiently from the soil. Because of these effects, nematodes significantly reduces crops productivity. These impacts make *M. incognita* a major concern for sustainable cropping systems (22).

Farmers adapted different tactics for the efficient management of PPNs, use of chemical pesticides are most common (15) due to their ability to quickly reduce PPNs infestation. Growing environmental and health concerns associated with synthetic chemical-based nematicides have prompted agricultural scientists to seek sustainable and

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eco-friendly alternatives to manage *M. incognita*. Among the promising approaches, integrated pest management (IPM) strategies have gained considerable attention due to their eco-friendly and sustainable nature. IPM relies on a combination of tactics, including microbial biopesticides, sustainable agronomic practices and plant-derived formulations (24). However, these methods often face limitations, such as inconsistent efficacy under variable conditions, non-optimized dosages, aliquots and undetermined shelf life. Among the alternatives, botanical leachates from medicinal plants have emerged as particularly promising due to their biodegradability, safety and diverse modes of action (11). Therefore, this study aimed to develop an eco-friendly, economically viable and commercially feasible solution for the efficient management of *M. incognita* by using lemongrass (*Cymbopogon citratus* DC.) and neem (*Azadirachta indica* A. Juss.) leaves.

MATERIALS AND METHODS

The study was conducted from September 2022 to February 2023 to accomplish the proposed research workgoal. The Nematode samples were collected from the Organic Agriculture experimental field of Amity University, Noida, Uttar Pradesh, India (28°29′46″ N latitude and 77°32′09″ E longitude, 212 m above sea level, mean rainfall: 700 mm/year annual, minimum and maximum temp 24 ºC and 28 ºC). Subsequent experiments were done at Biological Control Laboratory, Amity University, Noida, Uttar Pradesh.

Nematode culture maintenance and extraction

Nematode populations were collected by excavating 25 cm pit near the periphery of the severely infested brinjal (*Solanum melongena)* plants. Soil and roots were gently separated and root-knots were rinsed with water to remove adhering soil particles. Adult females were extracted from root-knots and examined under a microscope. After examination, the females were transferred to a new healthy brinjal seedling (Hybrid BS6-793) grown in nematode free soil. These host brinjal plants were maintained in our Lab. for 3-months. Following the incubation period, plants were uprooted and affected brinjal roots were thoroughly washed under running water. Nematode egg masses were then collected from the roots and transferred to glass petri dishes (8 cm dia) containing distilled water (27). The dishes were stored at 4 °C to facilitate the emergence of Second-stage juvenile (J2). Newly emerged J2 larvae were collected on the next day and used in the bioassay studies (1).

Aqueous leachate preparation of neem and lemongrass leaves

A. indica and *C. citratus* leaves (Figures 1-4) were collected from field of our Amity University. The leaves were washed thoroughly and air dried under shade. 100 g leaves were ground using a pestle and mortar. One L distilled water were added to the ground leaves. The resulting neem and lemongrass mixture was separately blended using a magnetic stirrer. The homogenised leachates were centrifuged at 5000 rpm for 15 min at 4 ºC. The supernatant was collected and filtered through double Whatman filter paper (Grade 1). Finally, leachates obtained (100 g leaf sample /1000 ml water) were considered as a 100 %. Six aliquots of each sample were prepared i.e.,10,20,40,60,80 and 100 %. All prepared leachates were used in bioassays within 24 h (19).

Figure 1. Lemon grass Plant

Figure 2. Lemon grass cut leaves

Figure 3. *Azadirachta. indica* plant

Figure 4. *Azadirachta. indica* leaves

GCMS (Gas chromatography -mass spectrometry) Analysis

For GC-MS profiling, neem and lemongrass leaves were washed and air dried at room temperature (25 °C) for 7 days. The dried leaves were powdered using a blender. To prepare the stock solution, 2 g powdered leaves were mixed with 4 ml methanol and incubated with shaking for 24 h. The extract was filtered through Whatman filter paper and stored at -20 °C. Gas chromatography -mass spectrometry GC-MS profiling was performed to identify the various compounds present in the extract using SHIMADZU GCMS QP2010 ULTRA (19).

Laboratory bioassays

A polystyrene culture plate containing 24 wells (2.5 ml capacity, 8.4 cm X 12.5 cm) was used to assess the effects of various aliquots of plant leachates on the mortality of the selected *M. incognita* species. For this, in each well, one ml distilled water (containing around 100 J2) was added. Bioassay were performed having five such replicates in each treatment. For *in-vitro* testing, 100 µl of each aliquot of neem and lemongrass leachates was applied. A control group was also maintained without any treatment. The effect of neem and lemongrass leachates was determined by counting live and dead nematodes under a microscope after 24,48 and 72 h (8).

Counting of nematodes

The number of live and dead nematodes was counted using a Magnus microscope at 10x magnification. A total 1100 µl of nematode rich culture mixed with leachates was placed on a glass slide for microscopic examination.

Stimulation /Inhibition impact of leachates

To evaluate the Stimulation /Inhibition impact of individual aliquots of both plants as compared to the control difference between mean value of treatment and control was calculated by using following formula (25):

$$X = \frac{A - B}{B} \times 100$$

A: Mortality rate after applying targeted aliquots of plant leachate
B : Mortality rate in control
X : Stimulation/Inhibition of the mortality percentage in contrast to negative control

Statistical analysis

Mortality data from all replicates of each treatment were analysed by Two Way Analysis of Variance (ANOVA), using Windostat software (version 8.5) developed by Indostat Services, Hyderabad, India. Least Significance Difference (LSD) at 5 % significant level. Subsequently, GraphPad (www.graphpad.com) was applied to conduct a paired Student's t-test analysis, allowing us to determine the two-tailed p-value of the responses.

RESULTS AND DISCUSSION

Botanicals long have been used as phyto-nematicides to mitigate the effects of PPNs since a long time (14,26). Many studies proved that it also increases the plant growth by controlling nematode infection (3). The efficacy of plant-derived nematicides is well established in literature emphasizing their dual function in suppressing PPNs populations and promoting plant growth by mitigating nematode-induced stress (20,21). Several studies also demonstrated the nematicidal properties of plants leachates against *M. incognita*. Joshi *et al.* (13) evaluated the effects of 8- plants: neem (*A. indica*) leaves, *Aloe barbadense* L. leaves, aak (*Calotropis gigantea* L.) leaves, sadabahar (*Vinca rosea* L.) leaves, jatropha (*Jatropha curcas* L.) leaves and seeds, garlic (*Allium sativum* L.) clove and onion (*Allium cepa* L.) bulb, on the mortality of *M. incognita* and observed significant nematicidal activity across all tested plant leachate.

(I). Neem leachates: In this study, we evaluated the nematicidal property of neem and lemongrass leaves aqueous leachate against *M. incognita* juveniles through *in-vitro* bioassays across varying concentrations (10 to 100 %) and exposure durations (24, 48 and 72 h) to seek an ecofriendly sustainable alternative of chemical nematicides. Our findings demonstrated a strong dose and time dependent mortality response from both the botanicals*A. indica* leachate exhibited significantly higher nematicidal activity as compared to lemongrass leachate across all tested concentrations and time points. 100 % leachates of neem at 72 h showed highest mortality (100±9.1; P value - 0.0001, t value - 47.6731) (Figure 5) followed by 80 % aliquots of lemongrass at 24 h (99±0.9; P value - 0.0001, t value 108.2361) (Table 1) and same percentage of neem at 72 h (99±2.7; P value - 0.0001 and t value - 35.9111). At the lower concentration of 10 % neem leaf leachate showed limited efficacy (4±2.1; P value - 0.0001, t value - 42.1651) at 24 h. (Table 1). These observations align with earlier studies, such as Danahap *et al*. (6) who reported up to 81 % egg hatching inhibition of significantly inhibited egg hatching of *Meloidogyne* spp by neem (*A. indica*) leaf extract at 6000 ppm.

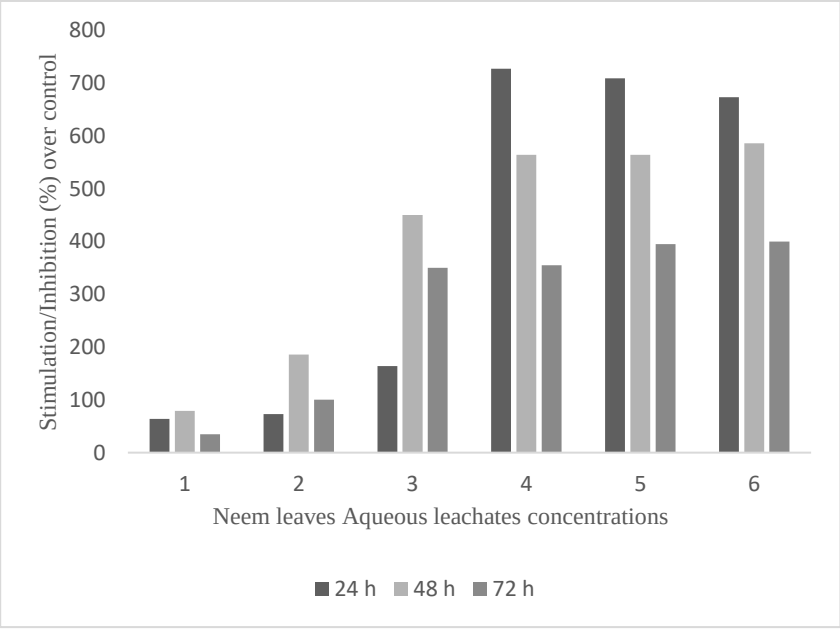

Figure 5. Stimulation/Inhibition (%) of mortality over control in Neem leaves
X-Axis: Stimulation/Inhibition (%) over control
Y-Axis: Six different concentrations of Neem leaves
1,2,3,4,5 and 6: 10,20,40,60,80 and 100 % concentration of aqueous *A. indica* leaves.

Table 1. Effects of aqueous leachates of *Azadirachta indica* on the mortality of *Meloidogyne incognita*

Concentration (%)	**Mortality (%) at Various Durations**		
	24 h (x)	**48 h (x)**	**72 h (x)**
10 (A)	4 (64)	25 (79)	27 (35)
20(A)	19 (73)	40 (186)	40 (100)
40 (A)	29 (164)	77 (450)	90 (350)
60 (A)	91 (727)	93 (564)	91 (355)
80 (A)	89 (709)	93 (564)	99 (395)
100 (A)	85 (673)	96 (586)	100 (400)
Control (B)	11	14	20

X = [(A-B)/B] * 100
A = Mortality rate after applying targeted aliquots of plant leachate
B = Mortality rate in control
X = Stimulation/Inhibition of the mortality percentage in contrast to negative control

(II). Lemongrass leachates: These are though more effective at low concentrations, also showed reduced activity at 10 % (72±1.1; P-0.0001, t value-39.5547), (Figure 6) It showed that neem was not effective at low concentrations, but it is most effective at highest concentrations reaffirming the concentration dependency of both botanicals. Nematotoxic properties of lemongrass has been used in many studies. Chavan *et al*. (5) observed the 100 % mortality of *Meloidogyne graminicola* Golden and Birchfield after 96 h of incubation with three essential oils i.e. basil oil, peppermint oil and lemongrass oil. Chahal *et al*. (4) attributed the nematicidal action of lemongrass to citral epoxide, which effectively inhibited egg hatching and juvenile survival. The nematicidal activity of plant leachates in this investigation was found to be dose-dependent, with mortality rates increasing with greater leachate concentrations and longer exposure times. Notably, the highest mortality rate of PPNs was observed at high doses after 72 h exposure. The nematicidal activity of six plants leachates (*Jatropha pandurifolia* L., *Polyalthia longifolia* Sonn., *Wedelia chinensis* L., *Nerium indicum* L., *Duranta repens* L. and *Cassia fistula* L.) was assessed by Asif *et al.* (2) against *M. incognita* through *in-vitro* analysis with exposure to several concentrations over 12,24 and 48 h durations. Plant leachates from the leaves of these 6-plant species showed highly promising mortality of 99-72 % after 48 h of exposure. Varied concentrations of aqueous leachates of leaves of *J. pandurifolia*, *P. longifolia*, and *W. chinensis* applied for different time durations were found to be highly efficient in inhibiting egg hatching and increasing juvenile mortality of *M. incognita.* Similarly, Khairan *et al.* (16) identified *n*-hexane in *A. sativum* extract as the most potent nematicide. These findings further highlighted the diverse mechanisms through which different plant leachates exert their nematicidal effects. Elevated activity may be due to the presence of various types of compounds in neem and lemongrass.

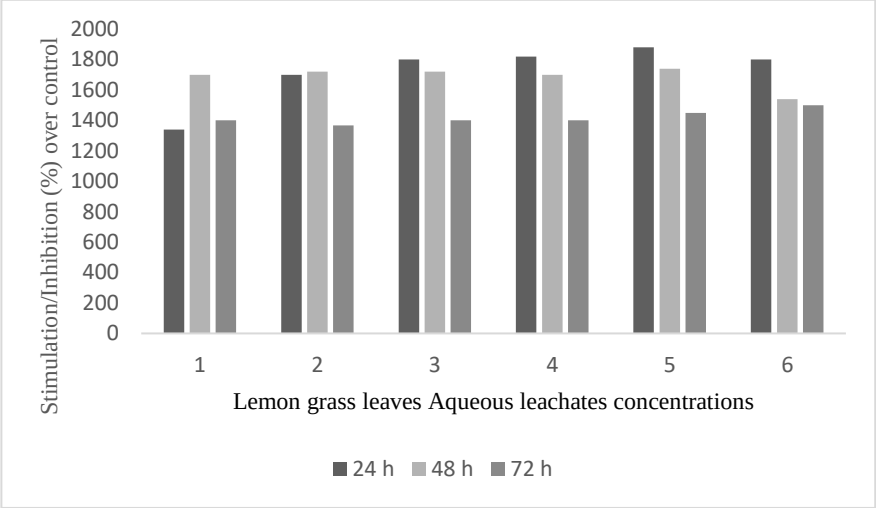

Figure 6. Stimulation/Inhibition (%) of mortality over control in Lemon grass leaves
X-Axis: Stimulation/Inhibition (%) over control
Y-Axis: Six different concentrations of Lemon grass leaves
1,2,3,4,5 and 6: 10,20,40,60,80 and 100 % concentration of aqueous *C. citratrus* leaves

Table 2. Effects of aqueous leachates of *Cymbopogon citratus* on the mortality of *Meloidogyne incognita*

Concentration (%)	**Mortality (%) at Various Durations**		
	24 h (x)	**48 h (x)**	**72 h (x)**
10 (A)	72 (1340)	90 (1700)	90 (1400)
20(A)	90 (1700)	91 (1720)	88 (1367)
40 (A)	95 (1800)	91 (1720)	90 (1400)
60 (A)	96 (1820)	90 (1700)	90 (1400)
80 (A)	99 (1880)	92 (1740)	93 (1450)
100 (A)	95 (1800)	82 (1540)	96 (1500)
Control (B)	5	5	6

X = [(A-B)/B] * 100
A = Mortality rate after applying targeted aliquots of plant leachate
B = Mortality rate in control
X = Stimulation/Inhibition (%) of mortality over control

GCMS: To understand the phytochemical basis of this activity, we performed GC-MS profiling of both botanicals. Chemical profiling of neem and lemongrass leaf extract showed the various types of compounds in these leachates used as nematicide against *M. incognita*. Profiling depicted the presences of alcohols, aldehyde, ketones, alkene, disaccharide, ester, monoterpene, terpenoids, triterpene, sesquiterpene alcohol etc. (Table 3). Chemical profiling of lemongrass showed 87 fractions, whereas, from neem 53 fractions were obtained. Compounds were selected on the basis of similarity index more than or equal to 90 (Table 3). Allelopathic effects of these compounds against PPNs were

Table 3. Chemical profiling of Leachates of *Azadirachta indica* and *Cymbopogon citratus*

#	Name of compound	Neem					Lemongrass				
		Retention Time	Area	Similarity Index	Retention Index	Allelopathic effect on PPNs	Retention Time	Area	Similarity Index	Retention Index	Allelopathic effect On PPNs
1.	2-Propyl-1-pentanol	5.627	622900	96	995	NT	5.613	190557	94	995	NT
2.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.559	396811	93	1269	YES	-	-	-	-	-
3.	Pyrrolidine, N-(3-methyl-3-butenyl)-	8.325	134397	91	1077	NT	-	-	-	-	-
4.	.beta.-D-Glucopyranoside, methyl	13.792	436337	90	1714	NT	-	-	-	-	-
5.	Phytol, acetate	16.227	1796518	90	2168	YES	16.225	2352459	92	2168	YES
6.	Phytol	18.945	7316786	98	2045	NT	18.943	893429	96	2045	YES
7.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	20.472	109504	90	1474	NT	-	-	-	-	-
8.	4,8,12,16-Tetramethylheptadecan-4-olide	21.078	132641	91	2258	NT	-	-	-	-	-
9.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	21.958	245851	90	1474	NT	-	-	-	-	-
10.	Diisooctyl phthalate	22.534	386550	93	2704	YES	-	-	-	-	-
11.	Squalene	24.601	205581	92	2914	YES	24.601	155390	92	2914	YES
12.	D-Limonene	-	-	-	-	-	5.654	621303	95	1018	YES
13.	Linalool	-	-	-	-	-	6.747	560416	97	1082	YES
14.	6-Octenal, 3,7-dimethyl-, (R)-	-	-	-	-	-	7.569	41334705	98	1125	NT
15.	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	-	-	-	-	-	7.749	165092	92	1196	NT
16.	Dodecanal	-	-	-	-	-	8.362	138128	90	1402	YES
17.	6-Octen-1-ol, 3,7-dimethyl-, (R)-	-	-	-	-	-	8.696	17183241	97	1179	NT
18.	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	-	-	-	-	-	9.06	20416790	95	1228	NT
19.	2,6-Octadienal, 3,7-dimethyl-, (E)-	-	-	-	-	-	9.299	162141	92	1174	NT
20.	6-Octen-1-ol, 3,7-dimethyl-, acetate	-	-	-	-	-	10.386	5782068	96	1302	NT
21.	p-Menthane-3,8-diol, cis-1,3,trans-1,4-	-	-	-	-	-	10.435	1542600	97	1320	NT
22.	Phenol, 2-methoxy-3-(2-propenyl)-	-	-	-	-	-	10.547	2750687	97	1392	NT
23.	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	-	-	-	-	-	10.774	3917973	94	1352	NT
24.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	-	-	-	-	-	11.045	3108123	97	1398	NT
25.	.beta.-copaene	-	-	-	-	-	12.295	4830661	94	1216	NT
26.	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	-	-	-	-	-	12.762	472376	95	1522	NT
27.	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	-	-	-	-	-	13.627	918505	95	1581	NT
28.	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	-	-	-	-	-	14.45	204057	92	1593	NT
29.	trans-Farnesol	-	-	-	-	-	14.979	492522	96	1710	NT
30.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-	-	-	-	-	16.675	617520	94	2045	NT
31.	Neryl (S)-2-methylbutanoate	-	-	-	-	-	17.334	263375	90	1586	YES
32.	trans-Geranylgeraniol	-	-	-	-	-	19.633	191080	92	2192	YES

PPN= plant-parasitic nematode
NT=Not determined

determined on the basis of literature (Table 3). Feng *et al*. (10) discussed the role of main secondary metabolites such as alkaloids, terpenoids, coumarins, glycosides and flavonoids, in which benzopyrone exhibits prominent nematicidal properties. Neem (*A. indica*) possesses various medicinal properties. Khan *et al*. (17) observed that GC-MS analysis of the chloroform fraction of neem leaf extract identified 7-compounds, including nonacosane (44.27 %) and tetratriacontane (13.43 %). Based on Literature review, they revealed that these compounds have antibacterial, antifungal, anticancer, antioxidant and anti-inflammatory activities, highlighting neem's potential as a source of bioactive agents (23). Faria *et al*. (9) observed essential oils from *C. citratus* (lemongrass) showed strong nematicidal activity against *Bursaphelenchus xylophilus*, with citral and geraniol outperforming emamectin benzoate. Hence, this study confirmed that the bioactive compounds of neem and lemongrass are responsible for nematotoxic properties. Thus, from all of these studies lemongrass and neem were used as a nematicide and in the present study, neem and lemongrass are showing the mortality > 90 %.

The use of neem and lemongrass as botanical nematicides offers a cost effective and ecofriendly sustainable approach for managing nematode infestation. Given that these plants are readily available and widely cultivated, their application in IPM programs can reduce reliance on synthetic nematicides, thereby, mitigating environmental risk and ensuring sustainability.

CONCLUSIONS

We found that the tested plant aqueous leachates, i.e., of neem and lemongrass, increased the mortality of PPNs significantly. This may be due to presence of 2-propyl-1-pentanol, phytol acetate, phytol and squalene in both leachates. These leachates may be applied as a soil amendment to reduce nematode infestations effectively. Proper use of these leachates aligns with the principles of sustainable agriculture efficient in minimizing chemical inputs. The farmers can achieve considerable nematode control, by integrating neem and lemongrass leachates into IPM programs.

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We did not exclude any author who substantially contributed to this manuscript. We have followed the ethical norms established by our respective institutions.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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