# Composition and nematicidal activity of the essential oil from *Piper longum* against root knot nematode

VIJAY KUMAR<sup>1</sup>, PARTHA CHANDRA MONDAL<sup>1</sup>, RAKESH KUMAR<sup>1</sup>, PANKAJ<sup>1</sup>, PARSHANT KAUSHIK<sup>1</sup>, NAJAM AKHTAR SHAKIL<sup>1</sup>, A P ABHISHEK GOWDA<sup>1</sup> and VIRENDRA SINGH RANA<sup>1</sup>\*

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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#### ABSTRACT

Piper longum L. (Piperaceae family), commonly known as long pepper, is used in traditional medicine and in recognized system of medicine in India for the treatment of inflammation, pain, epilepsy, arthritis, cancer, ulcer, and asthma in addition to pest control activity. Its fruits are rich in essential oil and possess different biological activities, but oil is not much explored against the root-knot nematodes. Thus, the essential oil from the fruits of P. longum was isolated by hydrodistillation method and analyzed by Gas chromatography-Flame ionisation detector and Mass spectrometry. Analysis showed that the oil is a complex mixture of volatile compounds. (Z)-β-Farnesene (25.08%), β-Caryophyllene (13.57%), α-Humulene (13.37%), 8-Heptadecene (9.28%), Heptadecane (7.07%), α-Patchoulene (5.44%), 3-Heptadecene (5.09%), γ-Elemene (4.37%), β-Humulene (3.69%) and Terpinen-4-ol (1.74%) were identified as main compounds in its oil. The essential oil was evaluated for its nematicidal activity against root-knot nematode at 62.5-1000 ppm concentrations in vitro conditions during 2020-21 at ICAR-Indian Agricultural Research Institute, New Delhi. Results showed promising nematicidal activity of the oil and have caused mortality of juvenile (J2), ranging from 2.33-88.0% after 24-96 hours of treatment compared to untreated control. The oil was further evaluated under the pot conditions in tomato (Solanum lycopercicum L.) (var. Pusa Ruby) at 3000 and 2000 ppm. Results showed a significant reduction in the number of galls/root of tomato with better plant growth in the essential oil based treatment as compared to the untreated control. These results suggest that the oil can be used for the development of nematicidal product for the protection of crops from the infestation of *M. incognita*.

**Keywords**: Essential oil, Gas chromatography, Mass spectrometry, Solanum lycopercicum

Plant parasitic nematodes are a major economically important biotic stress in agriculture crops which cause about 12.3% losses annually in 40 crops worldwide (Andrés et al. 2012, Singh et al. 2015, Kumar et al. 2020) and 20.4% in India (Kumar et al. 2020). Meloidogyne incognita, a root knot nematode also affects number of agricultural crops and causes huge economic losses (Kumar et al. 2020). Currently, synthetic nematicides are used for the management of the nematodes in field crops, but due to their persistence and toxicity to the non-targeted organisms, environment and human beings, the use of some of them has been banned or restricted and may be banned in the future (Desaeger et al. 2020, Rana 2022). The recent focus on nematicides is due to a response to the increasing regulatory pressure on hazardous products worldwide, and precisely the fact that some of the most effective nematicides, including methyl bromide, fenamiphos and aldicarb have become severely restricted (Desaeger et al. 2020). These issues are encouraging researchers to find out the effective, safe and

<sup>1</sup>ICAR-Indian Agricultural Research Institute, New Delhi. \*Corresponding author email: ranavs2000@yahoo.com

economical alternative methods of nematode management specially from the plant species possessing pest control property (Andrés et al. 2012). Different essential oils, extracts and compounds are reported to possess promising nematicidal activity against the nematodes under in vitro conditions (Ibrahim et al. 2006, Zasada et al. 2006, Andrés et al. 2012, Ntalli and Caboni 2012, El-Nagdi et al. 2014, Varughese et al. 2016, Avato et al. 2017, Ozdemir et al. 2018). Recent study showed that the essential oils and their major compounds showed promising nematicidal activity against Meloidogyne graminicola and M. incognita in both in vitro and field conditions (Ajith et al. 2020, 2021 and 2022, Gowda et al. 2022a,b). Keeping above in view, the present study was aimed to determine the chemical composition of the essential oil from the fruits of Piper longum L. and its nematicidal activity against Meloidogyne incognita under in vitro and pot conditions.

#### MATERIALS AND METHODS

The present study was carried out at Division of Nematology and Division of Agricultural Chemicals, ICAR-Indian Agricultural Research Institute (IARI), New Delhi during 2020–21. The dried fruits of *Piper longum* were purchased from the local markets of New Delhi, India. The dried fruits (500 g) were powdered using a mixer grinder. The powdered dried fruits were hydrodistilled using Clevenger type apparatus for 4 hours and the distillate containing essential oil was obtained. The essential oil from the distillate was extracted in diethyl ether and the ethereal layer was dried over anhydrous sodium sulphate. The diethyl ether was removed and the pure oil was stored at 4–8°C until further analysis.

Analysis of essential oil: The essential oil was analyzed by Gas chromatography-Flame ionisation detector (GC-FID, Shimadzu GC 2010 Plus) equipped with auto sampler and a capillary column, SH Rtx-5 (30 m  $\times$  0.25 mm  $\times$  0.25 µm film thickness). Nitrogen was used as carrier gas (1.0 mL/min) while injector temperature and Flame ionization detection (FID) temperature were 250°C and 260°C. The column temperature was held at 60°C, then increased up to 250°C at 3°C/min. The sample was injected (0.2 µl, 1000 ppm) in split mode (split ratio 1:20). The relative percentages of the individual components were calculated based on GC peak area (FID response) of the mean value of two injections without using correction factors.

The essential oil was also analysed by Gas chromatography-Mass spectrometry (GC-MS, Thermo DSQ) equipped with a capillary column, DB-5 (30 m  $\times$  0.25 mm, film thickness 0.25 µm) for the identification of the compounds. Helium was used as carrier gas (1.0 ml/min). The injector temperature was kept at 260°C. The column temperature was held at 60°C, and then increased up to 230°C at 3°C/min. The sample was injected (0.2 µl, 1000 ppm) in split mode (split ratio 1:20). The column was coupled directly to the quadrupole mass spectrometer at EI mode at 70 eV with the mass range from 28–400 a.m.u at 1 scan/s. The identification of individual compounds was carried out based on retention time and Kovat's Index (KI)

using a homologous series of n-alkanes ( $C_8$ - $C_{20}$ , Sigma-Aldrich) by comparing their mass spectra with NIST Mass Spectral Library (Ver. 2, 2005), co-injection with authentic samples and literature (Adams 2007).

#### Nematicidal activity

Preparation of test solution: The stock solution (1.0%) of the essential oil (*piper* oil) was prepared by mixing with Tween-80 (3.0 g) and distilled water using a lab stirrer at 1000 rpm at room temperature for 30 minutes. Different concentrations (62.5, 125, 250, 500 and 1000 ppm) of essential oil were prepared by serial dilutions of the stock solution. Tween-80 (3.0%) was used as a negative control and velum prime (1 ml/litre, Fluopyrum 34.48% sc) as a positive control.

Isolation of nematodes (J2): The roots of tomato (S. lycopersicum var. Pusa Ruby) infected with M. incognita were collected from the sick plot maintained at the Division of Nematology, ICAR-IARI, New-Delhi, India. The roots were washed with tap water and egg masses were separated and incubated at  $25 \pm 2^{\circ}$ C for hatching up to 120 hours. Juveniles were collected every 24 hours up to 120 hours, concentrated and used for bioassay.

In vitro bioassay: In vitro bioassay, nematode suspension (100 J2/1.0 ml) was taken in a culture plate (24 well) and 1 ml of different concentrations (62.5, 125, 250, 500 and 1000 ppm) of test solution of the oil were added and mixed. Tween-80 in distilled water (3.0%) was used as a control. The experiments were carried out in triplicates. Observations for mortality of J2 were recorded at 24, 48, 72 and 96 h at laboratory conditions.

Pot experiment: The seeds of *S. lycopersicum* (var. Pusa Ruby), obtained from Division of Vegetable Science, IARI, New Delhi, India were sterilized with sodium hypochlorite (1.5%) and sown in a tray filled with peat. Approximately, 25 days old seedlings were transplanted

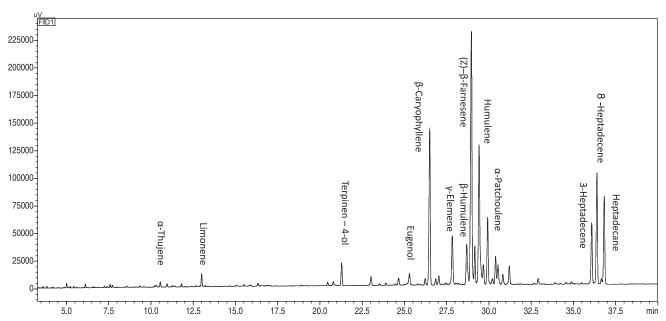


Fig 1 Total ion chromatogram of Piper longum oil with identified main compounds.

Table 1 Chemical constituents of the essential oil derived from *Piper longum* 

Compound	KI*	KI	Amount			
Manatamana hudua aguhang			(%)			
Monoterpene hydrocarbons	930	020	0.27			
α-Thujene		929	0.27			
Camphene	954	953	0.18			
Limonene	1029	1028	0.78			
Oxygenated monoterpenes	1006	1007	0.22			
Linalool	1096	1097	0.23			
Camphor	1146	1145	0.24			
Borneol	1169	1167	0.22			
Terpinen-4-ol	1177	1176	1.74			
Eugenol	1359	1359	0.93			
Sesquiterpene hydrocarbons						
δ-Elemene	1338	1337	0.10			
α-Cubebene	1351	1350	0.52			
α-Copaene	1376	1375	0.09			
β-Elemene	1390	1390	0.49			
β-Caryophyllene	1417	1415	13.57			
Bergamotene	1434	1433	0.71			
γ-Elemene	1436	1436	4.37			
β-Humulene	1438	1439	3.69			
(Z)-β-Farnesene	1442	1440	25.08			
αHumulene	1454	1452	13.37			
α- Patchoulene	1456	1457	5.44			
Pentadecane	1500	1502	1.77			
α- Bisabolene	1505	1506	1.15			
γ-Cadinene	1513	1511	0.91			
γ-Bisabolene	1515	1514	1.35			
Oxygenated sesquiterpenes						
Caryophyllene oxide	1583	1580	0.41			
Humulene epoxide-II	1608	1606	0.15			
Aliphatic hydrocarbons						
1-Tridecene	1291	1290	0.10			
3-Heptadecene	-	1679	5.09			
8-Heptadecene	-	1687	9.28			
Heptadecane	1700	1698	7.07			
Oxygenated aromatic compounds						
Acetophenone	1065	1063	0.11			
Methyl 3-phenylpropanoate	_	1258	0.64			
Oxygenated aromatic compounds			0.75			
Monoterpene hydrocarbons			1.23			
Oxygenated monoterpenes			3.36			
Sesquiterpene hydrocarbons			70.81			
Oxygenated sesquiterpenes			0.56			
Aliphatic hydrocarbons	21.54					
Total percentage of identified con	98.25					
Total percentage of identified compounds						

<sup>\*</sup>KI, Kovat's index calculated using homologous series on n-alkanes ( $C_8$ - $C_{20}$  Sigma). (*Source*: Adams 2007).

to six-inch pots containing sterilized soil (1.25 kg/pot). Two higher concentrations (2000 and 3000 ppm) of the essential oil were chosen and applied using soil drenching and root dip methods in a single application. In soil drench method, 120.0 ml of each of 2000 and 3000 ppm of test solution, Tween-80 (3.0%) and velum prime (1 ml/litre) were mixed with soil in a pot, separately. Similarly, in the root dipping method, individual seedlings of tomato were dipped in 10 ml of test solution, separately for 20 minutes before transplanting in plastic pots. The Tween-80 (3%) and velum prime were used as negative and positive controls. The seedlings (one seedling/pot) were transplanted in the plastic pots. The root zone of seedlings in each pot was then inoculated with nematodes (250 J2/1 ml), 5 days after transplantation and the experiments were carried out in triplicates. Observations for plant growth parameters (shoot and root length, shoot and root weight) as well as nematode infestation parameters (number of galls in roots, number of egg masses in roots, eggs/egg mass and reproduction factor) were recorded after 60 days of the experiment.

Statistical analysis: The numerical data of the experiments was square-root transformed before analysis. Analysis of variance (ANOVA) and probit analysis was performed using (SPSS 16.0) on the transformed data. Comparisons of relevant means were made using Tukey's significance test values at the 5% significance level.

### RESULTS AND DISCUSSION

Analysis of essential oil: In the analysis, dried fruits of P. longum were found to contain about 0.49% (dry weight basis) essential oil. The essential oil obtained was analyzed by GC-FID and GC-MS, which showed the presence of 31 compounds, representing 98.25% of the oil (Table 1 and Fig 1). The oil was found to be rich in sesquiterpene hydrocarbons (70.81%), followed by aliphatic hydrocarbons (21.54%), oxygenated monoterpenes (3.36%), monoterpene hydrocarbons (1.23%), oxygenated aromatic compounds (0.75%) and oxygenated sesquiterpenes (0.56%). (Z)-β-Farnesene (25.08%) was identified as a major compound in the oil followed by  $\beta$ -Caryophyllene (13.57%),  $\alpha$ -Humulene (13.37%), 8-Heptadecene (9.28%), Heptadecane (7.07%), α-Patchoulene (5.44%), 3-Heptadecene (5.09%), γ-Elemene (4.37%), β-Humulene (3.69%) and Terpinen-4-ol (1.74%). In a previous study by Varughese et al. (2016), the essential oil yield from the dried fruits of Piper longum was found to be 0.1% and the oil was found to be rich in monoterpene compounds.

In vitro *bioassay*: The essential oil from the fruits of *P. longum* evaluated at 62.5–1000 ppm concentrations, showed promising concentration dependent nematicidal activity against J2 of *M. incognita* under *in vitro* conditions. The essential oil showed highest  $(46.0\pm1.15-88.0\pm1.15\%)$  mortality of J2 at 1000 ppm from 24–96 hours of exposure (Table 2) followed by  $28.0\pm1.15-71.33\pm1.76\%$  mortality at 500 ppm with same duration of exposure. The result of probit analysis also revealed that LC<sub>50</sub> of the oil ranged from 994.79–363.13 ppm after 24–96 hours of exposure

Table 2 Effect of the essential oil from Piper longum on the mortality of J2 of M. incognita under in vitro conditions

Treatment	Conc.	J2 mortality (%)					
	(ppm)	24 h	48 h	72 h	96 h		
Piper oil	1000	$46.00 \pm 1.15^{b}$	$52.00 \pm 1.15^{b}$	$72.67 \pm 0.67^{b}$	$88.00 \pm 1.15^{b}$		
	500	$28.00 \pm 1.15^{c}$	$37.00 \pm 1.15^{c}$	$50.00 \pm 1.15^{c}$	$71.33 \pm 1.76^{\circ}$		
	250	$15.33 \pm 0.88^{d}$	$24.00 \pm 1.15^{d}$	$26.33 \pm 1.20^d$	$44.00 \pm 1.15^{d}$		
	125	$5.67 \pm 0.88^{e}$	$10.67 \pm 0.88^{e}$	$12.00 \pm 1.15^{e}$	$34.00 \pm 1.15^{e}$		
	62.5	$2.33\pm0.33^{\rm f}$	$4.00\pm0.58^f$	$8.33\pm0.88^f$	$13.33 \pm 0.67^{\rm f}$		
Tween-80 (3%)		$0.00\pm0.00^g$	$0.00 \pm 0.00^{g}$	$0.00\pm0.00^g$	$0.00\pm0.00^g$		
SDW		$0.00\pm0.00^g$	$0.00 \pm 0.00^{g}$	$0.00\pm0.00^g$	$0.00\pm0.00^g$		
VP		$66.00 \pm 0.00^{a}$	$80.00 \pm 0.00^a$	$90.00 \pm 0.00^{a}$	$99.00 \pm 0.00^a$		
LSD (P≤0.05)		2.21	2.39	2.44	2.91		
LC <sub>50</sub> (PO) (ppm)		994.794	875.647	625.554	363.131		
LC <sub>50</sub> (VP) (ppm)		-	-	-	2.76		

Values given above in the table are the mean of three replicates  $\pm$  SEm. Same alphabet letters in a column are not significantly (P<0.05) different according to Tukey's HSD. SDW, Sterile distilled water, VP, Velum prime® (500 g a.i./ha).

(Table 2). The mortality of the J2 were found highest (88.0±1.15%) at 1000 ppm concentration and 96 hours of exposure, compared to Tween-80, in which no mortality was recorded (Table 2). The nematicidal activity of the essential oil was found to be encouraging at 1000 ppm but was less than velum prime. However, higher concentration can give better nematicidal activity as shown by a commercial nematicide, velum prime. Earlier studies also showed that essential oils are effective against root knot nematodes. The essential oils from *Cymbopogon flexuosus*, *Syzygium aromaticum* and *Cymbopogon martini*, and their major compound (eugenol, citral and geraniol) showed promising mortality of J2 of *Meloidogyne graminicola* infecting the rice in field (Ajith *et al.* 2020). *Mentha longifolia* oil was also found lethal to J2 of *M. graminicola* with high mortality

(99.25%) at 1000 ppm after 96 hours of exposure (Gowda *et al.* 2022a,b). These results confirmed the efficacy the essential oil against nematodes and encouraged to evaluate the oil further in pot experiment.

Pot experiment: Based on the results from in vitro experiments, two higher concentrations (2000 and 3000 ppm) of P. longum oil were further evaluated in the pot experiments to establish the use of oil in management of M. incognita under open pot conditions using tomato, S. lycopersicum (var. Pusa Ruby) with its single application in soil drenching and root dip methods for 60 days. Results (Table 3) showed that the plant growth parameters such as root weight, shoot weight, root length and shoot length were significantly improved in essential oil treatments as compared to the control (Fig 2, 3). Plant growth parameters

Table 3 Effect of the essential oil on *S. lycopersicum* (tomato) growth parameters inoculated with J2 of *M. incognita* in pot experiment with soil drench and root dip applications

Treatment	Plant growth parameters						Number of galls/root			
	Soil drenching			Root dipping			Soil	Root		
	Shoot length (cm)	Fresh shoot weight (g)	Root length (cm)	Fresh root weight (g)	Shoot length (cm)	Fresh shoot weight (g)	Root length (cm)	Fresh root weight (g)	drenching	dipping
PO-3000	102.33 ± 1.45 <sup>a</sup>	122.33 ± 1.45 <sup>a</sup>	26.33 ± 0.88 <sup>b</sup>	15.00 ± 0.58 <sup>b</sup>	91.33 ± 0.67 <sup>b</sup>	107.33 ± 1.45 <sup>a</sup>	26.00 ± 0.58 <sup>b</sup>	14.33 ± 0.33 <sup>b</sup>	8.00 ± 0.58°	11.00 ± 0.58°
PO-2000	$94.00 \pm 1.15^{b}$	113.00 ± 1.53 <sup>b</sup>	$24.33 \pm 0.33^{b}$	$11.33 \pm 0.67^{\circ}$	86.00 ± 1.15°	$98.00 \pm 1.15^{b}$	$25.00 \pm 0.58^{b}$	$9.67 \pm 0.33^{\circ}$	$12.67 \pm 0.67^{b}$	$16.00 \pm 1.15^{b}$
VP	97.67 ± 1.45 <sup>b</sup>	$107.33 \pm 1.76^{\circ}$	$29.00 \pm 0.58^{a}$	15.33 ± 0.33 <sup>b</sup>	96.00 ± 1.15 <sup>a</sup>	$105.33 \pm 1.76^{a}$	$30.00 \pm 0.58^{a}$	$15.00 \pm 0.58^{b}$	$5.33 \pm 0.67^{d}$	$7.67 \pm 0.88^{d}$
UTIC	60.00 ± 1.15°	$67.67 \pm 1.45^{d}$	$21.67 \pm 0.88^{c}$	$18.00 \pm 0.58^{a}$	$60.00 \pm 1.15^{d}$	67.67 ± 1.45°	$21.67 \pm 0.88^{c}$	$18.00 \pm 0.58^{a}$	$32.00 \pm 1.15^{a}$	$32.00 \pm 1.15^{a}$
LSD (P≤0.05)	4.28	5.07	2.31	1.80	3.44	4.80	2.17	1.54	2.6	3.17

Value given above in the table is the mean of three replicates  $\pm$  Sem.. Same alphabet letters in a column is not significantly (P<0.05) different according to Tukey's HSD. PO, *Piper* oil; UTIC, Untreated nematode inoculated control; VP, Velum prime® (500 g a.i./ha).



Fig 2 Photographs showing the gall formation in tomato roots infected with *M. incognita* (Mi) after 60 days. (A), Untreated control (only Mi); (B), Velum prime + Mi; (C), *Piper* oil + Mi.



Fig 3 Effect of the essential oil on morphological parameters of tomato plant (A), Velum prime; (B), *Piper* oil at 3000 ppm and; C, Untreated control.

were found better in soil drenching compared to root dip application at 3000 ppm of oil than velum prime treatment. The positive effect on the plant growth parameters can be attributed to the reduction in nematode infestation parameters in the roots of tomato treated with essential oil and velum prime than the negative control treatment. There was significant reduction (75.0%) in number of galls in roots/plant at 3000 ppm concentration in soil drenching application and 65.62% reduction in root dip application (Table 3). Ozdemir *et al.* (2018) has also shown the positive

impact of essential oils from Artemisia absinthium, Lavandula officinalis, Mentha arvensis, Thymus serpyllum, Piper nigrum, Citrus bergamia and Eucalyptus citriodora on the plant growth parameters as well as on the reduction of galls in the roots. Another study reported that root gall formation was significantly reduced by both drenching and fumigation treatments with essential oils of Artemisia herba-alba, Rosmarinus officinalis and Thymus satureioides, whereas the essential oil of Citrus sinensis resulted in a significant suppression of galling indices at 200 µg/kg soil (Avato et al. 2017).

This study suggests that the essential oil of *P. longum* oil has the nematicidal potential and can be used for the management of *M. incognita* in tomato using soil drenching application at 3000 ppm. These results are based on single application of essential oil in soil drenching and root dip methods. The multiple applications of the essential oil either by drenching method or drip irrigation can give better protection of the tomato crops from nematodes with reduced dose due to continuous soil fumigation by essential oil.

Meloidogyne incognita is one of the key plant-parasitic nematode especially in horticultural crops including tomatoes. Medicinal and aromatic plants are the main sources of natural and eco-friendly alternatives to the synthetic nematicides. This study suggests that the essential oil from Piper longum has the potential to be bionematicidal against M. incognita, especially when applied in the soil drenching method. However, field trials of its essential oil need to be carried out to establish its efficacy at the field level against M. incognita.

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