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Nematicidal activity of bay leaf (*Laurus nobilis* L.) essential oil and its components against *Meloidogyne incognita*

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Abstract
In vitro studies were conducted to evaluate the nematicidal activity of bay leaf essential oil, its fractions, isolated and derivatized compounds for egg hatching and juvenile mortality of second stage juveniles of *M. incognita* at different concentrations (0.20-1.50 mg mL^{-1}) and durations (24-96 hrs) in Department of Plant Pathology, Punjab Agricultural University, during the year 2016-17. Bay leaf essential oil was partitioned into non-polar and polar fractions. Eugenol, 1, 8-Cineole and α-Pinene were isolated using column chromatography. α-Pinene and eugenol were chemically derivatized into campholenic aldehyde and eugenol epoxide respectively using metachloroperbenzoic acid. Bay leaf essential oil, its fractions, α-Pinene, 1,8-Cineole, eugenol, eugenol epoxide and campholenic aldehyde showed effective egg hatch inhibition and increased juvenile mortality at all concentrations and durations. Among the various components tested, bay leaf essential oil showed highest egg hatch inhibition and mortality. It showed complete egg hatch inhibition at 1.00 mg mL^{-1} after 72 hrs and complete mortality was observed at 0.80 mg mL^{-1} after 96 hrs. Both the activities were concentration and time dependent. The present studies revealed that bay leaf essential oil is effective against *M. incognita* and can be explored further for its nematicidal properties.

Keywords: egg hatching, hydrodistillation, juvenile, *Laurus nobilis*, root knot nematode

Introduction
Laurus nobilis L. is evergreen shrub, belonging to family Lauraceae comprises 32 genera and about 2,000-2,500 species. It is native to southern Mediterranean regions. It is also known as sweet bay, bay laurel, Grecian laurel and bay tree [1]. Bay is a small tree, having alternate, narrowly oblong-lanceolate leaves with small flowers and the black ovoid fruit [2]. Dried or fresh leaves are commonly used as household culinary herb while the essential oil of leaf is mostly used for flavors and fragrances [3-4]. The main components in essential oil are 1, 8-Cineole (33.4 %), linalool (16.0 %), *α*-Terpinyl acetate (13.8%), sabinene (6.91 %), methyl eugenol (5.32 %), *α*-Pinene (4.39 %) and β-Pinene (3.52 %) [5]. The bay essential oil serves as antioxidant [2], antifungal [6], antibacterial [7] and insecticidal agent [8].
Meloidogyne species are polyphagous plant parasites causing serious problems both to the quality and quantity of crops [9]. Root-knot nematodes spend part of their life in soil either as eggs or as second-stage larvae, then enter the roots and establish feeding sites in susceptible hosts, inducing root swelling with a characteristic knotty appearance. Root galling can significantly limit water, nutrient uptake leading to malnutrition, chlorosis, stunting, causing considerable qualitative and quantitative losses in several crop plants. At present, the major control method for nematodes is based on the use of chemical nematicides, but alternative management strategies like natural plant nematicides must be adopted due to the ban on soil fumigants, environmental and human health concerns and development of resistance to chemicals [10].
Essential oils are aromatic oily extracts obtained from plant parts such as buds, flowers, seeds, leaves, barks, roots and fruits [11]. They are also known as fragrant, volatile and aromatic oil with pharmaceuticals and flavor enhancing properties [12-14]. Essential oils, various extracts, compounds isolated and derivatized are known to be good nematicides.
In continuation of all earlier work [15-19] on nematicidal activity of essential oils, the present study was conducted to evaluate the nematicidal potential of bay leaf essential oil, its fractions, isolated compounds-α-Pinene (1), 1,8-Cineole (2), eugenol (3) and derivatives (4-5) against *Meloidogyne incognita*. The work comprises egg hatch inhibition and juvenile mortality studies.

2. Materials and Methods

The experiment was conducted in Nematology laboratory of Department of Plant Pathology at Punjab Agricultural University, Ludhiana, Punjab during early summer (Feb-March) of 2016-17.

2.1 Procurement of raw material

The bay leaves were purchased from local market. 300 g of bay leaves were grounded to powder and dipped in water (5 L) overnight in 10 L round flat-bottomed flask. The essential oil was extracted by hydrodistillation using Clevenger's apparatus. Bay leaf essential oil was partitioned thrice using diethyl ether (3 × 50 mL) and the diethyl ether layer was distilled and material was dried over anhydrous sodium sulfate to remove traces of water present if any. The process was repeated several times to get sufficient quantity of essential oil.

2.2 GC-MS analysis

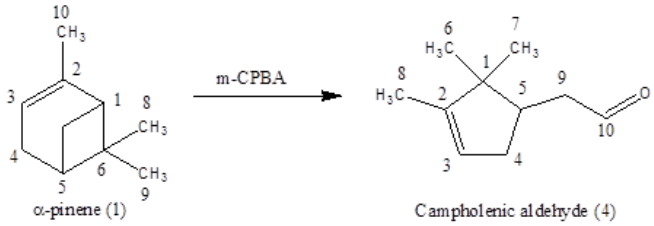
Bay leaf essential oil was analyzed using GC-MS (QP2010 Plus, Shimadzu, Japan), equipped with an Rtx-5 MS capillary column (30.0 m × 0.25 mm i.d., 0.25 µm film thickness). Peak identification was carried out by comparison of the mass spectra with mass spectra data available on database of NIST08, WILEY8 and Flavor and Fragrance libraries [20].

2.3 Column chromatography of bay leaf essential oil

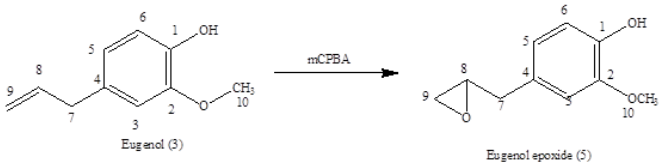
Bay leaf oil was subjected to column chromatography to have non-polar and polar fractions. The column was packed with silica gel (60-120 mesh size) activated at 110ºC for 1 hr. Bay leaf oil (8 g) was adsorbed on a small amount of silica gel turning into a dry powder. Column was eluted with petroleum ether and dichloromethane separately to obtain its non-polar and polar fractions respectively. Extensive column chromatography was carried out to isolate pure compounds. Column was eluted with solvents of increasing polarity. α-Pinene (1, 1.20 g) was obtained in hexane fraction, 1,8-Cineole (2, 0.90 g) in hexane: dichloromethane (5 %) and eugenol (3, 2.00 g) in hexane: dichloromethane (10 %) fraction. The purity of compounds was monitored by thin layer chromatography. The structural elucidation of the isolated and transformed compounds was carried out using spectroscopic techniques (Table 2).

2.4 Chemical transformations

2.4.1 Reaction of α-Pinene with m-CPBA: To the solution of α-Pinene (1, 2 g) in dichloromethane (40 mL), meta-chloroperbenzoicacid (4.5 g) was added to it in round bottom flask. The mixture was stirred for 5 hrs at room temperature. After the completion of reaction, product was extracted with sodium thiosulfate followed by sodium bicarbonate solution. Evaporation of the organic layer afforded a mixture of compounds (1.9 g). The major pure compound was isolated by column chromatography and identified as campholenic aldehyde (4, 0.8 g) on the basis of spectral data.



2.4.2 Reaction of eugenol with m-CPBA: Eugenol (3, 1 g) dissolved in dichloromethane was taken in round bottomed flask. To this, m-CPBA (2 g) was added. The reaction mixture was stirred for 3hrs at room temperature. It was washed with saturated solution of sodium thiosulfate and then with saturated sodium bicarbonate solution. Evaporation of organic layer under reduced pressure afforded a mixture of products which were separated by column chromatography. The product was identified as eugenol epoxide (5, 0.4 g) on the basis of spectral data.



2.5 Techniques for characterization of compounds

The purity of the isolated and derivatized compounds was ascertained by thin layer chromatoplates coated with silica gel G. The chromatoplates were developed in benzene: ethyl acetate (19:1) and iodine was used as the visualizing reagent. IR spectra were recorded on Perkin Elmer, Model RX-1 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AC (400 MHz) or mentioned otherwise as solutions (in $CDCl_3$) using TMS as an internal reference.

2.6 Preparation of test concentrations

Concentrations to be used were standardized by preliminary trials and concentrations ranging from 0.20 to 1.50mg mL^{-1} were found to be effective for carrying out nematicidal activity. The stock solution of concentration 1.5mg mL^{-1} was prepared by dissolving 0.15 g of each component (essential oil, non- polar fraction, polar fraction, α-Pinene, campholenic aldehyde, 1,8-Cineole, eugenol and eugenol epoxide) separately in 100 mL of water along with Tween 80 as emulsifier. The serial dilutions were made using distilled water as required. Distilled water was used as control and each treatment was replicated thrice.

2.7 Preparation of culture

Pure culture of *M. incognita* was raised by single egg mass technique and multiplied on brinjal, a susceptible host for root knot nematode. For mass multiplication of *M. incognita* culture, the soil was autoclaved at 15 psi pressure and 121ºC for at least 30 min and filled in earthen pots. Three week old seedlings were planted in the pots and inoculated with freshly hatched 2nd stage juveniles (J_2) collected from pure culture of egg masses. After sixty days of inoculation the egg masses were collected and used for bioassay studies on egg hatching and juvenile mortality of *M. incognita.*

2.8 Hatching test

For egg hatch inhibition studies, the infected plants of brinjal were uprooted and carefully washed. The egg masses were isolated from roots by forceps and collected in Petri dishes containing water. Five egg masses, with an average of 200-250 eggs per egg mass, were placed in 5 mL of each concentration (0.2-1.50mg mL^{-1}) and control (water only). The plates were covered with solid lid and kept in an incubator at 27^0C temperature. Hatched juveniles were counted on 24, 48, 72 and 96 hrs after incubation under light microscope. The percentage inhibition was calculated by the formula [21].

$$\text{Percent egg hatch inhibition} = \frac{\text{No. of nematodes in control - No. of nematodes in treatment}}{\text{No. of nematodes in control}} \times 100$$

2.9 Mortality test

For mortality test, egg masses were picked using sterilized forceps from heavily infected roots. These egg masses were washed in distilled water using 15 mesh sieve containing crossed layers of tissue paper to obtain freshly hatched juveniles. After 24 hrs, nematodes were collected in a beaker and allowed to settle down. Excess water was decanted off. The number of nematode juveniles was adjusted to 50 J_2/mL using light microscope. Counted numbers of juveniles were transferred to Petri dishes containing each concentration of test solution as well as control. The plates were covered with solid lid and kept in an incubator. After 24, 48, 72 and 96 hrs of exposure, number of dead/alive nematodes were counted. The nematodes were considered dead if found motionless when probed with fine needle. The motionless juveniles were placed in distilled water for 24 hrs to observe their survival. The percent mortality was calculated using the formula [22]

$$\text{Percent Mortality} = \frac{\text{Total no. of dead nematode juveniles}}{\text{Total no. of nematode juveniles (alive+dead)}} \times 100$$

2.10 Statistical analysis

Percent egg hatch inhibition and mortality data were subjected to statistical analysis using the sine arc transformations. The interactions of compounds, concentrations and days were tested at P = 0.05 %.

3. Results and Discussion

Bay leaf essential oil was reddish brown in colour having refractive index, specific gravity, pH and optical rotation of 0.971, 1.52, 6.9 and -2^0 respectively. The essential oil was insoluble in water, sparingly soluble in hexane and completely soluble in acetone and ethanol. GC-MS analysis of bay leaf essential oil showed the presence of 21 compounds. The major components present in essential oil were eugenol (63.57 %), α-Pinene (7.68 %) and 1,8-Cineole (3.37 %) whereas isoeugenol (10.59 %), spathulenol (3.74 %), isospathulenol (1.29 %) and caryophyllene (1.29 %) were the minor components (Table 1). Campholenic aldehyde and eugenol epoxide were prepared in good yield from α-Pinene and eugenol respectively using m-CPBA reagent.

3.1 Characterization of compounds

IR spectrum of compound (1) showed band (cm^{-1}) at 3024, 2984, 2920 and 2837 due to C-H stretching of methylene group along with stretching of C=C at 1657. ^{1}H NMR spectrum showed two singlets at δ 0.86 and δ 1.29 due to 3H at C-8 and C-9, respectively. A multiplet was present at δ 5.20-5.22 confirmed the vinyl group at C-3. Ten signals at δ 47.03 (C_1), 144.50 (C_2), 116.03 (C_3), 31.46 (C_4), 37.97 (C_5), 40.70 (C_6), 31.26 (C_7), 26.35 (C_8), 22.98 (C_9), 20.80 (C_{10}) in ^{13}C NMR indicated the compound to be a monoterpene. The spectral data on compared with literature [23] confirmed the compound to be α-Pinene (1). The compound (2) showed IR bands at 2947 and 2925 cm^{-1} due to C-H stretching of methylene, along with band at 1169 and 1150 cm^{-1} due to ether linkage. In ^{1}H NMR spectrum two doublets at δ 1.26 (6H, *J*= 6.96 Hz) corresponds to methyl groups at C_9 and C_{10}. The ^{13}C NMR showed ten signals at δ 72.70 (C_1), 37.40 (C_2), 24.20 (C_3), 39.70 (C_4), 24.20 (C_5), 37.40 (C_6), 25.50 (C_7), 76.80 (C_8), 25.50 (C_9) and 25.50 (C_{10}). The analysis of data confirmed the compound (2) to be 1,8-Cineole. IR spectrum of the compound (3) showed broad band at 3514 cm^{-1} due to hydroxyl group and bands at 1149, 1122 and 1034 cm^{-1} due to C-O stretching. The data was supported by ^{1}H NMR spectrum that showed a singlet at δ 5.44 due to hydroxyl group (exchangeable with D_2O) and singlet at δ 3.69 (3H, C_{10}) indicating the presence of methoxy group. A. In addition to this, two multiplets typical of a vinyl group was observed at δ 5.75- 5.85 and δ 4.88-4.95. The presence of ten signals in ^{13}C NMR signals at δ143.94 (C_1), 146.51 (C_2), 111.19 (C_3), 131.96 (C_4), 121.11 (C_5), 114.35 (C_6), 39.93 (C_7), 137.88 (C_8), 115.45 (C_9) and 55.88 (C_{10}) confirmed the compound be a eugenol (3) [24].

The reaction of α-Pinene with m-CPBA resulted in the formation of compound (4). The analysis of spectral data showed the IR band (cm^{-1}) at 1726 indicating the presence of aldehyde group. The ^{1}H NMR spectrum showed a triplet at δ 9.80 (*J*= 2.3 Hz) and a doublet at δ 5.25 (*J* =1.1 Hz) due to -CHO group and vinyl group, respectively. The appearance of ten signals in the ^{13}C NMR spectra at δ 46.92(C_1), 147.98 (C_2), 121.57 (C_3), 35.53 (C_4), 44.20 (C_5), 29.70 (C_6), 25.62 (C_7), 20.03 (C_8), 45.11 (C_9) and 203.06 (C_{10}) confirmed the compound (4) to be campholenic aldehyde. The formation of eugenol epoxide was confirmed by the analysis of spectral data which showed the IR bands at 1255, 1268 and 1149 cm^{-1} corresponding to C-O stretching. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) was similar in all respect to that of eugenol except for the presence of two multiplets at δ 3.11-3.15 (1H, C_8) and δ 2.54-2.56 (2H, C_8) and absence of multiplet signals at δ 5.75-5.85 and δ 4.88-4.95 pertaining to protons of C-8 and C-9, indicating the presence of ether linkage between C-8 and C-9. In ^{13}C NMR the absence of signals due to double bond at C-8 and C-9 and appearance of two new signals at 52.79 (C_8) and 46.86 (C_9) confirmed the formation of epoxide between these two carbons [24]. The spectral details of all isolated and derivatized compounds are given in Table 2.

3.2 Egg hatch inhibition studies

Bay leaf essential oil, its fractions, compounds isolated (α-Pinene, 1, 8-Cineole and eugenol) and transformed (campholenic aldehyde and eugenol epoxide) were found to inhibit egg hatching of *M. incognita*. All concentrations of tested components showed maximum reduction in egg hatch count after 96 hrs (Table 3). In case of bay leaf essential oil and polar fraction, complete egg hatch inhibition was observed at concentration of 1.00 mg mL^{-1}whereas non-polar fraction showed 79 percent egg hatch inhibition at 1.50 mg mL^{-1}.Amongst the isolated compounds, eugenol was most active as complete egg hatch inhibition was found at 1.25mg mL^{-1}whereas α-Pinene was least effective as it showed 79 % egg hatch inhibition at same concentration. Campholenic aldehyde, oxidation product of α-Pinene was more effective as compared to parent compound as 82 percent egg hatch inhibition was observed at higher concentration (1.50 mg mL^{-1}). Eugenol was more effective as compared to eugenol epoxide as it showed complete egg hatch inhibition at 1.25 mg mL^{-1}concentration whereas eugenol epoxide showed 94 percent egg hatch inhibition at maximum concentration (Fig. 1). Thus decreasing order of egg hatch inhibition was as follows

Bay leaf essential oil > Polar fraction > Eugenol > Eugenol epoxide > 1, 8-Cineole > Campholenic aldehyde > α-Pinene > Non-polar fraction

3.3 Mortality studies

The effect of bay leaf essential oil, its fractions, isolated and transformed compounds on percent mortality of J_2 of *M.*

incognita at different concentrations and durations was studied (Table 4). More than 50 percent mortality was observed even after 24 hrs at 0.20 mg $mL^{-1}$for all components except non-polar fraction. The data revealed that there was significant increase in percent juvenile mortality with increase in concentration at highest time duration of 96 hrs. Bay leaf essential oil showed complete mortality at 0.80mg mL^{-1} whereas polar fraction exhibited complete mortality at 1.00 mg mL^{-1} concentration. Non-polar fraction was found to be least effective as less than 50 percent mortality was observed at 0.20 mg mL^{-1} at lowest time exposure. Eugenol was most effective among isolated compounds as complete mortality was recorded at 1.25 mg mL^{-1}. Eugenol was more effective as compared to its epoxide whereas in case of α-Pinene and campholenic aldehyde, campholenic aldehyde showed 92 percent juvenile mortality which was more than parent compound as it showed 92 percent juvenile mortality at 1.50 mg mL^{-1}concentration. The statistical analysis showed a significant increase in nematicidal activity with increase in concentration and duration in all the components (Fig. 2). The decreasing order of activity against *M. incognita* followed the order

Bay leaf essential oil > Polar fraction > Eugenol > Eugenol epoxide > 1, 8-Cineole > Campholenic aldehyde > α-Pinene > Non-polar fraction

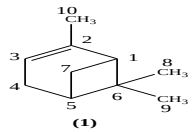
The results of present study were in consonance with previous studies which revealed that essential oil extracted from lemon [15] and vetiver grass [16] were more effective as nematicides as compared to its non-polar and polar fractions against *M. incognita*. It has been reported earlier that the essential oils of *Pectis oligocephala* and *P. apodocephala* exhibited significant nematicidal and larvicidal activity against *Aedes aegypti* [25]. The essential oils of *Chamaespartium tridentatum, Origanum vulgare, Satureja montana, Thymbra capitata* and *Thymus caespititius* also showed significant percent egg hatch inhibition and juvenile mortality against *M. incognita* [26].

Monoterpenes isolated from the essential oils also showed the significant nematicidal activity. Nerol and menthol isolated from essential oil proved to be more toxic to male, female and juvenile nematodes than β-citronellol [27]. Citral, a major component of lemongrass essential oil, proved most toxic to nematodes as compared to its derivatives prepared [15]. In the present study, isolated compounds (α-Pinene, 1, 8-Cineole and eugenol) and derivatives (Campholenic aldehyde and eugenol epoxide) were also found to be effective against root knot nematodes. It has been reported that eugenol, geraniol and menthol possessed 91, 90, 84 percent nematicidal activity respectively at 0.25mg mL^{-1} [28]. The survey of literature revealed that the oxygenated and phenolic monoterpenes proved to be stronger nematicides than other monoterpenoids [29]. The higher nematicidal activity of eugenol as compared to other isolated monoterpenes might be due to the presence of phenolic group in eugenol. Nematicidal activities of compounds with hydroxyl group or ether group are stronger than those with acetyl or carbonyl group [30]. This might be the reason for the lower activity of campholenic aldehyde.

Table 1: GC-MS data of bay leaf essential oil

S. No	Name	Retention Time (min)	Area (%)
1	α-Pinene	5.006	7.68
2	1,8-Cineole	11.850	3.37
3	Sabinol	12.092	0.22
4	Eugenol	16.605	63.57
5	Isoeugenol	16.747	10.59
6	α-Copaene	16.989	0.38
7	Methyleugenol	17.594	1.00
8	Caryophyllene	18.161	1.29
9	Aromedendrin	18.651	1.10
10	Leden	20.014	1.10
11	Germacrene B	20.131	0.48
12	Cadinene	20.679	0.45
13	Ledol	22.029	0.23
14	Spathulenol	22.264	3.74
15	Caryophyllene oxide	22.351	0.62
16	Globulol	22.451	1.15
17	Epiglobulol	22.657	0.69
18	Isospathulenol	23.572	1.03
19	α-Cadinol	24.091	0.35
20	Anethole	41.861	0.57
21	Acetyleugenol	45.656	0.37

Table 2: Spectroscopic data of isolated and derivatized compounds (1-5)

Compounds	IR (cm^{-1})	^{1}H NMR (δ)	^{13}C NMR (δ)
 (1)	3024, 2984, 2920, 2837, 1657	0.86 (3H, s, C_8), 1.29 (3H, s, C_9), 1.68-1.69 (3H, s, C_{10}) and 5.20-5.22 (1H, m, C_3)	47.03 (C_1), 144.50 (C_2), 116.03(C_3), 31.46(C_4), 37.97(C_5), 40.70 (C_6), 31.26 (C_7), 26.35 (C_8), 22.98 (C_9) and 20.80 (C_{10})

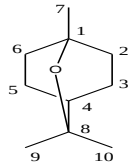
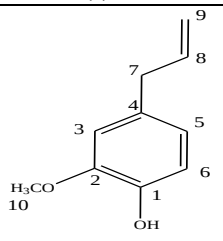
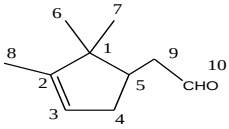
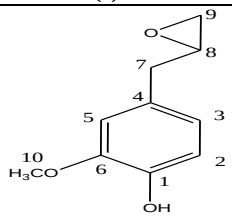
 (2)	2947, 2925, 2885, 2854, 1169, 1150, 844	1.26 (6H,d, *J*= 6.9 Hz, C_9 and C_{10}), 1.31(3H, s, C_7), 1.52-1.53(2H each, m, C_3 and C_5), 1.65-1.67 (2H each, m, C_2 and C_6) and 1.74-1.75 (m, 1H, C_4)	72.7(C_1), 37.40(C_2), 24.2(C_3), 39.7(C_4), 24.2(C_5), 37.4(C_6), 25.5(C_7), 76.8(C_8), 25.5 (C_9 and C_{10})
 (3)	3514, 3003, 2938, 2842, 1637, 1612, 1514, 1432, 1366, 1234, 1149, 1122	3.16 (2H, d, C_7), 3.69 (3H, s, C_{10}), 4.88-4.95 (2H, m, C_9), 5.44 (1H, s, C_1), 5.75-5.85 (1H, m, C_8), 6.51-6.54 (2H each, m, C_3 and C_5) and 6.68-6.71 (1H, m, C_6)	143.94(C_1), 146.51 (C_2), 111.19 (C_3), 131.96 (C_4), 121.11(C_5), 114.35 (C_6), 39.93 (C_7), 137.88(C_8), 115.45 (C_9) and 55.88(C_{10})
 (4)	2956, 2927, 2867, 1726, 1680	9.80 (1H, t, *J*=2.3 Hz, C_{10}), 5.25(1H, d, *J*=1.1Hz, C_3), 1.61-1.66(3H, m, C_8), 1.00 (3H, s, C_6) and 0.79 (3H, s, C_7)	46.92(C_1), 147.98(C_2), 121.57 (C_3), 35.53(C_4), 44.20(C_5), 29.70(C_6), 25.62(C_7), 20.03(C_8), 45.11(C_9) and 203.06(C_{10})
 (5)	3405, 2957, 2926, 2849, 1515, 1271, 1237	2.54-2.56 (2H, m, C_9), 3.11-3.15 (1H, m, C_8), 2.79 (2H, t, *J*=8.5 Hz, C_7), 3.86 (3H, s, C_{10}), 5.66 (1H, s, C_1), 6.66 (1H, s, C_3), 6.71-6.76 (1H, m, C_5) and 6.85 (1H, d, *J*=7.9 Hz, C_6)	146.53 (C_1), 144.40 (C_2), 111.62 (C_3), 129.02 (C_4), 121.64 (C_5), 114.40 (C_6), 38.36 (C_7), 52.79 (C_8), 46.86 (C_9), 55.91 (C_{10})

Table 3: Effect of bay leaf essential oil, its fractions and compounds isolated and transformed on percent egg hatch inhibition of *M. incognita* at different concentrations and durations.

Compounds	Duration (Hrs)	Average percent hatch inhibition at different concentration (mg mL^{-1})						
		0.20	0.40	0.60	0.80	1.00	1.25	1.50
Bay leaf essential oil	24	71.00±(0.56)$^{a-c}$	73.00(±0.32)$^{hj-n}$	78.00(±0.11)$^{sx-y}$	82.00(±0.09)$^{h-n}$	84.00(±0.26)$^{cd-h}$	90.00(±0.05)$^{e-l}$	96.00(±0.31)^{ad}
	48	74.00(±0.18)^{de}	80.00(±0.04)$^{l-p}$	84.00(±0.01)$^{p-r}$	90.00(±0.03)$^{c-j}$	94.00(±0.13)$^{t-w}$	98.00(±0.11)$^{uv-x}$	100.00(±0.00)^{a}
	72	84.00(±0.31)$^{d-i}$	87.00(±0.06)$^{p-st}$	92.00(±0.02)$^{b-ij}$	97.00(±0.01)$^{fg-k}$	100.00(±0.00)^{a}	100.00(±0.00)^{a}	100.00(±0.00)^{a}
	96	87.00(±0.25)^{opq}	90.00(±0.13)$^{d-h}$	96.00(±0.04)$^{c-i}$	99.00(±0.01)$^{f-h}$	100.00(±0.00)^{a}	100.00(±0.00)^{a}	100.00(±0.00)^{a}
Non-polar fraction	24	42.00(±0.24)$^{l-o}$	48.00(±0.36)^{lmn}	53.00(±0.14)$^{k-o}$	57.00(±0.54)$^{h-m}$	61.00(±0.10)$^{n-v}$	66.00(±0.01)$^{ij-o}$	69.00(±0.09)$^{uv-z}$
	48	46.00(±0.05)$^{ij-n}$	53.00(±0.14)$^{ef-l}$	57.00(±0.32)$^{u-w}$	61.00(±0.07)$^{j-l}$	64.00(±0.08)$^{bc-g}$	68.00(±0.01)^{abc}	71.00(±0.03)$^{l-pq}$
	72	51.00(±0.31)^{nr}	56.00(±0.21)$^{g-l}$	60.00(±0.25)$^{m-r}$	63.00(±0.06)$^{rt-z}$	67.00(±0.03)$^{as-t}$	72.00(±0.07)$^{n-v}$	76.00(±0.06)$^{j-no}$
	96	54.00(±0.01)$^{i-m}$	59.00(±0.25)$^{b-e}$	62.00(±0.05)$^{e-i}$	68.00(±0.04)^{ijk}	73.00(±0.07)$^{bc-g}$	75.00(±0.03)$^{ef-h}$	79.00(±0.10)$^{k-op}$
Polar fraction	24	66.00(±0.21)$^{u-y}$	71.00(±0.01)$^{ef-i}$	76.00(±0.05)$^{j-l}$	79.00(±0.07)^{nop}	82.00(±0.40)$^{f-k}$	87.00(±0.03)^{uvw}	98.00(±0.01)$^{j-l}$
	48	72.00(±0.01)$^{w-y}$	79.00(±0.05)$^{e-g}$	84.00(±0.09)$^{c-f}$	89.00(±0.39)$^{v-z}$	92.00(±0.28)$^{b-hi}$	96.00(±0.07)$^{c-h}$	100.00(±0.00)^{a}
	72	81.00(±0.05)$^{i-lm}$	85.00(±0.24)^{def}	91.00(±0.06)$^{p-v}$	94.00(±0.40)$^{e-g}$	99.00(±0.04)$^{g-l}$	100.00(±0.00)^{a}	100.00(±0.00)^{a}
	96	84.00(±0.35)^{ghi}	89.00(±0.12)$^{c-j}$	93.00(±0.18)$^{b-g}$	96.00(±0.17)$^{p-r}$	100.00(±0.00)^{a}	100.00(±0.00)^{a}	100.00(±0.00)^{a}
α-Pinene	24	51.00(±0.02)$^{b-ef}$	56.00(±0.25)$^{d-ij}$	56.00(±0.01)$^{i-o}$	60.00(±0.08)$^{p-r}$	64.00(±0.07)$^{t-y}$	68.00(±0.14)$^{v-yz}$	72.00(±0.06)$^{fg-l}$
	48	54.00(±0.36)$^{e-j}$	57.00(±0.05)$^{a-d}$	62.00(±0.07)$^{g-o}$	66.00(±0.03)$^{i-l}$	69.00(±0.12)$^{j-o}$	71.00(±0.09)$^{a-d}$	75.00(±0.01)^{yz}
	72	55.00(±0.10)$^{n-p}$	59.00(±0.19)$^{a-e}$	67.00(±0.14)^{tuv}	70.00(±0.05)$^{ej-r}$	73.00(±0.16)$^{u-w}$	76.00(±0.17)$^{qr-v}$	81.00(±0.68)$^{c-i}$
	96	58.00(±0.18)$^{i-l}$	62.00(±0.09)$^{n-r}$	70.00(±0.20)$^{j-p}$	73.00(±0.12)$^{h-m}$	75.00(±0.10)^{wxy}	79.00(±0.05)$^{a-c}$	82.00(±0.07)$^{f-l}$
Campholenic aldehyde	24	55.00(±0.16)$^{u-x}$	58.00(±0.01)$^{i-m}$	61.00(±0.25)$^{n-q}$	65.00(±0.10)$^{a-d}$	67.00(±0.14)$^{v-z}$	69.00(±0.19)$^{u-x}$	71.00(±0.05)$^{u-w}$
	48	57.00(±0.05)$^{v-y}$	59.00(±0.04)$^{b-f}$	65.00(±0.08)$^{k-p}$	68.00(±0.03)$^{v-z}$	70.00(±0.05)$^{g-i}$	74.00(±0.07)$^{d-o}$	75.00(±0.02)$^{f-h}$
	72	59.00(±0.10)$^{h-n}$	63.00(±0.30)$^{j-r}$	68.00(±0.04)$^{e-g}$	73.00(±0.36)$^{st-x}$	75.00(±0.07)$^{f-j}$	77.00(±0.01)$^{a-e}$	78.00(±0.02)$^{i-l}$
	96	64.00±(0.12)$^{d-j}$	68.00(±0.08)$^{f-ij}$	71.00(±0.25)$^{b-f}$	77.00(±0.07)$^{r-t}$	78.00(±0.04)$^{st-w}$	80.00(±0.07)$^{o-rs}$	82.00(±0.20)$^{i-m}$
1,8-Cineole	24	57.00(±0.01)$^{c-h}$	59.00(±0.14)$^{de-i}$	63.00(±0.11)$^{e-h}$	66.00(±0.02)^{ghi}	71.00(±0.10)$^{e-k}$	73.00(±0.10)$^{p-s}$	78.00(±0.06)$^{c-j}$
	48	62.00(±0.12)$^{a-f}$	67.00(±0.12)$^{d-g}$	71.00(±0.14)$^{m-p}$	76.00(±0.08)^{def}	79.00(±0.07)$^{b-e}$	81.00(±0.01)^{ijk}	84.00(±0.10)$^{p-v}$
	72	69.00(±0.02)$^{m-r}$	71.00(±0.07)$^{f-i}$	76.00(±0.10)$^{q-u}$	80.00(±0.15)^{tuv}	82.00(±0.20)^{opq}	86.00(±0.09)$^{f-jk}$	90.00(±0.10)$^{b-gh}$
	96	72.00(±0.04)$^{c-e}$	74.00(±0.12)$^{b-g}$	77.00(±0.10)^{cde}	83.00(±0.06)$^{t-v}$	85.00(±0.31)$^{a-ef}$	89.00(±0.05)^{hij}	93.00(±0.02)$^{s-w}$
Eugenol	24	64.00(±0.10)$^{s-w}$	68.00(±0.20)$^{h-l}$	72.00(±0.10)$^{l-o}$	74.00(±0.05)$^{s-w}$	79.00(±0.32)$^{mn-r}$	86.00(±0.10)^{qrs}	95.00(±0.06)$^{e-j}$
	48	73.00(±0.01)$^{e-j}$	79.00(±0.08)$^{c-h}$	81.00(±0.07)$^{p-t}$	85.00(±0.14)$^{a-jk}$	89.00(±0.01)$^{n-r}$	93.00(±0.14)pq	99.00(±0.05)$^{q-t}$
	72	79.00(±0.12)$^{f-p}$	82.00(±0.01)$^{c-g}$	87.00(±0.10)^{lmn}	89.00(±0.06)^{abc}	91.00(±0.01)$^{n-s}$	99.00(±0.06)^{vwx}	100.00(±0.00)^{a}
	96	81.00(±0.08)^{dl}	84.00(±0.07)$^{ab-j}$	90.00(±0.10)$^{v-z}$	91.00(±0.14)$^{u-x}$	95.00(±0.02)$^{n-q}$	100.00(±0.00)^{a}	100.00(±0.00)^{a}
Eugenol epoxide	24	58.00(±0.04)$^{b-h}$	61.00(±0.08)^{stu}	66.00(±0.01)$^{d-j}$	70.00(±0.21)^{lmn}	74.00(±0.10)$^{m-s}$	79.00(±0.09)$^{i-m}$	82.00(±0.01)$^{ij-m}$
	48	62.00(±0.14)$^{k-r}$	65.00(±0.06)$^{p-r}$	69.00(±0.01)^{def}	73.00(±0.05)$^{a-d}$	78.00(±0.8)^{nop}	83.00(±0.10)$^{w-z}$	85.00(±0.12)$^{p-r}$
	72	67.00(±0.10)^{ef}	70.00(±0.12)$^{c-g}$	73.00(±0.05)$^{l-q}$	78.00(±0.07)$^{d-i}$	83.00(±0.14)^{fgh}	88.00(±0.05)^{def}	90.00(±0.20)$^{j-o}$
	96	72.00(±0.14)$^{g-n}$	74.00(±0.10)^{pqr}	79.00(±0.06)$^{j-m}$	82.00(±0.04)$^{n-r}$	88.00(±0.11)$^{s-v}$	93.00(±0.04)$^{k-m}$	94.00(±0.04)$^{s-u}$

Values in the same column followed by different letter(s) are significantly different according to Duncan's test (P < 0.05). Values in parenthesis show ± standard error.

Table 4: Effect of bay leaf essential oil, its fractions, compounds isolated and transformed on percent mortality of *M. incognita* at different concentrations and durations.

Compounds	Duration (Hrs)	Average percent mortality at different concentration (mg mL^{-1})						
		0.20	0.40	0.60	0.80	1.00	1.25	1.50
Bay leaf essential oil	24	72.00(±0.17)$^{e-h}$	76.00(±0.08)$^{a-d}$	78.00(±0.27)^{eh}	84.00(±0.13)$^{jl-p}$	89.00(±0.30)$^{r-u}$	94.00(± .06)^{fgh}	96.00(±0.21)$^{r-uv}$
	48	74.00(±0.22)$^{x-z}$	79.00(±0.11)$^{df-i}$	83.00(±0.11)$^{ac-f}$	88.00(±030)^{pq}-t	92.00(±1.15)$^{ik-n}$	98.00(±0.09)^{dg}	100.00(±.00)^{a}
	72	80.00(±0.03)$^{p-r}$	85.00(±0.18)^{ghi}	89.00(±0.06)$^{e-f}$	94.00(±0.10)$^{ik-o}$	99.00(±0.01)$^{b-d}$	100.00(±0.00) ^{a}	100.00(±0.00) ^{a}
	96	89.00(±0.02) $^{w-z}$	92.00(±0.12)$^{q-t}$	97.00(±0.89)$^{uv-z}$	100.00(±0.00)^{a}	100.00(±0.00)^{a}	100.00(±0.00)^{a}	100.00(±0.00)^{a}
Non-polar fraction	24	48.00(±0.08) $^{ac-f}$	51.00(±0.55)^{cde}	55.00(±0.39)^{pq}	59.00(±0.20)$^{s-v}$	64.00(±0.04)$^{n-rs}$	69.00(±0.29)$^{p-r}$	73.00(±0.09)$^{d-h}$
	48	52.00(±0.21)$^{b-de}$	56.00(±0.60)$^{tu-z}$	59.00(±0.15)$^{f-h}$	63.00(±0.31)$^{c-f}$	66.00(±0.31)$^{st-w}$	70.00(±0.07)$^{k-n}$	77.00(±0.12)^{abc}
	72	57.00(±0.39)^{mno}	61.00(±0.02)$^{i-l}$	65.00(±0.01)$^{e-f}$	70.00(±0.12)$^{ij-p}$	74.00(±0.39)$^{c-j}$	79.00(±0.02)$^{r-v}$	84.00(±0.01)$^{op-u}$
	96	61.00(±0.10)$^{b-gh}$	64.00(±036)$^{d-f}$	67.00(±0.02)^{lmn}	72.00(±0.17)$^{cd-h}$	79.00(±0.03)^{rst}	85.00(±.36)$^{ef-l}$	89.00(±0.84)$^{r-t}$
Polar fraction	24	69.00(±0.16)^{ghi}	74.00(±0.03)$^{c-f}$	77.00(±0.44)$^{n-rs}$	84.00(±0.10)$^{b-d}$	87.00(±0.43)$^{s-x}$	91.00(±0.03)$^{k-p}$	95.00(±05)^{dfg}
	48	71.00(±0.18)$^{q-t}$	75.00(±0.12)$^{uv-x}$	80.00(±0.05)$^{l-n}$	86.00(±0.38)$^{d-hi}$	87.00(±0.44)^{wx}	92.00(±0.10)$^{w-z}$	97.00(±0.05)$^{n-s}$
	72	77.00(±0.31)^{rst}	81.00(±0.10)^{de}	88.00(±0.32)$^{ij-o}$	91.00(±0.14)$^{cd-g}$	94.00(±0.10)^{mno}	98.00(±0.17)$^{y-z}$	100.00(±0.00)^{a}
	96	80.00(±0.16) ^{kl}	88.00(±0.16)^{xy}	94.00(±0.36)$^{l-no}$	97.00(±0.24) $^{r-t}$	99.00(±0.09)$^{a-b}$	100.00(±0.00) ^{a}	100.00(±0.00)^{a}
α-Pinene	24	53.00(±0.44)$^{ij-n}$	58.00(±0.16)^{def}	62.00(±0.03)^{hij}	67.00(±0.01)$^{wx-z}$	74.00(±0.05)$^{j-o}$	79.00(±0.24)^{jk}	83.00(±0.12)$^{s-x}$
	48	56.00(±0.14)$^{e-j}$	59.00(±0.43)$^{n-p}$	64.00(±0.31)^{opq}	69.00(±0.10)$^{i-o}$	76.00(±0.38)$^{r-u}$	80.00(±0.25)$^{ij-mn}$	85.00(±0.47)$^{tu-v}$
	72	58.00(±0.08)^{ghi}	61.00(±0.14)$^{q-uv}$	67.00(±0.14)$^{ef-jk}$	72.00(±0.08)$^{w-x}$	77.00(±0.12)^{klm}	81.00(±0.08)^{uv}	87.00(±0.17)^{ij}
	96	62.00(±0.21)$^{t-w}$	64.00(±0.04)^{pqr}	69.00(±0.39)$^{l-o}$	73.00(±0.14)^{ghi}	80.00(±0.32)$^{b-f}$	84.00(±0.05)$^{i-n}$	90.00(±0.10)^{hi}
Campholenic aldehyde	24	55.00(±0.05)^{de}	58.00(±0.04)$^{q-tu}$	61.00(±0.07)$^{r-xy}$	66.00(±0.17)$^{w-yz}$	73.00(±0.14)$^{qr-t}$	79.00(±0.07)$^{fg-l}$	84.00(±0.07)$^{ab-g}$
	48	56.00(±0.23)^{de}	61.00(±0.21)$^{g-l}$	65.00(±0.41)^{wxy}	71.00(±0.14)$^{i-s}$	77.00(±0.08)$^{a-de}$	83.00(±0.07)$^{e-kl}$	88.00(±0.03)$^{j-o}$
	72	60.00(±0.02)^{mn}	65.00(±0.39)^{hij}	69.00(±0.04)$^{s-v}$	77.00(±0.07)$^{e-jk}$	82.00(±0.10)$^{ab-l}$	86.00(±0.47)^{efg}	90.00(±0.08)$^{a-f}$
	96	64.00(±0.01)$^{j-m}$	68.00(±0.10)^{ghi}	72.00(±0.21)$^{p-s}$	81.00(±0.31)^{yz}	86.00(±0.11)^{abc}	88.00(±0.05)$^{i-o}$	92.00(±0.07)$^{l-r}$
1,8-Cineole	24	59.00(±0.08)$^{q-t}$	67.00(±0.07)$^{rs-y}$	71.00(±0.04)^{fgh}	76.00(±0.07)$^{i-n}$	81.00(±0.02)$^{w-z}$	83.00(±0.06)$^{o-v}$	86.00(±0.22)$^{pq-y}$
	48	64.00(±0.14)$^{ef-j}$	69.00(±0.17)$^{c-h}$	73.00(±0.08)$^{l-p}$	78.00(±0.04)$^{j-m}$	83.00(±0.13)$^{r-t}$	86.00(±0.07)$^{m-rs}$	89.00(±0.23)$^{h-m}$
	72	68.00(±0.04)$^{b-g}$	72.00(±0.32)^{ab}	77.00(±0.15)$^{s-x}$	79.00(±0.05)$^{f-j}$	85.00(±0.14)^{ln}	87.00(±0.27)$^{g-k}$	91.00(±0.24)^{ghi}
	96	73.00(±0.14)$^{l-r}$	76.00(±0.36)$^{p-t}$	81.00(±0.14)$^{a-df}$	86.00(±0.06)$^{i-pr}$	89.00(±0.21)^{klm}	93.00(±0.06)$^{a-j}$	95.00(±0.38)^{jk}
Eugenol	24	64.00(±0.12)^{stu}	71.00(±0.19)$^{c-g}$	76.00(±0.04)$^{n-u}$	82.00(±0.08)$^{p-st}$	85.00(±0.22)^{abc}	90.00(±0.03)$^{hi-o}$	93.00(±0.01)$^{n-p}$
	48	69.00(±0.22)^{qrs}	73.00(±0.13)st	79.00(±0.18)$^{e-gh}$	84.00(±0.06)$^{k-m}$	87.00(±0.28)$^{p-r}$	91.00(±0.11)$^{g-kl}$	95.00(±0.01)$^{m-p}$
	72	72.00(±0.20)$^{l-p}$	78.00(±0.21)$^{m-o}$	83.00(±0.16)$^{r-t}$	86.00(±0.37)$^{p-u}$	92.00(±0.11)$^{d-h}$	95.00(±0.21)^{cde}	99.00(±0.05)$^{a-jl}$
	96	83.00(±0.24)^{uvw}	87.00(±0.08)$^{d-j}$	90.00(±0.08)$^{l-op}$	95.00(±0.41)$^{i-l}$	98.00(±0.11)$^{s-v}$	100.00(±0.00)^{a}	100.00(±0.00)^{a}
Eugenol epoxide	24	63.00(±0.14)$^{a-d}$	67.00(±0.21)^{jkl}	70.00(±0.05)$^{o-r}$	75.00(±0.14)^{ghi}	79.00(±0.20)$^{f-i}$	84.00(±0.11)$^{ab-g}$	87.00(±0.10)^{xyz}
	48	65.00(±0.14)$^{d-j}$	70.00(±0.15)$^{q-s}$	74.00(±0.22)^{opq}	78.00(±0.05)$^{b-e}$	83.00(±0.04)$^{p-t}$	88.00(±0.15)$^{j-q}$	90.00(±1.01)$^{w-yz}$
	72	69.00(±0.05)$^{s-v}$	73.00(±0.12)$^{j-m}$	77.00(±0.14)$^{o-u}$	81.00(±0.32)$^{j-m}$	89.00(±0.22)^{tuv}	91.00(±0.14)$^{a-c}$	93.00(±0.18)^{ijk}
	96	75.00(±0.14)$^{k-op}$	79.00(±0.11)^{cde}	84.00(±0.25)$^{a-ef}$	87.00(±0.54)^{hij}	93.00(±0.23)^{yz}	92.00(±0.11)$^{o-y}$	95.00(±0.21)^{def}

Values in the same column followed by different letter(s) are significantly different according to Duncan's test (P < 0.05). Values in parenthesis show ± standard error

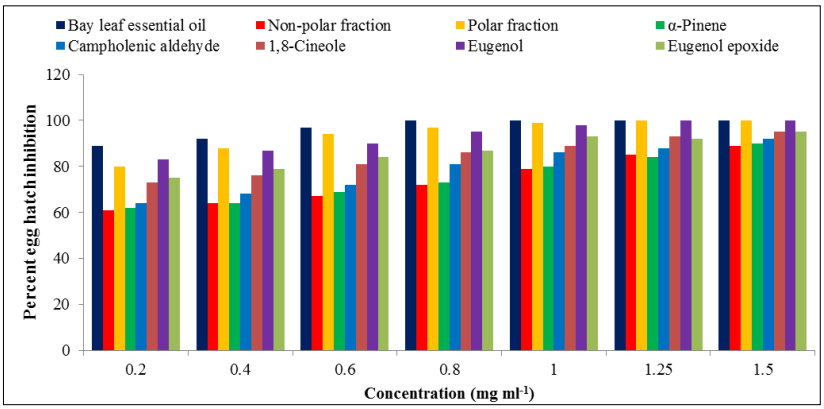


Fig 1: Comparative study of bay leaf oil, its fractions, compounds isolated and derivatized on egg hatch inhibition of *M. incognita* at different concentrations after 96 hrs

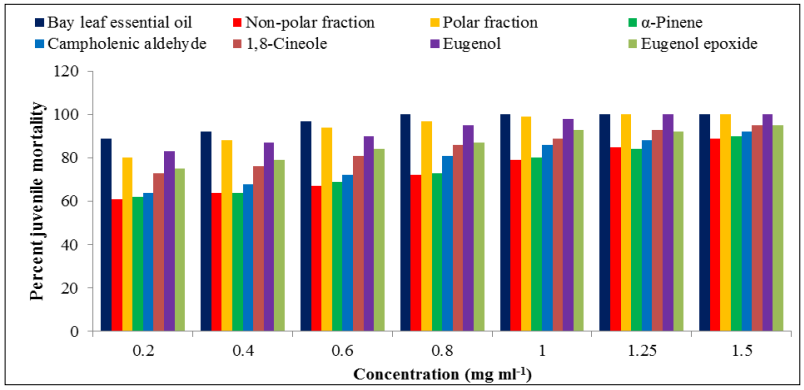


Fig 2: Comparative study of bay leaf oil, its fractions and compounds isolated and derivatized on J_2 mortality of *M. incognita* at different concentrations after 96 hrs

4. Conclusions

It can be concluded that bay leaf essential oil, isolated and derivatized compounds possessed significant nematicidal activity against *M. incognita*. Future research should be focused on micro plot and field experiments, along with identification of more active compounds responsible for their nematicidal activity. Health concerns and hazardous effects of synthetic chemicals as nematicides pave a way for identifying new class of pesticides from natural plants to replace the dangerous and expensive chemicals used at presently.

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