

## Chemical composition and fumigation toxicity of essential oil from *Murraya paniculata* L. (Jack) against almond moth, *Cadra cautella* (Walker)

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### ABSTRACT

Essential oils from plants are being studied for their insecticidal activities as alternative to synthetic insecticides and fumigants. Particularly in storage pest management use of fumigants is inevitable and considering the ban on methyl bromide and resistance development against phosphine by several pests, there is urgent need to identify alternative chemicals. Almond moth, *Cadra cautella* is one of the key lepidopteran pest of several commodities.

We investigated the fumigation toxicity of essential oil from the leaves of Orange Jasmine, *Murraya paniculata*, a shrub against *C. cautella*. Extraction by hydrodistillation and Gas Chromatography-Mass Spectrometry profiling of essential oil revealed Aromadendrene (23.9 %), Germacrene-D (23.3 %),  $\beta$ -caryophyllene (17.2 %) and Humulene (4.28 %) as major constituents. The fumigant toxicity bioassay against egg, larval and adult stages of *C. cautella* showed that *M. paniculata* essential oil possessed fumigant activity with LD<sub>50</sub> value of 57.48, 41.52  $\mu$ l/L and 38.40 at 35.708  $\mu$ l/L, 22.56 and 18.88  $\mu$ l/L, respectively, after 48 and 72 h of the exposure period. These bioassays demonstrated that *M. paniculata* leaf essential oil possessed the fumigation toxicity against insects and can be considered as a potential candidate for future studies.

**Keywords:** Almond moth, bioassay, *Cadra cautella*, essential oil, fumigation toxicity, insects, *Murraya paniculata*

### INTRODUCTION

Essential oils, commonly referred to as volatile plant secondary metabolites (23), are widely studied for their insecticidal and other properties against various insect pests (10,24,43). These possess broad-spectrum activities including contact, fumigant and growth inhibition against stored product pests and also field crop pests. Special attention is given to screen these oils against various pests to identify the potential source of insecticidal plants and compounds. Wide array of phytochemical present in different parts of *Murraya paniculata* L. (Jack) (Family: Rutaceae) plant (Fig. 1) possess various biological activities. It possesses diverse medicinal activities like antioxidant (41), Antinociceptive, anti-inflammatory (51), anti-obesity (18), diarrhea (45), antibacterial (47), antifungal, antihyperglycemic (14) etc. Moreover, the solvent extracts from its leaves also possess contact toxicity against pulse beetle, *Callosobruchus maculatus* (Fabricius, 1775) adults (12,17,28), larvicidal activity against *Culex quinquefasciatus* Say, 1823 (22,38,39). Essential oil from different parts of *Murraya* species (*M. paniculata*, *M. exotica*, *M. koenigii*, *M. tetramera*, *M. euchres-tifolia*, *M. kwangsiensis* and *M. alata*) have been extracted and characterized (6,10,30,34,37,42,52).

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Figure 1. Photographs of Orange Jasmine, *Murraya paniculata* plant



Figure 2. Adult and larva of almond moth, *Cadra cautella*

*Cadra cautella* (Walk.) (Lepidoptera: Pyralidae) (Fig. 2) is a key pest of several storage commodities in tropical and subtropical climate. It is major pest of figs, rough rice, dry fruits, wheat, barley, sorghum, soybean and oilseeds etc. Recently in India it has been reported as emerging pest of stored garlic (19) and tree bean, *Parkia Roxburghii* (32). The management of this pest in storage mainly depends on fumigation with phosphine and contact insecticides as another fumigant, methyl bromide, which has been banned for use due to its effect on ozone layer. Moreover phosphine and contact insecticides are less effective due to resistance development (35,36). There is no report on fumigation toxicity

of *M. paniculata* essential oil against *C. cautella*. The present study was aimed to extract, characterize and determine fumigation toxicity of essential oil from Orange Jasmine, *M. paniculata* against major storage pest, *C. cautella*.

## MATERIALS AND METHODS

### Insect rearing

The culture of Almond moth, *C. cautella* was maintained in the laboratory at controlled conditions (photoperiod 16L: 8D, Temperature  $26\pm 2$  °C and  $65\pm 5$  % RH) on wheat flour mixed with 5 % yeast for regular supply of test insect (12). Freshly emerged male and female adults (1:1 proportion) were released in oviposition jar (17x14 cm) covered with wire gauze at the bottom to collect the eggs. Freshly laid eggs were kept in petri plate with the wheat flour and after 3-4 days, hatched larvae were shifted to glass jar (25x18 cm) containing wheat flour mixed with 5 % yeast. The adults were supplemented with 10 % honey solution. The collected eggs were cleaned from the scales and were used for further bioassay. The uniform aged larvae (3<sup>rd</sup> instar) and adults (two days old) were obtained from fresh cultures.

### Plant material and extraction of essential oil

The fresh leaves of *M. paniculata* were collected, cleaned and air dried at room temperature ( $25\pm 3$  °C). Essential oil was extracted through hydrodistillation by using Clevenger's apparatus (11). Leaves (10 kg) were collected and extracted in two batches each consisting of 500 g fresh leaves with 1000 ml of distilled water. The extraction was done for 3-4 h and essential oil was collected on the graduated tube of the Clevenger's apparatus. The mixture of essential oil with water was collected and then partitioned with diethyl ether by using a separatory funnel. The essential oil thus obtained was passed through anhydrous sodium sulfate to remove traces of water and the quantity of the essential oil was recorded (0.30075 ml /kg of fresh leaves). The pure essential oil was transferred to airtight vials and stored in Refrigerator (4 °C) for further use in bioassay.

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The quantitative and qualitative analysis of essential oil was carried out using Focus-DSQ GC/MS (Thermo) equipped with TG-5MS capillary column (30 m x 0.25 mm i.d.; film thickness 0.25 µm). The injector temperature was 250 °C, helium as carrier gas at a flow-rate of 1 ml/min and the injection volume was 0.2 ml (1000 ppm in hexane), respectively. Initially column temperature was 60 °C and programmed at 3 °C/min to 250 °C and held for 5 min with split ratio of 1:20. The MS transfer line and source temperatures were 260 °C and 230 °C, respectively. The GC column was coupled directly to a single quadrupole mass spectrometer in EI mode at 70 eV with the mass range of 30-400 a.m.u at 1 scan/s. The individual compounds were identified based on retention time and retention index using a homologous series of n-alkanes (C<sub>8</sub>-C<sub>20</sub>, Sigma-Aldrich) by comparing their mass spectra with NIST Mass Spectral Library (Ver. 2, 2005).

### Fumigation toxicity

Fumigant toxicity of essential oil was evaluated against various stages of *C. cautella* based on the method reported earlier (5,20,31) with some modifications. Briefly, Whatman filter paper (1x1 cm<sup>2</sup>) was impregnated with the required quantity of essential oil to yield doses 4 to 80 µl/ L for preliminary screening. Based on preliminary screening five doses viz., 16, 20, 28, 36 and 60 µl/L air were used for toxicity analysis. The

filter paper was attached to the underside of the lid of 250 ml capacity flat bottom flask with the help of thread. After impregnation flasks were immediately closed to avoid evaporation loss. For each concentration and control 30 eggs, larvae and adults were released in the flask, separately, and replicated thrice. Mortality was recorded after 24, 48 and 72 h of exposure time. The dead larvae were confirmed by touching the larva with soft camel brush to ascertain their movement. To record egg mortality, the hatching of eggs was observed after exposure period. Before recording the mortality, sufficient aeration for 6 h was allowed for recovery.

#### Statistical analysis

To determine LC<sub>50</sub> and LC<sub>90</sub>, the corrected mortality was calculated according to Abbott's formula (1). The mortality data was subjected to probit analysis (13) using PoloPlus 2.0 software (LeOra Software, California, United States). The data from different doses and exposure time was subjected to analysis of variance (ANOVA) (one-way ANOVA) after angular transformation. The means were separated using Tukey's test at 5 % level of significance.

## RESULTS AND DISCUSSION

#### GC-MS profile of essential oil

The essential oils and their chemical constituents are potential ecofriendly alternatives to synthetic insecticides because of their low mammalian toxicity, medicinal value and biodegradability. The ease in application and role of certain phytochemicals for managing insecticide-resistant pests (4,44) makes them a potential candidate for future biopesticide. The efficacy of essential oils derived from different plant sources against different stages of *C. cauttella* has been investigated (7,8,15,48). The insecticidal properties of essential oils viz., ovicidal, antifeedant, repellents, contact and fumigation toxicity are considered to be due to various volatile constituents belonging to different chemical classes viz., terpenes, terpenoid, sesquiterpenes, alkaloids etc. The GC-MS analysis of *M. paniculata* leaf essential oil in the present study revealed the presence of 26 compounds with varying relative proportion ranging from 0.01 to 23.90 % (Table 1, Fig 3). Aromadendrene (23.9 %), a sesquiterpenoid, was major constituent along with other sesquiterpenes viz., Germacrene-D (23.3 %),  $\beta$ - caryophyllene (17.2 %) and Humulene (4.28 %) (Fig 4).

The variations in essential oil constituents of same plant from different locations is thought to be due to geographical variations (42,43). Caryophyllene oxide (16.6 %), caryophyllene (11.8 %), spathulenol (10.2 %), elemene (8.9 %), germacrene D (6.9 %), and methylene-6-4-(1- propenylidene) cyclooctene (6.4 %) have been reported as major constituents of *M. paniculata* essential oil from Bangladesh (10). Similarly (26) reported caryophyllene (23.3 %), spathulenol (16.1 %), (E) bergamotene (9.3 %), (E)-nerolidol (4.6 %), and elemene (3.3 %) as major constituents from China. Fruit essential oil of *M. paniculata* was rich in (E)-nerolidol (25.7 %) and linalool (18.7 %) (42).

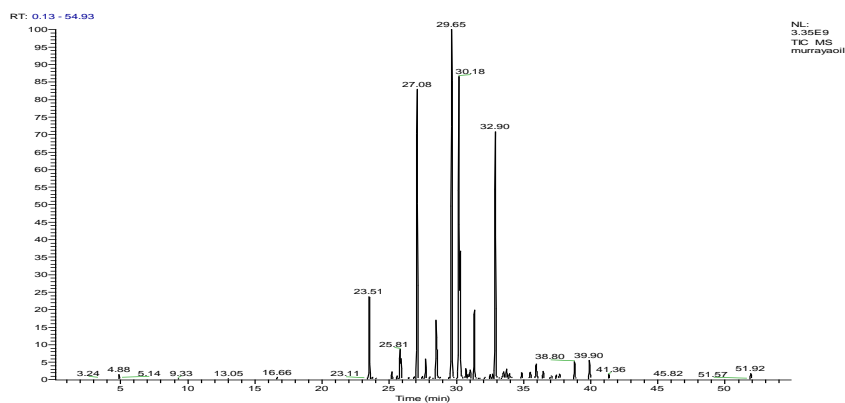


Figure 3. Chromatogram of the essential oil analysis through Gas chromatography-Mass spectrometry

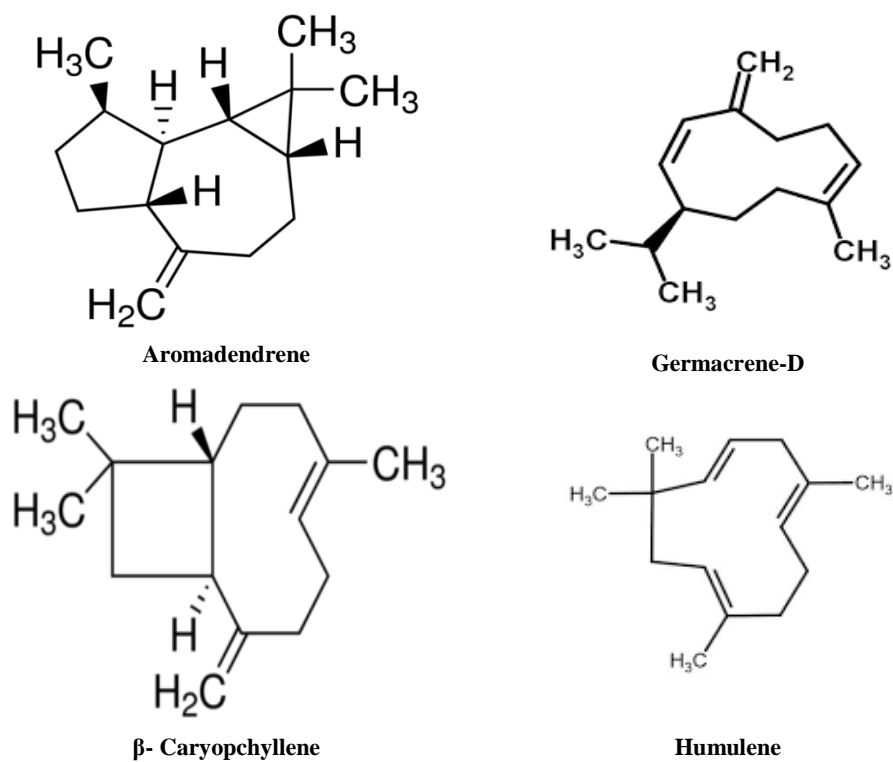


Figure 4. Chemical structures of major components of *Murraya paniculata* leaf essential oil

Table 1. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *M. paniculata* leaf essential oil

Sr. No.	Compound	Rt	KI	Area (%)
1	3-Hexenol	4.88	859	0.17
2	<i>p</i> -Cymene	8.9	1024	0.04
3	Linalool	13.05	1096	0.07
4	Neral	13.74	1238	0.01
5	Azulene	16.66	1298	0.12
6	$\delta$ -Elemene	23.51	1338	4.06
7	$\alpha$ -Cubenene	24.04	1351	0.09
8	$\alpha$ -Copaene	25.19	1376	0.37
9	$\beta$ -Bourbonene	25.59	1388	0.16
10	$\beta$ -Caryophyllene	27.08	1417	17.2
11	$\alpha$ -Bergamotene	27.71	1434	1.02
12	Humulene	28.49	1436	4.28
13	Aromadendrene	29.65	1441	23.9
14	Germacrene-D	30.18	1481	23.3
15	$\alpha$ -Zingiberene	30.71	1493	0.57
16	$\zeta$ -Muurolene	31.03	1500	0.89
17	$\beta$ -Bisabolene	31.33	1505	3.42
18	Germacrene-b	32.7	1561	0.25
19	trans-nerolidol	32.9	1563	13.2
20	Spathulenol	33.51	1578	0.59
21	Caryophylleneoxide	33.74	1583	0.66
22	tau-Cadinol	35.93	1640	1.25
23	Cubenol	33.95	1646	0.47
24	$\alpha$ -Cadinol	36.46	1654	0.47
25	$\beta$ -Bisabolol	37.07	1675	0.31
26	( <i>Z</i> )- $\alpha$ -trans-bergamotol	39.9	1690	1.24

Note: The identification of compounds was done based on retention time and retention index using a homologous series of *n*-alkanes (C<sub>8</sub>-C<sub>20</sub>, Sigma-Aldrich) by comparing their mass spectra with NIST Mass Spectral Library (Ver. 2, 2005). KI: Kovats Index.

#### Fumigation toxicity of essential oil against *C. cautella*

The evaluation of fumigant toxicity against eggs of *C. cautella* revealed non-significant differences in egg mortality (%) after 24 h ( $F=2.929$ ,  $P=0.059$ ), but significant difference were observed after 48 h ( $F=8.096$ ,  $P<0.05$ ) and 72 h ( $F=23.256$ ,  $P<0.05$ ) of the exposure time (Fig. 5). Similarly, larval mortality was also time-dependent and it significantly differed at various concentration (Fig. 5) after 24 h of the exposure time ( $F=5.722$ ,  $P<0.05$ ). After 48 h, the larval mortality ranged from 23.33-60 % which was significantly higher at 36 and 60  $\mu\text{L/L}$  concentrations ( $F=57.240$ ,  $P<0.05$ ). Mortality after 72 h exposure time was significantly higher at 60  $\mu\text{L/L}$  concentrations ( $F=7.0356$ ,  $P<0.05$ ) the mortality ranged between 17.57-63.06 % (Fig. 5). The mortality for adults also significantly differed at various concentrations at 24 h ( $F=21.764$ ,  $P<0.05$ ), 48 h ( $F=22.847$ ,  $P<0.05$ ) and at 72 h ( $F=26.911$ ,  $P<0.05$ ). The comparative fumigant toxicity among different stages revealed that adults was the most susceptible stage with the lowest LC<sub>50</sub> and LC<sub>90</sub> after 72 h (18.88  $\mu\text{L/L}$  air and 31.36  $\mu\text{L/L}$  air, respectively) followed by 48 h (LC<sub>50</sub> 22.56  $\mu\text{L/L}$  air and LC<sub>90</sub> 61.68  $\mu\text{L/L}$  air). Egg stage was more tolerant than

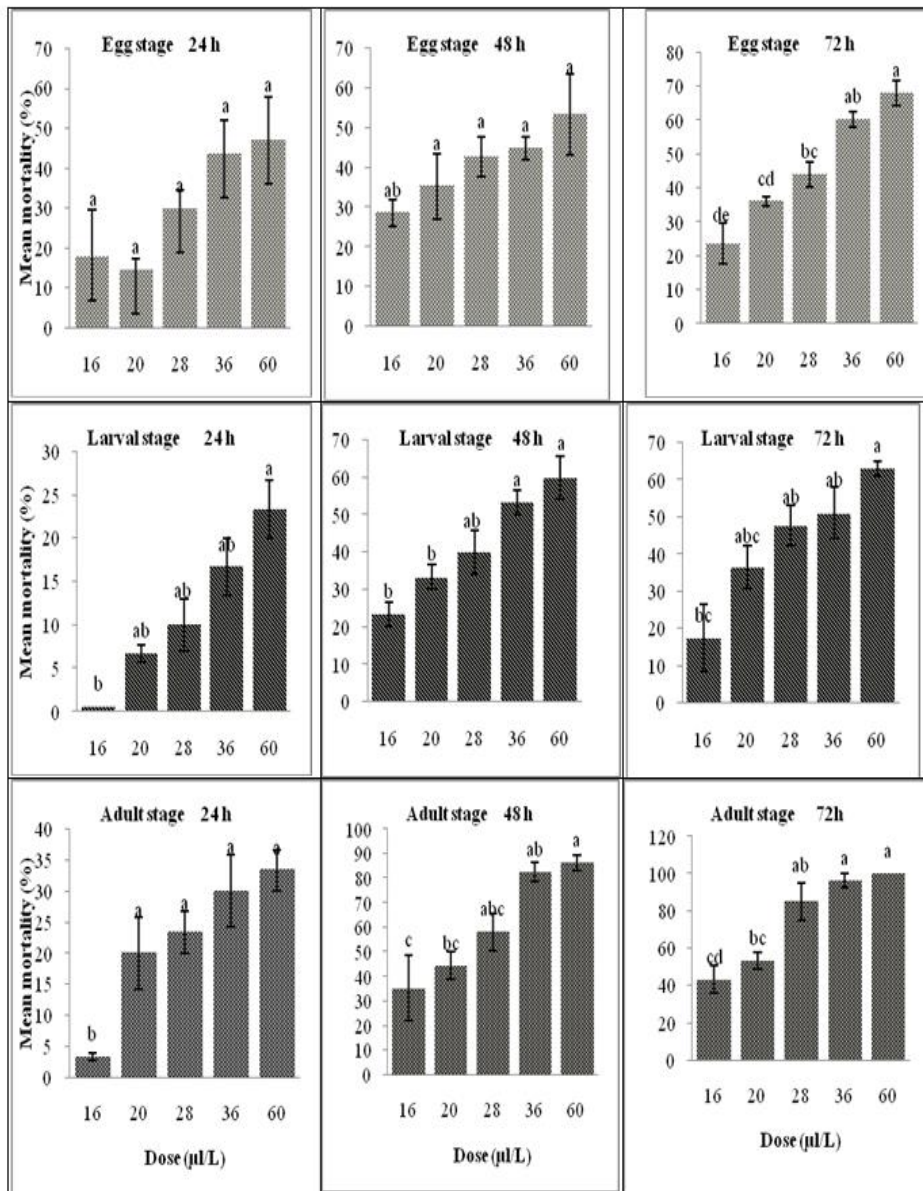


Figure 5. Mortality (%) of different stages of *Cadra cautella* due to fumigant toxicity of *Murraya paniculata* leaf essential oil. The bars represent mean percent mortality of three replications for each stage viz., Egg stage, Larval stage, and Adult stage. A replication consists of a fumigation flask with thirty individuals of each stage. Bars displayed with different letters shows significantly different mean mortality (%) at  $p < 0.05$  by Tukey's test

larvae and adults (LC<sub>50</sub> value 41.52 µl/L and 57.48 µl/L after 72 and 48 h of exposure time, respectively) (Table 2). Contrast to our results, egg stage has been reported as most susceptible stage against *Ricinus communis* oils (2). Egg stage of several storage insects is comparatively tolerant, whereas, adult stage is most susceptible to fumigants. The tolerance in eggs may be due to its unique eggshell structure (9). Similarly, Goyal *et al.* (15) reported that essential oil from *Lantana camera* had LC<sub>50</sub> of 48.56 µl/L against early stage eggs, whereas, 77.75 µl/L against late eggs, 29-39 µl/L against larval and 15.23 and 11.21 µl/L against early and late stage adults, respectively, of *C. cautella*.

Table 2. LC<sub>50</sub> and LC<sub>90</sub> (µl/L air) values of essential oils according to exposure periods against *C. cautella*

Stage/exposure period (h)	LC <sub>50</sub>	Fiducial limit	LC <sub>90</sub>	Fiducial limit	Slope (±S.E.)	X <sup>2</sup>
<b>Eggs</b>						
48	57.48	42.28-130.64	722.36	235.08-27182.4	1.17±0.32	0.58
72	41.52	32.4-65.4	131.64	77.28-742.76	2.56±0.4	3.83
<b>Larva</b>						
48	38.4	32.84-48.12	215.56	128.64-594.44	1.71±0.29	1.89
72	35.708	25.96-69.4	166.64	79.12-7051.0	1.92±0.29	5.82
<b>Adult</b>						
48	22.56	17.92-27.0	61.68	46.6-108.52	2.94±0.300	7.13
72	18.88	17.52-20.12	31.36	28.92-35.08	5.83±0.609	2.61

Note: For all stages after 24 h exposure period, the mortality at higher dose was < 50%, hence, lethal concentration (LC<sub>50</sub>, LC<sub>90</sub>) could not be calculated.

The fumigant toxicity exhibited by essential oil of *M. paniculata* was due to the presence of several compounds (Table 1). Although there are no earlier reports on fumigation toxicity of *M. paniculata* essential oil, but the species close to *M. exotica* reported to possess fumigant toxicity against adults of *Sitophilus zeamais* and *Tribolium castaneum* with LC<sub>50</sub> values of 8.29 and 6.84 mg/L, respectively, and contact toxicity with LD<sub>50</sub> values of 11.41 and 20.94 µg/adult, respectively (24). Essential oil from *M. paniculata* is repellent to black garden ant, *Lasius niger* (27), *Tribolium castaneum* (52) and *C. maculatus* (16).

As mentioned earlier, the biocidal activities of essential oils are due to the chemical constituents which may impart toxicity alone or in combination with other minor components. The present investigation revealed that the *M. paniculata* essential oil was rich in sesquiterpenes *viz.*, aromadendrene, germacrene-D, β-caryophyllene, humulene etc. similarly essential oil from *Ocotea glomerata* (Lauraceae) is rich in aromadendrene (17.3±0.6 %) and β-caryophyllene (14.6±0.3 %) reported to possess fumigant activity against two-spotted spider mite, *Tetranychus urticae* with LC<sub>50</sub> 1.32 % and the toxicity of β-caryophyllene was 33-folds higher (29). β-caryophyllene and germacrene D are major components of *Premna angolensis* and *P. quadrifolia* leaf essential oil. Oils from both plants proved toxic to *Sitotroga cerealella* adults with 100 % and 88.85 % mortality at 15 µl/ml dose (3). Although major components of essential oils are responsible for insecticidal activity but their minor components may also play role as synergists. The minor components identified in present study *viz.*, p-cymene, linalool, δ-Elementene, caryophyllene oxide etc. also possess fumigant toxicity (25,33,40). Moreover, the mode of

action of these compounds or essential oils remains obscure, the perception of sesquiterpene like germacrene D by olfactory neurons of insects like *Heliothis virescens*, *Helicoverpa armigera*, *H. assulta* (46) and its knockdown effect (through fumigation toxicity) in mosquito species viz., *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti* have been reported (21).

## CONCLUSIONS

The, GC-MS profile of essential oil from *M. paniculata* leaves showed that it is rich in sesquiterpenes viz., aromadendrene, germacrene-D,  $\beta$ -caryophyllene, humulene etc. The LD<sub>50</sub> values of essential oil against egg, larval and adult stages were 57.48 and 41.52  $\mu$ l/L, 38.40 and 35.708  $\mu$ l/L, 22.56 and 18.88  $\mu$ l/L, respectively, after 48 and 72 h. The studies suggested that the fumigation toxicity observed against different life stages of *C. cautella* will be useful for further exploration of this plant to develop ecofriendly alternate pesticide for its management.

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## CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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