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Characterization of secondary metabolites from the leaves of curry leaf (*Murraya koenigii* L.) essential oils with insecticidal activities against stored product insects

Naduvilthara U. Visakh[a,*], Berin Pathrose[a], Arunaksharan Narayanankutty[b]

[a] Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Thrissur, 680656, Kerala, India

[b] Division of Cell and Molecular Biology, PG & Research, Department of Zoology, St. Joseph's College (Autonomous), Devagiri, Calicut, 673008, Kerala, India

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ABSTRACT

Curry leaf, a significant aromatic shrub belonging to the Rutaceae family, is indigenous to the Indian subcontinent and is widely renowned for its application as a flavor enhancer. Due to specific flavor and fragrance, the leaves of *M. koenigii* have been recognized for their potential contributions to human health. Curry leaf essential oils have recently received much attention due to their numerous benefits and minimal risk to non-target organisms. This work focused on the comprehensive characterization of secondary metabolites present in the essential oil extracted from curry leaves and aims to evaluate its insecticidal potential in terms of contact, fumigant, and repellent activities against red flour beetle (*Tribolium castaneum*) and pulse beetle (*Callasobruchus maculatus*) for first time. Chemical characterization through GC-MS/MS showed that the main chemical compounds within curry leaf essential oil were caryophyllene (28.47%), α-guaiene (13.35%), and α-phellandrene (12.26%). Notably, results from various insecticidal bioassays showed substantial effects upon contact (LC_{50} at 24 h, 10.56 mg/cm^2 for *T. castaneum*, 20.80 mg/cm^2 for *C. maculatus*) and fumigant toxicities (LC_{50} at 24 h, 23.93 mg/L air for *T. castaneum*, 12.96 mg/L air for *C. chinensis*), alongside repellent activities (at 5 mg/cm^2, a mean PR of 90% to *T. castaneum* and at 5 mg/cm^2, *C. maculatus* demonstrated 80% PR, targeting both insects. Furthermore, a phytotoxicity assessment was performed on stored paddy grains, and neither seedling development nor germination rates were negatively affected. The essential oils extracted from curry leaves exhibit considerable potential as botanical insecticides that are effective against stored-product insects and environmentally benign.

1. Introduction

Out of 5.5 million insect species on earth, more than 20,000 are causing damage to one-third of the world's total food production. Insect pests attacking stored grains are known to annihilate a significant portion of food grains worldwide. The infestation of insects causes substantial losses to stored products with a high extent of damage. In India, about 20–25% of food grains are damaged quantitatively and qualitatively by storage insect pests, of which coleopteran insects cause two-thirds (Srivastava and Subramanian, 2016). Among coleopteran storage insect pests, red flour beetles and pulse bruchids are the two most detrimental insect species attacking cereals and pulses, respectively, with worldwide distribution (Usha et al., 2011).

* Corresponding author.
E-mail address: visakhnu17palazhi@gmail.com (N.U. Visakh).

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Tribolium castaneum, commonly known as the red flour beetle, is a major secondary stored grain insect pest belonging to the family Tenebrionidae. It is a prominent pest that damages stored products such as stored grains, wheat and rice flour. For households, farmers, and food processing industries, *T. castaneum* beetle infestations and the resulting damage can be severe. *T. castaneum* beetle larvae are voracious feeders and can quickly cause significant damage to food products by devouring the protein-rich portions of flour or the germ, bran, and endosperm of grains (Wanna and Wongsawas, 2022). By devouring the most nutrient-dense portions, they can lower the nutritional value of dried grains and flour, rendering them unfit for consumption by humans or other animals. Food commodities that have been infested may also develop contamination from beetles, their larvae, waste products, and exoskeletons that have been shed, leading to a loss of quality and possible hazards to health. In addition, *T. castaneum* beetles in stored products might result in losses for farmers and food processing facilities due to the requirement to remove infected items or pay additional expenditures for pesticide treatments (Morrison et al., 2021).

Similarly, stored grain legumes suffer more significant losses due to damage by the pulse beetles, *C. maculatus* (F.) (Coleoptera: Chrysomelidae). The grains may be hollowed entirely out by feeding of the larvae, and the characteristic emergence holes are evident only after the adult leaves the grains. Infestation of pulse beetle often destroys within six months. Pulse beetles cause 90–100% damage in stored seeds, resulting in poor germination. Its infestation initiates in the field and continues in the storehouses. The grubs eat the seed's endosperm, leaving only the thin outer covering or seed coat unfit for sowing (Kedia et al., 2015). Such bruchids are managed primarily with fumigation and surface treatments with insecticides. So far, malathion and deltamethrin have been the only insecticides widely used in storage godowns for surface treatment of walls and gunny bags. In India, the development of resistance to malathion in stored product insects viz., *T. castaneum* (Herbst), *Sitophilus oryzae* (L.), *Rhizopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.) had been known long back (Omkar, 2018).

To manage these key stored product insect pests, chemical insecticides are commonly used. The pesticides for preventing storage losses result in many hazards, and alternative eco-friendly measures are essential. However, insecticide resistance, pest resurgence, and residues cause hazards and failure of the pest management practice. In contrast, botanicals or plant-based natural products are eco-friendly and exploited against storage pests without affecting the quality of grains. Fumigation is one of the primary management practices against stored grain pests worldwide. Presently aluminium phosphide is the only available fumigant for storage pest management. But, restrictions are imposed on its use, and its effectiveness could be improved due to the development of resistance. An alternative to aluminium phosphide is required, and essential oils will provide an eco-friendly option. One of the eco-friendly and economic approaches is using plant products as grain protectants (Rolania and Bhargawa, 2015). Using botanicals for the protection of stored grains is a widely adopted practice. Many plant products, such as plant extracts and essential oils, have been evaluated for their insecticidal properties (Isman, 2000; Kumar, 2016).

Moreover, the significance of essential oils resides in their active role as a pivotal plant product in insecticidal evaluations, wherein their efficacious properties are rigorously assessed. Essential oils comprise a complex mixture of secondary metabolites, including terpenes, phenolics, aldehydes, ketones, alcohols, esters, and other volatile organic compounds (Shaaban et al., 2012). These secondary metabolites often serve ecological roles such as defense mechanisms against herbivores and pathogens, and they contribute to the characteristic aroma, flavor, and potential biological activities of essential oils (Narayanankutty et al., 2022; Kuttithodi et al., 2023). These essential oils are extracted from different plant parts, including leaves, flowers, stems, and roots. Due to their broad biological activity, these oils have been utilized for diverse purposes, including aromatherapy, cosmetics, and natural remedies (Albaqami et al., 2022). They have been used for millennia for their varied bioactivities. Plant secondary molecules, which give essential oils their distinctive aroma, may also have insecticidal characteristics, making them useful against a wide range of insects (Mossa, 2016). While synthetic chemicals currently dominate the repellent landscape, essential oils have emerged as promising candidates with the potential to offer practical and environmentally safer alternatives. Additionally, incorporating natural repellents into stored product pest management systems can address concerns related to chemical residues and insect presence in produce, catering to consumer preferences for pesticide-free products. Nevertheless, ongoing research is steering toward their application at reduced dosages as repellents to maintain equilibrium between detrimental and advantageous insect populations on crops and mitigate the uncertain side effects associated with essential oils (Bett et al., 2017).

In this respect, curry leaf, botanically known as *Murraya koenigii*, is a tropical and subtropical plant indigenous to India. Although curry leaves are often used in cooking due to its distinctive flavor and aroma are well known, there needs to be more understanding of the broader applications and possible health advantages. The essential oil extracted from curry leaves exhibits diverse biological properties. The yield and chemical composition of essential oils are subject to variability based on factors such as the plant portion utilized, the extraction process employed, and the concentration or dose applied, thereby influencing the biological properties of curry leaves oil. Specifically, the investigations have mainly centered on essential oil extraction, focusing on various biological activities such as antimicrobial, antioxidant, anti-inflammatory, hypoglycemic, and anti-cancer activities (Kumar et al., 2013; Rath and Priyadarshanee, 2017).

When exploring the insecticidal activities of curry leaf essential oils, limited research has been conducted on the repellent and fumigant toxicities of *M. koenigii* essential oil against insect pests. Some notable studies include the contact toxicity bioassay of *M. koenigii* essential oil against larva of *Spodoptera litura* (Fabricius) and *Spilosoma obliqua* (Walker) (Thodsare et al., 2014), the contact and repellent bioassay of *M. koenigii* oil against pulse beetles (Paranagama et al., 2002). Mang'era et al. (2021) described the *M. koenigii* phytochemical leaf extract disrupted growth and development in *Anopheles gambiae* mosquito larvae. Investigations into the effect of curry leaf essential oils have revealed their acaricidal activity against *Rhipicephalus* (*Boophilus*) *microplus*, particularly those strains resistant to synthetic pyrethroids (Singh et al., 2015). The efficacies of curry leaf essential oils against various stored product insects is still an avenue that remains largely uninvestigated. In the present context, an attempt has been made to study the detailed chemical characterization and different insecticidal activities of curry leaf essential oils against *T. castaneum* and *C. maculatus* for the first time.

2. Materials and methods

2.1. Collection and extraction of essential oil

Curry leaf (*Murraya koenigii*) plants in the College of Agriculture, Vellanikkara, India (10.5452° N, 76.2853° E) served as the source of plant material for essential oil extraction. Leaves were collected during January 2022 and shade-dried. Employing a Clevenger-type apparatus, we extracted essential oil from 100 g of shade-dried leaves through hydro-distillation at a temperature of 100 °C for 5–6 h. Extracted oils were passed through anhydrous sodium sulfate to remove traces of moisture in the oil. The yield of essential was calculated based on the equation,

$$\text{Yield (\%, v/w)} = \text{VEO} / \text{WD} \times 100$$

Where VEO is the volume of dried essential oil, and WD is the dry weight of curry leaf used for extraction (Visakh et al., 2022b). The oil was then stored in glass bottles (amber-colored) at 4 °C until further use for characterization and bioassays.

2.2. Test insects used for the experiments

Rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and the bruchid, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae), were used as test insects in the study (Visakh et al., 2022a). Flour beetles were reared in wheat flour with 2.5% (w/w) brewer's yeast, and bruchids were reared on cowpea. Adult insects were released onto plastic bottles (30 cm × 15 cm) containing the respective rearing media. After allowing five days for oviposition, the adults were removed from the bottles to obtain test insects of uniform age. The rearing conditions were 30 ± 2 °C and 86 ± 2% RH. Two week old adults of red flour beetles and 3–5 day old adults of bruchids were used for experiments.

2.3. GC-MS/MS analysis of essential oil

The researchers used a TSQ 8000 Evo (Thermo Fisher Scientific, USA) triple quadrupole GC-MS system for the chemical characterization of the curry leaf essential oil (Visakh et al., 2022a). The gas chromatograph was equipped with an autosampler. To separate the components of essential oil, a TG-1MS column was used. The injector was operated at a split ratio of 1:200, and helium was the carrier gas employed. Xcalibur 1.1 was utilized to evaluate the mass spectra obtained and compared with the NIST library to identify the essential oil components. The peak area computed for each compound was used to calculate the relative content of each constituent. The temperature of the oven and injector was gradually ramped, and the spectra were scanned within the range of 35–500 M/Z ratio. A C_7–C_{30} n-alkane mixture was co-injected to calculate the retention indices.

2.4. Contact toxicity bioassay

The residual film method assessed the toxicity of the essential oils (Bagade et al., 2021; Visakh et al., 2023). Various concentrations of the essential oils were prepared in acetone (HPLC grade) to prepare a residual film, and 1 ml of each concentration was poured onto a Petri plate using a micropipette. The Petri plates were rotated to get an even film of the essential oil on the Petri plates. Five concentrations ranging from 5 to 25 mg/cm^2 were chosen for bioassay against *T. castaneum,* and concentrations ranging from 10 to 50 mg/cm^2 were selected for bioassay against *C. maculatus* after preliminary bioassays. After allowing acetone to evaporate, ten adults of both beetle species were released into the Petri plates. A Petri plate coated with acetone alone was used as a control. A positive control (malathion 50 EC) was also employed to compare the curry leaf essential oil efficacies. Mortality of the insects was recorded after 24 and 48 h. The experiment was replicated thrice for each concentration, and the mortality was corrected using Abbott's formula (Abbott, 1925) and calculated the LC_{50} and LC_{90} values.

2.5. Fumigant toxicity bioassay

Curry leaf essential oils were tested for their fumigant toxicity against the two beetle pests as per the protocol of Wagan et al. (2022). Filter paper discs (2 cm diameter) were impregnated with essential oil at various concentrations. The filter paper discs were then hung from the lids of 250 ml glass bottles. Concentrations ranging from 10 to 50 mg/L air (five concentrations) were tested against red flour beetles, and concentrations from 5 to 25 mg/L air (five concentrations) were tested against pulse beetles. Flasks without essential oils served as control. Tight sealing of the bottle lids was ensured to prevent the leakage of essential oils. LC_{50} and LC_{90} values were computed from the insect mortality data collected after 24 and 48 h. Abbott's formula was used to correct the mortality. Similar to the contact toxicity assay, three replications were maintained at each concentration.

2.6. Repellent activity bioassay

Modified area preference bioassay was tested to check the repellent activity of *M. koenigii* essential oil (Visakh et al., 2022b). A 9 cm diameter Whatman filter paper was divided into two equal pieces. The repellent effectiveness against both insect species was evaluated at concentrations ranging from 0.5 to 5 mg/cm^2 500 μL of each prepared concentration was applied to one-half of the Whatman filter paper. Acetone was applied to the remaining half as control. After 10 min, the two halves were taped together to form a circular disc and kept in a 9 cm Petri dish to allow filter paper to dry. Ten adult beetles were released into the middle of the experiment arena, and the total number of insects in each disc half was recorded at an interval of 2, 4, 6, 12, and 24 h. As in other bioassays, each concentration was replicated thrice.

The formula PR = $(N_C - N_T)/(N_C + N_T) \times 100$ was used to calculate the percent repellency of the curry leaf essential oil against flour beetles and pulse beetles (Chen et al., 2018). PR stands for percent repellence, Nc for the number of insects in the control half, and N_T for the number of test insects in the treatment half in the equation (Chen et al., 2018). Based on the calculated percent repellence, the results were grouped into classes ranging from class 0 (0–0.1 percent repellence) to class V (80–100 percent repellence) to assess the repellent toxicity of curry leaf essential oil.

2.7. Phytotoxicity studies of curry leaf essential oil

The phytotoxic activity was assessed on the germination and radical elongation of paddy seeds (Jaya et al., 2014). These seeds are infested with various stored grain pests, including red flour beetles. It is expected to use these seeds in phytotoxicity assays because they are easily germinable. In the test, the seeds were soaked for half an hour with different concentrations (100, 250, and 500 μg/ml) of curry leaf essential oil (in 0.01% Tween 80). After soaking, the seeds were placed on filter paper in Petri dishes and kept at 25 °C ± 2 °C in the dark. Over a period of five days, the germination of the seeds was observed every 24 h. The root of a seed was considered germinated when it began to protrude. Furthermore, the researchers measured the roots' length up to 120 h (the fifth day) after sowing. Each test was repeated three times with ten seeds in each Petri dish. A positive control (in distilled water only) and a negative control (in 0.01% Tween 80 solution) were kept to normalize the data. The results were recorded as the mean ± standard deviation for germination and root length.

2.8. Data analysis

We employed ANOVA to analyze the results obtained from contact, fumigant, repellency and phytotoxicity studies statistically and Tukey's HSD was used to compare the means. The Polo Plus application was used to calculate the LC_{50} and LC_{90} values for the contact and fumigant toxicity bioassays.

3. Results

3.1. Yield and chemical composition of essential oil by GC-MS/MS

The hydrodistillation of *M. koenigii* (curry leaf) resulted in an essential oil yield of 0.78% ± 0.054 (v/v). A total of 25 chemical compounds were identified from the essential oil, representing the entire composition (Fig. 1, Table 1). The major components in the *M. koenigii* essential oil were Caryophyllene (28.47%), α-guaiene (13.35%), α-phellandrene (12.26%), Humulene (8.90%), Guaia-1 (10),11-diene (5.97%), and α-ocimene (4.67%), accounting for their respective percentages.

3.2. Contact toxicity

The contact toxicity bioassay using the residual film technique revealed that different doses of *M. koenigii* (curry leaf) essential oil showed significant toxicity to adult insects of the red flour beetle and cowpea weevil (Table 2). Fig. 2 shows that as the concentration of the substance increases, the mortality rate of the *T. castaneum* also increases. At the highest concentration tested (25 mg/cm²), the mortality rate was 90% after 24 h of exposure and 96.66% after 48 h of exposure. Similarly, at a dosage of 20 mg/cm², the mortality rate was 93.33% after 24 h of exposure and 96.66% after 48 h of exposure. The lower concentrations tested also showed significant mortality rates compared to the control group (Fig. 2). Fig. 3 shows that the substance tested substantially affects the mortality rate of *C. maculatus* beetles. As the concentration of the substance increases, the mortality rate of the beetles also increases. At the highest concentration tested (50 mg/cm²), the mortality rate was 96.66% after 24 h of exposure and 96.66% after 48 h of exposure. Similarly, the mortality rate was also 70% after 24 h of exposure and 96.66% after 48 h of exposure at a dosage of 40 mg/cm² (Fig. 3).

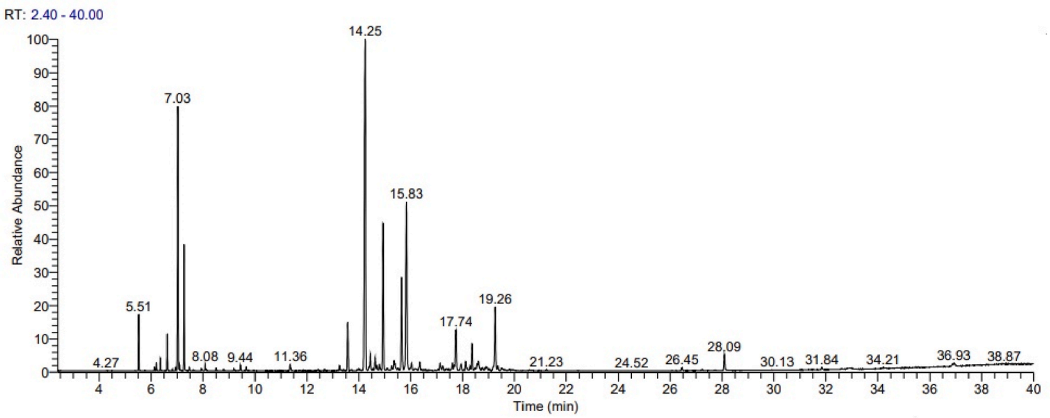

Fig. 1. GC-MS chromatograms of *M. koenigii* essential oil.

Table 1
Chemical composition of *M. koenigii* essential oil.

Peak no.	RT[a]	Chemical compounds	RI[b]	RI[c]	%RA[d]	Identification method
1	5.51	α-pinene	956	957	2.08	MS, RI
2	6.36	α-myrcene	992	983	0.49	MS, RI
3	6.62	Thuja-2,4 (10)-diene	1001	955	1.39	MS, RI
4	7.03	α-phellandrene	1009	1008	12.26	MS, RI
5	7.27	α-ocimene	1035	1047	4.67	MS, RI
6	13.57	β-Elemene	1396	1388	2.68	MS, RI
7	14.24	Caryophyllene	1406	1404	28.47	MS, RI
8	14.45	α-bergamotene	1415	1416	1.12	MS, RI
9	14.63	Alloaromadendrene	1423	1459	0.84	MS, RI
10	14.94	Humulene	1446	1449	8.90	MS, RI
11	15.36	β-guaiene	1453	1478	1.08	MS, RI
12	15.64	Guaia-1 (10),11-diene	1454	1488	5.97	MS, RI
13	15.83	α-guaiene	1458	1456	13.35	MS, RI
14	16.04	β-Copaene	1461	1449	0.64	MS, RI
15	16.35	α-Copaene	1464	1391	0.71	MS, RI
16	17.13	Farnesol	1504	1490	0.58	MS, RI
17	17.60	Spathulenol	1571	1566	0.54	MS, RI
18	17.74	Caryophyllene oxide	1581	1578	3.56	MS, RI
19	17.94	Guaiol	1585	1588	0.61	MS, RI
20	18.12	α-Eudesmol	1598	1631	0.73	MS, RI
21	18.37	Selina-6-en-4-ol	1613	1641	1.67	MS, RI
22	18.61	Cubenol	1642	1643	1.07	MS, RI
23	19.26	Humulane-1,6-dien-3-ol	1687	1596	4.77	MS, RI
24	28.09	Phytol	2012	2099	1.17	MS, RI
25	36.93	Ethyl iso-allocholate	3074	3194	0.65	MS, RI
Total compounds identified (%)					100.00	

[a] Retention time.
[b] Retention index calculated on TG-1MS capillary column.
[c] Retention index in literature.
[d] Relative area % (peak area relative to the total peak area).

Table 2
Contact toxicity of *M. koenigii* essential oils against *T. castaneum* and *C. maculatus* at different exposure times.

Toxicant	Exposure time (h)	LC_{50}[a] (mg/cm^2)	LC_{90}[a] (mg/cm^2)	Slope ± SEM[b]	χ^2 (df)
T. castaneum					
M. koenigii essential oil	24	10.56 (8.98–12.07)	21.18 (17.95–27.17)	4.24 ± 0.58	0.540 (3)
	48	7.31 (5.59–8.79)	17.82 (14.61–24.30)	3.31 ± 0.53	0.120 (3)
Malathion[c] 50 EC	24	0.28 (0.16–0.37)	1.8 (1.1–6.3)	1.12 ± 0.31	0.120 (3)
C. maculatus					
M. koenigii essential oil	24	20.80 (17.54–23.95)	43.99 (36.81–57.88)	3.94 ± 0.55	1.168 (3)
	48	3.95 (0.11–8.25)	23.68 (15.16–46.73)	1.65 ± 0.57	1.690 (3)
Malathion[c] 50 EC	24	0.026 (0.010–0.042)	0.10 (0.057–0.70)	1.23 ± 0.42	2.50 (3)

χ^2: chi square.
[a] Values in parenthesis represent lower and upper confidence limit.
[b] SEM: Standard error of mean.
[c] Positive control.

Probit analysis determined that the LC_{50} and LC_{90} values of *M. koenigii* essential oil against red flour beetle adults after 24 h of exposure were 10.56 and 21.18 mg/cm^2, respectively (Table 2). A positive control (malathion 50 EC) showed that lower LC_{50} and LC_{90} values against red flour beetle adults after 24 h of exposure were 0.28 and 1.8 mg/cm^2, respectively. Similarly, the LC_{50} and LC_{90} values of *M. koenigii* essential oil against red flour beetle adults after 48 h were 7.31 and 17.82 mg/cm^2, respectively. For *C. maculatus* adults, the LC_{50} and LC_{90} values after 24 h of exposure were 20.80 and 43.99 mg/cm^2, respectively. The malathion 50 EC determined that lower LC_{50} and LC_{90} values against *C. maculatus* after 24 h of exposure were 0.026 and 0.10 mg/cm^2, respectively. Likewise, the LC_{50} and LC_{90} values for *C. maculatus* adults after 48 h of exposure were 3.95 and 23.68 mg/cm^2, respectively. The mortality rate increased with longer exposure periods and higher concentrations for both insect pests. *M. koenigii* essential oil exhibited higher contact toxicity against *T. castaneum* adults compared to *C. maculatus* adults at lower dosages (Table 2). These results suggest that the curry lea essential oils tested have significant contact toxicity against red flour beetle and pulse beetle and could be used as an effective insecticide for controlling the infestations of these pests.

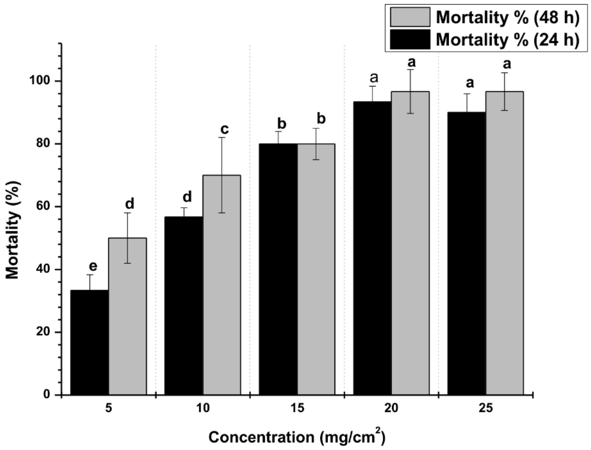

Fig. 2. Contact toxicity of *M. koenigii* essential oils against *T. castaneum* (same letters are not significantly different, P < 0.05).

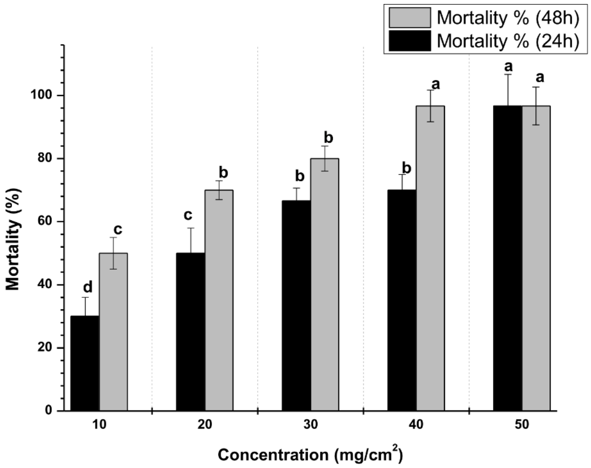

Fig. 3. Contact toxicity of *M. koenigii* essential oils against *C. maculatus* (same letters are not significantly different, P < 0.05).

3.3. Fumigant toxicity

Murraya koenigii (curry leaf) essential oil exhibited significant fumigant toxicity against both stored grain insect pests. The essential oil showed strong fumigant activity against *C. maculatus* (pulse beetle) adults at several doses (1–10 mg/L air). Additionally, fumigation with *M. koenigii* essential oil was also found to be toxic to *T. castaneum* (red flour beetle) adults (Table 3). Fig. 4 suggests that the substance tested has significant fumigant toxicity against *T. castaneum* beetles. As the concentration of the substance increases, the mortality rate of the beetles also increases. At the highest concentration tested (50 mg/L air), the mortality rate was 90% after 24 h of exposure and 96.66% after 48 h of exposure. Similarly, at a concentration of 40 mg/L air, the mortality rate was 93.33% after 24 h of exposure and 96.66% after 48 h of exposure (Fig. 4.). Fig. 5 suggests that the substance tested has significant fumigant toxicity against *C. maculatus* beetles. As the concentration of the substance increases, the mortality rate of the beetles also increases. At the highest concentration tested (25 mg/L air), the mortality rate was 96.66% after 24 h of exposure and 96.66% after 48 h of exposure. Similarly, at a concentration of 20 mg/L air, the mortality rate was 70% after 24 h of exposure and 95% after 48 h of exposure (Fig. 5.).

Table 3
Fumigant activity of *M. koenigii* essential oils against *T. castaneum* and *C. maculatus* at different exposure times.

Test insects	Exposure time (h)	LC_{50} [a] (mg/L air)	LC_{90}[a] (mg/L air)	Slope ± SEM[b]	X^2 (df)
T. castaneum	24	23.93 (15.29–33.61)	58.12 (39.374–206.2)	3.326 ± 0.516	1.396 (3)
	48	17.47 (9.21–23.54)	40.17 (28.34–97.37)	3.541 ± 0.526	4.331 (3)
C. maculatus	24	12.26 (9.73–14.92)	28.07 (20.32–69.05)	3.521 ± 0.531	3.670 (3)
	48	7.55 (5.88–9.84)	18.69 (15.64–25.61)	3.243 ± 0.527	0.589 (3)

χ^2: chi square.
[a] Values in parenthesis represent lower and upper confidence limit.
[b] SEM: Standard error of mean.

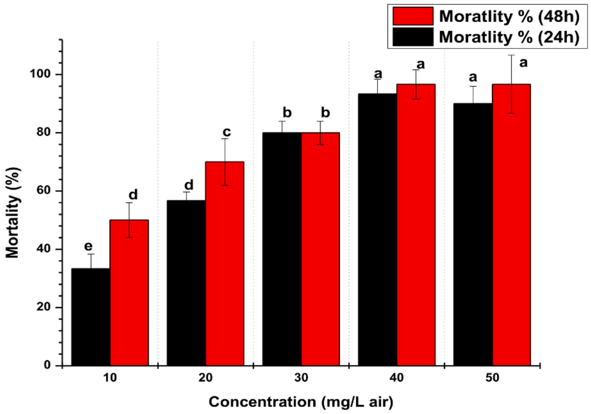

Fig. 4. Fumigant toxicity of *M. koenigii* essential oils against *T. castaneum* (same letters are not significantly different, $P < 0.05$).

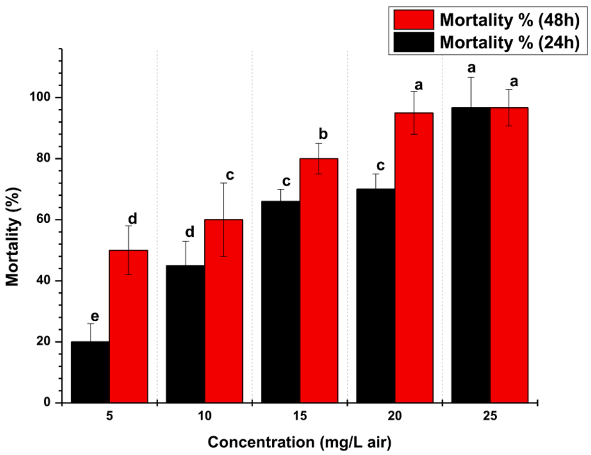

Fig. 5. Fumigant toxicity of *M. koenigii* essential oils against *C. maculatus* (same letters are not significantly different, $P < 0.05$).

The lethal concentrations (LC_{50} and LC_{90}) of *M. koenigii* essential oil were determined using a fumigant toxicity bioassay and the accompanying 95% confidence intervals (Table 3). Probit analysis was used to calculate the LC_{50} and LC_{90} values for each exposure period. LC_{50} values for red flour beetles were 23.93 mg/L air and 17.47 mg/L after 24 h and 48 h of exposure, respectively. Similarly, 50% mortality was observed in *C. maculatus* adults at concentrations of 12 mg/L air and 7.55 mg/L air after 24 and 48 h of exposure, respectively. The results showed that the lethal concentrations decreased with increasing exposure times, indicating a time-dependent effect of *M. koenigii* essential oil. *C. maculatus* adults were more vulnerable to *M. koenigii* essential oil than *T. castaneum* adults, as evidenced by the lower LC_{50} values (Table 3). These findings suggest that the substance tested has potential as an effective fumigant insecticide for controlling infestations of *T. castaneum* and *C. maculatus* beetles.

3.4. Repellent activity

The red flour beetle, *T. castaneum* and the cowpea weevil, *C. maculatus* were both shown to be strongly repelled by the essential oils of *M. koenigii*. The repellent activity of curry leaf essential oils depended on the dose, as shown in Table 4. Adult red flour beetle and pulse beetle were significantly repelled by *M. koenigii* essential oil at all doses and exposure durations ($P > 0.05$, Tukey's HSD tests). The mean percentage repellency (PR) values for *T. castaneum* ranged from over 60% (Class IV) at the lower dose (0.5 mg/cm^2) at 2 h–24 h post-exposure. In contrast, *M. koenigii* essential oils repelled only 37.3% (Class II) of pulse beetle adults at the lower dose (0.5 mg/cm^2) at 2 h–24 h after exposure. At a greater concentration (5 mg/cm^2) of *M. koenigii* essential oil, which was considerably repellent to *T. castaneum*, a mean PR of 90% (Class V) was seen. At the maximum applied dose (5 mg/cm^2), adult *C. maculatus* demonstrated 80% PR (Class V). Overall, a moderate level of repellency (Class II-IV) was observed for *M. koenigii* essential oils against *C. maculatus* adults 2 h–24 h after exposure, while *M. koenigii* essential oils exhibited strong repellency (Class IV-V) against *T. castaneum* adults. There was a significant difference in the repellent activities of *M. koenigii* essential oil against *T. castaneum* and *C. maculatus*, with the former showing higher sensitivity to *M. koenigii* essential oil compared to the latter (Table 4).

3.5. Phytotoxicity of curry leaf essential oils

The effects of curry leaf essential oil at various concentrations (100, 250, and 500 μg/mL) on paddy seed germination at different periods (48 h, 72 h, 96 h, and 120 h) are shown in Tables 5 and 6. In addition to the curry leaf essential oil treatment, distilled water

Table 4
Repellent activity of *M. koenigii* essential oils against *T. castaneum* and *C. maculatus* at different exposure times.

	Dose (mg/cm^2)	Repellence percent of treatments after					Mean repellence	Repellentclass
		2 h	4 h	6 h	12 h	24 h		
T. castaneum	0.5	50 ± 13.3[a]	60.0 ± 6.6[a]	56.7 ± 11.5[a]	66.7 ± 6.6[a]	73.3 ± 11.5[a]	61.3 ± 3.7[b]	IV
	1.5	53.3 ± 17.6[a]	66.7 ± 13.3[a]	73.3 ± 11.5[a]	76.7 ± 6.6[a]	80.0 ± 13.3[a]	70.0 ± 4.5[ab]	IV
	2.5	60 ± 13.3[a]	76.7 ± 6.6[a]	70 ± 13.3[a]	83.3 ± 6.6[a]	86.7 ± 17.6[a]	75.3 ± 3.8[ab]	IV
	3.5	76.7 ± 13.3[a]	80.0 ± 11.5[a]	83.3 ± 17.6[a]	93.3 ± 11.5[a]	90.0 ± 13.3[a]	84.6 ± 2.7[ab]	V
	5	83.3 ± 6.6[a]	86.7 ± 13.3[a]	90 ± 13.3[a]	93.3 ± 13.3[a]	96.7 ± 6.6[a]	90.0 ± 3.4[a]	V
	F value	1.09	0.67	0.75	0.81	1.02	2.72	
	P value	0.63	0.37	0.51	0.35	0.31	0.01	
	d.f	4	4	4	4	4	20	
C. maculatus	0.5	13.3 ± 6.6[a]	33.3 ± 13.3[a]	33.3 ± 6.6[a]	50.0 ± 6.6[a]	56.7 ± 11.5[a]	37.3 ± 6.4[c]	II
	1.5	30.0 ± 11.5[a]	33.3 ± 11.5[a]	50.0 ± 13.3[a]	73.3 ± 17.6[a]	76.7 ± 11.5[a]	52.6 ± 4.2[bc]	III
	2.5	46.7 ± 6.6[a]	50.0 ± 6.6[a]	63.3 ± 6.6[a]	80.0 ± 6.6[a]	86.7 ± 6.6[a]	65.3 ± 3.5[abc]	IV
	3.5	53.3 ± 13.3[a]	60.0 ± 13.3[a]	66.7 ± 11.5[a]	76.7 ± 11.5[a]	90.0 ± 17.6[a]	69.3 ± 6.7[ab]	IV
	5	60.0 ± 11.5[a]	76.7 ± 6.6[a]	80.0 ± 6.6[a]	86.7 ± 6.6[a]	96.7 ± 13.3[a]	80.0 ± 7.4[a]	V
	F value	3.37	1.35	1.64	3.54	5.14	6.06	
	P value	0.05	0.11	0.25	0.17	0.01	0.005	
	d.f	4	4	4	4	4	20	

*Means within the same column followed by same letter are not significantly different ($p < 0.05$).

Table 5
Effect of curry leaf essential oil on germination (%) of seeds of treated paddy seeds.

Concentration (μg/mL)	Seed germination (%) of treatments after			
	48 h	72 h	96 h	120 h
100	60.0 ± 5.6a	73.3 ± 3.9a	80.0 ± 3.2a	93.3 ± 11.2a
250	66.7 ± 1.9a	76.7 ± 1.8a	83.3 ± 1.9a	90.0 ± 1.9a
500	66.7 ± 6.3a	76.7 ± 3.2a	86.7 ± 3.0a	90.0 ± 1.9a
[a]Control	70.0 ± 5.6a	73.3 ± 3.2a	90.0 ± 6.2a	93.3 ± 6.2a
[b]Positive control	73.3 ± 3.0a	80.0 ± 1.8a	83.3 ± 6.2a	96.7 ± 1.8a

*Means within the same column followed by same letter are not significantly different ($p < 0.05$).
[a] 0.01 % Tween 80.
[b] Distilled water.

Table 6
Effect of curry leaf essential oil on seedling radicle growth of treated paddy.

Concentration (μg/mL)	Seedling growth of treatments after			
	48 h	72 h	96 h	120 h
	Radicle (cm)			
100	2.2 ± 0.54[a]	2.5 ± 0.41[a]	4.1 ± 0.70[a]	4.2 ± 0.42[a]
250	2.3 ± 0.14[a]	3.2 ± 0.52[a]	3.7 ± 0.32[a]	4.3 ± 0.54[a]
500	1.8 ± 0.83[a]	2.7 ± 0.54[a]	3.8 ± 0.43[a]	4.1 ± 0.89[a]
[a]Control	2.1 ± 0.83[a]	3.1 ± 0.70[a]	3.5 ± 0.54[a]	4.0 ± 0.54[a]
[b]Positive control	2.0 ± 0.43[a]	2.7 ± 0.44[a]	3.8 ± 0.70[a]	4.3 ± 0.03[a]

*Means within the same column followed by same letter are not significantly different ($p < 0.05$).
[a] 0.01 % Tween 80.
[b] Distilled water.

treatment was used as a positive control. The data indicate that the germination of paddy seeds treated with curry leaf essential oil is comparable to the control and favorable control treatments at all concentrations and time intervals. The germination percentage of treated seeds and control seeds is not significantly different ($p < 0.05$). Based on the study's results, curry leaf essential oil application at the tested concentrations did not adversely affect paddy seed germination.

Curry leaf essential oil was tested at various concentrations (100, 250, and 500 μg/mL) on the growth of paddy seedlings over a period of 120 h. After 48, 72, 96, and 120 h of treatment, the radicle (root) length was measured. Compared with the control and positive controls (distilled water and 0.01% Tween 80, respectively), essential oil had no significant effect on the radicle length of paddy seeds. In summary, the results indicate that curry leaf essential oil has no or little phytotoxic effect on the growth of paddy seedlings, and this effect is concentration-dependent.

4. Discussion

Indeed, a key area of research in pest management is the hunt for environmentally suitable substitutes for synthetic pesticides. Studies have been conducted on the insecticidal and insect-repellent activities of essential oils derived from aromatic plants against various insect pests. Because essential oils are very volatile, they can control stored insects through different insecticidal actions, which is one benefit of employing them as pesticides (Abdelgaleil, 2020; Isman, 2000; Nerio et al., 2010). The danger of infestation can be decreased by using essential oils as repellents to keep insects away from grains or other goods that are being kept (Said-et al., 2017).

According to the GC-MS/MS study, the essential oils extracted from *M. koenigii* contained significant amounts of caryophyllene (28.47%), guaiene (13.35%), phellandrene (12.26%), humulene (8.90%), guaia-1 (10),11-diene (5.97%), and ocimene (4.67%). These results are in line with other investigations (Rao et al., 2011; Taylor et al., 2014; Verma et al., 2012), which also characterized similar phytochemical substances such as caryophyllene, α-pinene, caryophyllene oxide, α-phellandrene, and humulene. It is significant to highlight that discrepancies in oil yields and chemical composition shown among studies may be explained by genotypic variances and environmental effects such as climate, extraction methods, collection time, soil composition, and extraction methods (Visakh et al., 2022a).

Adult *T. castaneum* and *C. maculatus* insects were shown to be resistant to the contact toxicity of *M. koenigii* essential oils. According to other research that revealed contact toxicity of *M. koenigii* essential oil against *T. castaneum* adult insects in particular, *M. koenigii* essential oils displayed substantial contact toxicity at low dosages against *C. maculatus* adults (Bagade et al., 2021). Saxena and Sayyed (2018) study on the significant contact toxicity of *M. koenigii* essential oil against pulse beetles lends weight to their findings (Saxena and Sayyed, 2018). The findings of other studies also supported the high contact toxicity of *M. koenigii* essential oil against a variety of insect pests, including *Sitophilus oryzae*, *Plutella xylostella, Rhyzopertha dominica*, and *T. castaneum* (Shawer et al., 2022; Kumar, 2016; Reddy et al., 2016). According to the study's findings, the bioactive compounds in the evaluated essential oils may interact with several insect target sites and make them toxic (Devi et al., 2020).

According to the fumigant toxicity testing results, *M. koenigii* essential oil significantly reduced red flour beetle and pulse beetle mortality after being exposed to various doses and exposure times. However, compared to adults of *T. castaneum, C. maculatus* was more sensitive to *M. koenigii* essential oil. These findings are in line with earlier research that showed *M. koenigii* essential oil to have fumigant capabilities against *T. castaneum* and other *Murraya* spp. to have fumigant characteristics against red flour beetle, *T. castaneum* (Kumar, 2016; Li et al., 2010; You et al., 2015). Similarly, in a study conducted on the fumigant toxicity of various citrus fruit essential oils against pulse beetles, which also belong to the same Rutaceae family as *M. koenigii*, *C. latiolia* essential oil showed an LC_{50} value of 10.02 mg/L air. While at the same 48-h exposure duration, the LC_{50} values for the essential oils from *C. reticulata, C. sinensis*, and *C. paradisi* were 12.68 mg/L air, 12.98 mg/L air, and 12.63 mg/L air, respectively (Dutra et al., 2016). According to this study, *M. koenigii* essential oil had an LC_{50} of 12.26 mg/L air against *C. maculatus* adults, which is better than that of other Citrus essential oils.

The repellent activity of *M. koenigii* essential oil was tested against stored grain insect pests, and it was found that the essential oil repelled both red flour beetles and pulse beetles in a dose-dependent manner. The essential oil extracted from *M. koenigii* leaves proved effective as a repellent against these insect pests. This is consistent with previous studies that have shown the repellency of *M. koenigii* essential oil against *T. castaneum* adults (Bagade et al., 2021; You et al., 2015) and *C. maculatus* (Saxena and Sayyed, 2018). Similar repellent properties have also been reported for essential oils from other plants such as *Plectranthus zeylanicus*, *Cinnamomum zeylanicum*, *Micromelum minutum*, and *Citrus maxima* against red flour beetle and pulse beetles (Balachandra et al., 2012; Paranagama and Gunasekera, 2011; Visakh et al., 2022b). Further studies should be conducted to evaluate the bioactivity of individual components of *M. koenigii* essential oil and their interactions with storage pests. Additionally, the level of repellency can directly impact the reduction of oviposition and development of adults (França et al., 2012), highlighting the potential of *M. koenigii* essential oil as a repellent against stored product pests such as red flour beetle and pulse beetles.

The present study examined the phytotoxic effects of curry leaf essential oil on paddy seeds. According to the results, all concentrations and time intervals of the essential oil tested did not inhibit paddy seed germination, indicating its safety in agriculture. The findings are consistent with previous studies showing the safety of different *Citrus* spp. essential oils belonging to the same family, Rutaceae, on germination of other plants, such as wheat (*Triticum aestivum*) (Visakh et al., 2022a).

Furthermore, there was no significant effect of the essential oil on the radicle length of treated paddy seeds, indicating it has no or little phytotoxic effect on paddy seedlings. The findings are consistent with previous studies reporting the safety of essential oils of *Ageratum conyzoides* L., *Coleus aromaticus* Benth. and *Hyptis suaveolens* (L.) on the growth of wheat (*Triticum aestivum*) seedlings (Jaya et al., 2014). Overall, this study suggests that curry leaf oil can be an alternative to synthetic pesticides to manage stored grain pests without affecting rice seeds' germination and growth. These results are consistent with prior research that found that curry leaf essential oil has insecticidal and repellant activities against various pests that attack stored grains, such as red flour beetles (*T. castaneum*) and pulse beetles (*C. maculatus* (Visakh et al., 2022b).

5. Conclusion

Efforts are being made to develop environmentally friendly alternatives for synthetic insecticides, and natural plant-derived substances such as essential oils have emerged as one promising option. Notably, our present studies on the insecticidal and repellent potential of curry leaf essential oils have been demonstrated against stored grain insect pests, making them an effective, economical, and storable grain protection treatment. The current study proves that curry leaf essential oil does not harm paddy seeds during germination or growth. In order to optimize their use, further research is necessary, including identifying the most effective oils, concentrations, and application methods for various pests and commodities, as well as evaluating the safety, long-term efficacy, and resistance potential of these compounds. Nevertheless, curry leaf essential oils can serve as an eco-friendly alternative to synthetic insecticides in sustainable agriculture pest management. In the context of advancing the practical utilization of essential oil-based bio-pesticides, it is imperative to undertake additional studies that concentrate on elucidating the interactions between secondary metabolites and the consequent toxicity towards non-target organisms.

CRediT authorship contribution statement

Naduvilthara U. Visakh: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. **Berin Pathrose:** Conceptualization, Project administration, Supervision, Validation, Writing – review & editing. **Arunaksharan Narayanankutty:** Conceptualization, Software, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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