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Research Article

EFFECT OF *LANTANA CAMARA* L. ESSENTIAL OIL: A NATURAL SOLUTION FOR MANAGING STORED GRAIN INSECTS

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ABSTRACT

Grain production increases annually, yet stored grain pests pose a persistent challenge to agriculture. While pesticides have traditionally been the primary solution, their improper and widespread use has led to the development of resistance in some pests, rendering them ineffective. Consequently, essential oils have emerged as a promising alternative to address this issue. In this research, the insecticidal and repellent properties of essential oil extracted from *L. camara* were assessed against significant stored grain pests viz., *Sitophilus oryzae* (rice weevil), (rice weevil), *Rhyzopertha dominica* (dhal beetle), and *Carpophilus dimidiatus* (peanut beetle), aiming to determine its potential as a viable method for safeguarding stored grains against the most prevalent pests. The results were promising, however, the underlying mechanism of action have not yet been fully understood. Allocating resources towards researching alternative methods of pest control can significantly enhance sustainable agricultural practices.

Keywords: Lantana, Essential oil, Pest management, Stored grain insects.

INTRODUCTION

The issue of food loss due to pest infestation during storage is a significant challenge across societies, both in developed and developing countries, resulting in substantial financial losses. Stored cereals serve as a vital food source for numerous insects, mites, and fungi, leading to quality degradation and causing net losses ranging from 9 to 20%. Approximately 1660 insect species worldwide are known to impact the quality of stored food products. Despite the severity of this problem, insufficient research funding is allocated to mitigate these losses. Since the 1960s, synthetic contact pesticides have been the primary method for controlling stored product pests. However, the use of these pesticides has faced increasing criticism due to concerns such as the emergence of resistance, elevated risks of environmentally and health-damaging residues, leading to their restricted use. Environmental worries and demands for food safety have highlighted the necessity for alternative approaches. Over the past few decades, plant essential oils have emerged as potential alternatives for various applications, including antimicrobial, antifungal, or herbicidal uses. Notably, essential oils also demonstrate promising properties for replacing synthetic insecticides. Isman and Grieneisen (2014) revealed a significant increase in the proportion of papers on botanicals among all papers on insecticides, indicating growing interest in essential oils as alternatives to synthetic pesticides. Essential oils possess characteristics such as high volatility, sensitivity to temperature and UV light degradation, rendering them less persistent in the environment than traditional pesticides. Additionally, most essential oils exhibit low mammalian toxicity compared to synthetic insecticides and are considered environmentally friendly. For example, studies have shown that eugenol is significantly less toxic than synthetic insecticides like pyrethrum and azinphosmethyl. Several studies have explored the efficacy of essential oils as alternative insecticides against the stored grain insects. Some have demonstrated high fumigation toxicity, while others have highlighted contact toxicity through topical application. Additionally, the repellency potential of essential oils against these insects has been analyzed, along with their ability to deter food consumption. Despite promising research findings, there have been few actual applications for protecting stored foodstuffs using essential oils. Moreover, there is still a need for systematic screening of potentially active oils under conditions that mimic storage realities and with standardized insect strains. Our

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study aims to address this gap by testing *L. camara* essential oil against important stored grain insects.

MATERIALS AND METHODS

Extraction of essential oil

L. camara leaves were collected from Valparai, Tamil Nadu (India). Leaves were processed, shade dried and hydro-distilled at higher temperature in order to isolate the active fraction from the oil glands present in the leaves. The granulated *L. camara* leaves were packed with a sufficient quantity of water and boiled. The influence of hot water and steam liberate essential fraction from the oil glands present in the plant tissue. The vapour mixture of water and oil is condensed by indirect cooling with water. The water content of essential oil is nullified by extraction with n-hexane. At this point, the essential oil separates from water and floats to top. The top layer was collected by using separating funnel and used for GC-MS-MS analysis and bioassay studies.

GC-MS-MS analysis

GC-MS-MS analysis was performed on a Varian 4000 MS coupled with a Varian 3800 GC, equipped with a cross linked 5% Phenyl 95% dimethyl polysiloxane VF-5MS capillary column (30 m x 0.25 mm i.d, film thickness, 250nm) and operating under the conditions as mentioned below: The oven temperature was programmed as 60°C (10 min), 60°C - 220°C (4°C/min), 220°C (10 min) and 220°C - 240°C (1°C/min). Injector and detector temperatures were maintained at 60°C and 240°C respectively. The amount of the sample injected was 1.0 µl in the splitless mode. Helium was used as carrier gas with a flow rate of 1ml/min.

Identification of phytocompounds

Interpretation on mass-spectrum of GC-MS-MS was conducted using the database of National Institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, molecular formula, retention time and retention indices of the components of the test materials were ascertained.

Bioassay of essential oil against stored grain pests

The experiment aimed to assess the effectiveness of *L. camara* essential oil as an insecticide against stored grain insects and to determine the concentration at which it exhibits optimal efficacy. Objective: To determine the efficacy of *L. camara* essential oil at different concentrations (ranging from 10 ppm to 30 ppm) on *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Carpophilus dimidiatus*. Experimental Setup: Location: ICFRE - Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu. Insects Used: Adult beetles of *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Carpophilus dimidiatus*, collected from a warehouse in Coimbatore. Bioassay Method: The bioassay was performed using filter paper discs (1 cm^2) infused with three different concentrations of *L. camara* essential oil (10 ppm, 20 ppm, and 30 ppm). Control: N-hexane was used as a control. Replications: Three replications were performed for each concentration to minimize errors. Observations: Knockdown Effect: The knockdown effect was observed continuously for 3 hours to determine the knockdown time (Kt_{50}), which is the time taken for 50 percent of the insects to become inactive or knocked down. Insecticidal Effect: Mortality rates were observed after 24 hours of treatment to determine the insecticidal effect. Statistical Analysis: LC_{50} Value: One-way ANOVA was performed to analyze the LC_{50} (lethal concentration 50) values. Kt50 Value: Kruskal-Wallis test was performed to analyze the Kt_{50} values. Tools Used: SPSS 25 (version 25) was used for statistical analysis.

RESULTS AND DISCUSSION

L. camara leaves extract analysis revealed the presence of triterpenoids, steroids, alkaloids, flavonoids, tannins etc. and the yield of esential oil is found to be a maximum of 0.44±0.02 percent in Valparai.The essential oils are being classified as potential biocontrol agents for pest and disease management. Extracts from the leaves tested to possess antimicrobial, fungicidal, insecticidal and nematicidal activity according to geographic origin of the plants. Those properties of natural chemicals could act an alternative pesticidal, and to be utilized on a large scale production and formulation of promising botanical insecticides. The essential oils, such as eugenol, linalool and geraniol give promising larvicidal activity. The monoterpenes E-anethol and E-nerolidol were found to be the active principles of the most toxic essential oils. Among monoterpenes they found that (1R) - (+) - a-pinene and (1S)-(-) - α-pinene were most toxic while menthone, 1, 8-cineole, linalool and terpineol were less toxic (Kaan, *et al*., 2016). Kanat and Alma (2004) reported that essential oils of wood turpentine, thyme herb, cypress berry and styrax are most effective in terms of mean mortality time against larvae of pine processionary moth.

The essential oil from leaves of *L. camara* was processed, extracted and purified and subjected to GCMS/MS analysis for metabolite profiling. The spectral character was interpreted on mass-spectrum of GC-MS-MS by using the database of National Institute Standard and Technology (NIST) having the reference of 62,000 profiles. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, molecular formula, retention time and retention indices of the components of the test materials were ascertained. Oil fraction analysed by GC/MS/MS has revealed major compounds of the said nature. About thirty six compounds (Table 1) were characterized from essential oil of *L.camara*, some of them like α- Copaene, Germacrene D & B, α – Cubebene, β – Elemene, α – Guaiene, α – humulene, Aromadendrene, β – Selinene, α – Selinene, Caryophyllene oxide, Nerolidol, Spathulenol and Delta – Cadinene, have expressed tritrophic interactions as reported by earlier findings as well as insecticidal activity in terms of larval mortality against teak defoliator (Table 2, Figure 1-2). Some of the compounds such as Isotridecanol, phytol, Lycopersen, Isochiapin B and Serverogenin acetate are known for their application in pharmacology (Abirami and Rajendran, 2011).

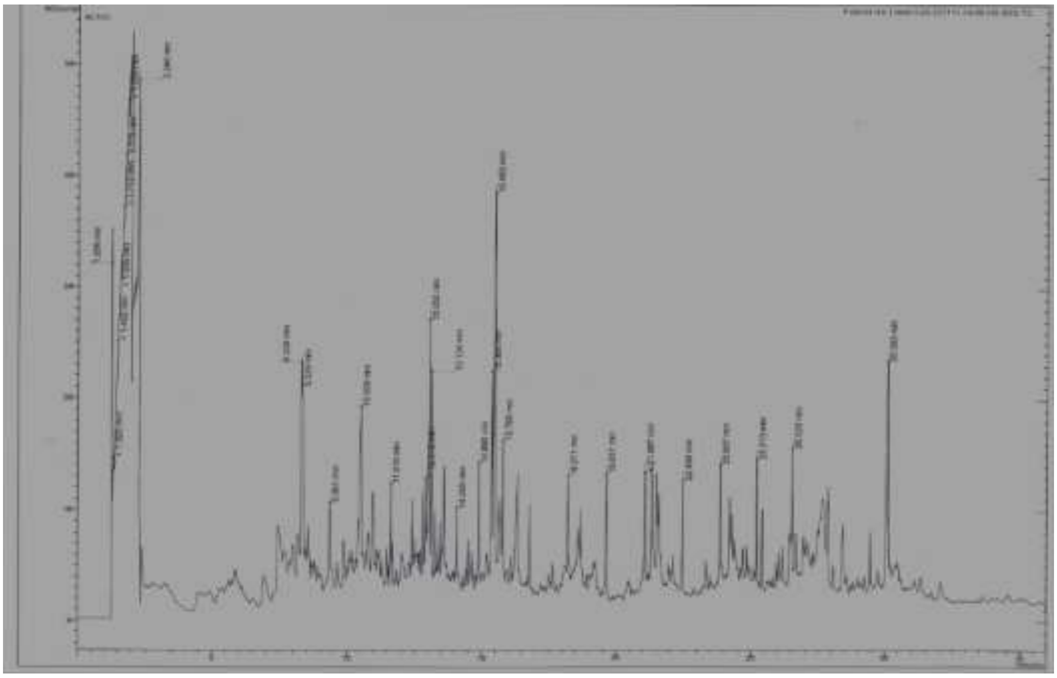
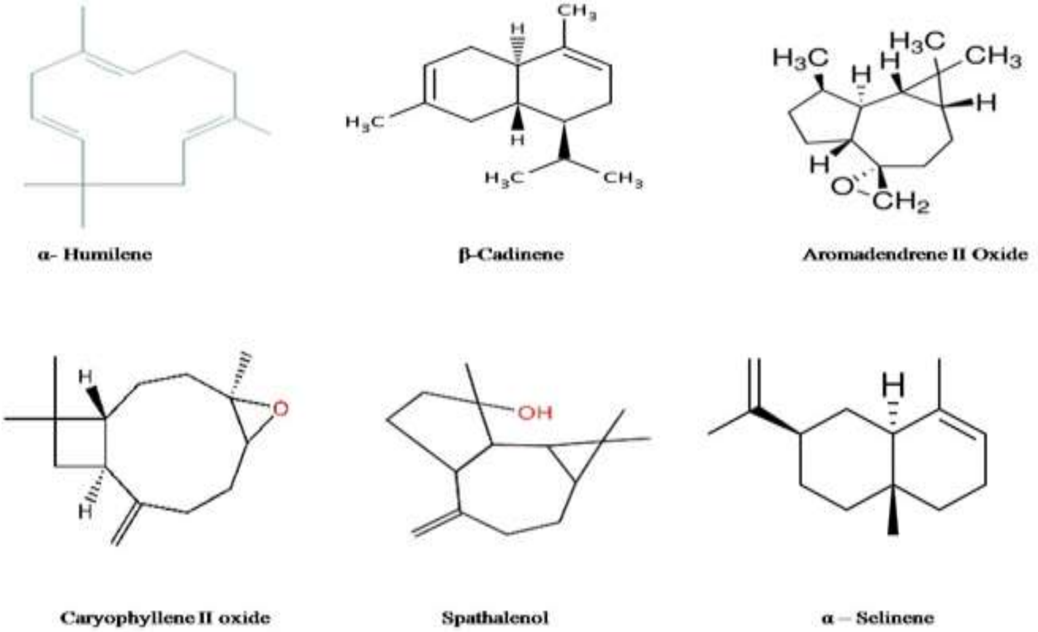


Figure 1. GC-MS-MS chromatogram of essential oil of *L. camara*.



α- Humilene β-Cadinene Aromadendrene II Oxide

Caryophyllene II oxide Spathalenol α – Selinene

Figure 2. Structure of individual compounds of *Lantana camara* essential oil.

The efficacy of essential oil at different concentration ranging from 10 ppm to 30 ppm was tested on three major stored grain insects viz., *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Carpophilus dimidiatus* in terms of insecticidal properties and knockdown effect. In this bioassay on the adult beetles of said insects, the knockdown time ($Kt_{50)}$ was ranged between 72 to 124 minutes when compared to control as 168 minutes to made 50 percent of beetles to become inactive (Table 3). The high mortality was observed on *S. oryzae* followed by *C. demidiatus* and *R. dominica* with the mean mortality of 15.33 to 19.66 within 24 hrs of treatment for all the treatment groups. The LC50 value was arrive as 7.196 ppm for *S. oryzae*; followed by 13.17ppm for *R. dominica* and 17.579 ppm for *C. dimidiatus* (Table 4). Significant mortality rate was observed in 10ppm of *L.camara* essential oil that confirms the low dose of essential oil of *L.camara* is fair enough to manage the stored grain insects. It was also observed that the cumulative mortality was observed to be increased with increase in concentration and the time taken to kill fifty percent of the population is ranged between 7 to 17 ppm. The feeding behaviour, knockdown effect and mortality rate depends on the dose of toxic metabolites in the essential oil of *L. camara* exposed to the insects. It is confirmed that the *L.camara* essential oil produces high impact on motility and feeding ability of the insects significantly which may be the concentration of secondary metabolites in the essential oil. The group of metabolites belongs to sesquiterpenes are distributed predominantly in oil but their concentrations are substantial to have an impact on insect pests.

Table 1. GC-MS/MS profiles of *Lantana camara* essential oil.

S.No	**Retention Time**	**Retention indices**	**Name of the compound**	**Peak Area**	**Molecular weight**	**Molecular formula**
1	26.486	802	Bicycloelemene	0.970	204	$C_{15}H_{24}$
2	27.005	818	α – Cubebene	4.226	204	$C_{15}H_{24}$
3	28.017	830	α – Copaene	1.187	204	$C_{15}H_{24}$
4	28.487	879	β – Elemene	2.404	204	$C_{15}H_{24}$
5	29.653	900	Bicyclo [5.2.0] Nonan, 2-Methy	4.509	204	$C_{15}H_{24}$
6	29.826	850	Germacrene B	3.179	204	$C_{15}H_{24}$
7	29.992	877	α – Guaiene	4.268	204	$C_{15}H_{24}$
8	30.704	855	α – Humulene	3.463	204	$C_{15}H_{24}$
9	30.820	906	Aromadendrene	6.257	204	$C_{15}H_{24}$
10	31.290	877	Napthalene	1.472	204	$C_{15}H_{24}$
11	31.499	905	Germacrene D	4.109	204	$C_{15}H_{24}$
12	31.754	912	β – Selinene	1.635	204	$C_{15}H_{24}$
13	31.839	884	Epi – Bicyclosesquiphellandren	0.977	204	$C_{15}H_{24}$
14	31.976	873	α – Selinene	6.747	204	$C_{15}H_{24}$
15	32.575	770	1-Hydroxy-1, 7-dimethyl-4-iso	2.119	222	$C_{15}H_{26}O$
16	32.636	911	β – Cadinene	3.600	204	$C_{15}H_{24}$
17	33.662	845	- Caryophyllene oxide	1.127	220	$C_{15}H_{24}O$
18	33.988	887	Nerolidol	4.841	222	$C_{15}H_{26}O$
19	34.924	833	Salvial – 4 (14) – en – 1 – one	2.862	220	$C_{15}H_{24}O$
20	34.980	877	Veridifloral	0.764	222	$C_{15}H_{26}O$
21	35.444	761	12-Oxabicyclo [9.1.0] dodeca – 3	2.296	220	$C_{15}H_{24}O$
22	35.574	777	1 – Napthalenamine, 4 – bromo	1.321	220	$C_{15}H_{24}O$
23	35.932	796	(-) – Spathulenol	4.087	220	$C_{15}H_{24}O$
24	36.064	839	Isospathulenol	1.632	220	$C_{15}H_{24}O$
25	36.208	830	Tetracyclo [6.3.2.0 (2,5) .0 (1,	1.188	220	$C_{15}H_{24}O$
26	36.303	856	Delta – Cadinene	2.399	204	$C_{15}H_{24}$
27	36.450	860	1-Napthalenol, 1, 2, 3, 4, 4a, 7	2.596	222	$C_{15}H_{26}O$
28	36.707	831	1R, 4S, 7S, 11R-2, 2, 4, 8 – Tetrame	3.211	204	$C_{15}H_{24}$
29	36.816	776	Alloaromadendrene Oxide – (2)	1.220	220	$C_{15}H_{24}O$
30	37.158	808	Aromadendrene Oxide– (2)	3.592	220	$C_{15}H_{24}O$
31	37.675	768	6-isopropenyl-4,8a-dimethyl-	1.407	220	$C_{15}H_{24}O$
32	38.122	750	4,4 – Dimethyl – 3 – (3 – methyl but -	5.599	202	$C_{15}H_{22}$
33	38.229	770	1H – Cycloprop [e] azulen – 7 – ol,	4.970	220	$C_{15}H_{24}O$
34	39.806	734	6-isopropenyl-4,8a-dimethyl-	0.952	220	$C_{15}H_{24}O$
35	41.920	948	Phthalic acid, butyl hexyl e	0.958	306	$C_{18}H_{26}O_4$
36	47.777	853	2-Hexadecen – 1 – ol, 3, 7, 11, 15-	1.859	296	$C_{20}H_{40}O$

Table 2. Biological properties of individual compounds of *L. camara* essential oil

S. No	**Compound name**	**Biological properties**
1	α- Copaene	Pest attractant
2	β – Elemene	Anti-carcinogenic
3	α – humulene	Anti-carcinogenic

4	Aromadendrene II oxide	Antimicrobial
5	β – Selinene	Anti-bacterial
6	α – Selinene	Has pharmacological uses
7	Caryophyllene II oxide	Antifungal, anti-inflammatory, analgesic and attractants to predators.
8	Spathulenol	Immunoinhibitory molecule.
9	Delta – Cadinene	Insecticide
		Abirami and Rajendran, 2011

This study evaluates the efficacy of *L. camara* essential oil against three prevalent grain insects: *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Carpophilus dimidiatus*, commonly found in Coimbatore, Tamil Nadu Warehouses. Notably, the essential oil exhibits significant insecticidal potential, with higher toxicity observed towards *S. oryzae* (rice weevil), followed by *R. dominica* (dhal beetle) and *C. dimidiatus* (peanut beetle). The calculated LC_{50} value stands at 7 ppm. Previous research by Demeter *et al*. (2021) investigated the toxicity of twenty-five essential oils on the granary weevil, *Sitophilus granarius*, revealing garlic essential oil to have a KD_{50} of 1.32mg/cm^2. Studies by Likhoshvai and Ratushny (2007), Yang *et al*. (2011), and Huang *et al*. (2000) have also described the toxicity of various essential oils on stored product pests like *Tenebrio molitor*, *Sitotroga cerealella*, *Tribolium castaneum*, and *Sitophilus zeamais*. The efficacy of essential oils can vary based on the target species, life stage, and exposure method. Several studies have highlighted the toxicity of essential oils from *M. avensis*, *G. procumbens*, and *E. dives*, as well as geraniol, the main compound of *T. vulgaris* essential oil, against stored product pests. Moreover, Yazdgerdian *et al*. (2015) found *G. procumbens* essential oil to be highly toxic to *S. oryzae*, aligning with the findings of this study. However, inconsistencies in protocols and lack of detailed compound descriptions hinder reliable comparisons across studies. Zohry *et al*. (2020) tested the toxicity of ten essential oils on *S. granarius* using a protocol similar to ours but provided limited information on essential oil compositions, impeding deeper comparisons. Future studies on the industrial potential of essential oils should adopt standardized protocols, considering media influences, and provide comprehensive essential oil composition descriptions. Despite numerous studies on essential oil toxicity against stored product pests, limited data exists on the mechanisms of their insecticidal effects as complex mixtures of molecules. Nonetheless, some studies have suggested potential mechanisms, such as the impact of α-caryophyllene on various enzyme activities in *Aphis gossypii* (Yu-qing, 2010). Essential oils' intricate compositions may lead to synergistic effects on insect physiology, necessitating holistic analyses of their impact. α-Phellandrene has been suggested to exert a neurotoxic effect on *Lucinia cuprina* (Chabaan *et al*., 2019), while diallyl disulfide has been shown to influence the digestion process of *Ephestia kuehniella* by reducing the activity of digestive enzymes (Shahriari *et al*., 2017). Furthermore, diallyl trisulfide, another significant compound found in garlic essential oil, has recently been identified as a regulator of the expression of the chitin synthase A gene, leading to alterations in morphology and inhibition of oviposition in *Sitotroga cerealella* (Sakhawat *et al*., 2020). Essential oils are complex mixtures of molecules, which may interact and synergize in their mechanisms of action. Therefore, it is crucial to analyze their impact on insects comprehensively, considering their effects on the insect as a whole.

Table 3. Effect of *Lantana camara* leaf essential oil on stored grain insects in terms of mortality and Knockdown effect.

Store product insects	**Concentration in ppm**	**Mortality @24hrs. Mean ±SE**	**Knockdown time (min) Kt_{50}**	**Percent mortality corresponding to Kt_{50}**
Rhyzoperthadominica	10	15.66 ± 0.33^b	124	28.6
	20	16.66 ± 0.33^b	96	71.4
	30	18.66 ± 0.33^d	72	100
Sitophilus oryzae	10	17.0 ± 0.55^c	116	36.4
	20	19.0 ± 0.57^e	92	68.2
	30	19.66 ± 0.33^e	72	100
Carpophilus dimidiatus	10	15.33±0.33^b	120	57.1
	20	18.33±0.33^d	96	85.7
	30	18.66±0.33^d	72	100
Control (n-hexane)		1.66±0.33^a	168	4.5
F		16.38	18.24	
df		9	9	

Significance	0.017	0.026

Mean followed by the same letter in column indicates that not significant at 5% level (P value<0.05) by DMRT (ANOVA)

Table 4. Probit analysis (SPSS version 25.) to arrive at LC_{50} value for the management of stored grain insects using essential oil of *L. camara.*

Stored grain insects	**LC_{50}**	**95% confidential limit**		**Chi square**	**Significance**
		LL	**UL**		
Sitophilus oryzae	7.196	6.07	9.87	17.429	0.000
Rhyzoperthadominica	13.179	10.82	18.77	14.030	0.000
Carpophilus dimidiatus	17.579	16.48	22.11	8.045	0.005

Firstly, given that mortality occurs shortly after insect introduction, there may be a strong selection effect favoring resistant individuals, resulting in only the more resistant individuals surviving after 24 hours. Secondly, the absorption of essential oils by the carrier material (filter paper) could diminish the bioavailability of active compounds, potentially reducing long-term efficacy. Lee *et al*. (2004) observed that the fumigation toxicity of certain essential oils is notably reduced in the presence of wheat due to absorption phenomena, suggesting a similar effect may occur in our experiments. Thirdly, our experiment was conducted at room temperature (30°C), where the rapid evaporation rate of essential oils may lead to a significant loss of active compounds within 6 hours. Heydarzade *et al*. (2012) highlighted the low persistence of essential oils from *Teucrium polium* and *Foeniculum vulgare,* where treated filter paper induced 99% mortality immediately but 0% after 30 hours, likely due to high volatility or rapid degradation of active compounds. Future studies should investigate the combined influence of evaporation and absorption by grains to demonstrate the persistence of toxicity over time. Additionally, it is crucial to include GC-MS analyses of treated grains to track the behavior and residual presence of essential oils both on the surface and inside the grains until the end of the experiment. This is essential for controlling insect pests that lay eggs within grains, creating a delay between treatment and potential contact with the insecticide by emerging individuals. Finally, the minimal difference in mortalities between exposure times could be attributed to the absence of accumulation of toxic compounds in the insects and their ability to metabolize them. Instances where differences were observed may be explained by a more physiological mode of action, inducing drying or inhibiting feeding, leading to a slower death pattern. Furthermore, to determine the viability of essential oils as alternatives to pesticides on an industrial scale, additional studies must be conducted, possibly in experimental granaries. These studies would aim to ascertain the amount of oil required per ton of grains and assess the practical applicability of such treatments. Additionally, the formulation of the essential oil is a crucial factor, as highlighted by Maes *et al*. (2019). In our experiments, dilutions were prepared using n-hexane, which differs significantly from actual industrial applications. These aspects warrant further detailed analysis and consideration.

CONCLUSION

The insecticidal effects and toxicity demonstrated by *L. camara* essential oil suggest its potential as a viable alternative to synthetic pesticides currently employed for grain beetle control. Given the variable composition of essential oils, this study underscores the significance of identifying the specific compounds within *L. camara* essential oil responsible for its insecticidal properties, as determined through GCMS analysis. Further research is warranted to elucidate the mechanism of action of the essential oil, including the potential contributions of minor components, both in insect and mammalian systems, to ensure its safe and effective industrial utilization.

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