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TOXICITY AND REPELLENT EFFECT OF THREE PLANT BASED ESSENTIAL OILS AGAINST THE RED FLOUR BEETLE *TRIBOLIUM CASTANEUM* (HERBST)

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ABSTRACT

In this study jasmine *(Jasminum officinale)*, lemon grass *(Cymbopogon citratus)*, and sandalwood *(Santalum album)* essential oils were investigated for their efficacy against the red flour beetle *Tribolium castaneum.* Sandalwood oil consistently showed the highest contact toxicity, with LD_{50} values decreasing over time. Jasmine and lemon grass oils ranked lower but still exhibited toxicity. The order of contact toxicity was sandalwood > jasmine > lemon grass at each exposure period. Fumigation treatments revealed sandalwood's initial superiority, though jasmine surpassed it at 48 hr. The order of fumigation toxicity shifted to jasmine > sandalwood > lemon grass at this extended exposure time. Repellency tests showed dose and time-dependent responses for both larval and adult stages, with occasional fluctuations. Sandalwood's significance differed for adult beetles between time intervals, and jasmine exhibited insignificance in its efficacy for larvae at certain intervals. This study provides insights into the potential of these essential oils as alternatives for pest control.

Key words: Jasmine, lemon grass, sandalwood, contact toxicity, fumigation toxicity, repellent activity, *Tribolium castaneum*, LD_{50} values, essential oils, mortality, exposure priods, adults, larvae

Before the invention of synthetic pesticides, people used to apply their own home remedies against harmful insects. People created insecticides from various plant items in their own unique ways. The usual technique was to crush the leaves of a deadly plant, dissolve them in water, and spray the resulting solution over crops (Ghosh, 2000). Plant extractions were once widely used commercially as insecticides, but around 1940, synthetic chemicals began to take the place of botanicals. The growing use of chemical pesticides in agriculture has had negative effects on the environment, society, and eco toxicology, which has prompted researchers to look for workable alternatives that are more environmentally friendly than synthetic chemicals. In this situation, both researchers and consumers are showing a lot of interest in the usage of insecticides based on plant extracts. Essential oils (EOs) are a possible substitute for other botanical extracts used as pesticides due to their widespread availability and comparative affordability.

Essential oils have intriguing characteristics that can be used in substitute of synthetic insecticides (Oliveira et al., 2017) in the present study, we evaluated the toxicity of essential oil (EO. Plants produce essential oils as secondary metabolites, and these compounds are crucial for plant defense (against both biotic and abiotic stressors) and signaling activities, as well as for luring pollinators and helpful insects (Smith and Barker, 2006; Walters, 2010; Zuzarte and Salgueiro, 2015). To prevent damage from insect pests, safe storage of grains and food products is a crucial issue (HAQ et al., 2005). Under ideal climatic and storage conditions, insect pests and mites steal about 9% of the world's grain production (Adams and Schulten, 1978; Fields, 2006; Rahman et al., 2009). One of the most common pests of stored grains is the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), which has been known to destroy a variety of goods including grains, flour, peas, beans, cacao, nuts, dried fruits, and spices (Campbell and Runnion, 2003). The present study was aimed to find out the toxicity and repellent effect of three plant based essential oils against the red flour beetle, *Tribolium castaneum*. Main objectives of this study are- To minimize the use of synthetic pesticides on stored grain as it has hazardous effect on human and animal health, increasing the use of plant based, environment friendly pesticides, determining the insecticidal effect of three essential oils against the main stored product pest in Bangladesh (*T. castaneum*).

MATERIALS AND METHODS

The test insect was grown as subcultures from the stock cultures of the University of Rajshahi's Entomology Laboratory in Rajshahi, Bangladesh. The adults that were harvested from the lab were kept in subculture in glass beakers with sterile feeding media.

The conventional diet medium of wheat flour combined with yeast was used to maintain the laboratory culture of *T. castaneum*. The whole-wheat flour was stored in a deep refrigerator for 48 hr. When the experiment was running, dried yeast powder was added in a standard 19:1 ratio together with whole-wheat flour (Zyromska-Rudzka, 1966). Flour was sieved using a stainer for the collection of adults. Adults were removed from the flour using a brush and a spoon, and then they were picked up and put in a petri dish. Three pure essential oils were purchased from market, jasmine (*Jasminum officinale*), lemon grass (*Cymbopogon citratus*) and sandalwood (*Santalum album*). In order to evaluate the contact toxicity bioassay, which is handled separately by the oils, newly hatched *T. castaneum* was used. For each replication, 10 red flour beetles were used with three replications. Pilot studies were conducted to determine dosages of oil that had a death rate of between 10 and 90% for the 4 to 7-day-old red flour beetles. The oil was weighed and diluted in acetone. The dose/ cm^2 was calculated by measuring the actual oil in a mixture of 1 ml and dividing the result by the surface area of the petri plate. On the perti dish that was being covered by the upper lid, a captive environment was created. Oil density was estimated and given in µlcm-2. A residual film delivery method was used to administer the medication (J.R. Busvine, 1960). Each dosage consisted of 1 ml of the recommended concentration applied in such a way that a thin film covered the petri plate (7 cm). After the solution had been air dried, it was applied to the petri dishes. A control experiment was maintained in which treatment was made with acetone. The mortality of the beetles were recorded after 3-, 6-, 24- and 48 hr of treatment. The doses for each oil were 0.124, 0.233, 0.337 and 0.407 µlcm-2. Glass vials (5 cm long by 2.5 cm diameter), capped with polypropylene stoppers was used for the fumigation toxicity bioassay.

Red flour beetles were transferred to the vials in groups of 10 adults. The vials were covered with markin cloth secured with adhesive tape. The doses used for each oils were 4.074, 2.037, 1.018 and 0.509 µlcm-2 and they were taken in vials separately. The vials containing the insects were then turned upside down over the vials containing oil and both of them were attached together with tape such that the oil vapours saturated the atmosphere of the containers containing the beetles. The control consisted of similar setup but without essential oils. The vials were placed at room temperature and mortality counts were made after 24 and 48 hr of the treatment. The bioassay for repellency was performed in a 9 cm petri dish. In the middle of the petri dish, 10 adults were released with three replications. The filter paper was divided in half and placed in a portion of the petri dish with prepared concentrations of essential oils, while the other half was just treated with acetone (the same technique was used for each concentration of each oil). A thin stick was attached with adhesive tape used to divide the treated area from the control area. For the experiment on repellency, the doses of each oil were 0.0049, 0.0098, 0.0196, 0.0392 and 0.0785 µlcm-2. For each concentration, the experiment was conducted three times, and the taxis of beetles were tracked. After one hr, counts of insects were made, followed by hourly counts for 5 hr.

The same experiment was conducted on larvae in their fourth instar using the same essential oil doses and methodology. The mortality data were corrected with the Abbott's (1925) formula: $P_r =$, here, Pt = corrected mortality (%), Po = observed mortality (%), Pc = control mortality (%). Then, using software the observed data were subjected to probit analysis in accordance with Busvine (1971). The % repellency (PR) was calculated to find out the intensity of activity of each oil. Negative values were used to describe attractant activity and positive ones to express repellency. Then each percent repellency was categorized comparing with that show a class for the value of PR. (Nerio et al., 2009; Obeng-Ofori, 1995). The following formula was used to compute % repellency. PR= (NC-NT)/ (NC+NT) ×100, here, PR= % repellency, NC= number of insect in the non-treated (control) area after the exposure interval, NT= number of insect in the treated area after the exposure interval. A two factor without replication ANOVA was performed to determine the dose response and time interval using Microsoft Excel.

RESULTS AND DISCUSSION

In the contact toxicity test evaluating the effects of sandal wood, lemon grass, and jasmine essential oils on *T. castaneum*, the LD_{50} values after 3 hr of treatment were found to be 0.552, 4.715, and 0.752, respectively. Sandal wood exhibited the highest activity, while lemon grass demonstrated the least potency at this time point. Subsequent assessments at 6 hr revealed LD_{50} values of 0.434, 1.263, and 0.437 for sandal wood, lemon grass, and jasmine, respectively, with sandal wood and jasmine demonstrating comparable activity. At the 24-hr mark, LD_{50} values of 0.264, 0.583, and 0.313 were observed for sandal wood, lemon grass, and jasmine, respectively, reinforcing the superior activity of sandal wood even after prolonged exposure. The trend persisted at 48 hr, where LD_{50} values of 0.183, 0.438, and 0.258 showcased

Table 1. Toxicity of essential oils against adult *T. castaneum*

Time of exposure	Oils	LD_{50}	95% confidence limits		Regression equation	χ^2 value (at 2df)
			Lower	Upper		
Contact treatment						
3h	Jasmine	0.752	0.312	1.810	Y = 3.371 + 1.885X	0.293
	Lemon grass	4.715	3.837	579.373	Y = 2.913 + 1.141X	0.345
	Sandal wood	0.552	0.318	0.959	Y = 3.392 + 2.234X	0.630
6h	Jasmine	0.437	0.302	0.633	Y = 3.512 + 2.334X	0.641
	Lemon grass	1.263	0.270	5.897	Y = 2.903 + 1.819X	0.518
	Sandal wood	0.434	0.284	0.662	Y = 3.707 + 2.046X	0.518
24h	Jasmine	0.313	0.247	0.396	Y = 3.808 + 2.387X	0.251
	Lemon grass	0.583	0.364	0.936	Y = 2.799 + 2.830 X	0.195
	Sandal wood	0.264	0.202	0.344	Y = 4.149 + 2.009X	0.623
48h	Jasmine	0.258	0.208	0.320	Y = 3.975 + 2.527X	0.323
	Lemon grass	0.438	0.339	0.565	Y = 2.787 + 3.422X	0.345
	Sandal wood	0.183	0.144	0.233	Y = 4.149 + 2.009X	0.715
Fumigation treatment						
24h	Jasmine	6.241	2.908	13.395	Y = 1.945+ 1.723X	0.107
	Lemon grass	16.283	2.643	100.293	Y = 2.242 + 1.252 X	2.992
	Sandal wood	3.993	2.324	6.862	Y = 2.378 + 1.643X	1.568
48h	Jasmine	0.365	2.065	4.703	Y = 2.270 + 1.823 X	6.268
	Lemon grass	8.763	2.478	30.986	Y = 2.684 + 1.208 X	0.335
	Sandal wood	1.381	1.602	3.617	Y = 2.826 + 1.574X	0.370

LD_{50}= Lethal dose 50%

sandal wood's sustained higher activity. There was an increasing activity of each oil over time, reaching their peak at 48 hr (Table 1).

In the fumigation treatments, the LD_{50} values after 24 hr revealed sandalwood's heightened activity with a value of 3.993, surpassing lemon grass (LD_{50} = 16.283) and jasmine (LD_{50} = 6.241). This trend persisted at the 48-hr mark, where sandalwood exhibited the highest activity with an LD_{50} of 1.381, followed by lemon grass (LD_{50} = 8.763) and jasmine (LD_{50} = 0.365). The order of activity across the oils consistently maintained the sequence of sandalwood > jasmine > lemon grass at both time points. Remarkably, the efficacy of each oil increased over time (Table 1).

In the evaluation of repellency against adult *T. castaneum*, jasmine oil exhibited significant repellent activity both between doses and time intervals. Lemon grass essential oil demonstrated substantial repellency in a dose-dependent manner. Sandalwood exhibited significant repellency between doses but showed insignificance between time intervals. With larvae ANOVA results indicated that jasmine oil's repellency was insignificant between doses but significant between time intervals. Sandalwood exhibited significant repellency at both 1% levels between doses and time intervals, showing its deterrent effect (Table 2).

Numerous researches have conducted on the effectiveness of essential oils (EOs) against *T. castaneum*, but there is still a lack of data comparing the EOs' effects on repellency and toxicity. This present study have concluded that sandal wood and jasmine essential oils can be more effective as contact and fumigant pesticide than lemon grass. Also in repellency test all the oils have significant repellent effect against *T. castaneum*. The nanoemulsion containing *Pimpinella anisum* essential oil exhibited toxicity towards red flour beetles (Hashem et al.). Essential oils extracted from *Laurelia sempervirens* and *Drimys winteri* exhibited strong repellent and insecticidal effects on red flour beetles (Zapata et al.). *Thymus persicus* essential oil shows potential as a fumigant for controlling stored-product insects such as red flour beetles and rice weevils (Saroukolai et al., 2010); *V. officinalis* essential oil showed the most toxicity to red flour beetles whilst *V. jatamansi* essential oil was the most effective against cigarette beetles (Feng et al.). Essential oils extracted from *Atractylodes lancea* and *A. argyi* exhibit significant contact toxicity, against red flour beetles (Guo et al., 2019). *Origanum* essential oil and its components have insecticidal properties and function as a repellent against red flour beetle adults, (Kim et al., 2010). Essential oils extracted from various *Cymbopogon* spp. such as *C. flexuosus*, *C. winterianus*, and *C. martini*, showed

Table 2. Repellency for essential oils against adult and larva of *T. castaneum*.

Test organism	Essential oils	Sources of Variations	SS	df	MS	F
Adult of *T. castaneum*	Jasmine	Between doses	5215.71	4	1303.92	16.14*
		Between time interval	5032.66	4	1258.16	15.57*
	Lemon grass	Between doses	19426.75	4	1656.15	27.05**
		Between time interval	6624.61	4	174.43	9.22*
	Sandal wood	Between doses	12788.04	4	3197.01	19.82*
		Between time interval	3662.51	4	915.62	5.67
Larva of *T. castaneum*	Jasmine	Between doses	232.60	4	58.15	1.90
		Between time interval	1751.67	4	437.91	14.35*
	Lemon grass	Between doses	17303.14	4	4325.78	28.26**
		Between time interval	6743.53	4	1685.88	11.01*
	Sandal wood	Between doses	4387.55	4	1096.89	54.35**
		Between time interval	3042.82	4	760.70	37.69**

SS= Sum of Squares, MS= Mean Square, df - Degrees of Freedom, F - F-ratio.* = Significant at $p<0.05$; ** = Significant at $p<0.01$

potent repellent effects on *T. castaneum*. The essential oils from these species exhibited potent repellent properties and can serve as effective tools for controlling *T. castaneum* (Devi et al., 2020). *Teucrium polium* essential oil demonstrates notable fumigant toxicity towards red flour beetle adults and show antifeedant effects (Ebadollahi and Taghinezhad, 2020). Essential oil extracted from aromatic plants has shown significant potential for IPM. Toxicity and antifeedant effects of essential oil isolated from aerial parts of *Teucrium polium* have been investigated against, *T. castaneum*.

The chemical profile of this oil was also assessed by gas chromatography-mass spectrometry (GC-MS. *Eucalyptus procera* essential oil is highly efficient with LT50 values of 16.137 hr for *T. castaneum* and 13.644 hr for *C. maculatus* at the highest quantities tested (Nauri-Ganbalani et al.). Calneem Ò oil exhibited significant toxicity and repellent effects on *T. castaneum*, resulting in over 90% mortality of the beetles within 72 hr when exposed to the highest dosage on grain. Calneem Ò oil treatments severely hindered the growth of eggs into adults on broken wheat (Adarkwah et al., 2010). Numerous studies have been conducted to determine the pesticidal properties of sandalwood essential oils. Sandalwood oil and its components can be used to environmentally control *A. gossypii* because of their insecticidal and insect-repellent qualities (Roh et al., 2015). Sandalwood oil showed substantial effectiveness against adult female two-spotted spider mites (TSSM) (Roh et al., 2011). Four essential oils, such as cardamom, coriander, rosemary, and sandalwood, exhibited adulticidal effects ranging from 68 to 98% against female house mosquitoes at a concentration of 1000 ppm (Kang et al.). An evaporating tablet containing jasmine, lemon grass, and sandalwood essential oils demonstrated 100% repellency against the house fly *Musca domestica* (Hazarika et al.). Present study has shown significant contact and fumigation toxicity effect of jasmine essential oil to control *T. castaneum*. A study determined that jasmine and peppermint essential oils had the lowest effectiveness as mosquito repellents (Khanna et al.). Jasmine Essential Oil (JEO) has the highest acaricidal capabilities against two-spotted spider mites, followed by Mustard Fixed Oil (MFO) and Lavender Essential Oil (LEO), (Farouk et al., 2021).

In this present study, lemon grass has no significant toxicity against red flour beetle in contact toxicity and fumigation bioassay. But, there are some researches on lemon grass oil to control *T. castaneum* and other insects applying different methods. The clove and lemon grass extracts showed a repellent effect on red flour beetles that increased with higher doses and longer exposure times. The highest doses achieved a 70% repellency impact 24 hr after application (Aboelhadid et al., 2021). Lemon grass essential oil demonstrated insecticidal activity against *A. ipsilon* (Moustafa et al., 2021). Lemon grass essential oil affects the behaviour, lipid composition, and enzymatic activity of cowpea weevils, which may effect their survival and reproductive activities (Alves et al., 2019). This study suggests that sandal wood and jasmine oil have the ability to act as grain protectants by eliminating different life stages of *T. castaneum* through contact and fumigant mechanisms. The results of these tests conducted in a laboratory context may not be directly applicable to large-scale insect control.

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AUTHOR CONTRIBUTION STATEMENT

Sakura Haque-collection of primary data, statistical analysis, and drafting the manuscript. Saiful Islam Faruki-Research Supervisor, and finalization of the manuscript.

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CONFLICT OF INTEREST

No conflict of interest.

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