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Toxic and repellent effect of Citronella essential oil against *Sitophilus oryzae* l. And *Tribolium castaneum* Herbst.

Istiak Mahfuz*, Shanjida Afrin, Beauty Khatun, and Shah H. A. Mahdi

Department of Zoology, University of Rajshahi, Bangladesh

Abstract: In the present investigation, the toxicity of Citronella essential oil was tested on two major stored grain pests, *Sitophilus oryzae* and *Tribolium castaneum* adults through contact and repellency bioassay as an alternative approach to that of the synthetic pesticide. Contact bioassay on *S. oryzae* showed LD_{50} values of 0.089, 0.064, and 0.650 µg/cm^2, whereas on *T. castaneum* the LD_{50} values were calculated as 0.121, 0.097, and 0.070 µg/cm^2 both after 24, 48, and 72 hours of exposure, respectively. The ANOVA of the repellency data showed no significant effect of time (P= 0.4962) and doses (P= 0.4505) against rice weevil, *Sitophilus oryzae.* In addition, the interaction between exposure time and doses is also insignificant against the weevil. In case of *T. castaneum,* repellency bioassay data showed no significant effect among exposure times (P =0.1008) and the doses (P = 0.0914) against the red flour beetle. The percent repulsion by the essential oil was found to be the highest against the doses of 0.157 and 0.062 µg/cm^2. Based on the present investigation it can be concluded that the citronella essential oil may act as a potent source of toxicant to control the red flour beetle, *T. castaneum* and rice weevil, *S. oryzae* which in turn can minimize the uncontrolled use of synthetic pesticides.

Keywords: Toxicity, Repellency, Citronella oil, *Sitophilus oryzae, Tribolium castaneum.*

Introduction

Agriculture is considered to be the prime source of livelihood for millions of people around the globe. Each year huge damage is caused to the stored grains, such as rice and wheat as a result of the severe infestation by insect pests. The most dominant group of storage pests are insects, the second is other arthropods like Acari or mites also function as a major pest to stored commodities (Khan & Mannan, 1991). The damage occurs as a result of causing weight loss and a decrease in the germination ability of grains. The culture of using synthetic chemicals for controlling pests has long been in practice. In the last 50 years, it has become the most widely used method for the regulation of pest populations, killing, and eradication of pests. However, repeated and uncontrolled use of these synthetic insecticides has serious drawbacks such as: developing pesticide-resistant insect species (Mills, 1983; Pacheco *et al.*, 1990; Sartori *et al.*, 1990; Subramanyam, 1995; Tyler *et al.*, 1983), causing serious health hazards to both man and animal through factors, such as direct toxicity to parasites, predators, pollinators, fish and man (Georghiou, 1990; Irshad & Gillani, 1990; Mahmud *et al.*, 2002; Zettler, 1991; Zettler & Cuperus, 1990)). As a result, naturally occurring plant product has been considered a potential alternative to synthetic chemicals for controlling stored product pests (Heyde *et al.*, 1984). They are well known for having a variety of properties against insect pests (Arthur, 1996). Several spices and herbs are found to be

*Corresponding author: istiak.mahfuz@ru.ac.bd

well known for possessing anti-insect activities (Tripathi *et al.*, 1999), particularly in the form of essential oils. Certain botanical oils have been proven and demonstrated as effective against stored commodities (Dunkel & Sears, 1998; Su, 1990). Besides, many researchers have studied the repellent, deterrent and other biological effects of some plant materials against stored product insets (Harish *et al.*, 2000; Kim *et al.*, 2003a,b; Tripathi *et al.*, 2002). The toxicity of a large number of essential oils and their constituents have been evaluated against a variety of stored product insects (Bouda *et al.*, 2001; Huang *et al.*, 2000a,b; Kéita *et al.*, 2001; Paranagama *et al.*, 2003; Tripathi *et al.*, 2002). A variety of plant essential oils has been proven to have some insecticidal and nematicidal activities and are reported to be lethal against a wide range of plant pathogenic fungi (Rice, 1995). The trend of using essential oils as a pest-control agent is very common in tropical countries (Rajapakse & Van Emden, 1997; Singh *et al.*, 1978).

The current investigation was conducted with the aim of screening citronella essential oils as repellents and fumigants against the adults of rice weevil, *S. oryzae* and red flour beetle, *T. castaneum*. These two pest species were chosen since they are cosmopolitan in distribution and easily available, and the essential oil was because of their insecticidal and repellent properties against similar insect species. Our ultimate goal is to minimize the use of synthetic insecticides and development of pesticide resistance using different methods.

Materials and Methods

Insects

The culture of the experimental beetles *i.e., S. oryzae* and *T. castaneum* was conducted in the Crop Protection and Toxicology laboratory of the Department of Zoology, University of Rajshahi, Bangladesh. The stock culture *S. oryzae* was maintained in room temperature at 32±5°C in large plastic jars. The room temperature was maintained at 32±5°C and 30±5°C for *T. castaneum,* without any light and humidity control to ensure the continuous supply of the beetles. For the culture of *T. castaneum*, the ratio of the wheat flour and Yeast was 19:1 which had to go through the sterilization process at 60°C for 24 hours and was kept unused for 15 days to allow its moisture content to equilibrate with the environment prior to being used as a food source. Eggs, larvae, pupae, and adults were separated at regular intervals, and the food medium was replaced after every three days by fresh food media to avoid contamination.

Essential Oil

Citronella essential oil from the plant *Cymbopogon nardus* was purchased from a handmade factory, Kanchan Industrial Estate, Saki Naka, Andheri (E), Mumbai 400072, India.

Contact bioassay

Series of dilutions of essential oils were prepared using acetone as solvent. Aliquots of 1 ml of the dilutions were applied into 5 cm diameter Petri dishes for contact bioassay (Busvine, 1971), at the dose of 0.102, 0.089, 0.076, 0.038 $\mu g/cm^2$ on *S. oryzae,* whereas 0.153, 0.127, 0.064, and 0.032 $\mu g/cm^2$ were applied on *T. castaneum*. The solvent was allowed to evaporate for half an hour and the insects were transferred to the treated Petri dishes. Control doses were set with acetone alone. Ten (10) adult beetles were used for each concentration and Ten (10) were used for the control. The Petri dishes were kept at room temperature and the mortality was recorded after 24, 48, and 72 hours of exposure.

Repellency bioassay

Repellency bioassay was conducted in Petri dishes of 9 cm diameter. Whatman filter paper (9cm diam.) was cut into 2 equal halves and both were set to the bottom of the Petri dish with the help of adhesive tape. One-half of the filter paper was treated with a particular dose of Citronella oil. This method was applied to all the concentrations *i.e.,* 0.125, 0.110, 0.094, and 0.047 μg/cm^2 for *S. oryzae* and 0.189, 0.157, 0.079, and 0.039 μg/cm^2 for *T. castaneum* and the other half remained untreated. The treated (Citronella oil) and non-treated (control) zone were separated by a thin stick, attached to the Petri dish by adhesive tape. Ten (10) beetles were released in the middle of each of the Petri dish for this bioassay and the whole experiments were set in 3 replicates. The experiment was repeated thrice for each dose and the taxis of the beetles were observed. The data *i.e.,* number of weevils present in treated and non-treated area of the Petri dish were then recorded at 1 hour interval up to 5 hours.

Analysis of data

Dose mortality test

The mortality percentage for contact bioassay was corrected using the Abbott's formula (Abbot, 1925; Busvine, 1971).

$$P_t = \frac{P_0 - P_c}{1oo - P_c} \times 100$$

Where P_t = Corrected mortality (%), P_0 = Observed mortality (%) and P_c = Control mortality (%).

The observed data were then subjected to probit analysis according to Busvine (1971) and Finney (1952) using software developed in the Department of Agricultural and Environmental Science, the University of Newcastle Upon Tyne, United Kingdom. The heterogeneity was tested by a chi-square test.

Repellency test

A two-way RM (Repeated Measures) ANOVA was performed to determine the dose-response and time duration by using Graph pad prism 6.0 software. Percent repulsion (PR) was calculated by using the following formula (Nerio *et al.*, 2009; Obeng-Ofori, 1995).

$$PR = \frac{NC-NT}{NC+NT} \times 100$$

Where, NC = Number of insects in the non-treated (Control) area after the exposure interval and NT= Number of insects in the treated area after the exposure interval. The percent repulsion was then categorized by using the Table 1.

Table 1. Repellency scale from less to the most repellent = 0 to V

Category/Class	PR (%)
0	< 0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

Results

Contact toxicity of Citronella oil

For *S. oryzae,* the doses of citronella oil used in contract treatment were 0.102, 0.089, 0.076, and 0.038 μg/cm^2. Dose mortality, 95% confidence limits, and LD_{50} values of citronella oils on adult *T. castaneum* after 24, 48, and 72 hours of contact treatment are shown in Tables 2 and 3.

For *T. castaneum* the doses of Citronella oil used in contact bioassay were 0.153, 0.127, 0.064, and 0.032 μg/cm^2. The LD_{50} values has been calculated as 0.121 μg/cm^2, 0.097 μg/cm^2, and 0.069 μg/cm^2 after 24, 48, and 72 hours respectively. Dose mortality, 95% confidence limits, and LD_{50} values of Citronella oil on adult *T. castaneum* after 24, 48, and 72 hours of contact treatment are shown in Tables 4 and 5.

Table 2. Dose mortality and LD_{50} values of Citronella oil on adult *S. oryzae* after different exposure time.

Exposure time (Hours)	Dose (μg/cm^2)	Corrected Mortality (%)	LD_{50} (μg/cm^2)
	0.102	60	
	0.089	50	
24	0.076	37	0.089
	0.038	23	
	Control	0	
	0.102	77	
	0.089	60	
48	0.076	43	0.064
	0.038	37	
	Control	0	
	0.102	80	
	0.089	63	
72	0.076	53	0.650
	0.038	50	
	Control	0	

Table 3. LD_{50}, 95% confidence limits and regression equation of essential oils against adult *S. oryzae* after different exposure time.

Exposure times (Hours)	LD_{50} (μg/cm^2)	95% conf. limits		Regression	χ^2 values (at 2df)
		Lower	Upper		
24	0.089	0.068	0.118	y=2.965+2.142x	0.818
48	0.064	0.048	0.084	y=3.365+2.053x	3.930
72	0.650	0.025	0.081	y=3.365+2.053x	2.968

Table 4. LD_{50} values of citronella oils on adult *T. castaneum* after 24, 48, and 72 hours of contact treatment.

Exposure time (Hours)	Dose (µg/cm^2)	Corrected Mortality (%)	LD_{50} (µg/cm^2)
	0.153	63	
	0.127	43	
24	0.064	33	0.121
	0.032	10	
	Control	0	
	0.153	70	
	0.127	53	
48	0.064	37	0.097
	0.032	17	
	Control	0	
	0.133	80	
	0.127	70	
72	0.064	47	0.067
	0.032	23	
	Control	0	

Table 5. The $LD_{50,}$ 95% confidence limits and the regression equations of Citronella oil against adult beetles of *T. castaneum* after 24, 48, and 72 hours of exposure.

Exposure times (Hours)	LD_{50}	95% conf. limits		Regression equation	χ^2 value (at 2df)
		Lower	Upper		
24	0.121	0.089	0.163	y=2.716+2.102x	1.730
48	0.097	0.074	0.129	y=3.023+1.999x	0.555
72	0.067	0.054	0.090	y=3.117+2.229x	0.189

Repellent effect of Citronella oil

The doses of Citronella oil showed highly significant repellent activity ($P<0.0001$) against the rice weevil *S. oryzae.* The exposure time of the beetle in selected doses have found no significant effect (P= 0.0594). However, ANOVA analysis did not show any significant interaction between exposure time and treatment doses (P= 0.5121). A histogram of dose-response and exposure time interval are shown in Figure 1. Category of repulsion found in *S. oryzae* is shown in Table 6.

The doses of Citronella oil showed no significant repellent activity (P=0.4505) against the *T. castaneum* adults. The exposure time of the beetle in selected doses were insignificant (P= 0.4962). ANOVA analysis showed no significant interaction between exposure time and treatment doses (P=0.8773). A histogram of dose-response and exposure time interval are shown in Figure 2.

The category of repulsion against *T. castaneum* was found to be class 0, III, 0, and I in case of the doses of 0.189, 0.157, 0.079, and 0.039 µg/cm^2, respectively, after 4 and 5 hours of exposure. It is interesting to observe that the highest effect was found in the dose of 0.157 µg/cm^2 (Table 7). It is also remarkable to observe that the higher doses (*i.e.,* 0.189 and 0.157) exhibited the highest repulsion compare to those of lower doses of Citronella oil.

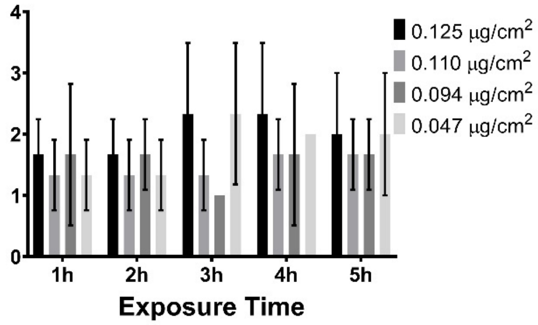

Fig. 1. Repellency effect of Citronella oil against *S. oryzae* in respective doses. Error bar indicates standard deviation (SD)

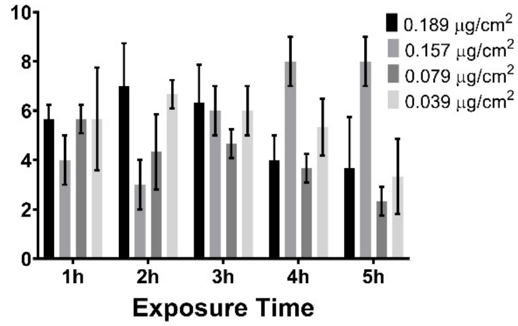

Fig. 2. Repellency effect of Citronella oil against *T. castaneum* adults in respective doses. Error bar indicates standard deviation (SD)

Table 6. Category of repulsion at different doses of Citronella oil against *S. oryzae.*

Dose (µg/cm^2)	Category of repulsion based on percent repellency				
	1h	2h	3h	4h	5h
0.125	0	0	0	0	0
0.110	0	0	0	0	0
0.094	0	0	0	0	0
0.047	0	0	0	0	0

Table 7. Category of repulsion at different doses of Citronella oil against *T. castaneum*

Dose (µg/cm^2)	Category of repulsion based on percent repellency				
	1h	2h	3h	4h	5h
0.189	I	II	0	0	0
0.157	0	0	I	III	III
0.079	I	0	I	0	0
0.039	I	II	I	I	0

Discussion

Insects are responsible for the physical, nutritional, and quality deterioration of stored products which are the most common reason for the post-harvest losses sustaining in the developing world leading to a detrimental impact on food security. Among the coleopteran pest beetles, the most damaging ones are found in the genus *Sitophilus* and

Tribolium. According to Tyler (1982), the amount of loss can vary according to the geographical distribution. The possible reasons working behind these variations are the lack of a standardized methodology and underlying environmental as well as climatic change (Tyler, 1982).

Monoterpenoids and Sesquiterpenoids which are the principal components of essential oils work as an alternative to classical fumigants (Papachristos & Stamopoulos, 2003). Later, the effectiveness of essential oils and their constituents having potentials similar to the fumigants was proven by the works of Huang *et al.*, 2000b; Lee *et al.*, 2001a, b; Tunç *et al.*, 2000. These components have a huge demand in the industrial markets for having certain potent biological activities in addition to their toxicity to insects (Isman, 2000; Kubo *et al.*, 1994; Weinzierl, 2000).

In this investigation, Citronella essential oil was applied against *S. oryzae* and *T. castaneum* adults through contact bioassay to measure its toxic effect and through repellency assay to find its repellent potential. The result of contract treatment shows that the oil was effective against both the insect pests. However, no evidence was found about research carried out to determine the repellent effect of Citronella oil against *S. oryzae*. Nattudurai *et al.* (2017) reported a toxic and repellent effect of *Atalantia monophylla* essential oil against *Callosobruchus maculatus* and *S. oryzae*. Significant repellent activity of *Pimenta pseudocaryophyllus* derivatives and also *Hyptis suaveolens* and *Hyptis spicigera (Laminaceae)* essential oils against *Sitophilus* spp were studied recently (Conti *et al.*, 2011; Ribeiro *et al.*, 2015). The current investigation on the repellent effect of Citronella oil on *S. oryzae* may lead to a potent source of botanical for controlling the concerned pest.

In the repellency bioassay, Cirtonella oil showed no significant effect against rice weevil, *S. oryzae* but against *T. castaneum*, it was highly significant after 4 & 5 hours of exposure. Guo *et al.* (2016) reported a highly significant effect of the essential oil from *Juniperus formosana* against *T. castaneum* which resemblance with the present repellent efficacy of Citronella oil. Using the area preference method, Caballero-Gallardo *et al.* (2011) showed highly effective repellent activities of some constituents of the essential oil viz. *Tagetes lucida, Lepechinia betonicifolia.* They also reported highly effective repulsion by the active compounds of Citronella essential oil which is in agreement with the present work. Al-Jabr (2006) reported the repellent effect of seven plant essential oils on *T. castaneum*. He reported a class III repulsion of Citronella oil against *T. castaneum* which is in agreement with the present study. Therefore, it can be concluded that this potent essential oil might have some potential use for the pest management of stored products.

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