International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 08-12-2022, Accepted: 24-12-2022, Published: 10-01-2023
Volume 8, Issue 1, 2023, Page No. 36-41

Repellent effect of lavender and rosemary essential oil against *Tribolium casteneum* and *Sitophyllus oryzae*

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Abstract

The current investigation was intended to control *T. castaneum* and *S. oryzae* using an alternative method rather than to use the traditional one. For this purpose, different doses (251.49, 125.75, 62.87, 31.44 and 15.72 µg/cm^2) of lavender and rosemary essential oils (EOs) was tested against *T. castaneum* and *S. oryzae*, to determine the repellent effect of these oils through repellency bioassay and the measurement of percent repulsion on selected doses at hourly interval up to 5 hours. Percent repellency of lavender EO showed highest repulsion of *T. castaneum* in lower doses *i.e.*, 31.44 and 15.72 µg/cm^2, and in *S. oryzae* very high repellent effect was found in 62.87 µg/cm^2. ANOVA data showed that the doses of lavender oil have significant (P=0.0017) effect on *S. oryzae*, although, in *T. castaneum* the effect of the doses is not significant (P=0.5639). The exposure time of *S. oryzae* in selected doses of lavender EO exhibited highly significant (P=0.0006) effect on repulsion, while in *T. castaneum* it was moderately significant (P=0.0162). Both in *T. castaneum* and *S. oryzae*, the interaction between exposure time and doses found to be not-significant. The percentage repellency of rosemary EO against *T. castaneum* was found to be highest in the doses of 62.87 and 31.44 µg/cm^2, whereas, in *S. oryzae* highest repulsion was found in 251.49 µg/cm^2. The ANOVA of repellency data showed a significant effect of time (P=0.0026), doses (P=0.0129), and interaction between time and doses (P=0.0112) against *T. castaneum* adults. In case of *S. oryzae*, exposure time, doses, and interaction were found to be not significant when tested with rosemary EO. With more research on the dose parameter, exposure time and active ingredient of the selected EOs, the current findings may lead to a very effective way and a potent source to control *T. castaneum* and *S. Oryzae* in storage which in turn can minimize the uncontrolled use of synthetic and/or chemical pesticides.

Keywords: repellent, essential oils, lavender, rosemary, *Tribolium*, *Sitophyllus*

Introduction

In the vast world of insects, there are some notorious as well as renowned individuals are in play either to damage or to be beneficial for our crop plant and stored grain products. Due to notorious nature of the insects, yearly a huge loss can be inflicted on the crop plant in the field as well as stored grain in the storages. Emery and Cousins [1] reported that between one quarter and one third of the world's grain crop is lost each year during storage due to insect attack. In addition, grain which is not lost is severely reduced in quality by insect damage. The protein content of feed grain is reduced by many grain pests due to the preferential eating of grain embryos, thereby reducing and lowering the viable seeds which germinate [1].

Currently, almost every country in the world uses synthetic pesticides in order to secure the safety of their stored food products. However, the uncontrolled and widespread use of these pesticides has been proven to be harmful to human health as well as raised pesticide resistant varieties of insects and other pests [2-4].

For all these drawbacks, the necessity of an alternative approach is desirable which is comparatively ecologically safer with no residual and toxic effects on non-target animals [5, 6]. Compared to synthetic pesticides, plant-oriented formulations like essential oils (EOs) are biodegradable and considered more sustainable and eco-friendlier [7, 8]. Hence, the EOs have received much attention and have been meticulously explored as natural insecticides against several stored product insects and acari [9-12].

Plant EOs are volatile and consist of a mixture of 20–60 constituents which gave their characteristic odor and flavor [13]. These oils contain monoterpenoid compounds that exert toxic effect on insects by destroying their nervous system [14, 15]. Currently, EOs and their major constituents from aromatic plants, mainly monoterpenes and susquiterpenoids are of special interest to industrial markets because of other potent biological activities in addition to their toxic and repellent effect on insects [16-18]. The practice of adding a little vegetable oil to stored rice or legumes for protection against stored-insect pests is well known and well established in oriental countries like China, India, and Indonesia. Recently, the practice of protecting stored products with oil has spread and has been adopted in Africa and South America.

Lavender essential oil is usually distilled from the plant *Lavandula angustifolia.* It is one of the most popular and multipurpose EOs used in aromatherapy [19]. Chemical constituents of this oil gave different efficiency and bioactivity control and had a wide range of toxicity against fungi, bacteria, insects, and non-insect pests [20-22]. Rosemary essential oil comes from an evergreen shrub, *Rosmarinus officinalis*, with needle-like leaves and a woody aroma [23, 24]. This plant is very well known for food seasoning, and one of the most popular aromatic and medicinal plants worldwide [24-26]. This essential oil has some potent bioactive compound which provides antimicrobial and antioxidant activities [27-29]. Rosemary essential oil also showed pesticidal activity against insects and acari [22, 30-32]. However, there is no reporting on the repellent activity of the essential oil from *Lavandula angustifolia* and *Rosmarinus officinalis* against stored grain product in Bangladesh. Thus, this work is undertaken to investigate the repellent activities of the above EOs against the two stored product insects *viz*. *T. castaneum* and *S. oryzae* for the first time.

Materials and Methods

Culture of selected insects

The red flour beetles, *T. castaneum* were reared at the crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. Whole wheat flour and Yeast were mixed at 19:1 ratio for the culture of *T. castaneum*. The food media was sterilized at 60°C for 24 hours. The sterilized food was not used until 15 days to allow its moisture content to equilibrate with the environment. *T. castaneum* culture was established in a room temperature at 32±5°C without any light and humidity control.

The wheat grain weevil, *S. oryzae* was collected from the stock culture maintained in same laboratory. The culture of *S. oryzae* was maintained in a plastic container with a standard food medium (wheat). The container was covered and kept at room temperature at 32 ± 5° C without any light and humidity control.

Essential Oils (EOs)

Lavender (*Lavendula. angustifolia*) and Rosemary (*Rosmarinus officinalis*) EOs were purchased from 'Tanah Essential Oil Co.', Brisbane, QLD, Australia. The country of origin of each oil was France.

Repellency bioassay

Repellency bioassay was carried out in a 9 cm Petri dish. Ten beetles were released in the middle of the Petri dish for this bioassay with three replications. The treated (Lavender oil) and non-treated (Control) wheat were separated by two thin sticks, attached to the Petri dish by adhesive tape and the same process is done against both insect, *T. castaneum and S. oryzae*. Similar methods were followed when conducting repellency bioassay with rosemary oil.

The doses of lavender and rosemary oil used in the repellency experiment were 251.49, 125.75, 62.87, 31.44 and 15.72 µg/cm^2. The experiment was repeated three times, and the taxis of the beetle was observed. The number of weevil present in the treated and non-treated areas of the Petri dish were then recorded at 1 hour interval up to 5 hours.

Analysis of repellency

A two-way RM (Repeated Measures) ANOVA was performed to determine the dose response and time duration by using GraphPad Prism 6.0 software. Percentage repellency (PR) was calculated using the following formula [33, 34].

$$PR=\left(\frac{NC-NT}{NC+NT}\right)\times 100$$

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 repellency was then categorized by using Table 1.

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

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

Repellent effect of lavender oil against *T. castaneum*

The doses of lavender oil showed repellent activity against *T. castaneum*. The exposure time of the beetle in selected doses have found to be significant (P=0.0162). ANOVA analysis showed no significant interaction between exposure time and treatment doses (P=0.3955). Histogram of dose response and exposure time interval is shown in Figure 1.

Percentage repellency showed that the highest repulsion effect was observed after 1 hour of exposure in selected doses and the lowest repulsion was found after the 5th hour (Table 2) suggesting that the beetle became adapted to the repellent after a long time of exposure. The category of repulsion in different doses (251.49, 125.75, 62.87, 31.44 and 15.72 µg/cm^2) is shown in Table 3.

Repellent effect of lavender oil against *S. oryzae*

The doses of lavender oil showed significant repellent activity (P=0.0017) against the wheat weevil, *S. oryzae*. The exposure time of the beetle in selected doses have found to be highly significant (P=0.0006). However, ANOVA analysis did not find any significant interaction between exposure time and treatment doses (P=0.9346). A histogram of dose response and exposure time interval is shown in Figure 2.

Percentage repellency showed that the highest repulsion effect was observed after 5 hours of exposure in selected doses (Table 2). The category of repulsion was found to be Class II, I, IV, II and IV in doses of 251.49, 125.75, 62.87, 31.44 and 15.72 µg/cm^2, respectively. It is interesting to observe that highest effect was found in the dose 62.87µg/cm^2, having the lowest repulsion (Class I) after 1 hour of exposure and the highest after 5 hours (Class IV) (Table 3).

Repellent effect of rosemary oil against *T. castaneum*

The doses of rosemary oil showed significant repellent activity (P=0.0129) against the *T. castaneum* adults. The exposure time of the beetle in selected doses have found to be highly significant (P=0.0026). ANOVA analysis showed a significant interaction between exposure time and treatment doses (P=0.0112). A histogram of dose response and exposure time interval is shown in Figure 3.

In *T. castaneum*, the percentage repellency showed that the highest repulsion effect was observed after 2 hours of exposure in selected doses (Table 4). The category of repulsion was found to be Class II, III, III, III and I in doses of 251.49, 125.75, 62.87, 31.44 and 15.72 µg/cm^2, respectively, after two hours of exposure. It is interesting to observe that the highest effect was found in the doses of 62.87 and 31.44 µg/cm^2 (Table 4). It is interesting to observe that the higher doses (*i.e.*, 251.49 and 125.75) exhibited the lowest repulsion compare to those of lower doses of rosemary oil.

Repellent effect of rosemary oil against *S. oryzae*

Percentage repellency of rosemary oil against *S. oryzae* adults showed that the highest repulsion effect was observed after 1 hour of exposure in selected doses. Data also showed that with the increase of time the repulsion decreased (Table 4). ANOVA analysis showed that the exposure time of the beetle in selected doses have no significant (P=0.7233) effect and the effect of different doses is also not significant (P=0.1652). No significant interaction was found between exposure time and treatment doses (P=0.3167), however, a significant difference was found among the replication suggesting experimental error. A histogram of dose response and exposure time interval is shown in Figure 4.

The category of repulsion in different doses (251.49, 125.75, 62.87, 31.44 and 15.72 µg/cm^2) against *S. oryzae* are shown in Table 5. It is interesting to observe that a high class of repulsion (Class III and II) effect was found in the doses of 251.49 and 125.75 µg/cm^2 respectively, compared to those of lower doses of rosemary oil.

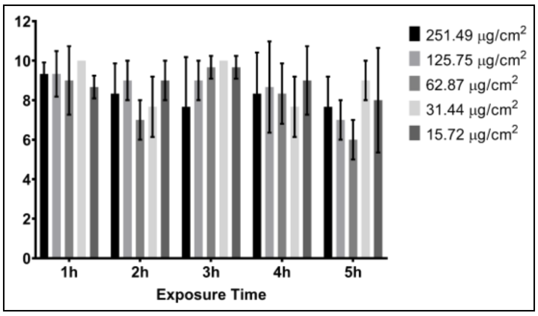

Fig 1: Repellent effect of lavender oil against *T. castaneum* (Hbst.) in respective doses. Error bar indicates standard deviation (SD).

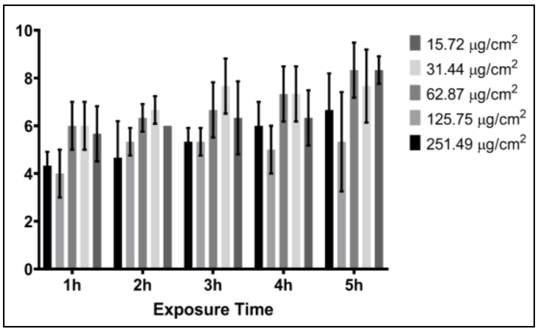

Fig 2: Repellent effect of lavender oil against *S. oryzae* in respective doses. Error bar indicates standard deviation (SD).

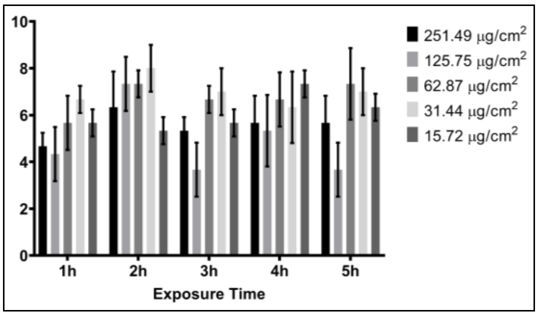

Fig 3: Repellent effect of rosemary oil against *T. casteneum* in respective doses. Error bar indicates standard deviation (SD).

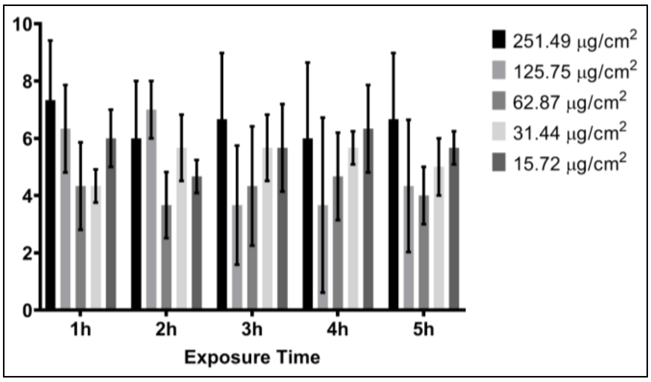

Fig 4: Repellence effect of rosemary essential oil against *S. oryzae* in respective doses. Error bar indicates standard deviation (SD).

Table 2: Percent repellency at different doses of lavender oil against *T. casteneum* and *S. oryzae*.

Insect Used	Dose µg/cm^2	Repellency (%)				
		1st	2nd	3rd	4th	5th
T. casteneum	251.49	86.67	66.67	53.33	66.67	53.33
	125.75	86.67	80.00	80.00	73.33	40.00
	62.87	80.00	40.00	93.33	66.67	20.00
	31.44	100.00	53.33	100.00	53.33	80.00
	15.72	73.33	80.00	93.33	80.00	60.00
S. oryzae	251.49	-13.40	-6.60	6.60	20	33.40
	125.75	-20	6.60	6.60	0	13.40
	62.87	20	26.60	33.40	44.60	66.60
	31.44	20	33.40	53.50	44.60	53.40
	15.72	13.40	20	26.60	26.60	66.60

Table 3: Category of repulsion at different doses of lavender oil against *T. casteneum* and *S. oryzae*.

Insect Used	Dose µg/cm^2	Category of repulsion				
		1st	2nd	3rd	4th	5th
T. casteneum	251.49	V	IV	III	IV	III
	125.75	V	IV	IV	IV	II
	62.87	IV	II	V	IV	I
	31.44	V	III	V	III	IV
	15.72	IV	IV	V	IV	III
S. oryzae	251.49	0	0	I	I	II
	125.75	0	I	I	0	I
	62.87	I	II	II	III	IV
	31.44	I	II	III	III	II
	15.72	I	II	II	II	IV

Table 4: Percent repellency at different doses of rosemary oil against *T. casteneum* and *S. oryzae*.

Insect Used	Dose (µg/cm^2)	Repellency (%)				
		1h	2h	3h	4h	5h
T. casteneum	251.49	-6.6	26.6	0	13.4	13.4
	125.75	-13.4	46.6	-26.6	6.6	-26-6
	62.87	13.4	46.6	40	33.4	46.6
	31.44	33.4	60	40	26.6	40
	15.72	13.4	6.6	13.4	46.6	26.6
S. oryzae	251.49	46.67	20.00	33.33	20.00	33.33
	125.75	26.67	40.00	-26.67	-26.67	-13.33
	62.87	-13.33	-26.67	-13.33	-6.67	-20.00
	31.44	-13.33	13.33	13.33	13.33	0.00
	15.72	20.00	-6.67	13.33	26.67	13.33

Table 5: Category of repulsion at different doses of rosemary oil against *T. casteneum* and *S. oryzae*.

Insect Used	Dose (μg/cm²)	Category of repulsion				
		1h	2h	3h	4h	5h
T. casteneum	251.49	0	II	0	I	I
	125.75	0	III	0	I	0
	62.87	I	III	II	II	III
	31.44	II	III	II	II	II
	15.72	I	I	I	III	II
S. oryzae	251.49	III	I	II	I	II
	125.75	II	II	0	0	0
	62.87	0	0	0	0	0
	31.44	0	I	I	I	0
	15.72	I	0	I	II	I

Discussion

Storage of foodstuffs as well as other commodities are essential for human habitation, and their protection from pests is inevitable to overcome economic damage and loss. Substantial losses occur every year in various stored products due to infestations by insects and other organisms in developing countries. Post-harvest losses of stored products are expressed in terms of direct weight and nutrient loss [35, 36]. Semple *et. al.* [37] gave estimated weight losses of various stored commodities in the ASEAN (Association of South East Asian Nations) members in detail. According to Tyler [38], estimates are extremely variable from commodity to commodity and from country to country or even different parts of the same country.

The use of pesticides is one of the means of preventing some losses during storage. However, the choice of pesticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food. As mentioned earlier, one alternative to synthetic insecticides is insecticidal plants. It was estimated that there are at least 250,000 different species of plant in the world today [39]. The figure could be as 500,000 [40]. It is also estimated that only about 10% of plant species have been examined chemically, so there is enormous scope for further work [40]. Hence, as an alternate source of protection against pests, different types of aromatic plant preparations such as powders, solvent extracts, essential oils, and whole plants are being investigated for their insecticidal activity including their action as repellents, anti-feedants and insect growth regulators [16, 41, 42].

In the present investigation, repellent effect of lavender and rosemary essential oil against *T. castaneum* and *S. oryzae* adults was carried out through repellent bioassay. A highly significant repellent effect of rosemary oil against *T. castaneum* was observed after 2 hours of exposure. Guo *et. al.* [12] reported highly significant repellent activities of the essential oil from *Juniperus formosana* against *T. castaneum* which agrees with our repellent efficacy of rosemary oil. Using the area preference method, Caballero-Gallardo *et. al.* [43] showed effective repellent activities of some constituents of the essential oils *viz*. *Tagetes lucida*, *Lepechinia betonicifolia*, *Lippia alba*, *Cananga odorata*, and *Rosmarinus officinalis.* In their research active compounds of rosemary essential oil also showed highly effective repulsion, which agrees with our current research. Al-Jabr [44] reported repellent effect of seven plant essential oils on *T. castaneum* (Coleoptera: Tenebrioidae). He reported a class III repulsion of rosemary oil against *T. castaneum*, which is in agreement with our current research. Several publications have reported significant repellent effect of essential oil from different plants on stored grain insects [45-47].

The result of the repellent effect of lavender essential oil was found to be significant against rice/wheat weevil, *S. oryzae*. Nattudurai *et. al.* [48] reported toxic and repellent effect of *Atalantia monophylla* essential oil on *Callosobruchus maculatus* and *S. oryzae*. Some recent studies showed significant repellent activity of *Pimenta pseudocaryophyllus* derivatives, and *Hyptis suaveolens* and *Hyptis spicigera (Lamiaceae)* essential oils against *Sitophilus* spp [20, 49].

Conclusion

The current investigation on the repellent effect of lavender and rosemary oil on *T. castaneum* and *S. oryzae* may lead to a potent source of botanical for controlling the concerned pest. Therefore, one can conclude that these potent EOs might be useful for the management and control of stored product beetles.

Competing interests

The author(s) declare that they have no competing interests.

Acknowledgements

The authors would like to thank the Chairman, Department of Zoology, University of Rajshahi, Bangladesh for providing necessary laboratory facilities to accomplish the research work.

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