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Repellent and Fumigant Effects of Essential Oil from Clove Buds *Syzygium aromaticum* L. against *Tribolium castaneum* (Herbest) (Coleoptera: Tenebrionidae)

M. M. Abo-El-Saad[1], A. M. Al Ajlan[2], M. A. Al-Eid[2] and I. A. Bou-Khowh[1]

1. Date Palm Research Center (Center of Excellence), King Faisal University, P.O. Box 400, Al-Hassa 31982, Saudi Arabia

2. College of Agriculture and Food Science, King Faisal University, P.O. Box 400, Al-Hassa 31982, Saudi Arabia

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Abstract: Essential oil from clove buds *Syzygium aromaticum* L. Merr. & Perry (Myrtaceae) was extracted using petroleum ether in Soxhlet apparatus. The resultant oil contained eugenol (48.92%), caryophyllene (18.55%), α-caryophyllene (3.25%), eugenol acetate (23%), *cis*-13-docosenamide (3.21%), presenting more than 96% of the oil. This oil was examined as a fumigant and repellent agent against adults of the red flour beetle, *Tribolium castaneum*. Data showed that various concentrations of the oil have revealed dramatic repellent activity against the tested insect, where it gave 100% repellency by 1.0, 0.8, and 0.2% clove oil at 4, 8, 10 min, respectively. The RT_{50} and RT_{95} were 1.1 and 8.0 min at 0.2% and 0.4, 2.6 min at 0.8%, respectively. In addition, fumigation assay has also exhibited strong fumigant activity toward the adults of *T. castaneum*. At 100 µL oil/L air, mortality was 75, 80 and 100% after 6, 7, and 8 days exposure period with LC_{50} and LC_{95} 17 and 70 µL/L air, respectively. The utilization of clove oil for its potential effects against stored product insect is discussed.

Key words: Essential oils, clove, *Syzygium aromaticum*, *Tribolium castaneum*, fumigation, repellency.

1. Introduction

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests causing quantitative and qualitative losses [1]. Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms in addition to direct toxicity to users [2, 3]. Thus, repellents, fumigants, feeding and insecticides of natural origin are rational alternatives to synthetic insecticides. In stored product insect control, essential oils may have numerous types of effects [4, 5]: they may have a fumigant activity [6, 7], they may penetrate inside the insect body as contact insecticides [8] and they may act as repellents [9-11].

Accordingly, essential oils are potential alternatives to current stored-grain fumigants and repellents because of their low toxicity to warm-blooded mammals and their high volatility [7, 12]. Pest control in many storage systems depends on fumigation with methyl bromide. The use of methyl bromide is being restricted and will be phased out by 2015 because of its potential to damage the ozone layer [13].

Many alternatives have been tested to replace methyl bromide fumigation for stored product and quarantine uses. During recent years, some plants have been receiving global attention and their secondary metabolites have been formulated as botanical pesticides for plant protection since they do not leave residues toxic to the environment, have lower toxicity to mammals and medicinal properties for human uses [14].

Corresponding author: M. M. Abo-El-Saad, professor, research field: insect toxicology. E-mail: maboelsaad@gmail.com.

In nature, essential oils play an important role in the protection of plants as antibacterials, antivirals, antifungals, insecticides and also as antifeedants against herbivores. At present, approximately 3,000 essential oils are known, 300 of which are commercially important, especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries [15].

The essential oil isolated from the clove buds, *Syzygium aromaticum* is widely used and well known for its medicinal properties. Traditional uses of clove oil include use in dental care, as an antiseptic and analgesic, where the undiluted oil may be rubbed on the gums to treat toothache. It is active against oral bacteria associated with dental caries and periodontal disease [16]. Hussein et al. [17] found that *S. aromaticum* extract was highly active at inhibiting replication of the hepatitis C virus. Kurokawa et al. [18] isolated and identified an anti-HSV compound, eugenin, from the extracts of *S. aromaticum*, which showed specificity in inhibiting HSV-1 DNA polymerase activity. Clove essential oil has been reported to show anticarcinogenic property [19].

The biological activity of *S. aromaticum* oil has been investigated against several pests. It was shown to inhibit the emergence of *Culex pipiens* larvae [20] and to display insecticidal activity against *Pediculus capitis* [21], *Anopheles dirus* mosquitoes [22] and some stored product insects and suppresses progeny development of *T. castaneum* and *Sitophilus zeamais* with isoeugenol being particularly active [23]. A previous study has confirmed that clove oil could be used as a novel fumigant against Japanese termites [24].

T. castaneum is a major pest of stored grains and grain products in the tropical countries. One of the most popular foods in Saudi Arabia and other tropics is dates which are attacked in storehouses by the red flour beetle *T. castaneum* as a major insect [25, 26]. As part of future strategies for stored-product insect control, essential oils with repellent and/or insecticidal properties should be studied. Therefore, the aim of the present work was conducted to evaluate the potential repellent and fumigant effects of essential oil extracted from clove buds, *S. aromaticum* against the red flour beetle, *T. castaneum* (Coleoptera: Tenebrionidae).

2. Materials and Methods

2.1 Extractions of Essential Oil

Essential oil from clove buds *S. aromaticum* was extracted according to the method of Wenqiang et al. [27]. Clove buds, 40-50 gm were ground with an electric blender to fine powder. The powder was packed in extraction thimbles cellulose 33 × 100 mm. Each packed material was then extracted in 200 mL of petroleum ether using a Soxhlet apparatus under cooling for 6 hours. After extraction, the extract was filtered and then evaporated using a rotary evaporator (at 70 °C and 100 rpm) under vacuum to avoid chemical degradation of extracted materials. The extracts were stored at 10 °C until further use.

2.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS QP 2010 model Shimadzu was used. The mass data were analyzed by a GCMS solution. The GC conditions were as follows: column, RTX(r), 30 m × 0.32 mm; 0.25 mm; He carrier gas flow rate, 1.4 mL/min; split less: temperature program, 3 min at 100 °C, 3 °C min gradient to 200 °C, 20 °C/min gradient to 280 °C; 13 min; injection temperature, 260 °C; detector temperature, 270 °C. The EI was 70eV. The NIST/EPA/NIH standard mass-spectrum data bank was used for reference.

2.3 Insects

Adults of *T. castaneum* were obtained from laboratory cultures maintained in dark incubators at 28-30 °C and 70-80% r.h. at the Date Palm Research Center, King Faisal University, Saudi Arabia.

2.4 Repellent Activity

The experimental method was performed as described by Jilani and Saxena [28] with some modifications. Whatman No.1 filter papers (diameter 9 cm) were cut in half. Test solutions were prepared by dissolving 2, 4, 6, 8 and 10 µL of *S. aromaticum* oil in 1 mL acetone. Each solution was applied to half a filter-paper disc as uniformly as possible with a micropipette. The other half of the filter paper was treated with acetone alone as a control. The treated and control half discs were dried in air for 5 min to evaporate the solvent completely. Treated and untreated halves were attached to their opposites using adhesive tape and placed in Petri dishes. Twenty unsexed adult (7-10 day old) flour beetles were released separately at the centre of each filter paper disc. Three replications were used for each concentration. Observations on the number of insects present on both the treated and untreated halves were recorded at different time intervals 2, 4, 6, 8, and 10 min. Percent repellency is defined as the percentage of insects that avoided the treated section and was calculated as described in methods of Ngoh et al. [29] as follows:

% Repellency = $100 - [(100 \times N\mathrm{t})(N\mathrm{t} + N\mathrm{c})]$

Nt = Number of insects in the treated side;

Nc = Number of insects in the control side.

2.5 Fumigant Activity

The fumigant activity of *S. aromaticum* oil was tested as described by Tripathi et al. [30] with some modification. For adults, 0, 20, 40, 60, 80 and 100 µL of *S. aromaticum* oil were dissolved in 1 mL acetone and applied to Whatman No.1 filter paper (diameter 9 cm) were then were dried in air for 5 min. Treated filter papers were attached with the lid of 1 L glass jars using adhesive tape. Twenty adults (10 days old) were then placed in 1 L glass jar containing 5 g of wheat flour with 15% broken grains. The jars were then sealed with air-tight lids. Three replicates of each control and treatment were performed. Mortality was observed every 24 h thereafter until end-point mortality, which was reached after 8 days. Probit analysis was used to estimate concentration (LC_{50}, and LC_{95}) and lethal time (LT_{50}, and LT_{95}) values as described by Finney [31].

2.6 Statistical Analysis

Data were analyzed using MSTAT-C software and means were compared by Duncan's multiple range tests at 5% level.

3. Results and Discussions

3.1 Chemical Analysis of S. aromaticum Oil

Essential oil from clove buds contained five major compounds: eugenol (48.92%), caryophyllene (18.55%), α-caryophyllene (3.25%), eugenol acetate (23%) and cis-13-docosenamide (3.21%), presenting more than 96% of the oil. In addition, the oil contained five other minor compounds: copaene (0.66%), cycloheptasiloxane (0.5%), caryophyllene oxide (0.61%), n-hexatriacontane (0.47%), n-dotriacontane (0.83%) (Table 1). Our results are consistent with data reported by Bensky et al. [32] showing that Eugenol comprises 72-90% of the essential oil extracted from cloves, and is the compound most responsible for the cloves' aroma. Furthermore, Wenqiang et al. [27] showed that the maximum content of eugenol in extracts oil from clove buds is 58.77%, whereas, the highest percentage of eugenol acetate reached to approximately 21%, which is also the main antioxidant ingredients in clove oil [33]. They also reported that the highest percentage of active antioxidant ingredients, eugenol together with eugenol acetate, in the extracted

Table 1 Chemical composition of *S. aromaticum* essential oil.

Compound	Retention index	Abundance (%)
Eugenol	1392	48.92
Copaene	1221	0.66
Caryophyllene	1494	18.55
α- Caryophyllene	1597	3.25
Eugenol acetate	1552	23.0
Cycloheptasiloxane	1447	0.5
Caryophyllene oxide	1507	0.61
cis-13-Docosenamide	2625	3.21
n-hexatriacontane	3600	0.47
n-Dotriacontane	3202	0.83

clove oil was 78% which are in agreement with our results showing eugenol together with eugenol acetate; reached to 72%.

3.2 Repellent Activity

The extracted essential oil from clove buds, *S. aromaticum* was tested as a repellent agent against adults of red flour beetle, *T. castaneum* which is a major insect in Saudi Arabia and other tropical countries [25, 26] attacking dates in storehouses causing significant loss. The filter paper assay exhibited the behavior of the insect once they were exposed to oil extract. At the onset, insects were agitated, moved frequently and showed exceptional excitement. Within 10 min, almost all experimental insects gathered together in one spot in the Petri dish. The number of insects in both treated and untreated area was recorded and the percent repellency was calculated as shown in Table 2. Fig. 1 shows that the extracted oil exhibited highly significant repellent effect against adults of *T. castaneum*. At 0.2, 0.6 and 1.0% of extracted oil, the percent repellency was 63%, 75%, 87% after 2 min of exposure and it reached 100% repellency at various concentrations after 10 min. The corresponding RT_{50} values were 1.1, 0.68 and 0.4 min. respectively at concentration 0.2, 0.6 and 0.8%, whereas, RT95 values were 8.0, 4.0 and 2.0 min respectively for the same exposure periods (Table 2). Thus, *S. aromaticum* oil has potential for use against some stored-product insects as a repellent agent. Present data are consistent with those reported by Ngoh et al. [29] who indicated that eugenol, methyl eugenol and isoeugenol are better toxicants and repellents to the insects than monoterpenes. Furthermore, Huang et al. [34] was tested insecticidal property of eugenol derivatives; eugenol, isoeugenol and methyleugenol against *T. castaneum*, the order of potency of these chemicals compared by the LD50 level was isoeugenol > eugenol > methyleugenol.

Recently, there have been many reports concerning the repellent properties of many kinds of essential oils; however, most of results came from artificial (*in vitro*) testing methods using filter paper, insect, animal or olfactometry, but some came from more realistic (*in vivo*) using animal, insect or human subjects [35]. The repellency effect of many essential oils included egunol from *S. aromaticum* against medicinal insects was evaluated by using an arm-in-cage test, where, it was found that eugenol posses strong effect as a repellent agent [22].

Based on our results, adults of *T. castaneum* were found to be repelled by *S. aromaticum* oil at very low concentration. We believe the strong repellent activity of *S. aromaticum* oil that is presented here could be due to eugenol the main constituent of the oil (48.92%). In previous research, this compound has shown to be powerful repellent towards many pests [22, 29, 34-36].

Table 2 Estimated RT_{50} and RT_{95} values of various concentrations of *S. aromaticum* essential oil against adults of *Tribolium castaneum*.

Conc. % (V/V)	RT_{50} (min)	RT_{95} (min)
0.2	1.10	8.0
0.4	0.90	6.8
0.6	0.68	4.0
0.8	0.40	2.6
1.0	ND	ND

RT = the time required of clove oil that provides 50% and 95% repellency; ND = not detected values because too far beyond the tested exposure ranges to be reliable.

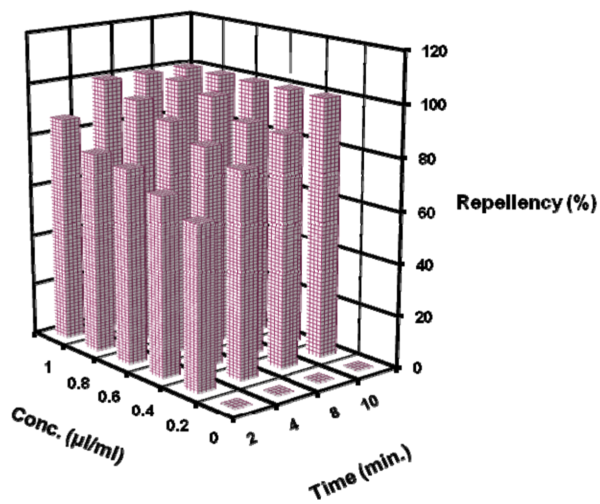

Fig. 1 Repellency percent of the red flour beetle, *Tribolium castaneum* adults exposed to *S. aromaticum* essential oil for various periods of times.

However, to clarify repellent activity of these pure constituents against *T. castaneum* future studies should be done.

3.3 Fumigant Activity

The analysis was extended to see whether *S. aromaticum* oil has a fumigant effect against adults of red flour beetle *T. castaneum.* Fig. 2 showed that the oil has a weak mortality up to three days from the exposure at various concentrations. The mortality reached 20% and 40% after 4 and 5 day of exposure at the concentration 100 µL/L air. However, by increasing the exposure period up to 7 and 8 days, significant mortality ($P < 0.05$) was achieved, it reached 80% and 100% respectively. The lethal concentrations values LC_{50} and LC_{95} were determined based on the method of Finney [31]. LC_{50} values were 156.89, 54.59 and 10.7 µL/L air after respectively 5, 6, and 8 days of exposure, whereas, LC_{95} values were 1,182.76, 357.7, and 254.87 µL/L air respectively for the same exposure periods (Table 3). Moreover, the median effective time causing mortality of 50% of tested insects (LT_{50}) at various concentrations (20, 40 and 100 µL/L air) were 7.2, 6.8 and 5.12 min respectively, whereas the LT_{95} were 16.8, 15.9 and 10.34 min. respectively (Table 4). The weak insect mortality of this oil for up to three days from the exposure at various concentrations against *T. castaneum* may be due to that the insect in the early days of exposure to essential oil is closing the spiracles, which reduces the opportunity for toxic substance to enter. So, this study it is clear indicated that the fumigation with natural substances is one of the most important ways to control stored-product pests without use of insecticides and the problems arising from its application.

The widespread use of synthetic insecticides has led to many negative consequences resulting in an increasing attention being given to natural products [36, 37]. Among biopesticides, botanical pesticides are experiencing a revival because some have favorable eco-toxicological properties (low human toxicity, rapid degradation and reduced environmental impact), which make them suitable insecticides for organic agriculture and store-products pests as well. In the case of stored-products pests, the biological activity of *S. aromaticum* oil has been investigated against several pests. It was shown to suppress progeny development

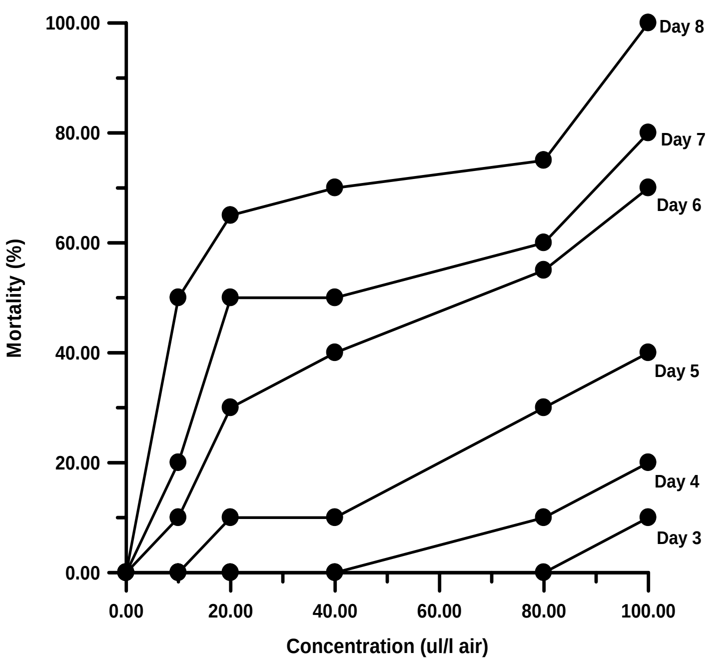

Fig. 2 Mortality percent of the red flour beetle, *Tribolium castaneum* adults exposed to *S. aromaticum* essential oil for various periods of times.

Table 3 LC50 and LC_{95} values of *S. aromaticum* essential oil against adults of *Tribolium castaneum* after exposed to various periods of time.

Exposure time (days)	LC50 (μL/L air) (95% fiducial limits)	LC95 (μL/L air) (95% fiducial limits)	Degrees of freedom	Slope (± SE)	Chi square (X^2)
5	156.89 (119.5-87.6)	1182.76 (2270.81-332.34)	3	1.96 ± 0.46	7.81
6	54.59 (95.54-36.96)	357.7 (282.1-165.3)	3	1.60 ± 0.38	7.81
7	35.27 (55.52-18.4)	329.48 (510.42-139.06)	3	1.29 ± 0.36	7.81
8	10.70 (21.93-0.254)	254.87 (167.60-92.5)	3	0.93 ± 0.36	7.81

LC = the lethal concentration for a 50% and 95% of treated insects.

Table 4 LT50 and LT_{95} values of various concentrations of *S. aromaticum* essential oil against adults of *Tribolium castaneum*.

Conc. μL/L	LT50 (min) (95% fiducial limits)	LT95 (min) (95% fiducial limits)	Degrees of freedom	Slope (± SE)	Chi square (X^2)
20	7.2 (8.5-6.6)	16.8 (14.5-8.5)	2	7.4 ± 2.01	5.99
40	6.8 (7.7-6.3)	15.9 (15.4-8.4)	2	1.96 ± 2.1	5.99
80	6.3 (7.2-5.6)	12.7 (17.1-8.6)	3	5.8 ± 1.36	7.81
100	5.12 (5.6-4.6)	10.34 (9.8-7.0)	4	6.79 ± 1.09	9.49

LT = the time required for a 50% and 95% kill.

of *T. castaneum* with isoeugenol being particularly active [23]. Our results are consistent with that data reported by Wang et al. [38] showing that some essential oils containing eugenol derivatives have strong fumigant activity against adults of *T. castaneum*.

It could be concluded that the oil extracted from clove buds has potential effects against *T. castaneum*, however, better understanding at the pure constituent level along with structure-activity relationships are required to develop *S. aromaticum* oil as a fumigant or repellent agent against stored-product insect pests. Further research on how to use *S. aromaticum* oil effectively for the control of insects in stored products is required.

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