Original article:

The Pesticidal Influence of Clove Extract Against the Rusty Flour Beetle, *Tribolium castaneum*

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Abstract

Background: The use of conventional insecticides to repel or kill insects leads to environmental pollution and harms human and animal health. Researchers are thus actively attempting to find natural ways of getting rid of insect pests in more environmentally sustainable ways. ***Objective:*** Behavioral response of the rusty flour beetle, *Tribolium castaneum,* to aqueous extract from clove was investigated here. ***Methods:*** In our study, the effect of the aqueous extract of cloves was tested for its potential to repel rusty flour beetle as the chemicals present in clove extract can trigger an olfactory response in this common pest. ***Results:*** Our findings indicate that the aqueous extract of cloves is a an effective repellant against the rusty flour beetle. ***Conclusion:*** This is a natural way to get rid of insect pests in more environmentally sustainable ways, as the use of traditional insecticides to repel or kill insects pollutes the environment and harms human and animal health.

Keywords: Rusty flour beetle, *Tribolium castaneum*, Clove plant, *Syzygium aromaticum,* Insect repellant

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Introduction

The world depends upon safe and healthy agricultural crops for food; to protect fields and post-harvest crops, synthetic chemicals have become pesticides of choice. However, they pose immense risks to human and animal health, as well as cause significant environmental pollution. Therefore, there is an urgent need to search for alternative methods of controlling agricultural pests.[1-3] Natural insect and/or pest repellants have low environmental impact and are safe for biota. These are generally natural extracts and secondary compounds derived from plants to fight against pest attacks.

A number of studies have reported testing plant extracts as pesticides. Taluker et al.[4] evaluated the potential of extracts from seeds of *Aphanamixis polystachya* to repel the rusty flour beetle, *Tribolium castaneum.* The authors demonstrated that the extract was highly repellent, although mildly nutritive, and was toxic to rusty flour beetles. Similarly, Islam et al.[5] studied the biological activity of the essential oil extracted from *Coriandrum sativum* L. against the eggs, larvae, and adult stages of the rusty flour beetle. Biological tests have shown that essential oils perform fumigation activity against eggs, and their toxicity increases gradually as concentrations are strengthened and repeated doses are applied. Coriander oil is reported to have a strong repellant activity against adult stages of the rusty flour beetle. Iqbal et al.[6] confirmed that ethanol extracted from sugar cane, *Acorus calamus*, and turmeric, *Curcuma longa*, can effectively repel the rusty flour beetle.

Elham et al.[7] experimented with the toxicity of acetylcholine seed extract against several types of insect pests to demonstrate that the extract had a repellant effect on the rusty flour beetle. Jema et al.[8] used essential vegetable oils from bay leaf plant, *Laurus nobilis,* to evaluate their repellant and toxicity effect against two main pests of stored

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products: the smaller grain bore, *Rhyzopertha dominica,* and the rusty flour beetle. They observed that volatile oils in the bay leaf plant not only repelled but were toxic to adult smaller grain bores and rusty flour beetles as well. Garcia et al.[9] reported essential oil from *Baccharis salicifolia* to be toxic to adult rusty flour beetle. Other than essential oils and extracts, direct use of powdered plant parts has been demonstrated to be effective against insecticides by Tripathi et al.,[10] who used a 100% clove powder at a dose of 1.5 g/50 g against rusty flour beetles. At a dose of 5g/100 g, powdered large cardamom, cinnamon, and clove completely inhibited the reproduction of *Callosobruchus maculatus* and the rusty flour beetle.

Methods

Insect collection and rearing: The rusty flour beetle was obtained from several sources (flour shop and a baker's warehouse) in Al-Qassim region of the Kingdom of Saudi Arabia. The rusty flour beetle was reared under laboratory conditions of 30°C temperature, 60% relative humidity, and 24 hours of complete darkness, following Al-Shuraym.[11] The experimental setups were prepared in 50 cm^3 glass jars that were filled with 1 kg of flour and 500 gm of yeast. Intact insects of the rusty flour beetle were isolated from an infested flour; 50 pairs were introduced into glass jars. The jars were covered with a mucilin cloth to prevent the escape of insects and to ensure adequate ventilation; the jar was fixed with a rubber strap.

Plant species and chemical composition: Flower buds of the carnation plant, clove, that is scientifically known as *Syzygium aromaticum,* were used in this study. Table 1 shows the major chemical constituents released in the clove bud that is generally used for a variety of purposes.

Table 1: Major chemical constituents and their average quantities in clove flower buds

Compound	Percentage
Furan, tetrahydro-3-methyl	2.5
2-propanone, methylhydrazone	5.6
Cyclopentane, methyl	4.0
Pyrrolidine, 2-butyl-1-methyl	0.1
2H-Pyran-2-one, tetrahydro-6,6-dimethyl	0.4
Eugenol	49.0
Copaene	0.5
Caryophyllene	7.5
Alpha-caryophyllene	1.4

Preparation of Clove Extract – Cold method: Fifty grams of cloves were added to 500 mL of distilled water and stirred on a magnetic stirrer at room temperature for half an hour. The solution was then filtered with a cloth to remove plant parts; the plants were then put in the centrifuge device at 3000 rpm for a period of 15 minutes to obtain a clear solution. The solution was poured into a glass Petri dish and placed in a drying oven at 35°C. Once all water had evaporated, the residue was scraped off the bottom of the Petri dish.[12] We obtained 0.2 grams of the residue that we scraped off after drying and placed it inside a graduated cylinder; the volume was then made up to a 100 mL total.

Experimental Design to Observe the Olfactory Response of Rusty Flour Beetle: The design of the experimental treatments was adapted from the study of Stamopoulos et al.[13] A custom-built small-scale device was prepared by using three plastic petri dishes for three treatments as shown in Figure 1. In our study, Dish 2 was prepared with two holes on the edge and was placed in the center of two other dishes, Dish 3 and 1. Each hole had a diameter of 6 mm; Dishes 1 and 3 were connected via plastic tubes of 75 mm length. In Dish 3, we had a treatment containing a semi-industrial environment made up of 5 grams of food (flour and yeast); this Petri dish had a fixed cover. On its inside, a piece of sponge measuring 1×1 cm was placed with the test substance (residue prepared before). This dish had one hole to connect it to one of the holes in Dish 2. Dish 1 was the control experiment that contained the same components as Dish 3, except that the test substance was not placed on the sponge.

The treatments: The aqueous extract of cloves (0.5μL) was placed in a graduated measuring flask, and a drop of acetone was added to it; the volume was made up to 1 mL with distilled water. This gave a final concentration of 0.05%. Following rigorous shaking, 1μL of the solution was placed on the sponge piece installed in the Petri Dish 3. In the control dish (Dish 1), no test material was placed. The number of intact rusty flour beetles was calculated in each dish after two hours had passed from the time that the set up was completed and insects were introduced in Dish 2. The rate of attraction and expulsion of the insects was calculated according to the following equation as described previously by Gunn & Cosway.[14]

Intensity of reaction =100□

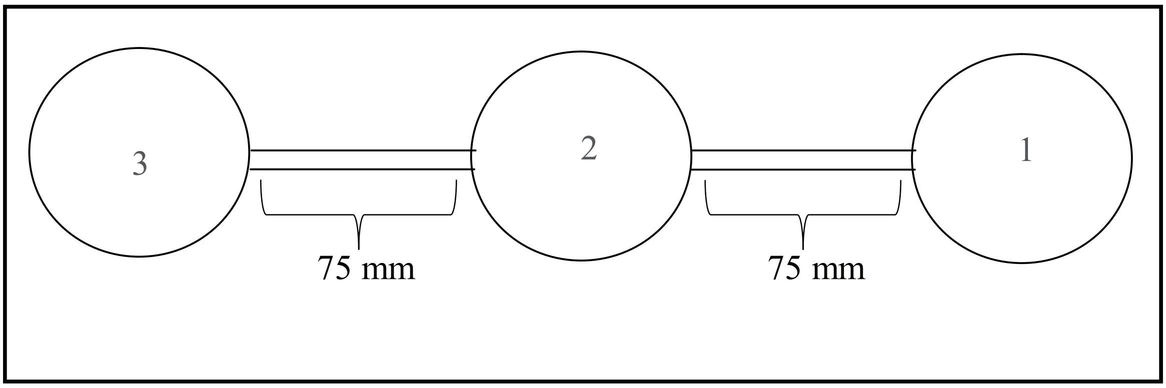

Figure 1. A custom-built device for the study comprising three petri dishes

Where, S is the number of insects attracted to a substance, A is the number of insects attracted to the control. The classification of attractiveness or repulsion of a material is done using the following criteria: <1% = non attractive repellent; 1-10% = very weakly attractive; 11-20% = weakly attractive; 21-40% = attractive; and ≤41% = very attractive.

Results

The results presented in Table 2 show that the aqueous extract of cloves led to an expulsion of the rusty flour beetle from the treatment in Dish 3; while some attraction of beetles was recorded in the control, i.e., Dish 1. Table 2 and Figure 2 depict the average ejection ratio according to the method of Gunn & Cosway.[14] Table 3 presents a summary of the response of the rusty flour beetle to the aqueous extract of cloves.

Table 2: Expulsion of the rusty flour beetle from the treatment in Dish 3 (aqueous extract of cloves)

Replicate number	S.	C.	S-C	S+C		Intensity of reaction (%)
Replicate 1	0	2	-2	2		-100%
Replicate 2	1	1	0	2		0%
Replicate 3	3	3	0	6		0%
Replicate 4	3	3	0	6		0%
Replicate 5	5	3	2	8		25%
Replicate 6	0	2	-2	2		-100%

S: The number of insects attracted to the scent of the test substance; C: The number of insects attracted to the control.

Table 3: Response of the rusty flour beetle to the aqueous extract of cloves

Replicate number		-	
Replicate 1	-100	-70.83	5016.89
Replicate 2	0	29.17	850.89
Replicate 3	0	29.17	850.89
Replicate 4	0	29.17	850.89
Replicate 5	25	54.17	2934.39
Replicate 6	-100	-70.83	5016.89
			15520.84

x□ Result of duplicate; x̄□ The result of the arithmetic mean; N: Replicate number

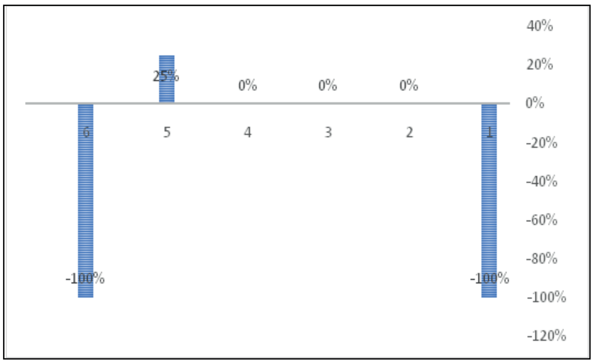

Figure 2. Intensity of reaction in percent with respect to the expulsion of insects by the aqueous extract of cloves

Discussion

The efficacy of allomones from the aqueous extract of cloves in repelling rusty flour beetle is clear from the results presented in Table 3. We tested the release of allomones with an olfactometer, which revealed repellent compounds to make up 29.17% of the total constituents in the aqueous extract of cloves. Our results are in agreement with previous research by Natalia et al.[15] that reported volatile oils to have a repellent effect on larvae and adult insects of the rusty flour beetle. On the contrary, Saim & Meelan[16] stated that the α-pinene complex present in laurel leaves has attractive properties. Numerous scientific studies have proven that insects have receptors that control their olfactory response, e.g., Jonsson & Anderson[17] demonstrated that the antennae of the cotton leaf worm are equipped with sensory filaments through which they can detect plant odors with high sensitivity. These odors are generally caused by volatile chemical compounds. Hansson et al.[18] studied olfactory receptor nerves in male scarab beetles, *Phyllopertha diversa,* that were capable of detecting and responding to volatile compounds emitted by green leaves, as well as the pheromones. The researchers indicated that the olfactory receptor nerves had the ability to distinguish between these volatile substances and molecules with a high degree of specificity and sensitivity. All these chemical compounds play an important role in the insect's behavior toward its plant hosts, which ensures its survival and reproduction in a given environment. This also explains the behavioral response of rusty flour beetles to many of the compounds that have been tested till now in our study, as well as others, such as essential oils, extracts, or terpenes. The different sensory properties are incurred by different organs distributed all over the body of the rusty flour beetle.

Conclusion

Pesticides extracted from plants have been demonstrated to repel insect pests; this is a safe and environmentally friendly method of preventing pest attack on agricultural crops at small and large scales. Based on our findings, we propose that aqueous extract of clove buds can effectively be used to protect storage warehouses and to keep insects away from stored food and prevent them from laying eggs.

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Ethical approval: The experiment was approved by the Departmental Ethical Committee for Care and Use of Animals.

Author's contribution: Both the authors contributed to the concept, design, literature search and drafting of the manuscript. They also revised and approved the final manuscript.

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