



Repellent potential of medicinal oils against *Tribolium castaneum* (Herbst) under laboratory conditions

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Abstract

Laboratory experiments were conducted to determine repellency of five local medicinal oils i.e., neem *Azadirachta indica* A. Juss., castor *Ricinus communis* L., rapeseed *Brassica napus* L., lettuce *Lactuca sativa* L. and chamomile *Anthemis cotula* L. against *Tribolium castaneum* Herbst, each applied at 0.1, 0.5- and 1.0-ml doses. A glass cylinder divided into A, B and C sections with one end close was used. Fifty grams of wheat flour was placed at A and C ends. Twenty freshly emerged *T. castaneum* was released at the center of cylinder (B). The data was recorded after one-, two- and three-days to count number of *T. castaneum* at A and C for calculating percentage repellency. All the oils showed repellent potential against *T. castaneum* as their repellency increased with dose and time exposure. After three days, 100% repellency of *T. castaneum* was recorded in 0.5- and 1.0-ml doses of neem oil, followed by 6.67±3.33% repellency at 0.1 ml neem oil. After three days, the maximum repellency of *T. castaneum* in rapeseed, castor, chamomile, and lettuce was 86.67±7.26, 76.67±6.01, 76.67±4.41, and 75.00±7.64%, respectively, all recorded at 1.0 ml dose. Overall, neem oil exhibited significantly more repellency of *T. castaneum*, whereas castor, lettuce, rapeseed and chamomile were found non-significant with each other. Among doses, although highest repellency was recorded at 1.0 ml dose, but not significantly different from 0.5 ml dose of the individual oil. Therefore, 0.5 ml should be applied per 50 grams of wheat flour to get effective repellency of *T. castaneum*.

Keywords: Beetle, Flour, Medicinal oils, Repellency

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1. INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae), is an important polyphagous insect pest of stored products characterized by its strong adaptability to diversified

environments. It can damage a wide range of cereals, grains and grain products^{1,2}. It is not only feeding on stored products but also contaminates them with their body parts and feces. The abdominal defense glands of this insect can secrete benzoquinones, that produce a pungent odor in the infected commodities and strongly depreciate the quality of the foodstuffs^{3,4}. Alone, *T. castaneum* can cause 5-30% infestation in stored commodities, thus the timely management of this noxious pest is required⁵.

Currently, the management of noxious stored grain pests is mainly dependent upon the usage of synthetic chemicals. However, injudicious, and heavy use of the chemicals in warehouses and agro-ecosystems have led to develop resistance among many serious pests including *T. castaneum*⁶. Moreover, synthetic pesticides' persistency in the environment has created many harmful impacts on humans and other vertebrates along with disturbance to ecosystem, and depletion of ozone layer^{7,8}.

During last century, microbial- and plant-based pesticides have been exploited on large scale against many harmful pests because of most of them possessed a rich quantity of secondary metabolites that are capable of to elicit insecticidal and repellent properties against many pests including insects^{9,10}. A wide variety of chemicals such as terpenoids, steroids, alkaloids, and phenolics are produced by the plants for their defense and same are reported to have important medicinal and insecticidal activities^{11,12,13}. Thus, essential oil derived from plants are considered as one of the key sources in the preparation of bio-pesticides^{14,15,16}, and can considered as a potential alternate of the synthetic pesticides due to their less persistency, reduced impact of the environment and non-target organisms including humans and their domesticated animals¹⁷.

Therefore, essential oils derived from many indigenous plants from various countries of the world have been tested and proved their effectiveness against insect pests, particularly *T. castaneum* has been reported to be sensitive against many of these essential oils and their active components^{18,19,20}. Mostly these botanical pesticides or their essential oils growth and metabolic activities of the targeted insects through enzymatic activities and physiological processes²¹. Accordingly, a quest for the development of botanical pesticides as alternative to synthetic insecticides has increased mainly because these botanicals are safe to humans and environmental¹⁴. Moreover, the understanding the biochemical properties of botanical pesticides against insect pests may provide safe management tools for keeping pest populations below threshold levels. Accordingly, this research was conducted to determine the repellent effect of various medicinal plant oils against *T. castaneum*.

2. MATERIALS AND METHODS

2.1 Studied Area

Study was carried out at Stored Grain Research Laboratory, Department of Entomology, Sindh Agriculture University, Tandojam during 2019-2020.

2.2 Samples collection

The culture of *T. castaneum* was collected from the laboratory-maintained stock at Stored Grain Research Laboratory, Department of Entomology. Further rearing of insects was carried out in the laboratory in fresh wheat flour in glass jars. Afterwards, freshly emerged adults obtained from this culture were used in the repellent bioassay.

2.3 Medicinal oils

Five medicinal oils i.e., Neem, *Azadirachta indica* A. Juss., Castor, *Ricinus communis* L., Rapeseed, *Brassica napus* L., Lettuce, *Lactuca sativa* L., Chamomile or Mayweed, *Anthemis cotula* L. were used in the study. Selection of medicinal oils was based on their medicinal potential, local availability and low cost. All the oils were obtained from the local market. Three doses i.e., 0.1 ml, 0.5 ml and 1.0 ml of each oil were used in the study.

2.4 Experimental setup, data collection and analysis

The experimental setup was adopted from Park *et al.*²² and Lee *et al.*²³ with slight modifications. A long cylinder, sealed from one end was divided into three areas (A, B, C), whereas the other end was sealed with thick cloth for ventilation and retention of *T. castaneum* inside the cylinder during the entire duration of experiment. The repellent efficacy of the essential oils and plant extracts against insects was evaluated according to the method used by Jo *et al.*²⁴. Each dose of the respective oil was applied on the filter paper

that was allowed to dry completely before placing them inside the cylinder. A control filter paper without oil was also used. The treated filter paper was introduced between A and B, whereas the control filter paper was placed between B and C (Fig. 1). Ten pairs of freshly emerged *T. castaneum* adults were released in the middle of B with help of a camel brush. The experiment was conducted under the temperature regime of $30\pm 5^\circ\text{C}$ and $60\pm 5\%$ relative humidity.

The data on repellence of medicinal oils against *T. castaneum* was recorded on daily basis for three days. At each data collections, wheat flour from portion C was carefully transferred into a glass tray to count the number of *T. castaneum* with the help of a camel brush. After data collection, the flour along with *T. castaneum* was gently placed back at the same place at C. Similar trend was followed at second and third day of the observations.

The repellent efficacy of medicinal oils was calculated using the following equation:

$$\text{Repellent efficacy (\%)} = \frac{N_c - N_a}{N_c + N_a} \times 100$$

Where, N_c is the number of insects in C, and N_a is the number of insects in A.

The experiment was arranged in a completely randomized design, where each treatment oil was replicated three times. Separate glass cylinders were used for the individual medicinal oil, where cylinders were thoroughly cleaned with tissue paper after performing each replication. The Analysis of Variance was used to analysis the data, whereas the means with significant differences were separated using the Least Square Difference (LSD) at 5% probability. All the analyses were performed through STATISTIX 8.1 computer software.

3. RESULTS AND DISCUSSIONS

All the medicinal oils used showed the repellent potential against *T. castaneum*. According to results, after one day, the highest repellency percentage of *T. castaneum* at 0.1 ml dose was recorded in neem oil ($53.33 \pm 6.67\%$), followed by castor oil ($36.66 \pm 12.02\%$), whereas the minimum repellency was observed in lettuce oil ($23.33 \pm 8.82\%$). A gradual rise was observed in the efficiency of medicinal oils to elicit repellent effect against *T. castaneum* with increasing their doses as the highest and lowest percentage repellency at 0.5 ml dose was recorded in neem oil ($58.33 \pm 7.26\%$), and chamomile oil ($33.33 \pm 8.82\%$), respectively. Similar trend in results was recorded at 1.0 ml dose of the oils as neem oil showed the highest repellency ($80.00 \pm 5.77\%$), followed by rapeseed oil ($60.00 \pm 5.77\%$), whereas the lowest repellency was recorded in castor and lettuce oils ($43.33 \pm 12.02\%$), followed by chamomile oil ($53.33 \pm 7.26\%$) (Fig. 2). Overall, after one day, there was no significant difference recorded in various doses of medicinal oils for their repellent potential against *T. castaneum* ($F = 0.41$, $P = 0.9087$).

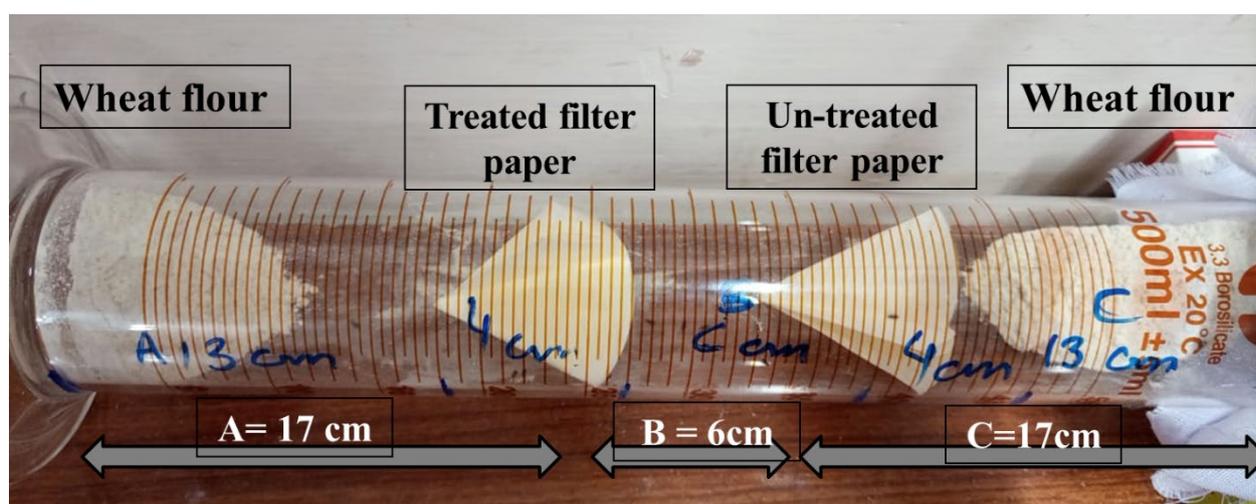


Fig. 1. Systematic diagram of cylinder trap for evaluating repellent efficacy of medicinal oils against *Tribolium castaneum*.

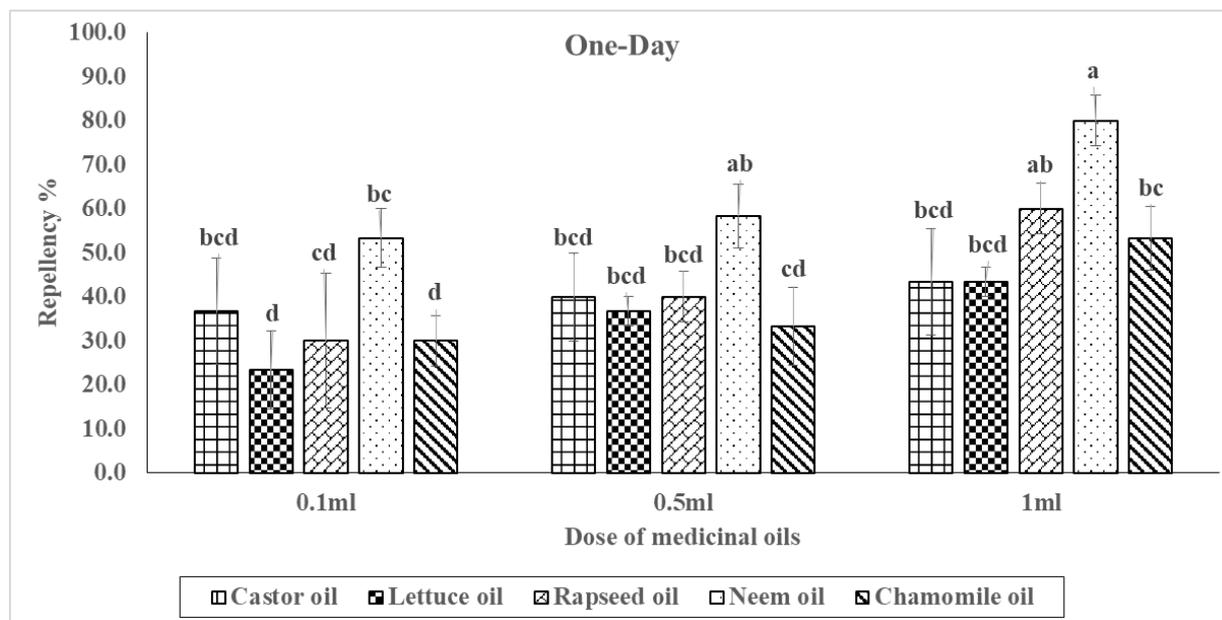


Fig. 2. Repellency percentage of different concentrations of medicinal oils against *Tribolium castaneum* after one day *Means followed by same letters are not significantly different (LSD = 24.54; $P < 0.05$)

Fig. 3 describes the results regarding the repellency percentage of *T. castaneum* due to the application of different doses i.e., 0.1, 0.5 and 1.0 ml of five medicinal oils after two-days. Like one-day, the combination effect of various doses of medicinal oils exhibited no significant difference ($F = 0.70$, $P = 0.6883$) in their repellency, however, a rise was recorded in the repellency of medicinal oils against *T. castaneum*. According to results, after two-days, the highest repellency at 0.1 ml dose was recorded in neem oil ($78.33 \pm 1.67\%$), followed by castor ($55.00 \pm 8.66\%$), whereas the lowest repellency of *T. castaneum* was recorded in lettuce ($38.33 \pm 8.82\%$) oils. A steady rise was witnessed in the efficacy of medicinal oils in their repellent effect against *T. castaneum* at 0.5- and 1.0-ml doses. Accordingly, after two-days at 1.0 ml dose, neem oil repelled $96.67 \pm 5.77\%$ *T. castaneum*, whereas the lowest repellency was recorded in chamomile oil ($55.00 \pm 7.26\%$). After three-days, a further repellency was recorded in different doses of all medicinal oils as 100% repellency was recorded in 0.5- and 1.0-ml doses of neem oil, whereas $96.67 \pm 3.33\%$ repellency was recorded at 0.1 ml dose of neem oil. Among remaining oils i.e., rapeseed, castor, chamomile and lettuce, the highest repellency recorded was 86.67 ± 7.26 , 76.67 ± 6.01 , 76.67 ± 4.41 , and $75.00 \pm 7.64\%$, respectively, all recorded at 1.0 ml dose (Fig. 4). Accordingly, like results of one and two-hours, statistically no significant ($F = 0.19$, $P = 0.9905$) difference was also recorded among various doses of medicinal plants after 24-hours of the application.

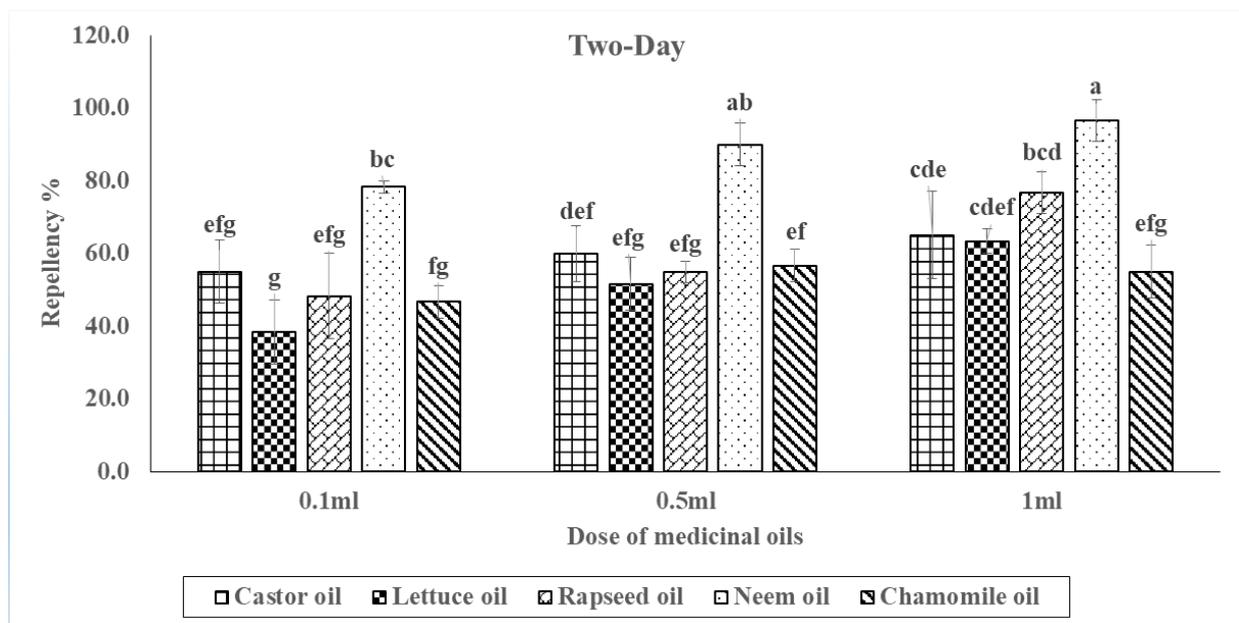


Fig. 3. Repellency percentage of different concentrations of medicinal oils against *Tribolium castaneum* after Two days

*Means followed by same letters are not significantly different (LSD = 18.13; $P < 0.05$)

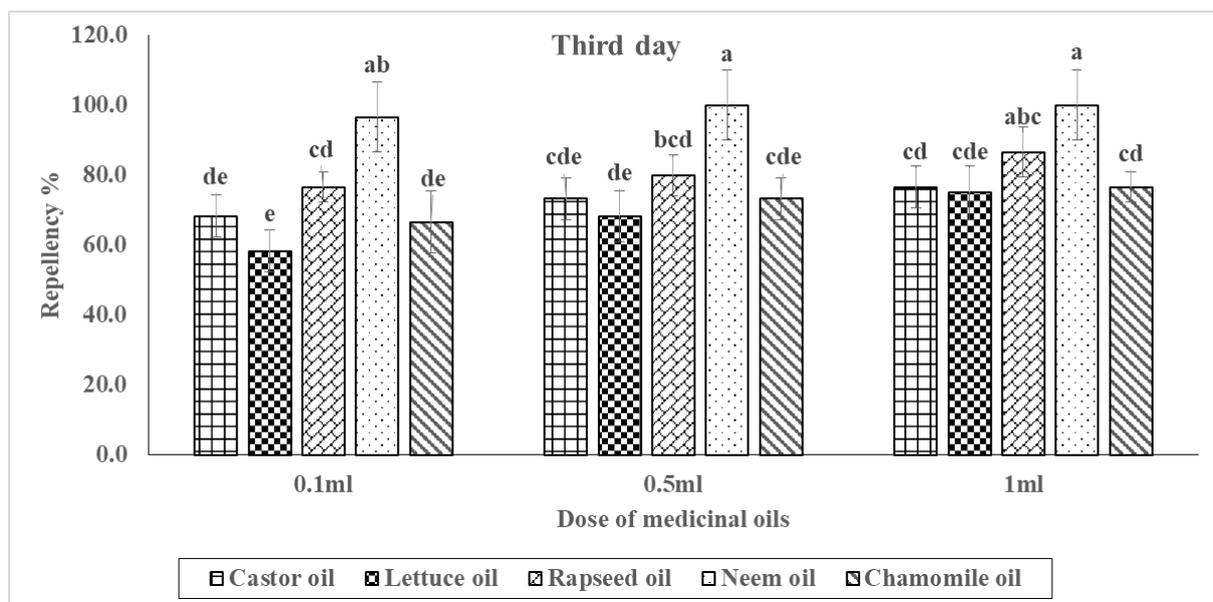


Fig. 4. Repellency percentage of different concentrations of medicinal oils against *Tribolium castaneum* after three days

*Means followed by same letters are not significantly different at the respective doses (LSD = 16.76; $P < 0.05$)

Fig. 5 showed the results regarding the overall repellent efficacy of various doses of medicinal oils at different time intervals i.e., one, two and three days against *T. castaneum*. At all the time intervals i.e., one day ($F = 8.65$, $P = 0.001$), two-days ($F = 5.62$, $P = 0.0017$) and three-days ($F = 4.37$, $P = 0.0067$), neem oil significantly showed the highest repellency percentage of 63.89 ± 5.26 , 88.33 ± 3.33 and $98.89 \pm 1.11\%$, respectively. All other oils showed no significant difference among themselves to repel *T. castaneum* at different time intervals when applied at various doses. The results also indicated a rise in the repellency of *T. castaneum* with the increase in dose of various medicinal oils. Accordingly, a significant difference was recorded in various doses applied of medicinal oils after one-day ($F = 3.36$, $P = 0.048$), two-days ($F = 5.89$, $P = 0.0069$) and three-days ($F = 4.38$, $P = 0.0214$) hours of application. According to results, after one-day, overall repellency of *T. castaneum* recorded at 1.0 ml dose of all medicinal oils was $56.00 \pm 4.58\%$, followed

by 0.5 ml (41.67±3.64%) and 0.1 (34.67±4.77%) ml doses. Similarly, after two- and three days of application, the highest repellency was recorded in 1.0 ml dose i.e., 71.33±4.24 and 83.00±3.34%, respectively, whereas the lowest repellency of *T. castaneum* was observed in 0.1-ml dose i.e., 53.33±4.70 and 73.33±4.16%, respectively. Moreover, the repellency percentage of beetles recorded at 0.5 ml dose after two- and three-days was 62.67±4.33 and 79.00±3.66%, respectively (Fig. 6).

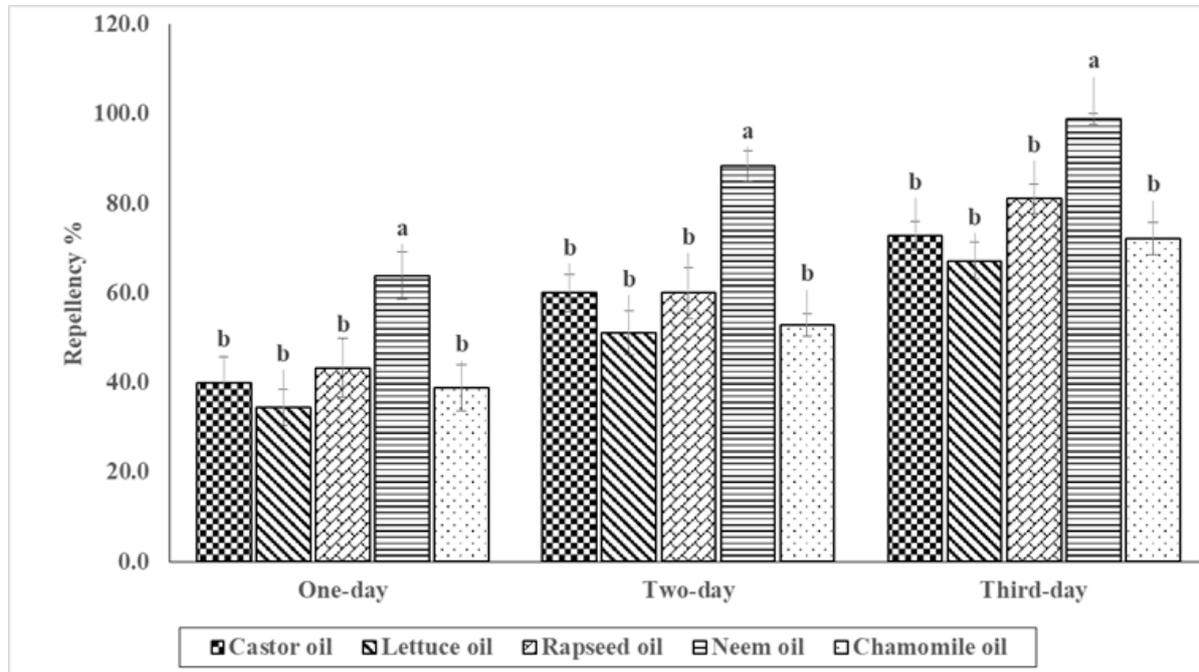


Fig. 5. Overall repellency percentage of various medicinal oils at different days intervals against *Tribolium castaneum*

*Means followed by same letters are not significantly different at their respective exposure time interval- LSD values @ P < 0.05

One-day = 14.171

Two-days = 10.473

Three-days = 9.6807

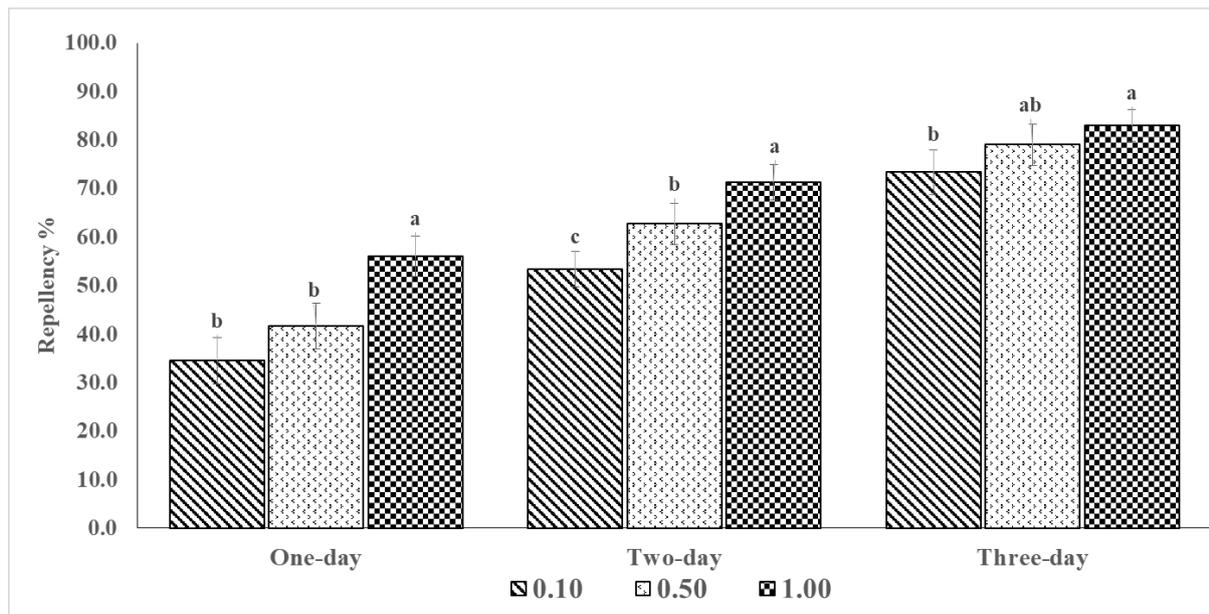


Fig. 6. Overall repellency percentage of different concentrations of medicinal oils at different days intervals against *Tribolium castaneum*

*Means followed by same letters are not significantly different at their respective exposure interval- LSD values @ P < 0.05; One-day = 10.977; Two-days = 8.1122; Three-days = 7.4986

Many plant extracts have also shown promising results on various biological parameters of *T. castaneum* with varying level of success. Generally, plants produced naturally occurring, volatile, complex secondary metabolites or compounds that possessed strong odor. Most of them are recognized for their bactericidal, fungicidal, virucidal, insecticidal and medicinal properties. Therefore, quest for the plant-based compounds to be use for the protection of stored products from damaging pests could lead to sustainable and healthy agriculture^{25,26}. Among plants, neem, *A. indica* is one of the widely studied plant as seed protectionist against many pests including more than 350 arthropod species, 12 nematode species, 15 fungi species, 3 viruses, and 2 snail species and one crustacean species^{27,28}. According to²⁹, extracts from various parts of neem has been successfully used for the management of storage, household and crop pests either used as fumigant or disinfectant in many countries of the world.

Among other plants, powders obtained from the rhizome of turmeric (*Curcuma longa* L.), and sweet flag (*Acorus calamus* L.) have elicited good results for lowering the population of *T. castaneum*³⁰. In another study,³¹ while screening forty Chinese local medicinal herbs found from thirty-two families for their bioactivity against *T. castaneum* and *Sitophilus Zeamais* L., mentioned that thirty of the herbs shown insecticidal properties against *T. castaneum*. Among the repellents tested against *T. castaneum*, powder of *Annona squamosa* L. has proven effective to protect the stored millet against *T. castaneum* when applied at the rate of 7.5g/25g (30% w/w).

Many plant based extracts and oils such as *Peganum Hamala* L.³², citrus essential oils³³, Tumha (*Citrus colocynthis* L.)³⁴, neem oil³⁵, *Curcuma longa* L. leaf oil, *Lippia rugose* L. (Lamiaceae), and *Hyptis spicigera* Lam. (Verbenaceae)³⁶, essential oils extracted from the rhizomes of *Zingiber zerumbet*, *Alpinia conchigera*, *C. zedoaria*³⁷, *Nicotiana tubacum* and *Salsola banyosma*³⁸ have shown insecticidal properties against *T. castaneum* with variable degree of success.

Thus, in continuation of the previous studies, all the medicinal oils used in the study i.e., neem, rapeseed, chamomile, castor, and lettuce showed promising repellent effects against *T. castaneum* and could be used as substances for stored grain protectants that are safe, nontoxic to humans, and eco-friendly^{39,40}.

4. CONCLUSIONS

All the medicinal oils showed repellency potential against *T. castaneum* as neem was found most effective showing 100% repellency of *T. castaneum* at 0.5- and 1.0-ml dose after three days. A rise in the repellency of medicinal oils was recorded with the dose and exposure time. Among doses, although highest repellency was recorded at 1.0 ml, but not significantly different from 0.5 ml of the individual oils.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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