



Evaluation of the Efficacy of Some Plant Oil Extracts in the Management of *Tribolium castaneum* (Herbst)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Four-way olfactometer was used to evaluate Laboratory repellency activities of *Azadirachta indica*, *Jatropha curcas* and *Dennitia tripetala* oil extracts on *Tribolium castaneum* at 10 μ l of each oil. Each arm of the olfactometer served as treatment arm with the 3 arms accommodating the 3 test substances, respectively and the 4th arm as control. Various concentrations (1, 2 and 3% V/V) of the essential oils were evaluated for fumigant and contact toxicity against *T. castaneum* at 8, 16 and 24 hours durations. The results obtained showed that *T. castaneum* spent significantly ($p < 0.05$) more time (min) in the control arm than the 3 arms accommodating the essential oils. Similarly, significantly ($p < 0.05$) more number of entries were made by the insect into the control arm compared with the test arms. The application of the essential oils significantly ($p < 0.05$) caused higher mortality at 8, 16 and 24hrs than the control for both fumigant and contact tests. The mortality of the *T. castaneum* increased with increase in the concentration of the essential oils. The 3% concentrations of the oil extracts significantly caused the highest mortality, 67.23 \pm 8.72, 69.22 \pm 18.74 and 79.55 \pm 9.29% by *A. indica*, *D. tripetala* and *J. curcas*, respectively after 24 hours application in fumigant test. The result also indicated that the contact application of *A. indica*, *D.*

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tripetala and *J. curcas* at 3% concentrations resulted in 63.07±6.55, 70.10±2.51 and 67.4±4.06% mortality of *T. castaneum*, respectively. The results suggest that the oil extracts from *A. indica*, *J. curcas* and *D. tripetala* can be used for effective management of *T. castaneum* infesting stored products.

Keywords: *Tribolium castaneum*; mortality; *Azadirachta indica*; *Jatropha curcas*; *Dennitia tripetala*.

1. INTRODUCTION

Red flour beetle, *Tribolium castaneum* is one of the most common and economically important secondary stored products insect pests. It is the most significant among about 100 insect species of the family Tenebrionidae (Order: coleopteran) causing huge losses to a wide range of stored food products [1,2,3]. The insect feed on the various stored products such as flour, cereals, legumes, spices, oilseeds, nuts, milled grains, baked products etc. [4,5]. *Tribolium castaneum* is a reddish brown insect with a survival temperature range between 22°C and 40°C but optimum temperature of 35°C and relative humidity of 75% [6].

The insect has been reported to be the most successive of the tenebrionidae due to its fast reproductive rate among other insect pests in addition to the habit of the adult to feed on eggs, larvae and pupae when population explosion and resultant competition occur [7,8,9]. *Tribolium castaneum* is regarded as a secondary colonizer because it develops more easily on broken grain kernels, flour or grains already infested by primary colonisers [10,11].

Adults and larvae of the insect are serious economic pests that cause quantitative and qualitative losses in tropical and subtropical regions [12,13,14,3,15,16].

The annual overall damage caused by *T. castaneum* has been estimated to be 10 – 40% of the total worldwide products [1]. The insect have been reported to be one of the most harmful insects of starch products in the world [6]. The infested stored products due to secretion of quinine compounds by *T. castaneum* have been reported to have foul smell and bitter taste [10,17,18,19,2,3,6].

Studies indicated that benzoquinone produced or secreted by *T. castaneum* may have toxic and carcinogenic effects on human and experimental animals [8]. Infestations of grains and stored products by insects promote growth of fungi including those that produce mycotoxins such as

aflatoxins and results in contamination of commodities with insect bodies and waste products. Aflatoxins are groups of secondary metabolites and are highly carcinogenic, produces by *A. flavus* and *A. parasiticus* [4,8].

Some of the synthetic chemicals (fumigant or contact) such as phosphine, methylbromide, lindane, malathion etc. are being used for the control of this insect pest [20;13,7,21].

However, some problems associated with the use of such conventional pesticides includes; rapid development of resistance by the targeted insect pests to the chemical, toxicity towards the non-targeted organisms (including humans) and undesirable environmental pollution [11,22,23,24,25,26, 8,27,28,16].

Hence, these problems posed by these conventional insecticides has discouraged the use of synthetic chemicals in the control of insect pests.

However, the continual quest for insecticides of plant origin may likely proffer solution to the malady posed by synthetic chemicals in the management of this stored product insect pests [29,18,30,31,32,33,34,35,36,37].

To address these problems of pest infestation and disease infection occasioned by *T. castaneum* on stored products resulting in both qualitative and quantitative losses (foul smell, bitter taste, reduction in weight/volume), there is a need to do more research on *A. indica*, *J. curcas* and *D. tripetala* that seems very promising as biopesticides for the control of *T. castaneum*.

The objectives of this study is to evaluate the efficacy of these plant extracts (*J. curcas*, *A. indica* and *D. tripetala*) for the management of *T. castaneum*.

2. MATERIALS AND METHODS

Test insect: *Tribolium castaneum* was obtained from the laboratory stock culture in the

Department of Crop Science, University of Calabar, and reared on untreated wheat flour purchased from Akim food stuff market in Calabar, Cross River State, Nigeria. The insect was cultured in transparent containers at room temperature.

After about five weeks of oviposition, the adults were removed by sieving the grains with a sieve of 2mm mesh. One week (7 days) old unsexed *T. castaneum* adult that subsequently emerged were collected from the laboratory colony and used for the experiment.

2.1 Plant Materials Collection and Extraction

Mature seeds of neem, *Azadirachta indica* were obtained from IBB Road, Calabar. While pepper fruit, *Dennitia tripetala* were purchased from Watt Market, Calabar. The location lies within Latitude 4°56'59.99"N and Longitude 8°19'18.00"E. The *Jatropha curcas* seeds were obtained from Ijegu Yala, Cross River State, Nigeria. It lies within Latitude 06° 28' 13"N and Longitude 8°34'29"E.

The plant materials were air dried on the shade for 3 days and 50g of the dried portion of each plant materials were grounded and used for oil extraction. The essential oil of each plant was extracted by soxhlet method. Fifty grams (50 g) of each plant powder weight into 500ml round bottom flask and 50ml of n – hexane added, the mixture was then heated, pure plant oil was obtained by evaporating the extracting solvent in a water bath at 80°C.

2.2 Laboratory Repellence Bioassay

Behavioral bioassay were performed in a four-armed olfactometer modified after Pettersson [38]. The olfactometer consisted of three layers of 6mm thick transparent perspex screwed together purchased from Rothamsted research, Harpenda, UK. Single bioassays were conducted and each was run for 10 minutes using a stop watch and the bioassay was replicated 10 times using a fresh insect and stimulus source in olfactometer. The first bioassay involved a control experiment in which all the 4 arms of the olfactometer contained 10µl of solvent (hexane) loaded on clear filter paper discs. This was followed by the repellence bioassay in which the three arms of the olfactometer contained the test compound (each essential oil serve as odour source) while the remaining one arm served as control and contained the solvent (hexane). Each

of the test arms had 10µl of one the essential oils as odour source.

2.3 Fumigant Toxicity

Solutions of each of the essential oils (*A. indica*, *J curcas* and *D. tripetala*) were prepared separately at 1, 2 and 3% of the oils respectively by distributing each essential oil at 0.2, 0.4 and 0.6ml in 20ml of n – hexane respectively and each applied to a filter paper strip for fumigant test. The treated filter paper stripe were allowed to dry for 3 minutes and placed against the wall of the 100ml flat bottom glass flask. Twenty *T. castaneum* were exposed to the treated paper strip and the bottle was sealed with screw caps. Hexane was used as a control and the treatments replicated three times. Adults were considered dead if appendages did not move when probed with a camel hair brush. The treatments (10) were arranged in completely randomized design and replicated 3 times.

2.4 Contact Toxicity

The contact toxicity of the essential oils against *T. castaneum* was evaluated using topical application. Three concentrations of the plant essential oils (1, 2 and 3%) were obtained by diluting each essential oil at 0.2, 0.4 and 0.6µl in 20ml of n – hexane respectively. Twenty adult insects were placed in a petri dish containing NO 1 whatman filter paper and treated with 1µl of each plant essential oils at the dorsal region of the insect. Adult (7 day old) insects treated with 1µl n – hexane served as control treatment. Both essential oil treated and control treatment were transferred to petri dishes on artificial diet and kept in the behavior room at room temperature. In each petri dish, 20g of wheat flour was added as food source for the insects. The experiment constituted 10 treatments replicated 3 times and arranged in Complete Randomized Design (CRD). An insect was considered dead when appendages do not move when probed with a camel hair brush [10].

2.5 Data Collection and Analysis

A computer program-for collecting and analyzing behavioral data with the four armed olfactometer (OLFA program) was used to collect repellency data. The data recorded included; time spent by the beetle in the different arms of the olfactometer and the number of visits made into each arms. The data obtained were subjected to one – way analysis of variance using Genstat 8.1

version software and means separated using Turkey's test at 5% probability level. Similarly, data obtained from fumigant action and contact toxicity of the experiment were transformed using arc sine transformation method before being subjected to analysis of variance. Means were separated using Duncan's multiple range test at 5% probability level.

3. RESULTS

3.1 Repellent Effects of Essential Oils against *Tribolium castaneum* in a Four-armed Olfactometer

The result of the time spent in the various arms of the olfactometer by *T. castaneum* is showed in Fig. 1. The result indicated significant ($p < 0.05$) difference in time spent by *T. castaneum* at the arms of the olfactometer. The result further revealed that *T. castaneum* spent significantly ($p < 0.05$) more time in the control arm (with hexane) than the other three arms treated with plant oil extracts. *T. castaneum* spent significantly ($p < 0.05$) longer time in the arm of the olfactometer treated with *A. indica* oil extracts than the arm treated with *D. tripetala* but statistically ($p < 0.05$) spent the same time in the arm treated with *J. curcas* oil extract. The duration spent in the olfactometer by *T. castaneum* in the arms treated with *D. tripetala* and *J. curcas* was also statistically ($p > 0.05$) the same.

The result of the number of the entries made by *T. castaneum* into various arms of the olfactometer is shown in Fig. 2. The analysis revealed significant difference ($p < 0.05$) in the number of entries made by *T. castaneum* into the arms of the olfactometer. Significantly ($p < 0.05$) more number of entries were made by *T. castaneum* into the control arm (with hexane) than that into the test arms (arms treated with *D. tripetala*, *J. curcas* and *A. indica* oil). *Tribolium castaneum* statistically ($p > 0.05$) made equal number of entries into the arms treated with *D. tripetala*, *J. curcas*, and *A. indica* oil extracts.

3.2 Fumigant Actions of Essential Oils against *Tribolium castaneum*

The result of the fumigant toxicity of essential oils on *T. castaneum* is shown in Fig. 3. The result indicated that at 8, 16 and 24 hours after the application of the various concentrations of the essential oils, there was a significantly ($p < 0.05$) higher mortality of *T. castaneum* than the control used except the use of 1% concentration of the essential oils at 8 hours after application. At 8 hours after the application of 1% concentration of the essential oils, the same rate of mortality of *T. castaneum* was experienced when compared with the control except 1% concentration of *D. tripetala* that caused significantly ($p < 0.05$) higher mortality of *T. castaneum* than the control. The application of 2% concentrations of essential oils

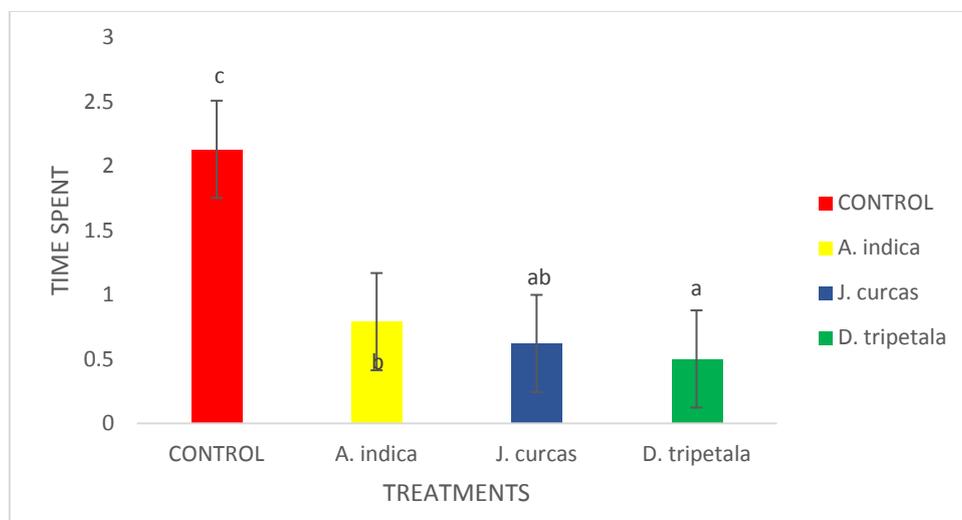


Fig. 1. Bioactivity of essential oils on the time spent by *Tribolium castaneum* in the arms of the olfactometer.

NOTE: Means capped with the same letter (s) Within the same week are not significantly ($p > 0.05$) different from each other according to Duncan's New Multiple Range Test (DNMRT).

HAP= Hours after application.

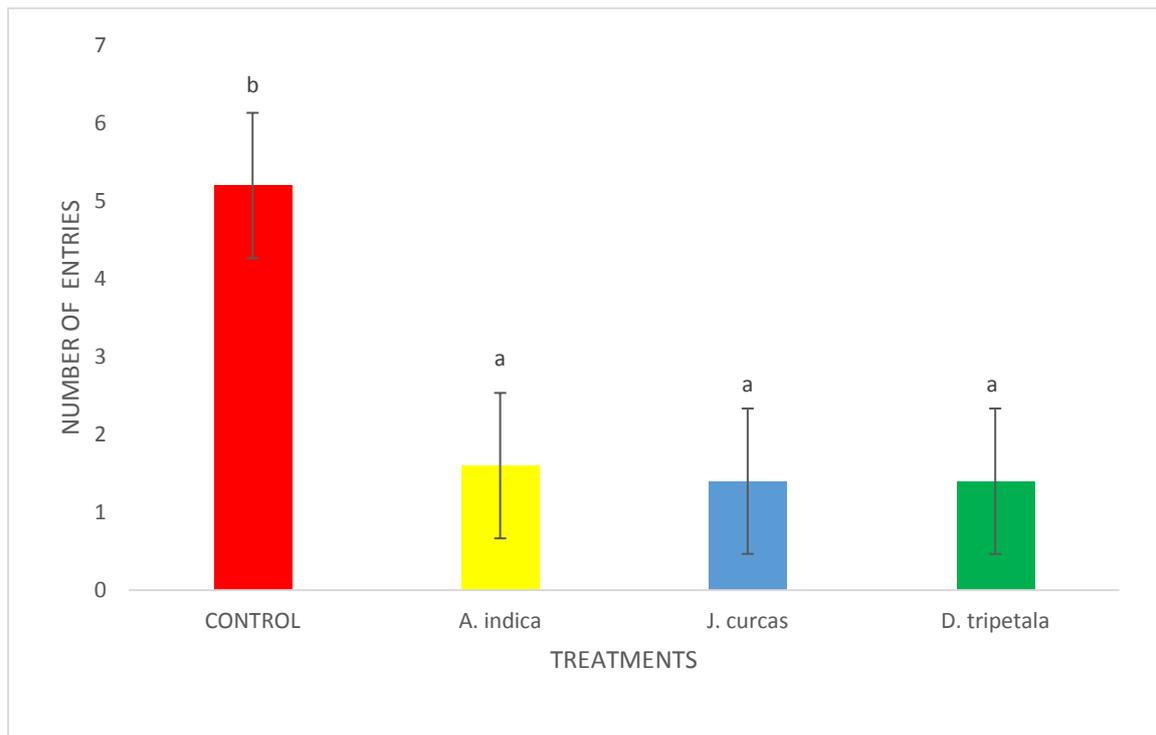


Fig. 2. Bioactivity of essential oils on the number of entries made by *Tribolium castaneum* into the arms of the olfactometer

NOTE: Means capped with the same letter (s) within the same week are not significantly ($p > 0.05$) different from each other according to Duncan's New Multiple Range Test (DNMRT).
HAP= Hours after application.

caused significantly ($p < 0.05$) higher mortality than 1% concentration and control after 8 hours of application except the application of 1% *D. tripetala*. The application of 3% of *J. curcas* and *D. tripetala* essential oils caused *T. castaneum* mortality that was significantly ($p < 0.05$) higher than any rate of application except, the use of 3% *A. indica* and 2% *J. curcas* oils.

Furthermore, the mortality of *T. castaneum*, 8 hours after the application of 2% concentration of *D. tripetala*, *A. indica*, *J. curcas* and 3% concentration of *A. indica* remains the same.

At 16 and 24 hours after the application of 3% concentrations of the three essential oils caused significantly ($p < 0.05$) the highest mortality of *T. castaneum* in the experiment which were statistically ($p > 0.05$) the same with each other.

Similarly, the application of 2% concentration of the oil extracts at both duration (16 hours and 24 hours) significantly ($p < 0.05$) caused higher mortality of *T. castaneum* than the application at

any other rate used except 1% of *J. curcas* that caused statistically ($p < 0.05$) the same mortality rate at 16 hours after application with that of 2% *D. tripetala*. Furthermore, 1% concentrations of all the oil extracts resulted in higher mortality of *T. castaneum* than that of the control.

3.3 Contact Effect of Essential Oils on *Tribolium castaneum* under Durations of Application

The result of the contact toxicity of essential oils against *T. castaneum* is presented in Fig. 4. The analysis showed that significant difference ($p < 0.05$) existed among treatments in all the duration of exposure. The application of the 3% concentration of the essential oils (*A. indica*, *J. curcas* and *D. tripetala*) had significantly ($p < 0.05$) the highest mortality rate in all the durations of application. The application of 2% concentration of the essential oils significantly ($p < 0.05$) had higher mortality than that of 1% concentration of the oils and control. The control treatment had significantly ($p < 0.05$) the least mortality rate across all the durations of application.

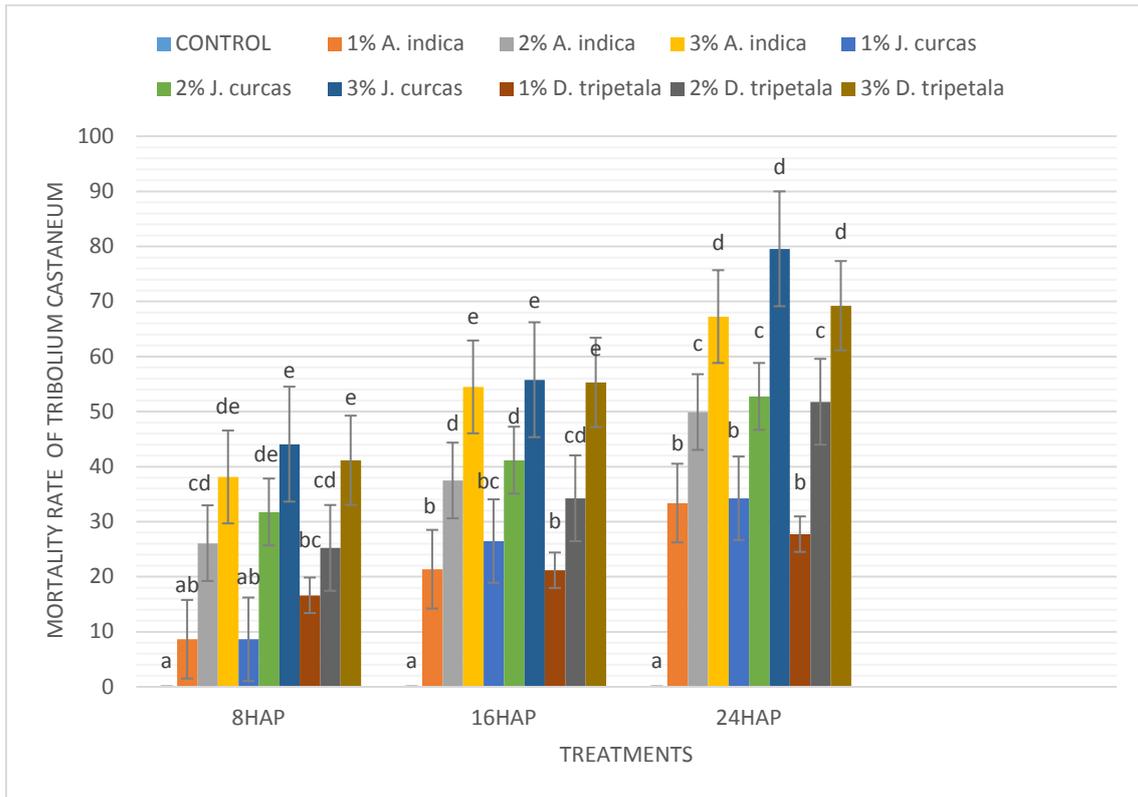


Fig. 3. Fumigant effect of essential oils on *Tribolium castaneum* mortality

NOTE: Means capped with the same letter (s) within the same week are not significantly ($p>0.05$) different from each other according to Duncan's New Multiple Range Test (DNMRT).
HAP= Hours after application.

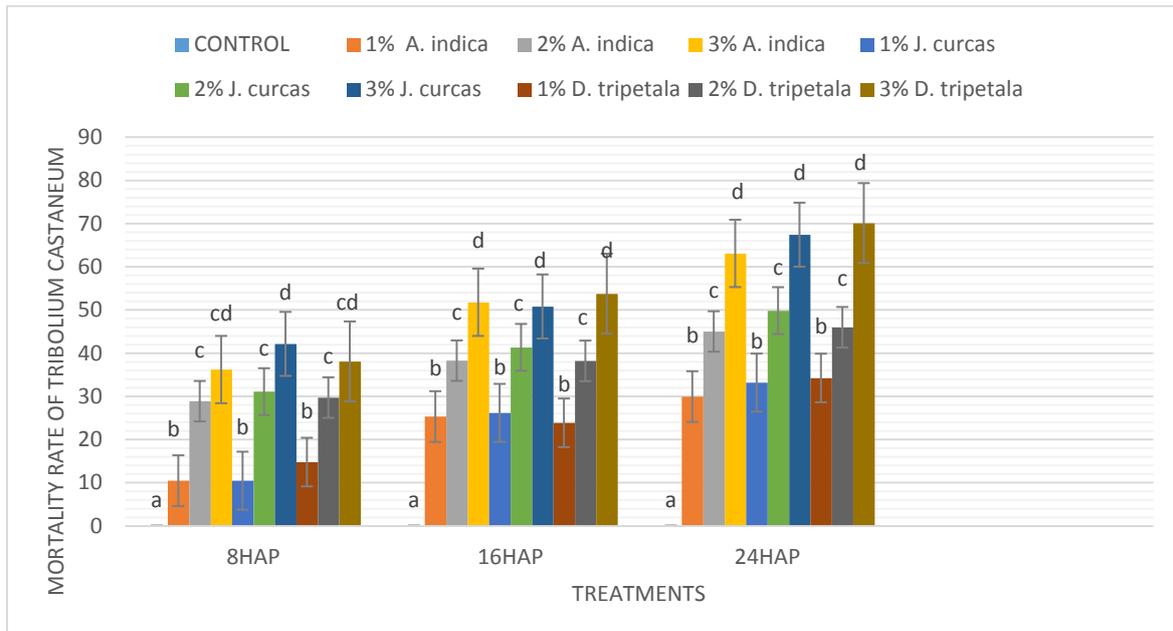


Fig. 4. Contact effect of essential oils on *Tribolium castaneum* mortality

NOTE: Means capped with the same letter (s) within the same week are not significantly ($p>0.05$) different from each other according to Duncan's New Multiple Range Test (DNMRT).
HAP= Hours after application.

4. DISCUSSION

The result of the study revealed that *A. indica*, *J. curcas* and *D. tripetala* oils are repellent to *T. castaneum*. These essential oils significantly reduced the time spent and number of entries made by *T. castaneum* into the oil-treated arms of the olfactometer than the control arm. This observation is in support of the findings of Parvin et al., [39]; Ukeh and Umoetok, [10]; Ahmad et al., [40] & Malekpour et al., [2]; Teke and Mutlu, [41]; that took responsibility for the repellency of the essential oils owing to the chemical composition of the oil impacting strong odour on the insect thereby driving them from the odour source. The results of the effects of fumigants and contact toxicity revealed greater mortality associated with fumigant and contact actions of various essential oils applied on *T. castaneum*. Increase in the concentration of the essential oils resulted in significantly higher mortality of the insect in all durations of the experiment. This observed action of the essential oil could be due to their ability to act easily in vapour phase and as such gaining access with each into the internal organs of insects. It could also be in line with the position of the scholars that states essential oils to be rich in volatiles and other metabolites which are protective substances against *T. castaneum* [42,40,39,17, 11,43,3,6].

Similarly, Ukeh et al. [11] also posited that since essential oil act by inhibiting insect acetylcholinesterase and this ultimately blocking the nerve function, applying higher concentrations of essential oil ultimately results in higher mortality of insects thereby bringing about less damage to stored produce and maintaining high purity state of our products devoid of significant contamination.

5. CONCLUSION

Significant differences were noted in the repellence property of the essential oils against *T. castaneum* and as such *A. indica*, *J. curcas* and *D. tripetala* could be regarded as repellants against the insects. Based on the result obtained from this experiment, it could be infer that the three essential oils significantly caused higher mortality of *T. castaneum* associated with fumigant and contact toxicity. The 3% concentration of the three essential oils gave the highest mortality of *T. castaneum*. Therefore, these three plant oil extracts could serve as great protectant against our stored products.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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