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EFFECT OF PRE-EXPOSURE ON THE INSECTICIDAL AND REPELLENCY PROPERTIES OF *Citrus paradisi* PEEL ESSENTIAL OIL AGAINST *Tribolium castaneum*

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ABSTRACT. Although the use of essential oils (EOs) for pest control has gained popularity due to their biodegradability and ecological safety, a major concern regarding their use is stability. Therefore, this aspect deserves empirical studies to enable potential end users to adopt research findings on the pesticidal potential of EOs. This research investigated the insecticidal and repellence impacts of Soxhlet-extracted grapefruit peel (*Citrus paradisi* Macfad. (Rutaceae) Lane) EO pre-exposed to air for 0, 0.5, 1 and 2 h (before introducing insects) on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The ethanol diluents were initially allowed to evaporate for 15 min in all treatments. Contact toxicity was tested on larval and adult stages at 5 and 15% concentrations of the EO, while repellency was tested at a 5% concentration. The impacts of EO on adult behavioural activity were also observed 24 h after exposure. Grapefruit EO was effective in controlling *T. castaneum* adults and larvae at higher doses. Exposure periods had an immediate significant effect on larvae and adult mortalities 1 and 6 h after the insects were introduced. At a 15% concentration, EO with a 0 h pre-exposure period had significantly higher adult and larva mortalities after 6 h than those of 0.5, 1 and 2 h pre-exposure. Regardless of oil pre-exposure, insects were repelled, especially in test periods between 12 and 30 min. Therefore, grapefruit EO could be formulated for increased stability when an immediate impact is needed.

Keywords: exposure periods; grapefruit peel; mortality; pest control; toxicity.

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INTRODUCTION

Traditionally, aromatic plants have been used for pest management of stored products. The powder and organic and inorganic extracts of different plant parts, including the leaves, stems and roots, have been shown to have insecticidal properties against stored product insect pests (Abdurruhman *et al.*, 2019; Mario *et al.*, 2023).

However, there has been renewed interest in the use of essential oils (EOs) rather than other botanical formulations because of their chemical complexity, diversity (Zhao *et al*., 2023), and pharmaceutical, medicinal, aesthetic and agricultural uses.

By definition, EOs are complex mixtures of volatile compounds that are common or abundant in aromatic plant parts, including leaves, flowers, roots, back, rhizomes, fruit and seeds (Dhifi *et al*., 2016). EOs contain one of several secondary metabolic materials that plants produce in addition to primary metabolites, which help them to stay healthy and maintain fitness under biotic and abiotic stress (Basaid *et al*., 2021).

Research into the use of EOs for insect pest management is becoming even more popular. This increasing trend is largely because EOs are considered environmentally friendly, safe for human health and biodegradable, and some are effective even at low doses even without direct contact with the target organism (Assadpour *et al*., 2023; Babarinde *et al*., 2015).

Essentially, EOs can be used to deter and repel insect pests, cause mortality, and influence the level of pest activity and behaviour. For example, EOs of over 100 plant species have shown repellent properties against culicids (de Oliveira *et al.,* 2020). The toxicity and repellent properties of EOs against important stored product pests, including *T. casternium*, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae), have also been investigated (Aisha *et al*., 2024; Matos *et al*., 2020; Nisar *et al*., 2022; Pang *et al.*, 2020; Peschiutta *et al.*, 2022).

A recent study by Bumbulytė *et al.* (2023) showed that EOs from six plants affected the movement behaviour of yellow mealworm (*Tenebrio molitor* L.) when tested in a Petri dish.

Waste products of plant organic materials that have EOs, such as the peels of Citrus spp., are good substitutes for synthetic food preservatives (Grover *et al.*, 2024). Their pesticidal potential has been well documented (Djebbi *et al.*, 2023; El Kasimi *et al.*, 2023). *Citrus paradisi* (Macfad) has been shown to have antimicrobial (D'Almeida *et al.*, 2022) and insecticidal properties against several stored product pests (Abbas *et al.*, 2012; El Houda *et al.*, 2020), including *Tribolium castaneum* (Heidari *et al.*, 2017).

The major components of *C. paradisi* peel EO include limonene, pinene, terpinene and cymene (Bourgou *et al*., 2012; El Houda *et al*., 2020).

Despite several studies on the use of EOs in pest management, limited research has looked at EO stability. Generally, EOs are known to be subjected to degradation and instability caused by extrinsic parameters, including light, temperature and available oxygen (Turek and Stintzing, 2013), and there is a need

to study changes in the effectiveness of EOs over time.

Tribolium castaneum is an important stored product pest of economic importance. It is commonly found in stored flour, and its presence in stored food directly affects the quantity and quality of the commodity (Ajiboye *et al*., 2023; Negi *et al*., 2022). It is considered a model insect because it has been widely used for genetic and insecticide resistance studies and the development of pest monitoring and management strategies (Campbell *et al*., 2022). As a model insect, it can be used to test EO stability over time. Therefore, the objective of this study was to test the insecticidal potential of EO from *C. paradisi* peels pre-exposed to air for different time periods against *T. castaneum*.

MATERIALS AND METHODS

Research site

The research was carried out in the Agronomy Laboratory of Osun State University, Ejigbo Campus, Osun State, Nigeria.

Insect population and rearing

Tribolium castaneum larvae and adults were obtained from a household-infested product, yam flour, in Ejigbo, Nigeria.

The insect was maintained on flour kept with perforated lids to promote adequate aeration, as previously described (Babarinde and Ogunkeyede, 2008). The lids were insect-proofed by lining them with air-permeable clothing materials. The test insect, *T. castaneum*, was maintained in the laboratory at 26±2℃ and 70±15% relative humidity.

Sources of *Citrus paradise* and essential oil extraction

Fresh grapefruits were obtained from small-scale farmers in Iperu-Remo, Ogun State, Nigeria, and the peels were carefully removed from the fruit using a sharp knife. The fresh grapefruit peels were air dried at room temperature until they were crisp, and then they were ground using a King of the Kitchen blender to increase the surface area. Grapefruit oil was extracted from the blended peels using a Soxhlet extractor. Ethanol was used as the solvent.

Twenty-gram samples were measured into a thimble (2.8 cm diameter and 10 cm height) and placed in glassware mounted on the Soxhlet extractor. Extraction was performed at 100℃ for about 35 min. The filtrate was air-dried or oven dried at 40℃ to remove the ethanol, thus retaining only the citrus EO.

Sample preparation

Two concentrations (C1: 5%, C2: 15% v/v) were prepared from the stock oil solution by diluting with ethanol to the required percentage, and absolute ethanol was used as the control.

Insect bioassays

Contact toxicity and impact on the activity level

Essential oil concentrations of 5 and 15% were used for the experiment. Exactly 100 µL EO mixture was applied to a 162 mL capacity plastic jar and exposed to air for 15 min to evaporate the ethanol in the treated samples. They were then further exposed to air separately (termed pre-exposure periods) for 0, 0.5, 1 and 2 h before use.

Ten adult *T. castaneum* were added to each treatment, including the control. The experiment was arranged in a completely randomised design. The number of live and dead insects was counted at 1, 6 and 24 h after introduction (HAI). The same experiment was repeated for the larval stage of the insect, and mortality was checked at 1, 6 and 48 HAI.

The percentage mortality (PM) was calculated using the following *Equation (1)*:

$$PM = \frac{Number\ of\ dead\ Insects}{Total\ number\ of\ insects} x \frac{100}{1} \quad (1)$$

The effect of the oil on the insects was presumed to reduce their activity level; therefore, we examined their walking activity.

The level of walking activity (WA)was tested at the adult stage of the insect. At 24 HAI, the number of insects walking per treatment was checked in 5-min intervals for 30 min, and the percentage level of activity (A) was determined using the following *Equation (2)*:

$$\%A = \frac{Total\ number\ of\ walking\ insects}{60} x \frac{100}{1} \quad (2)$$

Insects were considered walking when they moved away from their original position during the observation period. The treatments were replicated four times.

Repellence activity

The area preference methodology previously described by Babarinde *et al*. (2017) was used with some modifications. The filter paper was folded, rather than cut in two. The solvent used was ethanol and not acetone, as described by the author, and the doses used were different. A 5% (v/v) EO concentration was used in this experiment. The filter paper was folded into equal parts, similar to Babarinde *et al*. (2014). Exactly 100 µL of EO mixture was added to one side of the filter paper, while absolute 100 µL ethanol was added to the other side as a control. This was exposed to air for 15 min to evaporate the ethanol and then placed in a 9-cm diameter Petri dish.

The four pre-exposure periods tested were 0, 0.5, 1 and 2 h. Ten *T. castaneum* adults were added to the centre of the repellency chamber (filter paper in the Petri dish) and allowed to choose between the treatment and control sides. The number of insects on the control side and those on the treated sides were recorded and used to calculate the repellency index.

The experiment was replicated four times, and the setups were arranged in a completely randomised design.

Repellency was recorded at 3, 12, 21 and 30 min, and the repellency chamber was rotated every 3 min to avoid directional bias. This experiment was also performed for the larval stage.

The repellency percentage was determined by counting the number of insects in the control and treated parts following the method used by Babarinde *et al*. (2016).

The repellency percentage (PR) values were computed using the following formula *Equation (3)*:

$$PR = \frac{NC - NT}{NC + NT}\ x\ 100/1 \quad (3)$$

where NC is the number of insects in the untreated area and NT is the number of insects in the treated area. Zero was used to replace the negative PR values.

Statistical analysis

The PM and PR were compared among all pre-exposure periods for adult and larval stages separately using the Kruskal–Wallis non-parametric test in library ("agricolae") in *R* (Mendiburu and Yaseen, 2020). A Kruskal–Wallis post-hoc test was performed and adjusted using the "Bonferroni".

The analyses were performed using the R statistical software package (R Core-Team, 2021).

An analysis of variance was used to compare the activity level at 5% probability, and the means were compared using an honestly significant difference (HSD) test.

The 15% concentration was statistically analysed using the non-parametric Kruskal–Wallis test because the data generated from that concentration were not normally distributed.

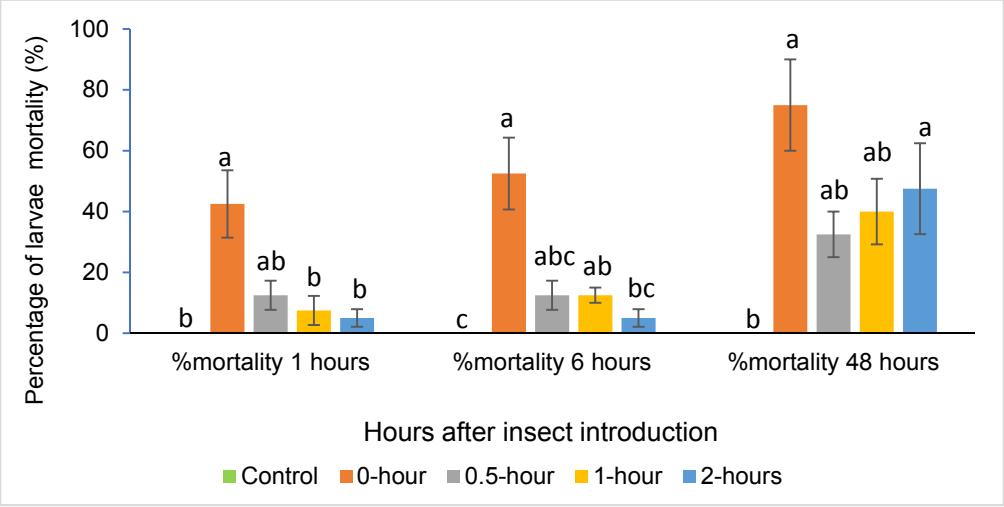
RESULTS

Percentage mortality of *T. castaneum* adults and larvae exposed to 5 and 15% grapefruit peel essential oil at different pre-exposure periods

When the larval stage of *T. castaneum* was introduced to a 5% EO concentration at different pre-exposure levels and the control, there were significant treatment effects at 1, 6 and 48 h after insect introduction (H=12.4, df=4, p=0.02; H=14.0, df=4, p=0.01; H=12.2, df=4, p=0.02, respectively *Figure 1*).

At 1 HAI, oil pre-exposed for 0 h had the highest mortality (42.5%), although this was not significantly different (p>0.05) from those pre-exposed for 0.5 h.

Other pre-exposure periods (1 and 2 h) resulted in a significantly lower mortality than that of oil exposed for 0 h (*Figure 1*).



The Kruskal–Wallis test results for 1, 6 and 48 h after exposure are: H=12.4, df=4, p=0.02; H=14.0, df=4, p=0.01; and H=12.2, df=4, p=0.02, respectively. Different lowercase letters above the bars indicate significant differences between the means

Figure 1 – Percentage mortality of *Tribolium castaneum* (Herbst) larvae introduced to 5% *Citrus paradisi* (Macfarlane) peel essential oil with control, 0, 0.5, 1 and 2 h pre-exposure periods and observed at 1, 6 and 48 h after insect exposure

At 6 HAI, oil pre-exposed for 0 h resulted in the highest mortality (52.5%), which was significantly different from that of oil pre-exposed for 2 h and the control (*Figure 1*).

At 48 HAI, oil pre-exposed for 0 h resulted in the highest mortality (75%), although it was not significantly different from that observed when oil was pre-exposed for 0.5, 1 or 2 h. No mortality was observed in the control (Figure 1).

Tribolium castaneum larvae introduced to a 15% EO concentration with different pre-exposure periods did not show any significant difference after 1 HAI ($H=4.7$, $df=4$, $p=0.32$).

However, at 6 HAI, mortality was highest (62.5%) in insects exposed to oil pre-exposed for 0 h, and this was significantly different ($p<0.05$) from those exposed to oil pre-exposed for 0.5, 1 and 2 h and the control (*Figure 2*).

After 48 h, the pre-exposure effects were neutralised since the percentage mortality was high regardless of the pre-exposure period (92.5–100%) and this was significantly different from the control, which resulted in 0% mortality.

At a 5% concentration, adult mortality was not significantly different ($p<0.05$) regardless of the pre-exposure period tested.

No adult died at 1 or 6 HAI, regardless of the pre-exposure period tested. Similarly, at 24 h, mortality was between 0 and 5%.

However, when a 15% EO concentration was applied, at 1 HAI, mortality was generally low regardless of the EO pre-exposure period tested (between 0 and 7.5%), although the percentage mortality was significantly ($p<0.05$) higher in the 0 h pre-exposure period than that of the control (*Figure 3*). However, at 6 HAI, mortality was significantly ($H=14.4$, $df=4$, $p=0.01$) influenced by the applied treatments. The 0 h pre-exposure period resulted in the highest mortality, and this was significantly different from that of other pre-exposure periods, including the control.

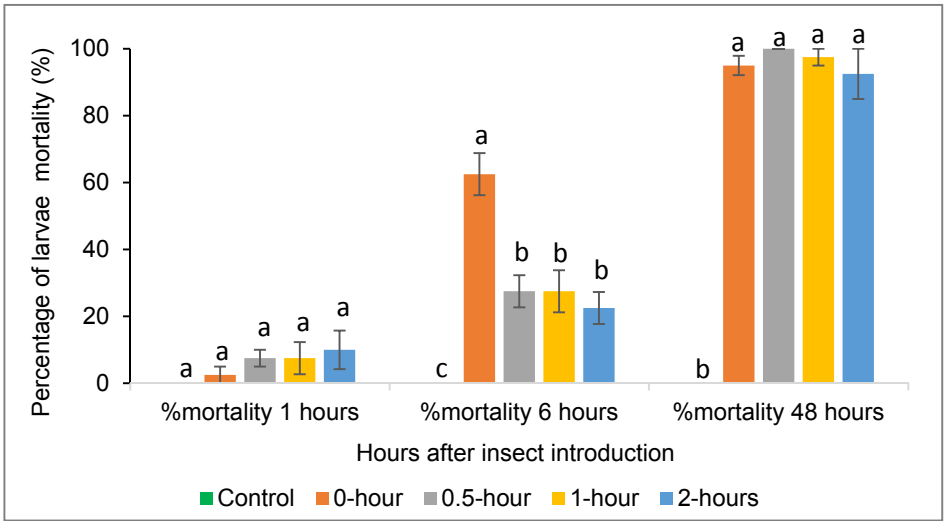
However, at 24 h, the pre-exposure effect was neutralised because although the pre-exposure period of 0 h resulted in the highest mortality (28%), it was not significantly ($p>0.5$) different from that of other pre-exposure periods, except for the control (*Figure 3*).

Effect of grapefruit peel essential oil pre-exposure periods on the activity level of *T. castaneum* adults

At a 5% concentration, the walking activity was 50, 8.0, 7.9, 2.1 and 4.6% after exposure to EOs from the control, 0, 0.5, 1 and 2 h pre-exposure periods, respectively. At a 15% concentration, the walking activity was 45, 0.5, 2.3, 0.6 and 0% after exposure to EOs from the control, 0, 0.5, 1 and 2 h pre-exposure periods, respectively.

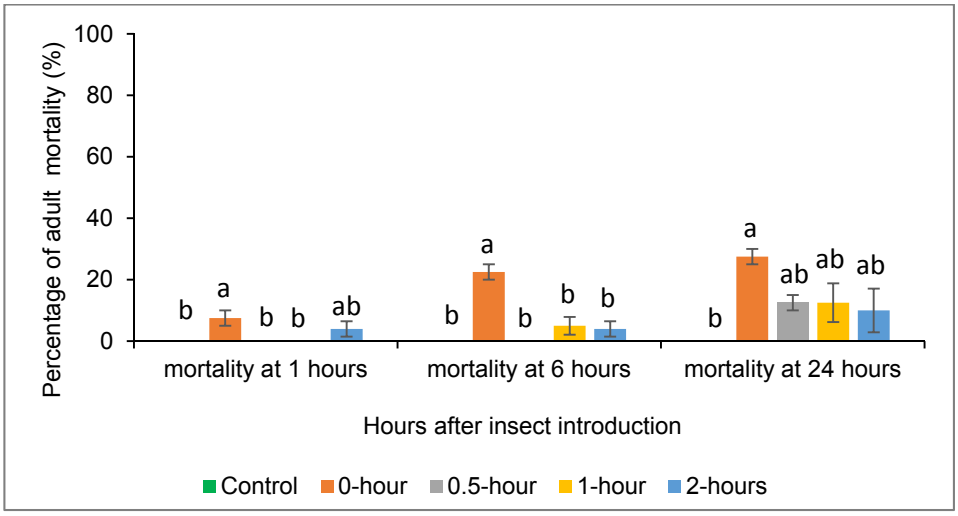
At 5 and 15% concentrations, there was a significant decrease ($F=38.0$, $df=4$, $p\leq0.01$ and $H=13.1$, $df=4$, $p=0.01$, respectively) in the activity level (walking) after exposure to EOs from the 0, 0.5, 1 and 2 h pre-exposure periods compared to the control (*Figure 4*).

For example, at a 5% concentration, the control had 50% walking activity, which was significantly higher than the 2–8% walking activity observed under other tested pre-exposure periods (*Figure 4*).



The Kruskal–Wallis test results for 1, 6 and 48 h after exposure are: H=4.7, df=4, p=0.32; H=15.3, df=4, p=0.01; and H=12.9, df=4, p=0.01, respectively. Different lowercase letters above the bars indicate significant differences between the means

Figure 2 – Percentage mortality of *Tribolium castaneum* (Herbst) larvae introduced to 15% *Citrus paradisi* (Macfarlane) peel essential oil with control, 0, 0.5, 1 and 2 h pre-exposure periods and observed at 1, 6 and 48 h after insect exposure



The Kruskal–Wallis test results for 1, 6 and 24 h after exposure are: H=10.1, df=4, p=0.04; H=14.4, df=4, p=0.01; and H=11.1, df=4, p=0.03, respectively. Different lowercase letters above the bars indicate significant differences between the means

Figure 3 – Percentage mortality of *Tribolium castaneum* (Herbst) adults introduced to 15% *Citrus paradisi* (Macfarlane) peel essential oil with control, 0, 0.5, 1 and 2 h pre-exposure periods and observed at 1, 6 and 48 h after insect exposure

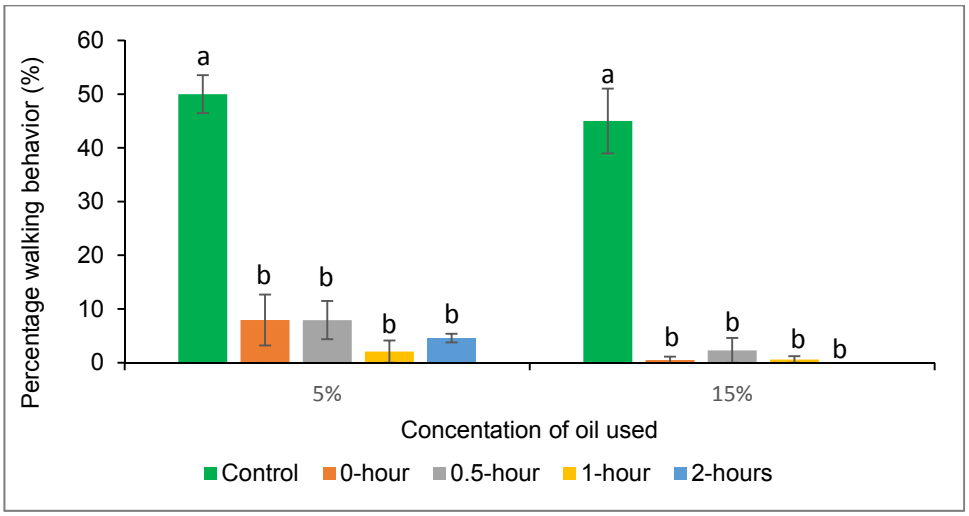


Figure 4 – Activity level of *Tribolium castaneum* (Herbst) adults exposed to 5 and 15% *Citrus paradisi* (Macfarlane) peel essential oil with control, 0, 0.5, 1, 2 h pre-exposure periods and observed 24 h after insect exposure (ANOVA, F=38.0, df=4, p≤0.01 and Kruskal–Wallis, H =13.1, df=4, p=0.01). Different lowercase letters above the bars indicate significant differences between the means

Percentage repellency of *T. castaneum* adults and larvae exposed to 5% *C. paradisi* peel essential oil with various pre-exposure periods

The grapefruit peel EO generally repelled (over 70%) *T. castaneum* larvae throughout the trial, regardless of the pre-exposure period tested. A similar PR was recorded for *T. castaneum* adults, but this was strictly at durations between 12 and 30 min (*Table 1* and *Table 2*). However, EO pre-exposure treatments did not significantly influence the level of repellency regardless of its duration or insect stage used (*Table 1* and *Table 2*).

DISCUSSION

Pre-exposure of *C. paradisi* peel EO to air before use had an immediate impact on its insecticidal property. Within the first 6 h, exposure periods had an inverse relationship with mortality. One hour after introducing *T. castaneum* larvae, EO pre-exposed for 0 h had a higher mortality (42.5%) than those pre-exposed for 1 or 2 h (<8%) at the same concentration of 5% EO.

Additionally, at a 15% concentration, at 6 HAI, EO pre-exposed for 0 h had a higher mortality than other longer pre-exposure periods for both larval and adult stages.

The possibility of EO changing and becoming less potent due to polymerisation and oxidative processes resulting from several extrinsic factors such as temperature, light and oxygen have been reported (Turek and Stintzing, 2013). The low level of mortality associated with pre-exposing EO may therefore be due to its high level of volatility and instability. In this research, the EO was pre-exposed for 0.5–2 h, a period that may be considered too minimal for any major changes to have taken place. This is subject to further research.

Table 1 – Percentage repellency (±SE) of *Tribolium castaneum* (Herbst) larvae exposed to *Citrus paradisi* (Macfarlane) peel essential oil with different pre-exposure periods

Exposure Period (h)	Duration (minutes)			
	3	12	21	30
2	75.0±9.6	55.0±9.6	75±9.6	75.0±12.6
1	60.0±16.3	75.0±5.0	80±0.0	75.0±5.0
0.5	45.0±9.6	75.0±12.6	75.0±15.0	80±8.2
0	75.0±9.6	75.0±5.0	90±10.0	85.0±5.0
Kruskal–Wallis test	H=4.59, df=3, p=0.21	H=3.59 df=3 p=0.31	H=1.71, df=3, p=0.63	H=1.17, df=3, p=0.76

The Kruskal–Wallis test results for 3, 12, 21 and 30 min are: H=4.59, df=3, p=0.21; H=3.59, df=3, p=0.31; H=1.71, df=3, p=0.63; and H=1.17, df=3, p=0.76, respectively

Table 2 – Percentage repellency (±SE) of *Tribolium castaneum* (Herbst) adults exposed to *Citrus paradisi* (Macfarlane) peel essential oil with different pre-exposure periods

Exposure Period (h)	Duration (minutes)			
	3	12	21	30
2	10.0±10.0	60±23.1	45±17.1	90±5.8
1	25.0±25.0	85.0±9.6	65.0±5.0	80.0±0.0
0.5	5.0±5.0	75.0±18.9	60.0±8.2	70±12.9
0	35.0±17.1	70.0±19.1	65.0±17.1	85.0±5.0
Kruskal–Wallis test	H = 2.7, df= 3, P=0.44.	H =0.4, df =3 P= 0.93	H =1.31, df= 3, P= 0.73	H = 3.05, df= 3, P= 0.38

The Kruskal–Wallis test results for 3, 12, 21 and 30 min are: H=2.70, df=3, p=0.44; H=0.40, df=3, p=0.93; H=1.31, df=3, p=0.73; and H=3.05, df=3, p=0.38, respectively

However, the fact that the EOs that were pre-exposed had access to air (jars not covered with a lid) before introducing insects might have increased their volatility. Khalili *et al*. (2015) showed that sealing influenced the effectiveness of thyme EO in inhibiting the growth of *Aspergillus flavus* Link and that encapsulation increased the shelf life of EOs. Therefore, the fragility of EO demands that it should be protected against degradation. Beyki *et al*. (2014) proposed that due to the volatility and instability of *Mentha piperita* L. EO against environmental factors, encapsulation could be used to improve its impact as this process is known to protect against the fragility of EO (El Asbahani *et al*., 2015).

Ordinarily, the level of volatility should be concentration dependent. Our study showed that, generally, mortality was also concentration dependent, with a higher concentration of oil resulting in greater mortality compared to those of lower concentrations regardless of the exposure level tested. Other studies have shown the impact of concentration on the volatility and pesticidal properties of EOs (Borotová *et al*., 2022; El-Aswad *et al*., 2023; El Kasimi *et al*., 2023; Gwiazdowska *et al*., 2022). The impact of pre-exposure on mortality of *T. castaneum* did not go beyond 6 h for larvae or adults in all durations tested, regardless of the concentration used, suggesting that pre-exposure resulted in

slow pesticidal action of the pre-exposed oil compared to those not pre-exposed.

The larval stage of the insect was more susceptible than the adult stage; for example, after 6 h, the larval and adult mortalities were 62.5 and 22.5%, respectively, at a 15% EO concentration with a 0 h pre-exposure period. A similar study showed that *T. castaneum* larvae were about 3.9 times more susceptible than adults when treated with the EO of *Tanacetum vulgare* L. (Asteraceae) (Kavallieratos *et al*., 2021). In addition, Babarinde *et al*. (2009) reported that larvae were more susceptible to *Ricinus communis* L. seed extract than adults. This, however, is contrary to the observations by Wang *et al*. (2006) and Huang *et al*. (2000), which showed high fumigant and contact toxicity, respectively, in adults compared to the larval stage of *T. castaneum*. The susceptibility of the larval stage of *T. castaneum* could be age dependent (Huang *et al*., 2000); therefore, the age and botanical type used might have resulted in the disparities observed between studies.

Just as it influenced mortality, the activity level was also influenced by EO. At 24 h, all live insects in the treated jars were less active than those in the control. We tested this by quantifying their walking activity. Walking activity was significantly lower in the EO-treated insects than in the control. This suggests that insects that were still alive were generally less active, regardless of the pre-exposure level tested. The reduced number of walking insects implies that more insects had become weak, suggesting that apart from mortality, this EO may have other secondary negative effects on their longevity, feeding and reproductive activities (Sandoval-Mojica and Capinera, 2011), thus negatively impacting the level of damage to stored grains or flour.

The ability of the EO to repel the adult and larval stages when tested at lower concentrations suggested that the repellent property of the EO was not negatively affected, regardless of the EO pre-exposure periods tested. This affirms the strong repellent property of *C. paradisi* against *T. castaneum*. A previous study confirmed the strong repellent potential of this EO against *Aedes aegypti* (Ugwu and Chime, 2023). Since larval and adult stages of *T. castaneum* are important pests of multiple food products and stored grains (Naseri and Majd-Marani, 2020; Upadhyay *et al*., 2018), preventing their infestation is a critical and fundamental control strategy. This means that this EO can be used as a repellent in the store as a preventive measure. In addition, this EO can be explored as a repellent in an integrated pest management strategy, even against field pests. However, formulating them for increased stability will enhance their effectiveness in pest control.

Further research is needed to analyse the impact of the quality and quantity of the EO components at various exposure periods using Gas Chromatography/Mass Spectrometry (GC-MS) and Gas Chromatography/Flame Ionisation detection (GC-FID). It may also be necessary to expand pre-exposure durations to days rather than just a few hours. Other factors, such as whether the jar was opened or closed with a lid, may also help to reach a more accurate conclusion on the impact of pre-exposure on insect mortality.

CONCLUSIONS

Soxhlet-extracted EO of *C. paradisi* peels was effective as a bioinsecticide against both *T. castaneum* adults and larvae. The larval stage was more susceptible than the adult stage. The EO pre-exposed for 0 h caused greater mortality of larvae and adult stages than the other pre-exposure periods at 6 HAI.

This suggests a need to improve the stability of the oil. The EO also reduced the walking activity of the insect regardless of the pre-exposure periods tested, implying that the insects were already weak. EO application also showed strong repellent properties against *T. castaneum*, suggesting their potential use in the integrated pest management of this pest.

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