TOXICOLOGICAL, REPELLENT, AND GROWTH INHIBITORY POTENTIAL OF PHYTOCHEMICAL BIO-PESTICIDES EXTRACTED FROM DIFFERENT PLANTS AGAINST *TRIBOLIUM CASTANEUM* AND *ORYZAEPHILUS SURINAMENSIS.*

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Saw-toothed grain beetle (Oryzaephilus surinamensis) and red flour beetle (Tribolium castaneum), are important cosmopolitan insect pests of cereals, dry fruits, and processed food during storage. Popular present researched activity, extract of some plants (*Piper nigrum, Syzygium aromaticum*, and *Ocimum basillicum*) was evaluated for their toxic, repellent, and growth inhibitory potential against red flour beetle and saw-toothed beetle. Mortality facts of exposed insects had been noticed following a 24-, 48-, and 72-hour period. Data was observed for larval, pupal, and adult emergence from the jars having these surviving insects. The finding revealed that *Piper nigrum* showed the highest repellency and toxicity in opposition to O. surinamensis and T. castaneum. *P. nigrum* also effected as growth inhibitory potential in opposition to O. surinamensis and T. castaneum. This shows an expected road for the improvement of designated biopesticides that can upset bother populaces without turning to the utilization of engineered synthetics and are ok for the climate.

Keywords: *Tribolium castaneum, Oryzaephilus surinamensis,* Biopesticides, Cosmopolitan, Toxicity, Repellent Effect, Growth Inhibitory Potential, Biopesticides.

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INTRODUCTION

The red flour beetle, Tribolium castaneum, is a pest bug that can infest flour mills, grain markets, food departments, and grocery stores. It is widely distributed worldwide. Plant stems, leaves, fruits, and bark frequently contain plant essential oil, a type of lipid. According to Hosseini et al. (2013), it begins in particular cells or groups of cells. It is considered to be among the most hazardous all-around pests of controlled and stored goods. (Bingham *et al*., 2017). Numerous studies indicate that T. castaneum gains resistance to synthetic insect repellents by altering the achievement point of essential enzymes like acetylcholinesterase and increasing metabolic activity, which in turn increases detoxifying enzymes. (Tripathi et al., 2009; Subaharan et al., 2021). The O. surinamensis saw-toothed grain beetle, is destructive and significant grain pest kept in bulk. This bug infests every type of stored food consumed by humans, containing dried fruits, cereal grains, quick meals, seeds, and nuts. (Hashem *et al*., 2012). In general, synthetic pesticides are used to manage stored items, which has several drawbacks. To expand the capacity season of specific new things while holding their general quality (Aloui et al., 2014). Against atherogenic, hostile to platelet oxidative, and mitigating exercises are additionally found in Flute player nigrum. (Kim et al., 2012; Child et al., 2012). Various books on the medicinal balm of P. nigrum (PNO) have uncovered that it has solid insecticidal viability against various vermin, including T. castaneum, the put away grain bother (Khani et al., 2012). O. Due to its uses in cooking, medicine, cosmetics, pharmacology, and food preparation, basillicum (basil; sweet basil; common basil) has received a lot of attention. (Politeo et al., 2007). The O. basillicum vital oils are said to have antifungal, bug repellent impacts. (2012, Sakalauskait et al.) The purpose of the study is to investigate the essential oils of globulus and basillicum leaves' ability to repel stored grain insect pests. Basilicum L. is a repellent plant with well-documented repellent properties and is readily available locally. The choice was sweet basil. Syzygium aromaticum, an unpredictable oil separated from clove buds, is widely used and very much perceived for its remedial impacts. (Bhowmic et al., 2012; Perez et al., 2010) The biological activity of S. aromaticum oil against a variety of pests has been studied. The purpose of this study is to investigate the insecticidal properties of S. aromaticum (L.) essential oil in relation to protecting stored grains from insect infestation.

MATERIALS AND METHODS

Collection and rearing of insects

Invaded tests of wheat, maize, and dried dates having populaces of O. surinamensis and T. castaneum that were assembled from the grain market and dried organic products shop. Insects had been sieved out from the infested sample and had been released on sterilized crushed wheat and uncapped dried dates for rearing the homogenous insect culture. Adults of saw-toothed beetle had been sieved out from the food medium after 4 days. Food medium having eggs would again be positioned in the pots and placed into an incubator at optimal growth situations for one month to obtain F_1 adults of homogenous age. These adults were used for insecticide bioassay studies.

Preparation of Plant Extracts

The leaves of the test plants had been gathered and washed in sterile water before being used. The leaves had been crushed in an electrical grinder once they had dried out in the shadows to produce a finely ground plant powder. A rotary shaker had been used to prepare the extract. For this purpose, 50 g plant powder had been dipped in 150 ml acetone in a conical flask which had been placed on rotary shook and were shaken for 72 hours at 220 rpm. The crude extracts were filtered and placed in clean, airtight container lids. Before being used, the samples were kept in the refrigerator. Different concentrations of 5, 10, 15, 20, 25, and 30% were created from each plant's stock solution.

Bioassay-1 Toxicity of extracts of four plants against red flour beetle and saw-toothed beetle under laboratory conditions

A completely randomised design was used to conduct the experiment (CRD) to reproduce

50 adults of the test insects three times utilizing all plant extracts. The impregnated filter paper method, developed by (Visetson, 1991), was used to determine the efficacy of each plant extract and synthetic pyrethroid in terms of mortality. After 2, 5, and 7 days of exposure, the data was recorded regularly.

Bioassay-2 Repellent effect of plant extracts against red flour beetle and saw-toothed beetle under laboratory conditions

The repellent effect was ascertained by applying the area preference approach. After being divided into two halves, each filter paper had one half treated with plant extracts. Following the evaporation of surplus solvent, the two components were combined and placed within petri dishes. Thirty test insect beetles were released in the centre of the treated filter sheets. The quantity of insects on the filter paper's treated and untreated halves had been counted after 24, 48, and 72 hours.

Bioassay-3 Plant Extracts Growth inhibitory activities against saw-toothed beetle red flour beetle and under laboratory conditions

Test beetles had been placed into jars with a specific food that had been prepared with various quantities of test substances. Adults, larvae, and pupae population growth inhibitory data had been recorded every 7 days until F_1 generation emerged.

Statistical Analysis

To compare the treatments, all of the obtained data on mortality and growth inhibition underwent analysis of variance testing (ANOVA). After completing the HSD Tuckey test at level of 5%, the means of significant treatments were compared.

RESULTS

The experiment was carried out in the Faisalabad Department of Entomology. The Grain Research Training & Storage Management Cell (GRTSM) at the Department of Entomology received the first culture of stored grain pests from the Grain Market in Faisalabad.

Repellent effect of some phytochemical biopesticides observed by different dose rates against *Tribolium castaneum* and *Oryzyphilius surinamensis*.

The highest means repellency of red flour beetle on *Piper nigrum* was observed by 30% concentrate (63.27) and 5% (32.14). The highest means repellency of red flour beetle on *Syzygium aromaticum* was observed by 30% concentrate (35.067) and 5% (9.267). The highest means repellency of red flour beetle on *Ocimum basillicum* was observed by 30% concentrate (21.054) and 5% (6.037). The highest means repellency of saw tooth beetle on *Piper nigrum* was observed by 30% concentrate (54.157) and 5% (33.357). The highest means repellency of saw tooth beetle on *Syzygium aromaticum* was observed by 30% concentrate (25.078) and 5% (5.035). The highest means repellency of saw tooth beetle on *Ocimum basillicum* was observed by 30% concentrate (24.067) and 5% (7.267).

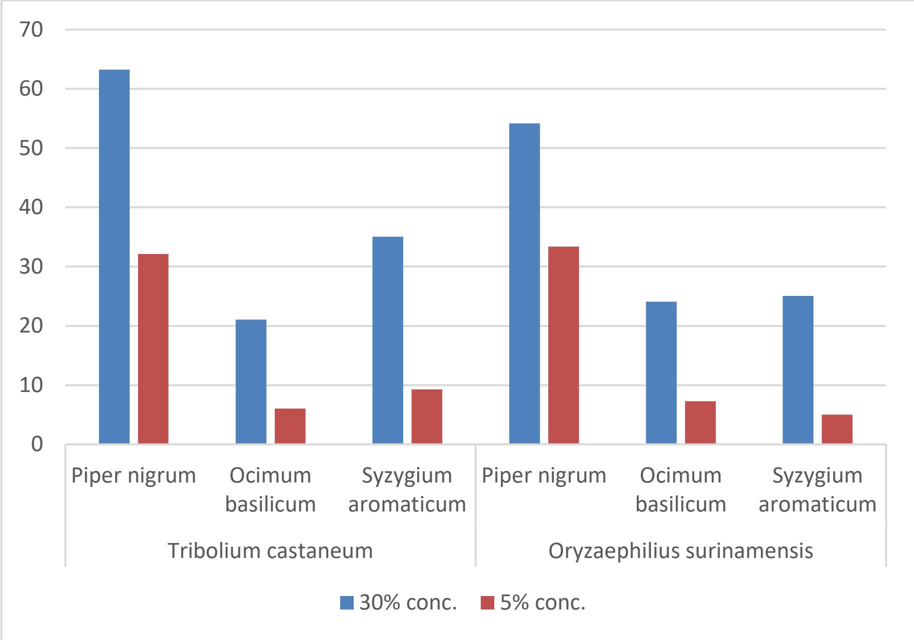

Figure 1. Repellent effect of some phytochemical biopesticides observed by different dose rates against *Tribolium castaneum* and *Oryzyphilius surinamensis*.

Mean toxicity of *Tribolium castaneum* and *Oryzyphilius surinamensis* against different dose rates of some phytochemical biopesticides.

The highest toxic effects on red flour beetle of *Piper nigrum* were observed by 30% concentrate (73.135) and 5% (45.356). The highest toxic effects on red flour beetle of *Syzygium aromaticum* were observed by 30% concentrate (40.055) and 5% (15.134). The highest toxic effects on red flour beetle of *Ocimum basillicum* were observed by 30% concentrate (39.037) and 5% (15.245).

The highest toxic effects on saw tooth beetle of *Piper nigrum* were observed by 30% concentrate (72.046) and 5% (45.578). The highest toxic effects on saw tooth beetle of *Syzygium aromaticum* were observed by 30% concentrate (32.057) and 5% (10.036). The highest toxic effects on saw tooth beetle of *Ocimum basillicum* were observed by 30% concentrate (36.067) and 5% (11.267).

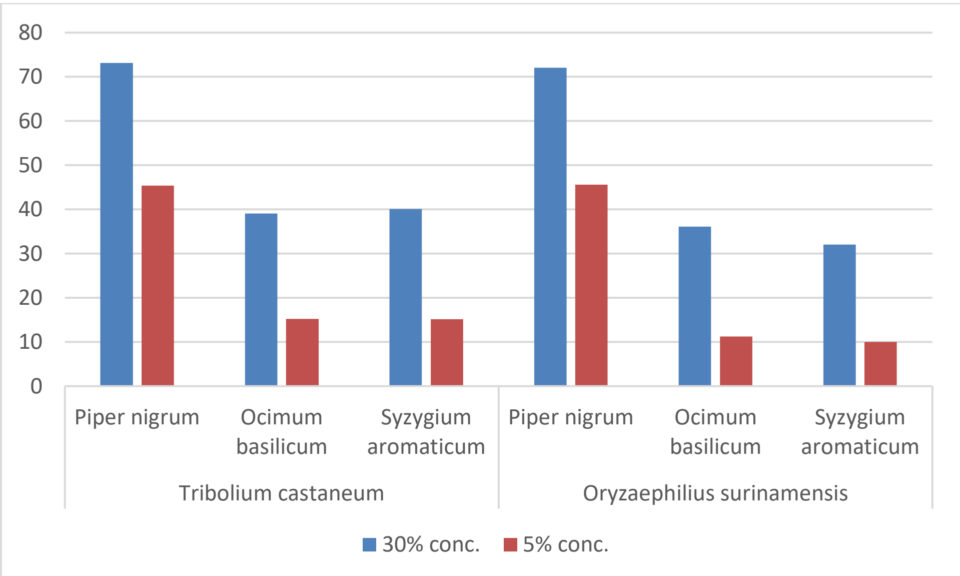

Figure 2. Mean toxicity of *Tribolium castaneum* and *Oryzyphilius surinamensis* against different dose rates of some phytochemical biopesticides.

Effects of adult exposure to various concentrations of some phytochemical biopesticides on the development of F_1 larvae, pupae, and adults in *Tribolium castaneum* and *Oryzaephilus surinamensis*.

The highest growth inhibitory potential of red flour beetle larvae on *Piper nigrum* was observed at 30% concentrate (81.180) and 5% (38.202), while in pupation at 30% concentrate (78.889) and 5% (34.167) and in adults at 30% concentrate (76.648) and 5% (32.967). The highest growth inhibitory potential of red flour beetle larvae on *S. aromaticum* was observed by 30% concentrate (79.946) and 5% (36.856), while in pupation at 30% concentrate (77.174) and 5% (35.326) and in adults at 30% concentrate (75.000) and 5% (34.066). The highest growth inhibitory potential of red flour beetle larvae on *O. basillicum* was observed by 30% concentrate (81.644) and 5% (41.096), while in pupation at 30% concentrate (77.534) and 5% (36.438) and in adults at 30% concentrate (73.913) and 5% (35.054).

The highest growth inhibitory potential of saw tooth beetle larvae on *Piper nigrum* was observed at 30% concentrate (82.967) and 5% (40.385), while in pupation at 30% concentrate (80.220) and 5% (38.462) and in adults at 30% concentrate (75.824) and 5% (35.440). The highest growth inhibitory potential of saw tooth beetle larvae on *S. aromaticum* was observed by 30% concentrate (81.868) and 5% (48.352), while in pupation at 30% concentrate (78.846) and 5% (41.209) and in adults at 30% concentrate (76.374) and 5% (38.736). The highest growth inhibitory potential of saw tooth beetle larvae on *O. basillicum* was observed by 30% concentrate (80.914) and 5% (39.785), while in pupation at 30% concentrate (77.297) and 5% (37.568) and in adults at 30% concentrate (76.087) and 5% (36.141).

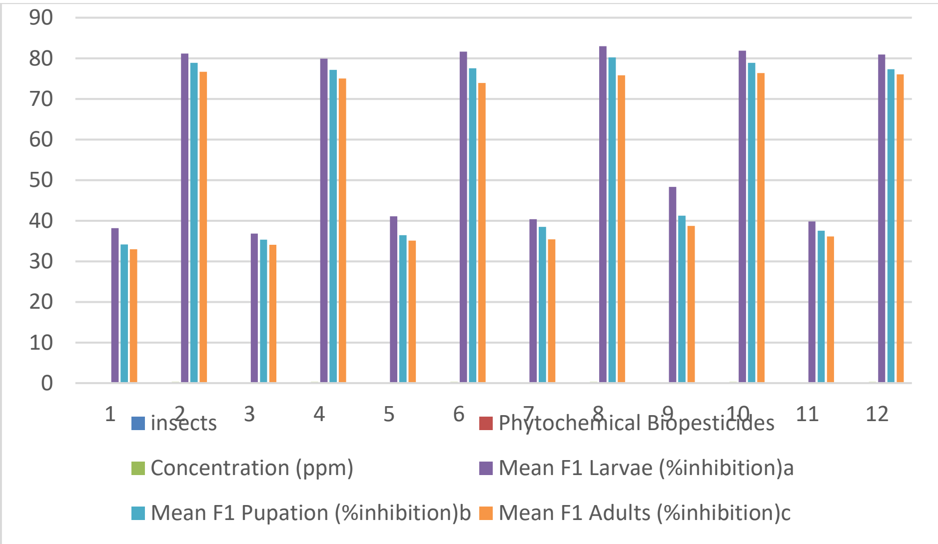

Figure 3. Effects of adult exposure to various concentrations of some phytochemical biopesticides on the development of F_1 larvae, pupae, and adults in *Tribolium castaneum* and *Oryzaephilus surinamensis*.

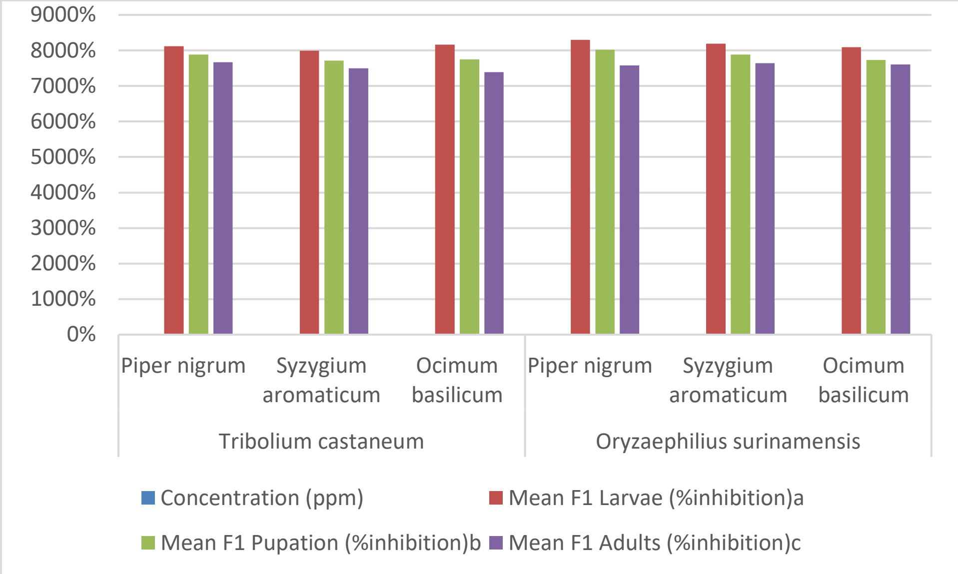

Figure 4. Effects of adult exposure to concentration (ppm30%) of some phytochemical biopesticides on development of F_1 larvae, pupae, and adults in *Tribolium castaneum* and *Oryzaephilus surinamensis*.

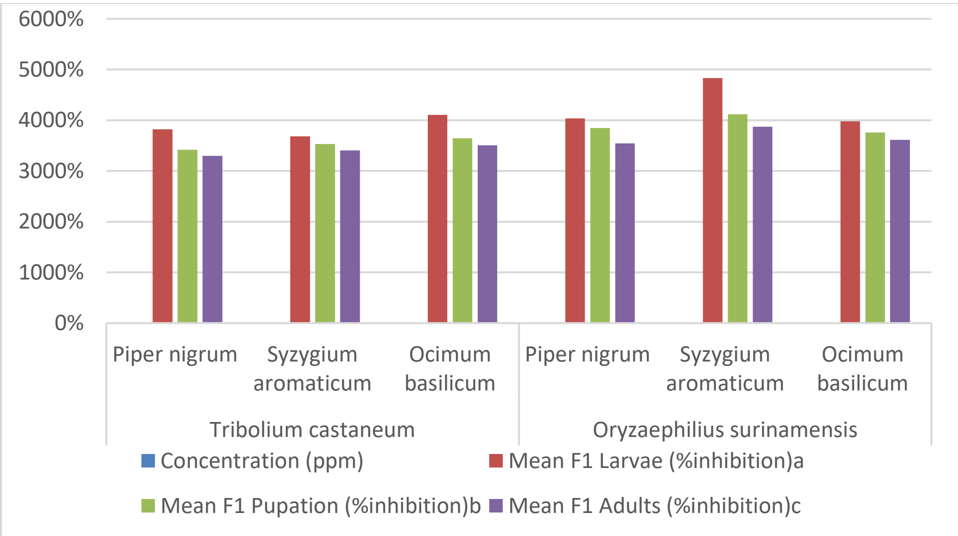

Figure 5. Effects of adult exposure to concentration (ppm30%) of some phytochemical biopesticides on development of F_1 larvae, pupae, and adults in *Tribolium castaneum* and *Oryzaephilus surinamensis.*

DISCUSSION

According the study's findings, black pepper (*Piper nigrum*) has an insecticidal impact on *Tribolium castaneum* at all treatment levels, even though the effects changed according to the length of exposure and the amount of powder. Another finding was made by (Updhyay and Ahmad *et al*., 2011), who outlined P. nigrum's extreme toxicity against a number of pests that prey on stored goods (T. castaneum) and provides grains with continuous protection. As per recent research, *T. castaneum* extracts were tested on the plant at five different doses for seven days (0.5, 1.0, 1.5, 2.0, and 2.5 ml) (Mary and Durga *et al*., 2017). It was discovered that the 2.5 ml concentration had the highest death mean rate. This was consistent with findings (Tripathi *et al*., 2009) and (Vijay-Kumar *et al*., 2015), who employed different ground spices and discovered that they had fumigant and poisonous effects, respectively. In this investigation, plant extracts were utilized at various quantities, and it was found that all of them, at greater concentrations—in this case, 1.5 percent—showed good repellent efficacy against the red flour beetle. Lower quantities have also demonstrated repelling action; however, it was less strong. These outcomes are comparable to those from India, in particular, where it was noted that numerous plant extracts displayed encouraging insect pest-repellant properties (Alim *et al*., 2017). According to (Al-Saadi *et al*., 2017), the LD50 values of an ethanol extract of *P. nigrum* against adult *T. castaneum* were both 0.73 and 0.34 mg/cm 2 for larvae. According to the findings of our investigation, repellency induced by all EOs against *O. surinamensis* is still effective 96 hours after the bioassay. The area test's second-strongest repellant was the *Piper nigrum* essential oil. After 12 hours, 96 hours, and 48 hours of the test, it had the second-highest repellency and third-highest repellency, respectively. O. surinamensis and T. castaneum, two pests of stored products, have been shown to be poisonous, repellent, and antifeedant against a variety of insect species (KIM et al., 2003; WAGAN et al., 2016, 2017a). After 30 minutes of exposure, the repellence tests conducted in this study revealed that the essential oils of S. aromaticum and A. sativum had good potential as chemicals against T. castaneum. Past exploration (Abo-El-Saad et al., 2011) proposes that the essential elements of clove oil, eugenol and caryophyllene, might be to be faulted for the oil's outstanding insecticidal properties. Smells, designed blend, and fundamental pieces of S. aromaticum and A. sativum EOs are additional elements that could deal with their suitability. As demonstrated by GC-MS (Abo-El-Saad et al., 2011), eugenol, which makes up 48.92 percent of clove oil, may be the clarification that S. aromaticum EO has solid areas for such properties (Abo-El-Saad et al., 2011). Furthermore, according to Boraei et al. (2016), eugenol (37.43 percent) was the essential fixing in the clove rejuvenating ointment. This fixing has been exhibited in different before assessments to be a fruitful bug trouble repellant (Shuit-Hung et al., 2002, Zapata et al., 2010). The effectiveness of the studied essential oils as repellents and toxins against other T. castaneum Herbst life stages, such as eggs, larvae, and pupae, will require further research, but this investigation may indicate that it is advantageous to control T. castaneum in order to protect stored grains and their products by using S. aromaticum as an irritant and P. dulcis and M. chamomilla essential oils for contact mortality. To determine the degree of safety in the future, we hope to evaluate the acetone residues on treated stored grains and their byproducts. The chemical components of the tested oils may be responsible for their efficacy, as proposed by (Zeng *et al*., 2010), who investigated the chemical makeup of clove oil and identified eighteen components (the most common and important being 2-methoxy-4-(2-propenyl)-phenol and Trans caryophyllene), tested T. castaneum and S. oryzae separately with clove oil and found that clove oil and 2-methoxy-4-(2-propenyl (2-propenyl) Compared to Trans-caryophyllene molecule, -phenol caused the highest rate of mortality, However, it was discovered that 2-methoxy-4- (2-propenyl) phenol was more effective at repelling than clove oil and other substances. Numerous authors have verified that the tested botanical volatile oils had an impact on various insect species, such as (Moawad and Al Gamdi *et al*., 2018), who demonstrated that *S. aromaticum* (L.) fumes had an insect-repellent effect and decreased the ability of *O. surinamensis* eggs to hatch (L.). The results generally showed that tested volatile oils might be employed in pest management as a substitute safe component rather than insecticide. Basil oil's strong adulticidal and larvicidal effects may be caused by its neurotoxic action, which prevents acetylcholine esterase from functioning (Dris *et al*., 2017). Basil oil is recognized to have strong repellent properties against *Phenacoccus solenopsis* and *Aphis gosypii* (Singh *et al*., 2012). The mortality rate increased to 90% when T. castaneum was treated with a nano emulsion. (Nuchuchua et al., 2009).

CONCLUSION

The phytochemical bio-pesticides derived from various plants exhibit diverse effects on Tribolium castaneum (Red Flour Beetle) and Oryzaephilus surinamensis (Saw-toothed

Grain Beetle). Here are the key conclusions: Toxicity: Cinnamomum cassia oil demonstrated significant toxicity against *T. castaneum*, with a sub-lethal concentration (LC20) of 0.172% v/v after 72 hours. Other oils, such as Pogostemon cablin, Murraya koenigii, and Foeniculum vulgare, also exhibited varying degrees of toxicity. Repellency: C. cassia and P. cablin oils displayed notable repellency. The index of repellency (IR) at 24 hours was 0.1 for C. cassia and 0.2 for P. cablin at 1% v/v concentration. Growth Inhibition: These bio-pesticides may hinder the growth of stored product pests, making them promising alternatives in pest management. In summary, botanicals hold potential as effective pesticides against stored grain pests, contributing to food security and minimizing post-harvest losses.

Authors' contributions statement: M. Sagheer, I. Qadeer designed, and completed the experiments; I. Qadeer prepared the draft; M. Sagheer reviewed and finalized the draft.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgment: We would like to thank all of the participants who volunteered their time in the study.

Ethical statement: This article does not contain any studies regarding humans or animals

Availability of data and material: We declare that the submitted manuscript is our work,
which has not been published before and is not currently being considered for publication elsewhere.

Code availability*:* Not applicable.

Consent to participate: All authors participated in this research study.

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