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BIOEFFICACY OF BOTANICAL ESSENTIAL OIL EXTRACTS ON *Sitophilus oryzae* L. (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Sitophilus oryzae infestation in stored products causes significant economic losses, and the severity of these post-harvest impacts underscores the urgent need for sustainable, efficient, and affordable alternative insecticides for stored product protection. In response, the well documented disadvantages of commonly used synthetic pesticides have driven a growing shift towards botanical insecticides, particularly those formulated from essential oils, as promising eco-friendly alternatives. In this study, three plant extracts of essential oil, which were *Allium sativum* (garlic), *Syzygium aromaticum* (clove), and *Cinnamomum verum* (cinnamon), were tested for their repellency and mortality effect on *S. oryzae*. The repellency and mortality testing of *S. oryzae* against different essential oils was carried out in a petri dish where filter paper was treated with various concentrations of essential oil. Repellency results by the *Sy. aromaticum* showed the highest repellency, with the mean percentage of repellency between 56.57-74.33% as compared to *C. verum* and *A. sativum*. No significant differences (one-way ANOVA, $P>0.05$) between concentrations of 1-4µl/ml, indicating stability and no variation in repellency effect after exposure to *Sy. aromaticum.* Total mortality of *S. oryzae* was reached on day 4 for *C. verum*, which was faster than *Sy. aromaticum* and *A. sativum* that occurred on day 5 for all tested concentrations. Thus, these extracts were seen as a suitable alternative to replace chemical insecticides in controlling stored product insects due to their efficacy and safer options for food products.

Keywords: Botanical extract; control; essential oil; rice weevil; *Sitophilus oryzae*

ABSTRAK

Serangan *Sitophilus oryzae* pada produk simpanan menyebabkan kerugian ekonomi yang ketara, dan tahap keterukan impak pascatuai ini menekankan keperluan mendesak terhadap racun serangga alternatif yang lestari, berkesan dan mampu milik bagi perlindungan produk simpanan. Sehubungan itu, kelemahan racun perosak sintetik yang digunakan secara meluas telah mendorong peralihan yang semakin meningkat ke arah racun serangga botani, khususnya yang dirumus daripada minyak pati, sebagai alternatif mesra alam yang berpotensi. Dalam kajian ini, tiga ekstrak tumbuhan minyak pati iaitu *Allium sativum* (bawang putih), *Syzygium* *aromaticum* (cengkih), dan *Cinnamomum verum* (kayu manis), telah diuji untuk kesan penghalau dan kesan kematian terhadap *S. oryzae*. Ujian penghalau dan kematian *S. oryzae* terhadap minyak pati yang berbeza telah dijalankan dalam piring petri yang mana kertas penapis dirawat dengan pelbagai kepekatan minyak pati. Keputusan ujian penghalau menggunakan *Sy. aromaticum* menunjukkan kesan penghalau tertinggi, dengan purata peratusan kesan penghalau antara 56.57-74.33% berbanding *C. verum* dan *A. sativum.* Tiada perbezaan yang signifikan (ANOVA sehala, *P>0.05*) antara kepekatan 1-4µl/ml menunjukkan kestabilan dan tiada variasi dalam kesan penghalau selepas terdedah kepada *Sy. aromaticum.* Kematian 100% *S. oryzae* dicapai pada hari ke-4 untuk *C. verum*, yang mana ianya lebih cepat daripada *Sy. aromaticum* dan *A. sativum*, yang berlaku pada hari ke-5 untuk semua kepekatan yang diuji. Oleh itu, ekstrak ini dilihat sebagai salah satu pilihan alternatif yang sesuai dalam menggantikan racun serangga kimia dalam mengawal serangga produk simpanan kerana keberkesanan dan pilihan yang selamat untuk produk makanan.

Kata kunci: Ekstrak botani; kawalan; minyak pati; kutu beras; *Sitophilus oryzae*

INTRODUCTION

Rice is known as the staple food for the world’s population. Asia cultivates 90% of the rice production, and only 7% exported from its origin country (Firdaus et al. 2020). However, 10-20% of agricultural products are destroyed due to infestation of storage insects annually (Syarifah Zulaikha et al. 2018). The condition is also worsened by improper management. The infestation of stored product insects such as rice weevils, *Sitophilus oryzae* is known as one of the challenges faced that cause a detrimental effect on our rice manufacturer and food storage industry (Kirk-Bradley et al. 2024). They destroy rice grains as the developed adults from grubs in the grains chew the seed coat and emerge within it. The feeding and life cycle behaviour of *S. oryzae* on rice causes grain weight loss, nutrient loss, seed viability, and rice contamination by fungi and mites (Okpile et al. 2021). Losses in the economic sector occurred as damaged products were discarded and were no longer suitable for sale, as they are associated with customer dissatisfaction.

In previous years, insect control programs at food plants were highly dependent on synthetic insecticides. Repetitive and overreliance on these insecticides have caused the occurrence of insecticide resistance among insect pests and vectors in the contemporary agriculture sector and public health, and many other drawbacks due to their application, thus requiring an alternative solution. Insecticide-based control was the cornerstone of the fight against agricultural pests and pathogens associated with them. Development of resistance towards insecticides occurred due to repetitive exposure to the same insecticide with the same mode of action repetitively until insects were able to withstand the exposure to the standard dose (Haddi et al. 2023). However, in recent years, massive applications of synthetic insecticides have been restricted and severely curtailed (Ngegba et al. 2022). A few factors contributing to the decline of synthetic insecticide usage recently are the implementation of new regulations for its application, consumers' demand for less chemical usage, the high cost, and new registration for insecticide replacement, even though it was undeniable that synthetic and conventional insecticides were proven to control stored-product pest successfully.

Before the development of synthetic insecticides, farmers primarily depended on plant-based remedies for storage insect management. This is because some plant products contain essential oil produced from the extraction process, which causes toxicity and repellent effects towards insects with less mammalian toxicity to applicators and reduced hazard to the environment (Pavela 2016). Therefore, in this study, we evaluate an alternative method using plant essential oil (EO) of *Allium sativum, Syzygium aromaticum*, and *Cinnamomum verum* to combat *S. oryzae* infestation in rice products. This study aims to determine the repellency and mortality activity of these three plant essential oils towards rice weevils, *S. oryzae*.

MATERIALS AND METHODS

Insect Rearing of *Sitophilus oryzae*

The collected *S. oryzae* from the infested rice bought from a local market located in Bagan Ajam, Butterworth, Pulau Pinang (5˚26'35.4N 100˚22'48.3E) were transferred into the rearing container containing 300 grams of new and uninfested rice. The rearing containers were then placed inside the climatic chamber (BINDER Constant climate chamber KMF115) with a temperature of 28±2°C and humidity of 80±5%. To ensure a constant supply of *S. oryzae* during the whole project period, new, sterilized, and clean rice was used to replace the rice biweekly.

Identification of Rice Weevil, *Sitophilus oryzae*

Analysis for the morphology of *Sitophilus oryzae* was carried out in the Medical Entomology Laboratory (109A), G08, using an Olympus SZX10 microscope. A distinct difference in the size of the pronotum acts as an indicator to distinguish male and female *S. oryzae*. Male *S. oryzae* rostrum was anteriorly narrowed, and posteriorly broader, bending slightly downwards with distally borne mouthparts (Devi et al. 2017), whereas in females, the rostrum is generally longer, slenderer, and more strongly curved than in males, facilitating penetration into kernels to deposit eggs (Rees 2004).

Extraction of Botanical Essential Oil

Extraction of botanical essential oil (EO) was carried out using *Allium sativum* (garlic), *Syzygium aromaticum* (clove), and *Cinnamomum verum* (cinnamon), which were bought locally and ground into powder form. Extraction of all the botanical products was carried out separately using distilled water as solvent via the hydrodistillation technique for a three to four-hour extraction time, following Katekar et al. (2023) methods with an alteration on the amount of plant samples. Fifty grams of *A. sativum* (garlic) were chopped into small pieces on a chopping board using a knife and placed into a boiling flask. The ratio of the mass of solute placed to the volume of water used for hydrodistillation was 1:5. The mixture was swirled before being placed onto the heating mantle and left to boil until it reached 100˚C of boiling water. Hydrosol containing the mixture of distillate and essential oil was collected and separated using a separation funnel for 24 hours to complete the separation process. The essential oil was then collected, and the distillate was discarded. The same procedure was then repeated to obtain *Sy. aromaticum* and *C. verum* essential oils.

Repellency Bioassay Test

Bioassay testing was performed by dividing a filter paper with a diameter of 9 cm (surface area 63.62 cm^2) into two equal halves with a surface area of 31.81 cm^2. One-half of the filter paper was treated using essential oil, while the other half was treated with distilled water that acted as a control arena, using a similar volume on both treatment sites (Aryal et al. 2023). Prior to testing of *S. oryzae* repellency, one-half of the filter paper was treated with 1, 2, 3, 4, and 5μL of *A. sativum* essential oil, respectively, while the other half was treated with distilled water as a control.

The concentration used is determined based on a pilot study, which begins with identifying the lowest concentration that produces a detectable repellency effect. The petri dish was then left closed for one minute before releasing ten female adults of *S. oryzae* to the center of the petri dish. The *S. oryzae* was considered repelled by essential oil when they moved towards the control side in the petri dish arena. The repellency activity of the essential oil against *S. oryzae* was recorded every 10 minutes for 60 minutes consecutively. Five replicates were prepared for each concentration of the essential oil. The same repellency testing procedure was applied to *Sy. aromaticum* and *C. verum* essential oils against *S. oryzae*.

Mortality Bioassay Test

Five different concentrations of *A. sativum*, *Sy. aromaticum* and *C. verum* essential oils, respectively, were tested on *S. oryzae*, in which each concentration consisted of five replicates. Tested concentrations against *S. oryzae* were 3, 5, 7, 9, and 11µL, respectively. The concentration used is determined based on a pilot study, which begins with identifying the lowest concentration that produces a detectable mortality effect. Ten adult female *S. oryzae* were selected for the testing. The filter paper inside the petri dish was first treated with 3 µL *A. sativum* essential oil, and 10 spikelets of rice were provided in each petri dish as a food source for *S. oryzae*, to prevent biased mortality of *S. oryzae*, due to starvation. Control sets were prepared using filter paper treated with distilled water, and 10 rice spikelets were prepared as well. Ten female *S. oryzae* were released onto the petri dish, and their mortality was recorded every day for five consecutive days (Kanimozhi et al. 2023). The same procedure for the mortality bioassay was carried out using *A. sativum, Sy. aromaticum* and *C. verum* essential oil against *S. oryzae*.

Statistical Analysis

All data obtained for repellency and mortality testing were subjected to the normality test using IBM SPSS 27.0 Version. Data were normalized using log transformation to fulfill the assumption of ANOVA. A quantitative test using one-way ANOVA was carried out to test the significance of the concentration used against *S. oryzae*. For testing the mortality analysis, a two-way ANOVA was performed using IBM SPSS Version 27.0.

RESULTS

Repellency Effect of Plant Essential Oils towards *S. oryzae*

There was an increasing pattern in the repellency of *A. sativum* essential oil on *S. oryzae* from 1µl to 3µl concentration. However, the 3µl of *A. sativum* concentration gave significantly higher and distinct repellency at 60% as compared to other concentrations, which imposed repellency against *S. oryzae* (post-hoc, $P<0.05$; Figure 1A). Meanwhile, the repellency activity dropped at 4µl and 5µl, which showed statistically distinct repellency results as compared to 3µl (post-hoc, $P<0.05$), which represented the highest repellency at 60%. Thus, it suggested that the best concentration for repellency against *S. oryzae* was 3µl.

Meanwhile, for *Sy. aromaticum* essential oil, the highest repellency was demonstrated in the use of 1µl, but no significant difference in repellency with other concentrations (post-hoc, $P>0.05$) except significantly higher than 5µl concentration (post-hoc, $P=0.045$). This data exhibited that 1µl of *Sy. aromaticum* was already sufficient to repel 74.33% of *S. oryzae* (Figure 1B). The concentration of 2µl C. verum showed the lowest significant difference in repelling *S. oryzae* as compared to 4µl of *C. verum* essential oil concentrations, which showed the significantly highest repellency (post-hoc, $P=0.001$) (Figure 1C).

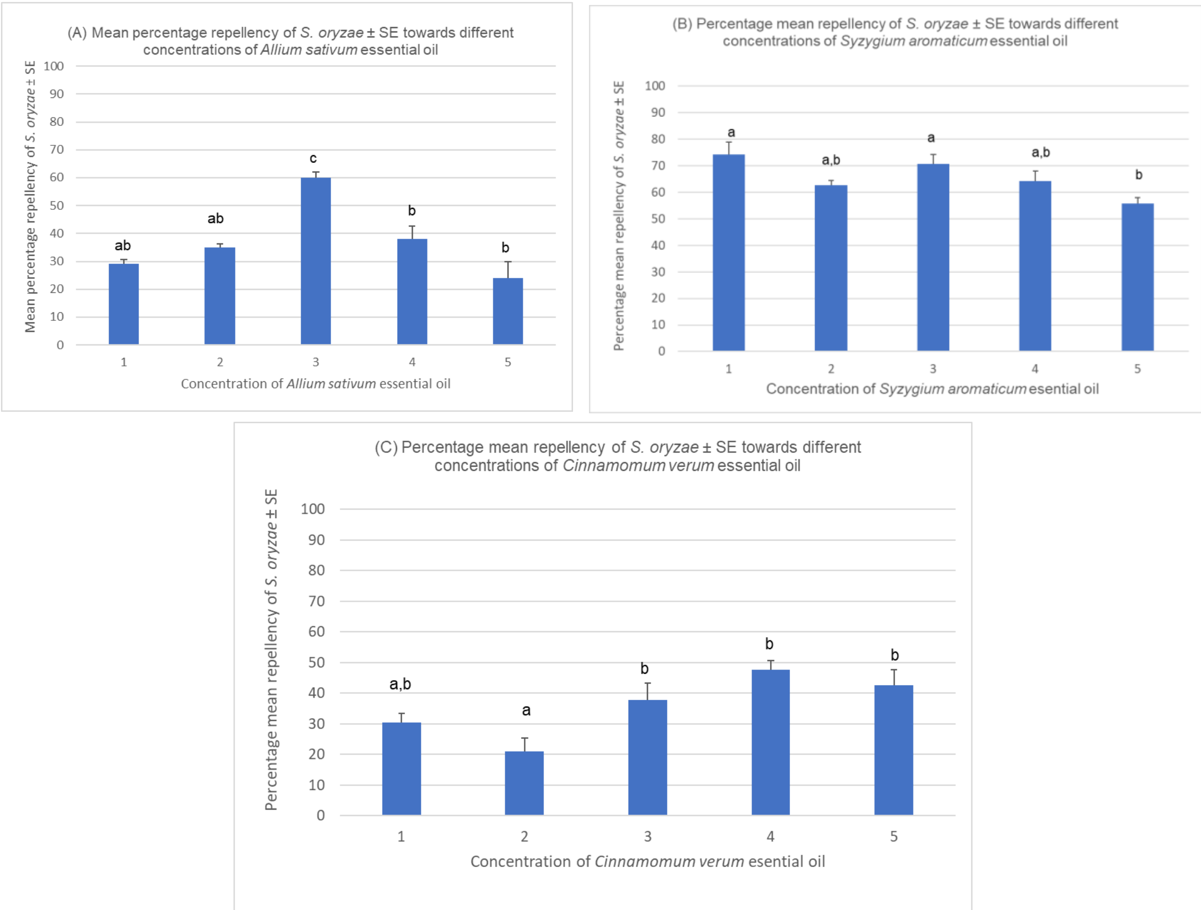

Figure 1. Mean percentage repellency of *S. oryzae* on (A) *Allium sativum*, (B) *Sygizium aromaticum*, and (C) *Cinnamomum verum* essential oils

Residual Mortality Effect of Plant Essential Oils on *S. oryzae*

Based on Figure 2, the mortality of *S. oryzae* was found to increase depending on the concentrations used and days of exposure. The two-way ANOVA analysis for *Allium sativum* essential oil involving the days of exposure and mortality of *S. oryzae* showed a significant difference between days of exposure (F=65.823, df= 4, *P=0.000*, *P<0.05*; Table 1), the concentrations *of A. sativum* essential oil used (F=19.792, df=4, *P=0.000*0, *P<0.05*) and their interactions (F=3.762, df=16, *P=0.000*, *P<0.05*) with the mortality of adult females *S. oryzae* tested. High mortality (70%) of *S. oryzae* was demonstrated on day 1 of exposure using 11 µl of concentration, which then reached 100% mortality on day 5 of exposure. Only one homogenous subset was obtained during days 4 and 5 of exposure among different concentrations (post-hoc, *P<0.05*), leading to the non-significant difference in mortality percentage of *S. oryzae*. These results suggest that concentration effects on the respective days have been stabilized, as compared to days 1, 2, and 3, where more distinct and significant mortality effects using different concentrations were seen on *S. oryzae*.

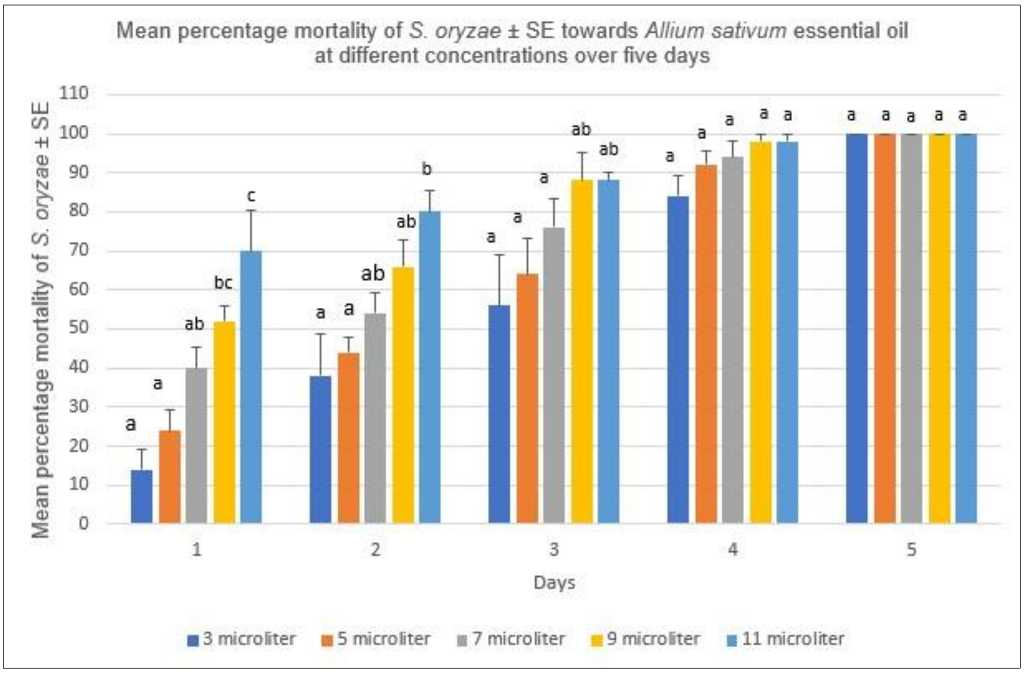

Figure 2. Mortality of *S. oryzae* exposed to *Allium sativum* essential oil at different concentrations over five days post-treatment

Table 1. Two-way ANOVA analysis between-subject effects of *Allium sativum, Sygizium aromaticum,* and *Cinnamomum verum* essential oils against mortality of *S. oryzae*

Source	**df**	**Mean Square**	**F-Value**	**Sig. Value (*P*)**
Allium sativum				
Intercept	1	514.84	9459.98	0.000
Day	4	3.58	65.82	0.000
Concentration	4	1.08	19.79	0.000
Day * Concentration	16	0.21	3.76	0.000
Error	100	0.05		
Sygizium aromaticum				
Intercept	1	614.32	11246.02	0.000
Day	4	1.20	22.50	0.000
Concentration	4	0.78	14.43	0.000
Day * Concentration	16	0.20	3.62	0.000
Error	100	0.05		
Cinnamomum verum				
Intercept	1	98.55	12267.46	0.000
Day	4	0.68	91.53	0.000
Concentration	4	0.11	14.95	0.000
Day * Concentration	16	0.06	8.02	0.000
Error	100	0.01		

On day 1, the results of 3µl and 5µl of *Sy. aromaticum* essential oil exposure showed significantly lower mortality percentage of *S. oryzae* as compared to the results for 7-11 µl concentrations of the same essential oil (Post-hoc, *P<0.05*; Figure 3). Results also showed instability of *S. oryzae* mortality percentage on the first day to the third day of observation for concentrations between 3 and 5µl (Figure 3). From day 3 up to day 5, there were no significant mean differences between various concentrations (*P<0.05*). The two-way ANOVA analysis between-subject effects for *Sy. aromaticum* essential oil showed a significant difference between days of exposure and concentrations (F=3.622, df=16, *P=0.000*; Table 1).

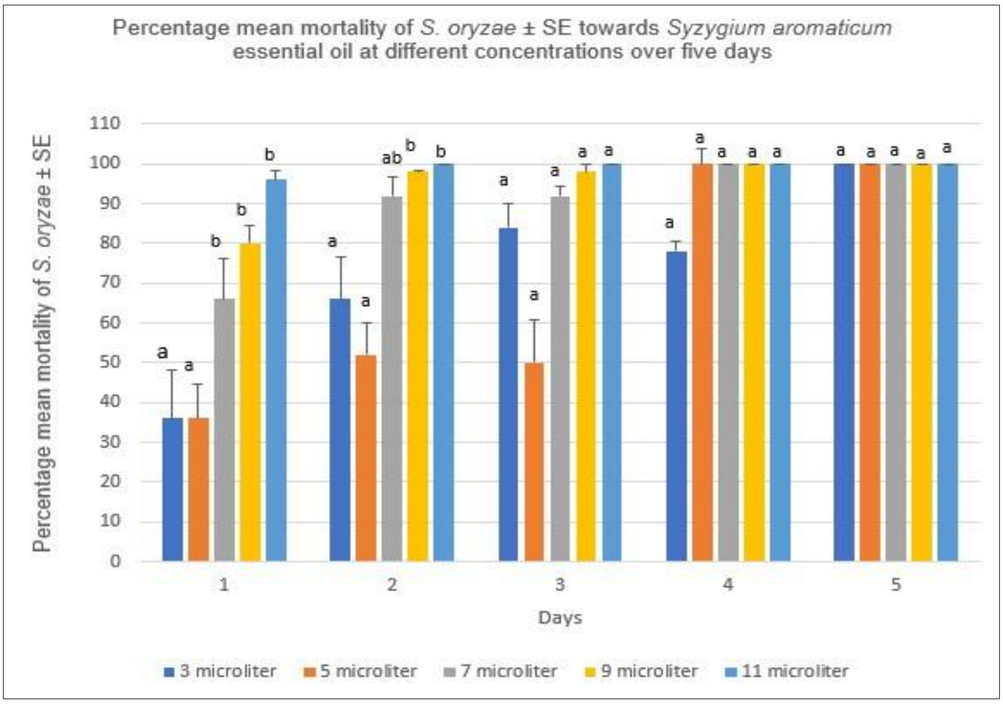

Figure 3. Mortality percentage of *S. oryzae* upon *Sygizium aromaticum* essential oil exposure at different concentrations over five days post-treatment

Based on Figure 4, exposure to 3µl of *C. verum* essential oil resulted in significantly lower mortality of *S. oryzae* with 22% mortality as compared to other concentrations (*P<0.05*). On day 2, the highest mortality was exhibited through the exposure using 11µl of *C. verum* which was found to be significantly different from other lower concentrations (post-hoc, *P<0.05*), with the mortality of 92%. Based on Table 1, the two-way ANOVA analysis of between-subject effects of *C. verum* essential oil involving days of exposure and mortality percentage of *S. oryzae* showed a significant effect between days of exposure (F=91.534, df= 4, *P=0.000*, *P<0.05*). *Cinnamomum verum* showed 100% mortality starting on day 3. On days 3, 4, and 5 of exposure, there were no significant differences in the mortality percentage of *S. oryzae* found between all concentrations of *C. verum*, as the mortality percentage almost reached the maximum of 100%. No mortality of *S. oryzae* among all control groups was observed among all control sets set up.

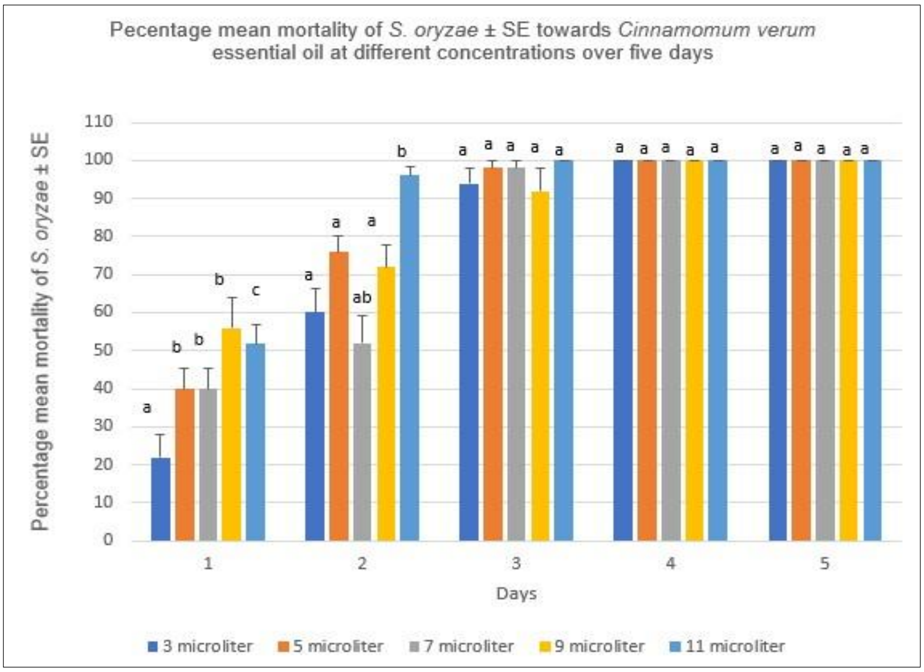

Figure 4. Mortality percentage of *S. oryzae* upon exposure to *Cinnamomum verum* essential oil at different concentrations over five days post-treatment

DISCUSSION

Plants with bioactive substances have been used widely and effectively against insect pests over time, especially in crop management sectors (Karabörklü & Ayvaz 2023). Farmers traditionally used botanical plant extracts to control postharvest pest insect infestation against grains while they were being stored (Ahmed et al. 2021). Variations in the mode of action by botanical plant extract containing essential oil also interact with insects' biochemical activity, physiological function, metabolic reaction, behavioural, and anatomy (Karabörklü & Ayvaz 2023).

In our study, *S. oryzae* showed a significantly higher repellency effect against *Sy. aromaticum*, even at a low dose of 1µl concentration. This might be due to the presence of terpenoids in *Sy. aromaticum* essential oil was effective in causing behavioural change in *S. oryzae* as suggested by Plata-Rueda et al. (2018), who found terpenoid constituents of cinnamon and clove essential oils had caused toxic effects and behavioral repellency response on grain weevil. Exposure of insects to essential oil will lead to changes in behavioural responses, such as leaving the toxic environment as soon as toxicants are present (Barson et al. 1992; Koul et al. 2013; Watson & Barson 1996). In *Sy. aromaticum* essential oil, volatile compounds such as eugenol, caryophyllene oxide, αhumulene, α-phellandrene, and α-pinene induced high repellency to *S. oryzae* and were associated with a respiratory effect similar to *S. granaries* (Plata-Rueda et al. 2018). Based on our observation, the repelling behaviour of *S. oryzae* can be seen during the first 10 minutes of exposure, which could be related to the respiration process.

A significantly lower repellency effect of *S. oryzae* against the essential oil at 1µl and 2µl concentrations may be due to the low application dosage (Yang et al. 2010). Low application dosage of essential oil reduces the protective effect of grains towards pest infestation as some of the active ingredients applied were lost to the surroundings, making its efficacy in repelling *S. oryzae* decreased (Lee et al. 2003; Kłyś et al. 2020; Pierrard 1986). All the repellency effect against *A. sativum* essential oil was due to the presence of diallyl disulfide and diallyl sulfide that induce the deterrence behaviour of *S. oryzae* (Rahman & Motoyama 2000). Repellent compounds such as allicin and other sulfide compounds were likely to be at the optimal level at 3µl, which makes the *S. oryzae* sensory system more sensitive to essential oil stimuli. A significant drop in repellency was observed after 3µl of concentration, probably due to the overwhelming sensory mechanism of insects. Instead of eliciting the repellency, the higher concentration causes sensory adaptation or desensitization, making insects less responsive to the essential oil, which also might occur in *C. verum.* In a more saturated area with treatment concentration, a more continuous interaction between molecules with the receptors of insects occurred (Zufall & Leinder-Zufall 2000). A significant peak of repellency was found during the exposure concentration of 4µl as compared to concentration of 2µl from *C. verum* essential oil because the intensity of essential oil odour in high concentration is postulated to be stronger and overwhelm the *S. oryzae* sensory mechanism.

In the study, a better effect of habituation or desensitization of *C. verum* essential oil to reach 100% mortality could be seen occurring at day 3 for all concentrations compared to other EOs. *Sitophilus oryzae* mortality against the exposure of essential oils showed that a certain concentration of the essential oils was no longer significantly affecting the mortality. This is probably due to the cumulative toxicity accumulated in its body, leading to the later lethal effects (de Araújo et al. 2017; Guedes et al. 2005). Besides, it could also be due to the physiological stress that occupies *S. oryzae,* where frequent and continuous firing or exposure to the essential oil, even at low concentrations, leads to chronic stress by *S. oryzae*, which later builds up and leads to death (de Araújo et al. 2017; Guedes et al. 2005). As for *Sy. aromaticum* and *C. verum*, some studies found that as the essential oil compromises with insects' respiratory system, it could lead to lower respiration rate, physiological stress, impair muscle activities, causing paralysis and eventually death from starvation (de Araújo et al. 2017; Guedes et al. 2005).

Syzygium aromaticum was associated with the terpenoid compounds present in it (Lazo-Tavera 1999). Terpenoids play a crucial role in the clove plant itself, maintaining good physiological and cellular membrane function, and act as a defensive role against pests (Boncan et al. 2020; Lazo-Tavera 1999). Thus, they cause mortality in *S. oryzae*. Whereas properties of essential oils in *A. sativum*, which were lipophilic in nature and were able to be inhaled and ingested, could indicate their rapid action and neurotoxic mode of action, leading to *S. oryzae* mortality (Enan 2001). It gives the *A. sativum* the capability to penetrate through the insect cuticle when in contact with this EO (Richards 1978). The 100% mortality that could be achieved on day 5 of exposure was reported due to the odour of *A. sativum* essential oil that was choking and exhibited toxic effects, which were disturbing to the respiratory activity of the weevils. As it might also affect the respiratory system of rice weevils, it later leads to asphyxiation and death (Adedire & Ajayi 1996; Ileke & Oni 2011).

CONCLUSION

All botanical essential oil extract treatments impose repellency and mortality effects towards adult females *S. oryzae*, indicating all essential oils used in this treatment exhibit insecticidal properties, which were useful for an effective pest control program. In the future, emphasis should be placed on the method of application of essential oils to control stored product pests on agricultural yield crops or food. The encapsulation method of essential oils helps to reduce adverse effects on food flavors, control their release rate, and reduce volatile substance degradation. Thus, besides reducing environmental impact and successfully controlling *S. oryzae*, it ensures that a safer, less hazardous application of the control method is delivered to reduce any adverse effects of chemical insecticide application.

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AUTHORS DECLARATION

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Conflict of Interest
No conflict of interest declared by authors.

Data Availability Statement
All data generated or analyzed during this study are included in this published article.

Authors' Contributions
The authors' contributions are as follows: NNMZ: Data curation, investigation, methodology, formal analysis, and writing original draft; WFZ: Conceptualization, data curation, formal analysis, funding acquisition, project administration, resources, supervision, validation, and writing review.

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