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EVALUATION OF PLANT ESSENTIAL OILS AGAINST *SITOPHILUS ORYZAE* LINNAEUS (COLEOPTERA: CURCULIONIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

Sitophilus oryzae Linnaeus, commonly known as rice weevil is a globally serious pest of stored grains, especially rice. Considering the target specific nature of botanicals and less hazardous effect than synthetic insecticides, the study was conducted to evaluate the effectiveness and appropriate dosage of four essential oils i.e., lemongrass (*Cymbopogon citratus* Stap.), eucalyptus (*Eucalyptus globulus* Labill.), peppermint (*Mentha piperita* L.), and tea tree (*Melaleuca alternifolia* L.) against *S. oryzae.* Each essential oil was used at 0.1, 0.5, and 1.0 ml doses on filter paper. A long glass cylinder (1000 ml size), divided into three (A, B, and C) sections, supplied with 20 g rice at ends, was used in the study. Ten freshly emerged adults of *S. oryzae* were released in the center of B, whereas treated and untreated filter paper was placed at A and C section, respectively. Observations on repellency and mortality percentage were recorded after 24 and 48 hours of exposure. The results indicated that all the tested essential oils exhibited insecticidal potential against *S. oryzae*. The insecticidal activity of all the essential oils increased with their application dose and exposure timing. Maximum mortality of *S. oryzae* after 48 hours was recorded in tea tree (76.67±3.33%) at 1.0 ml dose, whereas maximum repellency was recorded with 0.1 ml lemongrass (76.67±12.02%) treatment. Overall, tea tree and lemongrass showed maximum insecticidal performance, whereas peppermint was the least effective. After 48 hours, the lowest [0.008 ml (0.001-0.019) and highest [0.018 ml (0.000-0.078) LD_{50} values against *S. oryzae* were recorded with lemongrass and peppermint essential oils, respectively. The LD_{50} values recorded for eucalyptus and tea tree treatment were 0.07 ml (0.000-0.059) and 0.009 ml (0.000-0.046), respectively. Therefore, it is suggested that the essential oils of either tea tree or lemongrass may be applied in the warehouses against *S. oryzae*, that may not only cause repellency but also mortality among them.

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INTRODUCTION

Sitophilus oryzae Linnaeus (Coleoptera: Curculionidae), commonly known as rice weevil, is an important and serious insect pest of rice and other stored cereals like wheat, maize, rice, sorghum, oat, pulses, and gram stored in godowns (Park et al., 2003). It has been considered as one of the most universal and devasting store product pest of rice all over the world (Bello et al., 2001; Djamin and Idris, 2012). After rice, wheat and maize are the most affected commodities due to the feeding of rice weevil. Therefore, rice weevil can cause loss in the weight of rice from 0.69 to 5.93 percent when they are stored for two to six months condition (Kudachi and Balikai, 2014).

Rice weevil is native from India and then spread throughout the world and now has a universal distribution (Koehler, 2008). Rice weevil is a major pest of cereals both in field before harvest and storage (Nyamador et al., 2017). One pair of *S. oryzae* can reproduce about one million of its species within a period of three months under favorable conditions and the adults are internal feeders and cause serious quantity and qualitative losses to the grains (Thomas et al., 2002).

Generally, rice weevil adults have long snout and body coloration black or brown, their body are 2 mm long. *S. oryzae* adults are short flyers with a lifespan of up to 2 years (Kanmani et al., 2021) It is a cosmopolitan pest where adults consume rice and make small holes to lay their eggs inside the kernel and cover them with sticky material. Normally, adult female lays 300-400 eggs throughout the life span. The egg time continued five to ten days while incubation period was recorded three to ten days. The egg hatching ability of rice weevil is affected by the female age (Hoy et al., 2000). The larval period was recorded twenty-three to thirty days in rice (Barberena and Aide, 2003; Nguyen et al., 2016). Larvae consume rice kernels ideally and finished the large percentage of vitamins and proteins while feeding of the endosperm (Bello et al., 2001). The pupal period is entirely completed inside the rice kernels, and it comes out after two to four days (Choudhury and Chakraborty, 2014; Lee et al., 2001). The total pupal period is counted as 6-9 days, whereas pre pupal period is noted as 1-2 days. Generally, the total life cycle of rice weevil is completed in a month that may be extended several months due to unfavorable conditions (Pimentel et al., 1997). It has been observed that rice weevil adult and larva both consume the rice grains and reduce carbohydrates contents and rice quality (Lucas and Riudavets, 2002). Only the larva consumes 14 mg of rice grain and adults consume 0.4 mg grain per day (Kemabonta and Falodu, 2013).

The most popular technique to prevent the infestation of stored grain pests including *S. oryzae* is the use of insecticides because of their quick action against the target pests (Wijayaratne and Rajapakse, 2018). However, insecticides are an unfavorable option due to their high costs, unavailability to small-scale farmers, residual impact, pest resistance, persistence, and harmful effect on non-target organisms including humans and their environment (Elhag, 2000). As a result, many researchers are recently focusing on using more rational options particularly the use of edible plant species as a resource for environmentally and human-safe, degradable pesticides against stored grain pests (Rahman and Talukder, 2006). Natural insecticides are derived from plants that contain different bio-active ingredients that may affect pests in different ways (Chaubey, 2019). Therefore, for controlling the stored grain pest populations, the use of essential oils and extract from many plant species are a favorable alternative of the synthetic pesticides as these oils are target specific with low residual effects (Isman, 2000; Sammour et al., 2018).

Therefore, considering the effectiveness and target specificity of the plant essential oils along with their less hazards impacts to humans and the environment, this study aimed to evaluate lemongrass (*Cymbopogon citratus)*, eucalyptus (*Eucalyptus globulus*), peppermint (*Mentha piperita)* and tea tree (*Melaleuca alternifolia*) essential oils against *S. oryzae* under laboratory conditions. The findings could help to determine the most effective essential oils along with its appropriate dosage that can be suggested to be applied against *S. oryzae* to reduce its losses to stored cereals.

MATERIALS AND METHODS

Location

The study was carried out at Stored Grain Research Laboratory, Department of Entomology, Sindh Agriculture University, Tandojam during 2022.

Collection and rearing of *Sitophilus oryzae*

The initial collection of rice weevils was conducted in the local godowns of Tandojam and Hyderabad, and their identification was based on their distinctive characteristics by experts from the department. Subsequently, additional rearing was carried out in the laboratory using rice placed in plastic jars covered with muslin cloth. Freshly emerged F1 generation males and females were employed for further experimentation.

Essential oils

Four essential oils were purchased from Chiltan, an ISO certified company used in the study mentioned below:

1. Lemongrass, *Cymbopogon citratus* Stap
2. Peppermint, *Mentha piperita* L.
3. Eucalyptus, *Eucalyptus globulus* Labill
4. Tea tree, *Melaleuca alternifolia* L.

Experimental setup, data collection and analysis

A long cylinder trap (1000 ml, 18.3 inch height, 2.35 inch diameter) trap was manufactured as described in the study of Park et al. (2018) and Lee et al. (2020) with slight modifications (Figure 3.1). The long cylinder was connected with feeding dishes divided into three parts (A, B, C) (Figure 1). At one end, it was sealed with calico or cloth to facilitate ventilation and to prevent insects from escaping.

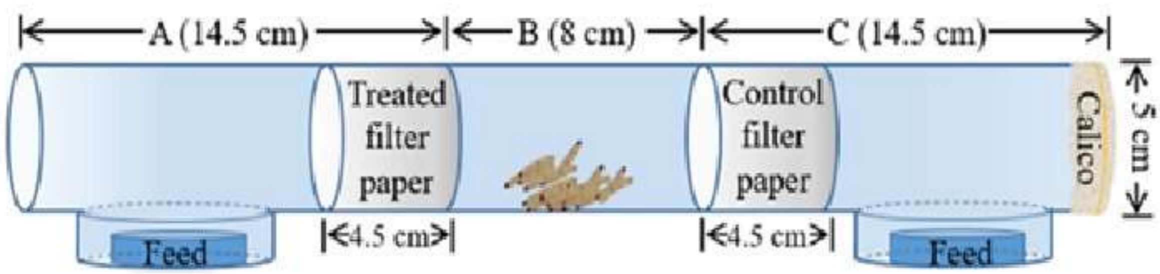

Figure 1: Experimental setup used in the study as adopted from Jo et al. (2013) and Khaskheli et al. (2021).

Methods of Jo et al. (2013) and Khaskheli et al. (2021) were used to evaluate the repellent efficacy of essential oils. In this experiment, three doses of essential oils such as 0.1, 0.5, and 1.0 ml were used against the adults of *S. oryzae*. The respective dose of the individual oil was applied on a filter paper that was then allowed to air-dry for 20 minutes. Afterwards, the treated filter paper with respective dose of a particular oil was placed between A and B, whereas controlled filter paper without any oil was kept between B and C (Figure 3.1). Ten freshly emerged F1 adults were then released separately in the middle of B with the help of an aspirator as the face of cylinder was closed with a fine muslin cloth at the end of section C to ensure the ventilation inside it. The entire experiment was conducted at the temperature of 30±2°C with 55±5% R.H. The completely randomized design was used to set up the experiment where three replications were managed for each essential oil treatment. A separate glass cylinder was used for each treatment, whereas the cylinder was thoroughly cleaned and wiped with the help of tissue paper after performing each replication.

The data was collected by counting the number of insects in C and A section after 24 and 48 hours of the application of essential oils to calculate the repellent efficacy and mortality percentage.

The percentage (%) repellent efficacy was calculated by using the following equation:

$$\text{Repellent efficacy } \% = \frac{\text{Nc} - \text{Na}}{\text{Nc} + \text{Na}} \times 100$$

Where, Nc is the number of insects in C, and Na is the number of insects in A.

The Percentage of mortality was calculated by using the following equation.

$$\text{Mortality } (\%) = \frac{\text{Number of dead adults}}{\text{Total number of adults released}} \times 100$$

The Analysis of Variance was used to analyze the obtained data on mortality and repellency of essential oils against the adults of *S. oryzae,* whereas means with significant differences were separated using the Least Square Difference (LSD) at 5% probability. All the analyses were performed through STATISTIX 8.1 computer software (Statistix, 2003). Moreover, Probit Analysis was used through SPSS (version 21, IBM Corp.) (IBM Corp. (2012) to calculate the LD_{50} values because it is most widely used and acceptable tool to calculate lethal doses or concentrations of pesticides, including essential oils, which can be used against the target pests.

RESULTS

The experiment on the insecticidal potential of various essential oils i.e., tea tree, lemongrass, eucalyptus, and peppermint oil against *S. oryzae* applied at 0.1 ml, 0.5 ml and 1.0 ml doses confirmed that all the tested essential oils exhibited insecticidal potential against *S. oryzae*. The insecticidal activity of all the essential oils increased with their application dose and exposure timing (Figure 2-6). Accordingly, Figure 2 shows the results regarding the percentage mortality of *S. oryzae* after 24 hours application of various essential oils applied at various doses. A highly significant difference was recorded among various essential oils (F =10.83, P < 0.001) applied at various doses (F= 22.08, P < 0.001) to elicit the *S. oryzae* mortality. According to the results, the maximum mortality (60.00 ± 5.77%) after 24 hours of application was recorded in tea tree treatment applied at 1.0 ml dose, followed by 50.00 ± 0.00% mortality recorded in the same treatment applied at 0.5 ml dose. Moreover, the maximum mortality recorded in remaining treatments i.e., eucalyptus, lemongrass, and peppermint were 46.67 ± 14.53, 30.00 ± 5.77, and 23.33 ± 3.33%, respectively, all recorded when these oils were applied at 1.0 ml dosage.

A rise in the mortality of *S. oryzae* was recorded at 48 hours of the application of various doses of essential oils, however, there was a significant difference among various oils (F= 22.65, P < 0.001) and doses (F= 61.48, P < 0.001) applied (Figure 3).

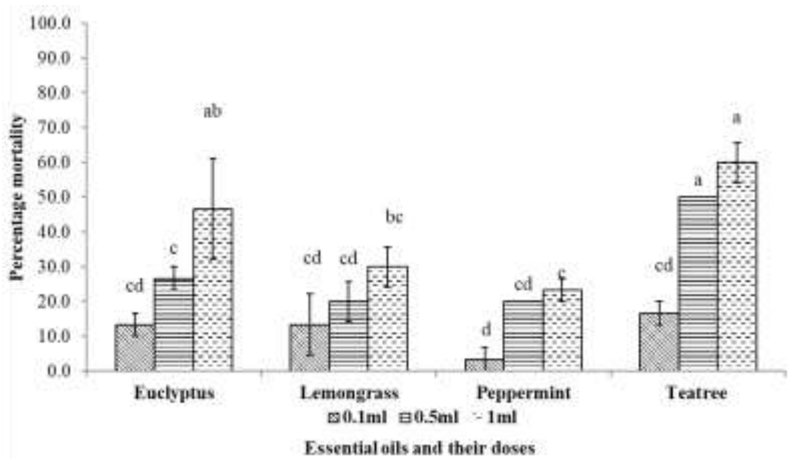

Figure 2: Impact of various essential oils on percentage mortality of *S. oryzae* after 24 hours.
*Means followed by different letters are significantly different from each other (LSD = 17.763, P < 0.05)

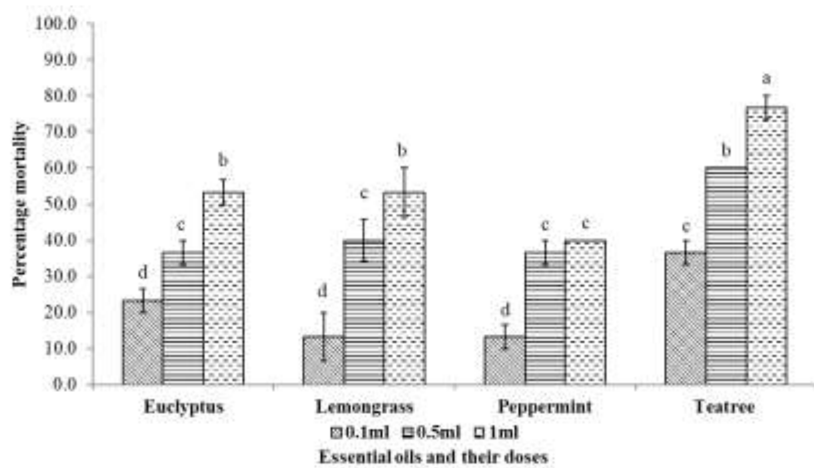

Figure 3: Impact of various essential oils on percentage mortality of *S. oryzae* after 48 hours.
*Means followed by different letters are significantly different from each other (LSD =12.871, P < 0.05).

According to the results, the maximum mortality (76.67 ± 3.33%) after 24 hours of the application was recorded in tea tree treatment applied at 1.0 ml dose, followed by 60.00 ± 0.00% mortality recorded in the same treatment applied at 0.5 ml dose. Furthermore, the highest mortality recorded in the remaining treatments i.e., eucalyptus, lemongrass, and peppermint were 53.33 ± 3.33, 53.33 ± 6.67 and 40.00 ± 0.00%, respectively, all recorded at 1.0 ml dose of these oils.

Similar to mortality results, 24 hours after the application, different essential oils showed significant difference (F = 7.96, P = 0.007) in their potential to repel *S. oryzae*, however, no significant difference (F = 2.22, P = 0.1302) was recorded in repellency of various doses of these oils (Figure 4). Accordingly, the highest repellency percentage of *S. oryzae* was recorded in lemongrass essential oil (73.33 ± 12.02 and 73.33 ± 6.67%) when applied at 0.1 and 0.5 ml doses. Moreover, the maximum repellency of *S. oryzae* recorded in eucalyptus, peppermint, and tea tree treatments was 53.3 3± 3.33, 60.00 ± 00, and 60.00 ± 5.77% applied at 0.5 ml and 0.1 ml doses, respectively. Afterwards, a decline in repellency percentage of *S. oryzae* was recorded in all the treatments at 1.0 ml dose because of the rise in mortality percentage in the respective treatments. Moreover, after 48 hours of the application of essential oils, maximum *S. oryzae* repellency (76.67 ± 12.02%) was recorded in lemongrass applied at 0.1 ml dose, followed by 60.00 ± 5.77% repellency in the same essential oil when applied at 0.5 ml dose (Figure 5).

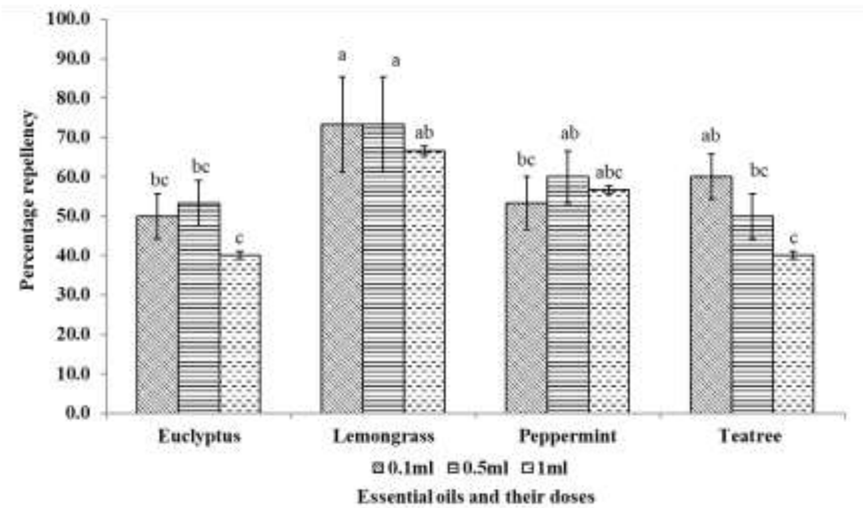

Figure 4: Impact of various essential oils on percentage repellency of *S. oryzae* after 24 hours.
*Means followed by different letters are significantly different from each other (LSD = 18.841, P < 0.05).

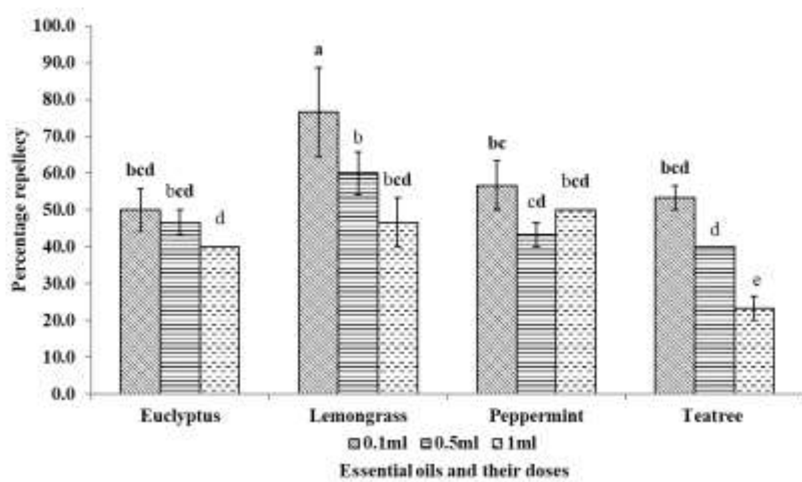

Figure 5: Impact of various essential oils on percentage repellency of *S. oryzae* after 48 hours.
*Means with different letters are significantly different from each other (LSD = 15.638, P < 0.05).

Moreover, after 48 hours the maximum repellency observed in the remaining treatments i.e., eucalyptus, peppermint, and tea tree were 50.00 ± 5.77, 56.67 ± 6.67 and 53.33 ± 3.33%, all recorded when these essential oils were applied at 0.1 ml dose.

Figure 6 illustrated overall comparisons regarding the impact of various essential oils applied at 0.1, 0.5, and 1.0 ml doses on the mortality and repellency of *S. oryzae.* A highly significant difference was recorded among the essential oils regarding their potential to cause mortality ($F = 8.11$, $P < 0.001$) and repellency in *S. oryzae* ($F = 11.10$, $P < 0.001$) adults. Overall, significantly the highest mortality of *S. oryzae* (50.00 ± 4.78%) was recorded in tea tree treatment, whereas the mortality percentage recorded in eucalyptus (33.33 ± 4.04%), lemongrass (28.33 ± 4.22%), and peppermint (22.78 ± 3.31%) was not significantly different from each other. On the other hand, significantly the highest (66.11 ± 3.80%) and lowest (44.44 ± 3.15%) repellency percentage of *S. oryzae* was recorded in lemongrass and tea tree treatments, whereas the repellency recorded in eucalyptus (46.67 ± 2.43%) and peppermint (53.33 ± 1.98%) was not significantly different from each other.

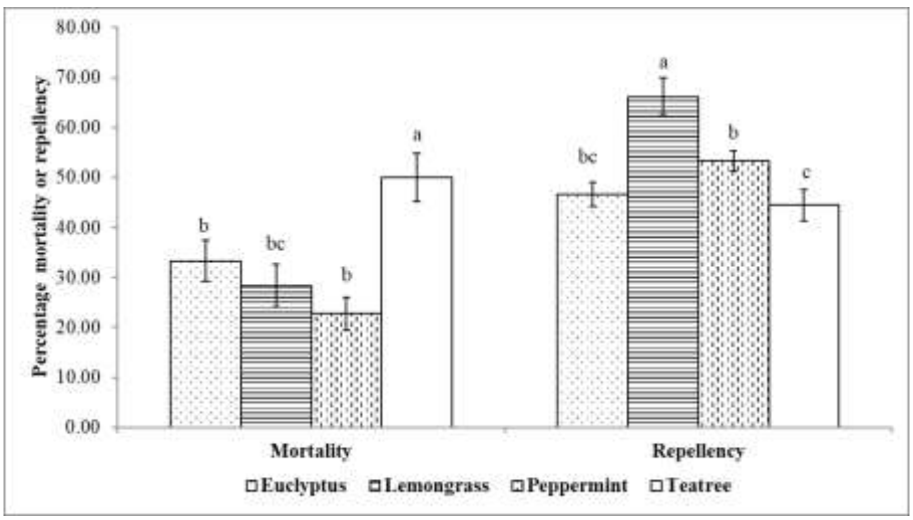

Figure 6: Overall mortality and repellency percentage of *Sitophilus oryzae* due to application of essential oils.
*Means with different letters are significantly different from each other LSD values @ $P < 0.05$ (Mortality = 11.636, repellency = 8.2545).

Table 1 describes the results regarding the LD_{50} values calculated for various essential oils used against *S. oryzae*. It is evident from the results that LD_{50} values of all the essential oils were time dependent, instead of dose dependent as LD_{50} values decrease at the data observation of 48 hours from 24 hours. Among all the essential oils, the lowest LD_{50} values after 24 hours was recorded in lemongrass [0.009 ml (0.000-0.046)], followed by eucalyptus [0.036 ml (0.014-0.062)] and tea tree [0.040 ml (0.005-0.076)], whereas the peppermint showed the highest LD_{50} values of 0.054 ml (0.004-0.121) against *S. oryzae.* After 48 hours, the lowest [0.008 ml (0.001-0.019) and highest [0.018 ml (0.000-0.078) LD_{50} values were recorded for lemongrass and peppermint essential oils, respectively. Moreover, LD_{50} values recorded for eucalyptus and tea tree treatment were 0.07 ml (0.000-0.059) and 0.009 ml (0.000-0.046), respectively.

DISCUSSION

It was observed in the study that all the essential oils i.e., eucalyptus, lemongrass, peppermint, and tea tree showed their insecticidal potential against *S. oryzae* with significant differences among them. Tea tree and lemon grass were found to be the two most effective oils to cause overall combined significant mortality and repellency, whereas peppermint oil was the least effective against *S. oryzae.* Moreover, insecticidal potential oils against *S. oryzae* increased with dosage and exposure timings. The results obtained in this study are supported with previous studies on botanical pesticides as the application of southern blue gum i.e., eucalyptus and tea tree were found more effective against *S. oryzae* than peppermint and basil, *Ocimum basilium*, as both these botanicals caused 100% mortality of the weevil's death at the lowest concentrations and shortest application times (Abdelgaleil et al., 2009; Padalia et al., 2015).

Table 1: LD_{50} values (mortality plus repellency) of various essential oils against *Sitophilus oryzae* after 48 hours of the application.

Essential oil	Hours	LD_{50} (ml)	Fiducial Limit (95%)	Slope ± SE	χ^2	Probability
Eucalyptus	24 hours	0.036	0.014-0.062	0.757 ± 0.110	8.610	0.282
	48 hours	0.017	0.000-0.059	0.757 ± 0.120	22.566	0.002
Lemon grass	24 hours	0.009	0.000-0.046	1.026 ± 0.169	36.720	0.000
	48 hours	0.008	0.001-0.019	1.142 ± 0.206	13.719	0.056
Peppermint	24 hours	0.054	0.004-0.121	0.737 ± 0.106	20.458	0.005
	48 hours	0.018	0.000-0.078	0.673 ± 0.114	31.812	0.000
Tea tree	24 hours	0.040	0.005-0.076	1.816 ± 0.200	35.515	0.000
	48 hours	0.009	0.000-0.046	1.026 ± 0.169	36.720	0.000

The insecticidal potentials of lemongrass and tea tree have also been observed against many stored grain pests including *S. oryzae.* While evaluating seven essential oils against *Callosobruchus maculatus* and *S. oryzae,* tea tree and cinnamon essential oils proved to be more lethal for both the pests as 90% mortality of *S. oryzae* was recorded with tea tree oil at 16.0 µl /50 ml air after 24 hours of the application (El-Salam and Ahmed, 2010). The studies on the combined effect of various irradiation doses of X-ray and γ rays with eucalyptus and tea tree against *S. oryzae* confirmed an increased mortality by 3 to 6 times with combined application as compared to individual application of the treatments (Hossain et al., 2021). As compared to γ rays, X-rays were found more effective when applied with combination of eucalyptus or tea tree essential oils with LD_{50} values of 746.02 and 737.1 Gy with Eucalyptus EO and 632.03 and 615.5 Gy with Tea tree EO, at dose rates of 0.76 and 0.19 kGy/h, respectively. Lemongrass has also been reported to be toxic towards *S. oryzae* as study of Uwamose et al. (2017) also reported that as compared to powder extracts, methanol extracts of lemongrass was found more toxic against the weevils. Accordingly, the LC_{50} and LT_{50} values calculated against *S. oryzae* were 2.16 mg/20 ml of methanol and 75.10 hours, respectively. A recent study also evaluated the essential oil of lemongrass grown in Serbia against four major stored grain pests i.e., adults of *S. oryzae, Acanthoscelides obtectus* and *Tribolium castaneum* along with larvae of *Plodia interpunctella* (Gvozdenac et al., 2021). The lemongrass essential oil showed strong repellent potential against adults of all *S. oryzae, A. obtectus and T. castaneum* when applied at 0.2 and 0.5% concentrations, whereas its repellent activity against *P. interpunctella* larvae comparatively lower, even at higher concentration (0.5%). Plata-Rueda et al. (2020) also reported that essential oils of lemongrass and its components have the potential to elicit toxic and repellent properties against *S. granarius*, another serious stored grain pest under genus *Sitophilus.* Their findings showed that as compared to 99.9% survivorship in untreated control, *S. granarius* survival decreased to 57.6, 43.1, and 25.9% when treated with lemongrass essential oil, citral and geranyl acetate (components of lemongrass), respectively. The reason for their lower survival in the treatments of essential oils and active components was reported to be due to the low respiration rates and movement (Plata-Rueda et al., 2020). Singh et al. (2018) evaluated various essential oils i.e., eucalyptus, lemongrass, and citrus, individually and in combination against *S. oryzae* and found that combination of eucalyptus + lemongrass was found to be more lethal against the weevils as a gradual increase in mortality was recorded. After 21 days of exposure, 96.67% mortality of *S. oryzae* was observed with the application of eucalyptus + lemongrass, whereas new progeny emergence was also low among the treatments i.e., 54.78.

The LD_{50} results indicated that lemon grass (0.008) and tea tree (0.009) exhibited the lowest values to cause

50% insecticidal (mortality and repellency) of the targeted *O. oryzae* as the LD_{50} reduced with the exposure timing. Similar type of results regarding the lower LD_{50} values with increased exposure timings were obtained by Abo Arab et al. (2022) while using basil oil and petit mandarin grain against *S. oryzae* and *Rhyzopertha dominica*. However, studies of Mishra et al. (2012) and Hernandez-Lambraño et al. (2015) reported the possession of insecticidal properties of different plant essential oils against stored grain pests including *S. oryzae* as their efficacy increases at higher doses.

Therefore, in continuation of above studies, all essential oils elicit promising mortality and repellent effect against *S. oryzae,* but essential oils of tea tree and lemongrass were found more effective to cause mortality, whereas the peppermint and eucalyptus were found to be repellent. Moreover, the mortality or repellence of *C. maculatus* adults increased with increasing dose of essential oils and their exposure timings.

CONCLUSION

All the essential oils used i.e., tea tree, lemongrass, eucalyptus, and peppermint exhibited insecticidal potential (mortality or repellency) against *S. oryzae* adults. Tea tree and lemongrass were the two most effective essential oils with lowest LD_{50} values, whereas peppermint was the least effective against *S. oryzae*; however it elicited good repellent potential for *S. oryzae* adults. The insecticidal potential for all the essential oils increased with higher dosage and extended exposure timings. Therefore, it is suggested that tea tree and lemon grass essential oils may be exploited against *S. oryzae* on large scale and practical conditions of warehouses to reduce its losses to cereals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

KR wrote manuscript and conducted research; AAG and LBR conceived idea and supervised research; JH edited manuscript; SM analyzed data; JGMS helped in data analysis; and ZP helped in data collection.

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