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INSECTICIDAL AND REPELLENT ACTIVITIES OF EUCALYPTUS OIL AGAINST LESSER GRAIN BORER *RHYZOPERTHA DOMINICA* (FABRICIUS)

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ABSTRACT

Lesser grain borer *Rhyzopertha dominica* (F.) is a destructive insect pest of stored grains. Both larvae and adults of the pest attack whole, sound grain and cause extensive damage. Larvae are internal feeders whereas adults are free living and bore into the commodity and can fly. Fumigation is the most useful and effective method to manage this insect pest. There are several insecticides available in the market to manage this pest but some insecticides have been banned because of environmental pollutions and for some insecticides this insect pest has developed resistance. Therefore efforts are required to find safe, effective and viable alternatives. Natural products are well known to have a range of useful biological properties against insect pests. Recently, there has been considerable interest in essential oils and extracts of medicinal and edible plant and herbs for the development of alternative insecticides and fumigants. This research was initiated to evaluate the fumigation toxicity and repellent action of *Eucalyptus globulus Labill* essential oil against adults of *R. dominica*. GC/MS analysis showed the major components of *E. globulus* essential oil as 1,8-cineole (22.35%), α-pinene (12.58%) limonene (4.01%), aristolene (3.35%), p-cymene (3.25%), trans-verbenol (3.02%), isosativene (2.85%), α-myrcene (2.15%) and terpinen-4-ol (2.10%). Fumigation toxicity and repellent activity showed that as the concentration and exposure time increased the mortality and repellency also increased. Maximum repellency of 62.0% was observed at 0.32μl/cm^2 concentration after 36 hours of exposure.

Keywords: Eucalyptus species, Fumigant toxicity, Insecticides and fumigants, Insect repellent activity, Lesser Grain Borer

INTRODUCTION

Increase in human population has led to several problems, especially in respect to food security. At present time, agriculture is facing challenge to produce more food and fibre to feed growing population. Although we have produced enough of food grains, but there is a huge gap between demand and supply ostensibly due to loss in food grains. Losses in grain will start from the field and continue during storage leading to extensive qualitative as well as quantitative loss **(Metcalf and Metcalf 1993; Sedlasek *et al.*, 2001)** much of this is due to insect pest attack **(WAGOV 2005)**. Globally, storage pests damage account to the extent of 10–40% of stored agricultural crops **(Raja *et al.*, 2001)**. The bostrichid beetle lesser grain borer, *Rhyzopertha dominica* is a highly destructive primary pest of stored grains including cereals, seeds and dried fruit. This insect is now established in several East and West African countries following recent accidental introductions from its previously more limited indigenous range in meso-America **(Golob 1988)**. Fumigation is the main strategy to manage the insect pests of stored grains. There are several insecticides reported for treating godown and containers and for managing stored product pests such as organophosphates and pyrethroids **(Kao *et al.*, 1992; Hashem *et al.*, 2012; Hung *et al.*, 1990)**. Methyl bromide and phosphine achieved great success in the management of these stored grain insect but methyl bromide is one of the substance responsible for ozone layer depletion and signatory countries of montreal protocol are being phasing out the production of such compounds and fumigation with phosphine has a long history of resistance development **(Hashem *et al.*, 2012; Collins *et al.*, 2000; Tyler *et al.*, 1983; Rajendran and Narasimhan 1994; Ren *et al.*, 1994; Collins 1998; Acda 2000; Ansell *et al.*, 1990)**. Although, fumigation is the only option for bulk storage of grains, there is limited scope of utilizing the fumigation technique for grain protection at small scale grain storage at farm sites in view of lack of trained manpower in conducting scientific fumigation and paraphernalia required for cover fumigation using toxic phosphine tablets. Hence, there is a need to develop alternative safe and economical non-chemical methods for controlling insect pests of stored products at small scale grain storage. Among the available options, essential oils and their constituents may have potential as alternative fumigants in view of their volatility and fumigant activities **(Shaaya *et al.*, 1997)**.

Essential oils are usually obtained *via* steam distillation of medicinal and aromatic plants, mostly used as fragrance and flavouring agents for aromatherapy and as herbal medicines. Essential oils from more than seventy five plant species have been studied for fumigant toxicity against insect pests of stored grains **(Rajendran and Sriranjini 2008)**. Among the essential oils, *Eucalyptus* oil is more useful as it is easily extractable and possesses a wide range of desirable properties worth exploiting for insect pests management. *Eucalyptus* is a naturally-green, tall tree covered with odorous branches full of essence-based centers and found all over the world. The oil has been used commercially in food, flavoring, perfumery, and in the pharmaceutical industries **(Aref *et al.*, 2015; Singh *et al.*, 2009)**. The essential oil from leaves of *Eucalyptus* species has been the object of several studies viz., antibacterial, anti-inflammatory, antioxidant, anti-hyperglycemic, anti-malarial, antifungal and analgesic activity **(Bendaoud *et al.*, 2009; Gray and Flat 1998; Yu Chang *et al.*, 2006)** .Considering the dual global necessity to achieve food security and food safety, more attention should be devoted to explore novel alternative treatments that are economically feasible, sustainable, user and environment friendly. In the view of above mentioned facts, present study was undertaken to elucidate the constituents of *Eucalyptus globulus* (Family: Myrtaceae) essential oil and to evaluate its fumigation toxicity against lesser grain borer, *R. dominica*.

MATERIAL AND METHODS

Collection and Culture of Insects

Insect culture of *Rhyzopertha dominica* was procured from storage laboratory, Division of Entomology, Indian Agricultural Research Institute, New Delhi, India. About 200-400 adults were released in the 1 L glass container having 400 grams of wheat mixed with 5% Brewer's yeast and jars were kept in rearing room maintained at 30±2^{0}C and 70 % R.H. After four to five days of oviposition, these adults were separated and put into a fresh rearing jar. A succession of jars having four to five days oviposition in each was maintained. On emergence of appreciable number of adults, the insects were sieved out and used for experiment or subsequent sub-culturing.

Essential oil

The essential oil of *E. globulus* used in this study was obtained from Central Institute of Medicinal and Aromatic Plants (CIMAP), Pantnagar, Uttrakhand, India.

Chemical analysis of essential oil

Gas Chromatography- Mass Spectroscopy (GC/MS) Analysis: The separation and identification of compounds in *E. globulus* essential oil was done by using Shimadzu QP 2010 Ultra GC-MS equipped with Rtx-5ms column measuring 30×0.25mm and NIST14 library. Helium with flow rate 1ml/min was used as carrier gas. 1µl volume of each sample was utilized. The injection temperature was maintained at 250°C. The oven temperature programme was set with initial temperature at 50°C and then it was increased to 250°C with 4°C/min ramping rate. The temperature for ion source was maintained at 200°C and the interface at 250°C.

Fumigant toxicity

To determine the fumigant toxicity of the *E. globulus* essential oil, flat bottom fumigation flasks of 250 ml with airtight stopper were used. Thirty adult insects (1–7 days old) were released in glass vial covered with muslin cloth and kept at the bottom of flask. Concentrations *i.e*., 50, 100, 150, 200, 250 and 300 µL/L of *E. globulus* essential oil were applied. Essential oil at desired concentrations was applied onto a piece of filter paper (Whatman No.1) of 2.5cm diameter size. The treated filter paper strip was hung inside the flask through a thread and then flask was tightly closed with a glass stopper. Untreated filter paper strips served as a control. The experiment was replicated five times and mortality was recorded at 3, 6,9,12 and 24h after exposure. After completion of exposure period the flask were opened and vials kept outside for two hours at room temperature in normal conditions. Observations were recorded on mortality of the insects. The treated insects when primed with a camel hair brush would be considered dead when no leg or antennal movements were observed. Per cent mortality of adult insects was recorded after taking into account control mortality using the Abbott's correction **(Abbott, 1925)**. Probit analysis was done to estimate the values of LT_{50} and LT_{99} **(Finney 1971)**.

Repellency of Essential oil

In order to study repellent activity, an experiment was carried out in 10cm diameter glass petri dishes following the method described by **Jilani and Saxena (1990)** with some modifications. Test solutions were prepared by dissolving 2, 4, 6 and 8 µl of *E. globulus* essential oil in 1 ml of acetone. The filter paper was cut into two equal pieces and each concentration was applied at half portion of filter paper uniform by using micro pipette. The other half of the filter paper was treated with acetone only as control. The treated halves were air-dried to evaporate the solvent completely. Treated and untreated halves were attached with tape and placed in petri dishes. Twenty adults of *R. dominica* were released at the center of the filter paper in petri dishes and then sealed firmly, each dose was replicated five times. The number of insects present on both the treated and untreated halves was recorded after 3, 6, 24, 36 hours.
Percentage repellency (PR) was calculated according to McDonald formula **(McDonald *et al.*, 1970)** as follows:

$$PR= [(Nc-Nt)/(Nc+Nt)]\ 100$$

Where: Nc: Numbers of insects in the control filter paper area.
Nt: number of insects in the treated filter paper area.

RESULTS AND DISCUSSION

Chemical composition of *Eucalyptus globulus* essential oil

Fifty three compounds (Table 1) were identified in the *E. globulus* essential oil. These include 1, 8-cineole (22.35%), α-pinene (12.58%), limonene (4.01%), aristolene (3.35%), p-cymene (3.25%), trans-verbenol (3.02%), isosativene (2.85%), α-myrcene (2.15%) and terpinen-4-ol (2.10%) as major components. Chemical composition of essential oils of different eucalyptus species have earlier been elucidated **(Derwich *et al*., 2009; Bignell *et al*., 1996; Khemira *et al.*, 2012)**. Among the chemical constituents of Eucalyptus oil, α-pinene and 1,8-cineole were identified as major components The insecticidal and repellency effects of 1,8-cineole, terpineol and α-pinene have been demonstrated by other researchers too **(Tapondjou *et al* ., 2005)**. Some researchers found 1,8-cineole to be highly repellent and toxic to *Sitophilus granarius* L., *S. zeamais*, *Tribolium confusum* and *Prostephanus truncates* (Horn) **(Obeng Ofori *et al.*, 1997)**. While, in some of the studies indicate that α-pinene and terpineol to possess potent repellent and toxic effects against *Tribolium confusum* **(Ojimelukwe and Adler 1999)**. Among various compounds of *E. globulus* essential oil, α-pinene and1,8-cineole was found as major ones. This compound is characteristic of the *Eucalyptus* genus and is mainly attributed to its insecticidal properties **(Batish *et al.*, 2008)**. The above findings suggest that acute toxicity of *E. globulus* essential oil and its constituent compounds especially α-pinene and 1,8-cineole are quite promising and have insecticidal and repellency effects against lesser grain borer *R. dominica*.

Table 1 Chemical compounds identified in chronological order using GCMS analysis of *Eucalyptus globulus* essential oil (major compounds are marked in bold form).

Peak	Compounds	*RT (min)	Area (%)
1	Isoledene	3.86	0.40
2	Humulene	4.92	0.51
3	α- Elemene	5.95	1.10
4	3-Carene	6.05	0.95
5	α –Pinene	7.05	12.58
6	Ocimene	7.12	0.30
7	α-Terpinene	7.25	0.40
8	α-Cubebene	7.38	0.50
9	Cis-ocimene	7.80	0.80
10	Gama-Cadinene	8.05	0.90
11	Camphene	8.25	1.10
12	Epizonarene	8.75	0.90
13	β-caryophyllene	8.90	0.70
14	β-Pinene	9.05	2.20
15	α-Thujone	9.12	1.10
16	Isocaryophillene	9.45	1.10
17	Patchoulene	9.75	0.08
18	Copaene	9.87	1.20
19	Ylangene	15.96	0.30
20	Seychellene	18.02	1.10
21	1,8-Cineole	20.56	22.35
22	Isosativene	21.12	2.85
23	Aristolene	21.35	3.35
24	Solanone	22.09	2.05
25	β-Phellandrene	22.80	1.18
26	α –Myrcene	23.22	2.15
27	Terpenehydrochlorite	23.45	1.21
28	p-Cymene	24.50	3.25
29	Terpenylformate	25.72	1.20
30	Sabinene	26.85	1.49
31	Limonene	28.35	4.01
32	Bornyl acetate	29.50	1.05
33	terpinyl acetate	30.08	2.10
34	Neryl acetate	30.65	1.10
35	α-terpineol	32.05	2.10
36	Terpinolene	34.64	0.91
37	Trans-verbenol	35.26	3.02
38	4- Caranol	36.50	0.19
39	Terpinen-4-ol	37.74	2.10
40	1-Octen-3-ol	38.06	0.10
41	p-Meth-1-en-4-ol cis	38.45	1.15
42	α –Eudesmol	39.15	0.17
43	Geraniol	40.35	0.98
44	Linalyl acetate	40.65	0.10
45	m- Mentha, 4-8 diene	40.60	1.19
46	Geranyl acetate	41.56	0.90
47	Carvacrol	41.80	0.70
48	Linalool	42.95	0.40
49	Terpinylisovalerate	43.10	0.80
50	Cis-linalool oxide	44.01	0.85
51	Piperitone	48.40	0.10
52	Borneol	49.50	0.10
53	Geraniol	52.12	0.13
Total			93.55

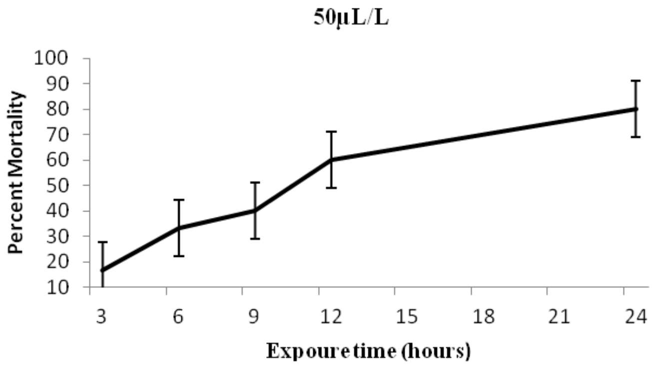
*RT: Retention time obtained by chromatogram

The insecticidal property of many essential oils is mainly attributed to monoterpenes which are typically volatile and are lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions. Due to their high volatility, they have fumigant and gaseous action which are very important in controlling the stored-product insects **(Aref *et al.*, 2015)**. Among various medicinal and aromatic plants, Eucalyptus possesses a wide range of desirable properties worth exploiting for pest management **(Boland *et al.*, 1991; FAO 1995)**. In fact, eucalyptus oil has been known for hundreds of years as antibacterial, antifungicidal and antiseptic in nature **(Kleinig and Kleinig 2006)**. Eucalyptus oil ranks superior in quality and has advantages over essential oil from other tree crops since it has multipurpose uses in perfumery, pharmaceutical and other industries **(Boland *et al.*, 1991; FAO 1995)**. The essential oils of eucalyptus species comprise of metabolic compositions such as terpenoids and phenols that have toxic effects against stored product pests **(Lee *et al.*, 2004; Tapondjou *et al.*, 2005)**.

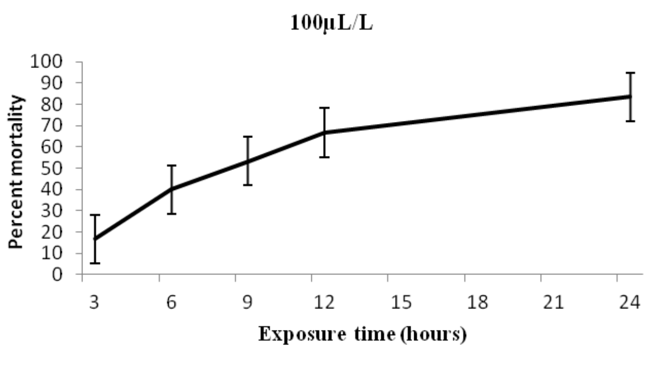
Fumigant toxicity

Essential oil of *E. globulus* was found to be toxic to adults of *R. dominica* when evaluated for its fumigant toxicity. In all cases, considerable differences in mortality of insects to essential oil vapour were observed with different concentrations and exposure period. The lowest concentration (50µL/L) yielded 80% mortality after 24 hours exposure. When concentration was increased to 100, 150 and 200 µL/L insect mortality was also increased to 83.3, 93.3 and 96.6% respectively after 24 hours exposure. The present study corroborate with previous finding which demonstrated the bio-efficacy of essential oils against major stored product insect pests for example the essential oils from *Eucalyptus camaldulensis*, *Eucalyptus intertexta* and *Eucalyptus sargentii* against three major stored product beetles, *Callosobruchus maculates* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* and the mortality increased significantly with increasing concentration against 1 to 7 days old adults **(Negahban and Moharramipour 2007)**.
In the present study fumigant effect of *Eucalyptus globulus* oil against *Rhyzopertha dominica* was found to be17.72µL/L and 58.31 µL/L air LT_{50} and LT_{95} respectively (Table 2). *Rhyzopertha dominica* mortality increased with exposure period of *Eucalyptus globulus* oil (fig 1a, 1b, 1c, 1d, 1e). Our finding are consistent with **Negahban and Moharramipour (2007)** observed LC_{50} 2.55 and 3.97 µl/l air against *C. maculatus*, 6.93 and 12.91 µl/l for *S. oryzae* and 11.59 and 33.50 µl/l air for *T. castaneum* with *Eucalyptus* essential oils. Similar insecticidal effects of *Lavandula stoechas* L. essential oil against *Tribolium castaneum* Herbst, *Lasioderma serricorne* (F.) and *Rhyzopertha dominica* were recorded at different concentrations and exposure times (24, 48 and 72h) by **Ebadollahi *et al.*, (2010)**.

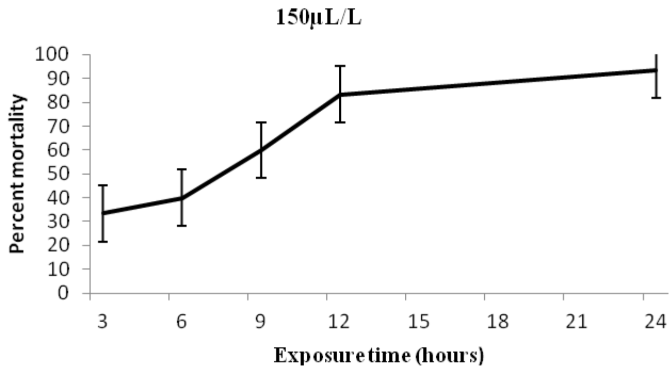
(1a)



(1b)



(1c)



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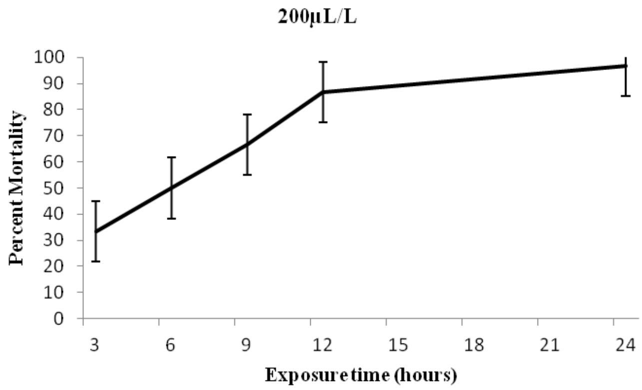

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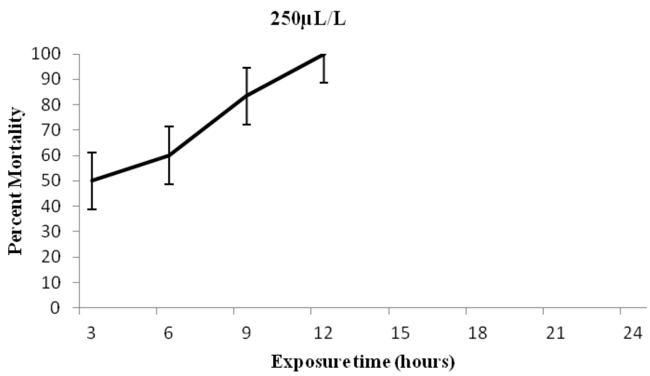

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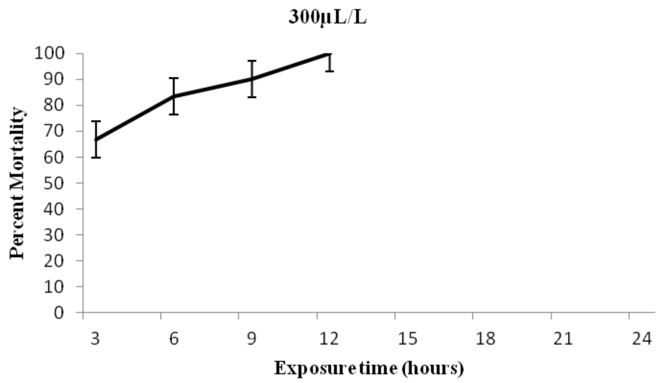

Figure 1a, 1b, 1c,1d, 1e,1f Percentage mortality of *Rhyzopertha dominica* exposed for various concentrations and exposure periods to *Eucalyptus globulus* essential oil at 27°C temperature and 65 percent relative humidity

Table 2 Fumigation toxicity of *Eucalyptus globulus* oil against *Rhyzopertha dominica*

Dose(µL)	LT_{50}(h) (95 %FL)	LT_{99}(h)(95%FL)	Slope ± SE	χ^2	DF	H
50	9.818(7.972-12.376)	136.953(68.894-507.74)	2.033±0.175	4.0737	3	1.3579
100	8.081 (7.268-8.96)	96.655(68.037-156.394)	2.159±0.178	0.702	3	0.234
150	5.826 (2.471-8.999)	62.695(25.349-4465.54)	2.254±0.184	19.657	3	6.552
200	5.15(3.198-6.78)	44.361(23.608-235.537)	2.488±0.197	10.463	3	3.487
250	3.414(1.242-4.915)	25.003(13.666-234.722)	2.69±0.237	13.909	3	4.636
300	2.19(0.271-3.534)	18.445(10.278-299.89)	2.514±0.279	11.358	3	3.786

Abbreviations: FL- Fiducial Limits, DF- degree of freedom, H- heterogeneity, χ^2 Chi-square. 95% lower and upper fiducial limits are shown in parenthesis.

Repellent activity

The repellent activity of *E. globulus* essential oil against *R. dominica* was found to be both dose dependent and time dependent. At the lowest concentration 0.08 µl/cm^2, the repellent activity was found to be 42.5% in 36 hours. When dose was increased to 0.16, 0.24, and 0.32 µl/cm^2 the repellent activities were found to be 43.5, 47.5 and 62.5% respectively after 36 hours of exposure (Table 3). The repellent activity had increased with the increase in concentration and exposure period.

Table 3 Repellent activity per cent of *Eucalyptus globulus* essential oil against *Rhyzopertha dominica*

Insect species	Dose (µl/cm^2)	Repellent percent: Duration of exposure(h) 3	6	24	36
Rhyzopertha dominica	0.08	17.5	35.0	41.00	42.5
	0.16	32.0	40.0	42.5	43.5
	0.24	38.5	42.0	45.5	47.5
	0.32	40.0	53.5	60.5	62.5

Role of essential oils from different plants have clearly been observed on various storage insects. In the present study chemical composition, fumigation toxicity and repellent activity of *E. globulus* essential oil was evaluated against the adults of *Rhyzopertha dominica*. The result revealed that *E. globulus* oil has significant repellency against *R. dominica*, similar finding was also observed by **Salvadores *et al.*, (2007)** with clove essential oil against *R. dominica*, *Sitophilus oryzae* and *T. castaneum* and **Benziet *et al.* (2009)** against the adults of *S. granarius*, *R. dominica*, and *T. castaneum* by *Schinus molle* L. essential oils. In another experiment **Khemira *et al.*, (2012)** reported the strong repellency of essential oil of *E. astringens* against *R. dominica* and *Oryzaephilus surinamensis*.

CONCLUSIONS

From this study, it is clear that *E. globulus* essential oil possess a wide spectrum of biological activities and having strong fumigant toxicity against *R. dominica* and provides a simple, cheap, and environment friendly alternative to pest control. Essential oils such as the one tested in this study can be commercially exploited as a fumigant for stored product insects and could be a potential candidate in the management of *R. dominica* and other stored product insect pests. However additional experiments are needed to exploit the individual effects of different constituents of essential oils of *Eucalyptus sp.* so as to augment ecofriendly methods of pest control in small grain storage structures.

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Conflict of interest: The work is presented in the manuscript with the consent of all authors. The authors declare that they have no conflict of interest.

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