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REPELLENT ACTIVITY OF *CYMBOPOGON CITRATUS* ESSENTIAL OIL AGAINST FOUR MAJOR STORED PRODUCT PESTS: *PLODIA INTERPUNCTELLA, SITOPHILUS ORYZAE, ACANTHOSCELIDES OBTECTUS* AND *TRIBOLIUM CASTANEUM*

SONJA GVOZDENAC[1], BILJANA KIPROVSKI[1], MILICA AĆIMOVIĆ[1], JOVANA STANKOVIĆ JEREMIĆ[2], MIRJANA CVETKOVIĆ[2], VOJISLAVA BURSIĆ[3], JELENA OVUKA[1]*

[1]Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksima Gorkog 30, Novi Sad, Serbia
[2]Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, Belgrade, Serbia
[3]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia
**Corresponding author: sonja.gvozdenac@ifvcns.ns.ac.rs*

SUMMARY

The purpose of this study is to assess the repellent activity of lemongrass (Cymbopogon citratus) essential oil (EO), grown in Serbia under greenhouse conditions, against four prevalent stored product pests: Plodia interpunctella (larvae), Sitophilus oryzae, Acanthoscelides obtectus and Tribolium castaneum (adults). The lemongrass EO repellency was tested using filter paper in Petri dishes and a Y-tube olfactometer. According to the repellency index (RI), the lemongrass EO repellency was divided into 5 classes. Prior to biotesting, the chemical characterization of lemongrass EO was performed and the following main compounds were detected: myrcene (31.0%), geranial (30.0%), and neral (23.6%). The C. citratus EO considered was found to exhibit the Class III repellent activity against P. interpunctella larvae only at the highest concentration (namely 0.5%). This is the very first report on the C. citratus EO repellent activity against this pest. The lemongrass EO examined showed strong repellency (Class IV) against S. oryzae (0.2% and 0.5% of EO), A. obtectus (0.1% and 0.2%), and T. castaneum (0.05-0.1%). Moreover, higher lemongrass EO concentrations (0.5%) were found to exhibit extreme repellency (Class V) against A. obtectus and T. castaneum. The results obtained were confirmed in the bioassays performed, indicating the great potential of lemongrass EO as a bio-repellent when applied in higher concentrations to all the insects considered, regardless of the exposure period.

Key words: *essential oil, lemongrass, biological activity, repellency, storage pests*

Abbreviations: essential oil (EO); relative humidity (r.h.); repellency index (RI)

INTRODUCTION

Stored product pests have unique attributes that justify the need for specific control measures. Chemically synthesized insecticides have been used for decades to control harmful insects in storage facilities. However, these compounds have caused a number of negative effects such as environmental pollution, food and feed residues, ozone depletion, insecticide resistance, as well as human and animal health issues (Zettler & Arthur, 2000; Prado, 2003; Goulson, 2013; Foo & Hameed, 2010). According to the Directive 128/2009, the EU member states are obliged to reduce the use of chemically synthesized pesticides and promote alternative means of pest control. Therefore, considerable efforts have been expended over the past years to use plant-based products (botanicals) for insect control (Liu et al., 2000), including the control of stored product pests. Among botanical products, the use of plant essential oils (EOs) has been proposed as an effective measure for insect control in stored grains by Bakkali et al. (2008) and Isman et al. (2011). In recent years, the primary focus has been placed on the EOs repellent activity against insect pests, the influence of EOs on insect behaviour and the potential of EOs to be used alongside the existing control measures for stored product pests (Koul, 2008; Caballero-Gallardo et al., 2011; Licciardello et al., 2013). Essential oils are considered the by-products of plant metabolism and are regarded as volatile secondary metabolites of plants (Stojanović et al., 2018a). They are obtained from various plant parts through various methods of distillation or extraction. Essential oils are composed of alcohols, aldehydes, aromatic phenolic compounds, esters, ethers, terpenoids, and other bioactive molecules (Dev, 1989; Sangwan et al., 2001; Stojanović et al., 2018b). Due to their volatile, odorous and lipophilic characteristics, the EOs can induce behavioural modifications in insects, directly disrupt specific physiological activities, cause mortality, and negatively affect insect reproduction (Prates & Santos, 2002). Moreover, EOs and their components cause indirect effects such as anti-feeding (i.e. feeding deterrence and inhibition) and repellency, which have been reported and well described by a number of researchers referring to storage insect pests (Padin et al., 2000; Papachristos & Stamopoulos, 2002; Wang et al., 2006; Moharramipour, 2008; Đukić et al., 2016; Lee at al., 2020; Devi et al., 2020).

Although a number of plant species have exhibited insecticidal and/or repellent activity, as mentioned by Sung-Wong et al. (2013) and Nenaah (2014), only a few have been and still are used on a commercial scale (namely azadirachtin, pyrethrum, rotenone, etc.), whereas some were even banned under the Directive 128/2009. All the considerations mentioned above emphasise the necessity for new bioactive pest control compounds of botanical origin.

Cymbopogon species belong to the grass family (*Poaceae*). They are perennial plants found in warm and tropical regions (Bertea & Maffei, 2010, Aćimović et al., 2019, 2020). The efficacy of Cymbopogon EOs against different agricultural pests has been confirmed in several previous studies (Olivero-Verbel et al., 2010; Hernandez-Lambraño et al., 2015; Wang et al., 2006). However, Bossou et al. (2015) reported significant differences in the Cymbopogon EO pest repellency depending on the plant species and concentrations applied. *Cymbopogon citratus* (DC. ex Nees) Stapf., i.e. lemongrass, is an important source of chemical metabolites in the pharmaceutical industry. Moreover, the toxic effects of lemongrass EO and its terpenoid compounds have been used for pest and insect control (Ketoh et al., 2000; Olivero-Verbel et al., 2010; Jiang et al., 2012). According to a number of authors (Carvalho de Sousa et al., 2004; Hanaa et al., 2012; Rocha et al., 2014), the predominant biologically active compounds in lemongrass EO are citral A (geranial) and B (neral). However, the spectra and content of bioactive compounds in the EO produced by an individual plant can differ depending on the environmental and growing conditions, plant phenophase, and other factors. Although the biological activity of lemongrass EO has not been fully clarified yet, it has been undoubtedly confirmed that lemongrass EO has the great potential to be used as an alternative means for stored pest control.

The Indian meal moth (*Plodia interunctella* Hübner; Lepidoptera: Pyralidae) is one of the most polyphagous pest species with cosmopolitan distribution. High adaptability, polyphagia and huge reproductive potential make this species one of the most challenging to control. The rice weevil (*Sitophilus oryzae* L; Coleoptera: Curculionidae) is considered one of the most threatening primary pests found in stored cereals. It causes quantitative and qualitative changes in stored commodities, as well as significant economic losses (Almaši et al., 2003). The bean weevil (*Acanthoscelides obtectus* Say Coleoptera: Chrysomelidae, Bruchinae) is a cosmopolitan and polyvoltine insect that infests beans and other legumes (Dobie et al., 1984) It is able to cause grave bean losses (up to 80%) after six to seven months of storage (Idi, 1994). The management of the bean weevil is of great importance, especially in developing countries where legumes represent a major food resource. The red flour beetle (*Tribolium castaneum* Herbst Coleoptera: Tenebrionidae) is one of the most destructive pests of milled products and a secondary pest of grain commodities. Its resistance to chemical fumigants has been detected in some regions, thus highlighting the need for an environmentally friendly control solution (Devi et al., 2020).

Although a large number of plants exhibit repellent activity against stored product pests, only a few are currently used on a commercial scale. Therefore, there is a constant need for the discovery and characterization of new active compounds and/or plants species with high application potential in pest management. The purpose of the present study is to assess the repellent activity of EO originating from locally grown *C. Citrates* against four major stored product pests: *P. interpunctella, S. oryzae, A. obtectus* and *T. castaneum.*

MATERIAL AND METHODS

Chemical analysis of *C. citratus* essential oil

The lemongrass used as raw material in this study was grown under controlled conditions in three different greenhouses over a period of three consecutive years (2018-2020). The experiment conducted was set up in Banatska Topola, Vojvodina, Serbia (45°40′N; 20°27′E; altitude 78 m). Owing to its origin and temperature requirements, lemongrass can only be grown in controlled environments under the existing agroecological conditions in Serbia. Lemongrass plants should be protected from freezing in the winter and from direct sunlight exposure and precipitation in the summer.

The harvested lemongrass material was dried in a solar dryer (2-3 days), which is in accordance with the recommendations for drying medicinal plants rich in essential oil, in order to prevent the evaporation of volatile components (Müller et al., 1997). The distillation of lemongrass EO was performed in an industrial distiller Inox Ltd. (Bački Petrovac, Serbia) used for commercial distillation, at the Department of Vegetable and Alternative Crops, the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The essential oils obtained from all the distillations performed were mixed and used for further analysis and bioassays.

The content and composition of the lemongrass EOs obtained were analysed using a gas chromatograph Agilent 6890 connected to a mass detector Agilent 5973MSD in a positive EI mode. The separation of individual compounds was performed with a capillary column Agilent 19091S-433 HP-5MS of the following features: 30 m of length, 0.25 mm of inner diameter, and 0.25 μm of film thickness. Helium was used as the carrier gas, with a flow rate of 0.1 mL/min measured at 210 °C. The column temperature was linearly programmed from 60 °C to 285 °C, with a temperature rise rate of 4.3 °C/min. The injector temperature was 250 °C, the source temperature was 200 °C, and the interface temperature was 250 °C (ion source emerge 70 eV). Mass measurements were performed in the range of 40-350 Daltons with 11.47 skunks per minute. The component identification was performed according to retention indices, comparing the mass spectra with the spectra of the Wiley and NIST libraries. All the experimental results were expressed as percentage (%) of the lemongrass essential oils obtained.

Rearing of test insect populations

The insects used in the experiment were reared under controlled conditions at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. *P. interpunctella* originates from the laboratory population reared for ~50 generations. The rearing was performed in glass jars (2.5 L) placed in a climate chamber at constant conditions (28 ±1ºC; r.h. 60 ±10%; photoperiod 14 L:10 D), using a standard laboratory diet specific for this species (prepared according to Silhacek & Miller, 1972). *S. oryzae* weevils were reared on wheat kernels for ~20 generations in glass jars (2.5 L) placed in a climate chamber at 26 ±1ºC, r.h. 6 ±10% and photoperiod 14 L:10 D). The *A. obtectus* population has been maintained in the laboratory at large size (about 1000 individuals) for more than 20 generations on common bean seeds (*Phaseolus vulgaris* L.) under constant conditions (27 ±1º C; r.h. 65 ±5%; photoperiod 16:8 (L:D)). The *T. confusum* experimental population was reared according to the method described by Davis & Bry (1985), in glass jars (2.5 L) on a substrate made of wheat, maize flour (ratio 1:1) and yeast (5%) under controlled conditions (26 ±1°C and r.h. 60 ±5%; photoperiod 14 L:10 D).

Repellency bioassays

The repellent activity was assessed in two separate bioassays: (1) a test involving filter paper in Petri dishes and (2) a test using a Y-tube olfactometer.

The first repellency test was performed as follows: the circles of the filter paper were cut into half discs and shortened in the centre in order to create a space between the cuts when placed in Petri dishes. The EO obtained was dissolved with emulsifier (Tween 80, 0.1%) and applied in a series of concentrations (0.05, 0.1, 0.2 and 0.5%). The EO solution (2 mL) was applied to one group of the half discs, whereas Tween 80 dissolved in water was applied to the other as the control group. After discs were dried, ten insect specimens (*P. intrepunctella* larvae, *S. oryzae*, *A. obtectus* and *T. castaneum* adults) were placed in the centre of the Petri dish. The distribution of insect individuals on the half discs was recorded after one and two hours. The experiment was performed in six replications with different application rates.

A Y-tube olfactometer was used for the olfactometric testing. It consisted of two glass arms, positioned at an angle of 45 ° and connected in a central tube. A rubber hose connects arms with glass vials (ø 4 mm) at the end. Filter paper cuts treated with 2 µL of EO solution (0.05, 0.1, 0.2 and 0.5%) dissolved with Tween 80 emulsifier (0.1%), were placed in the vials at one end, whereas the paper cuts treated with only Tween 80 emulsifier dissolved in water were placed in the control vial. A separate test was performed for each EO concentration. Ten insect specimens (*P.*

intrepunctella larvae, *S. oryzae*, *A. obtectus* and *T. castaneum* adults) were released into the central tube. After one and two hours, the number of individuals in each arm was recorded.
The repellency index (RI) was calculated according to the following formula:

$$RI= (C-T/C+T)*100$$

RI - repellency index
C – the number of individuals on the control half disk / arm
T – the number of individuals on the treated half disk / arm

The classification of repellent activity was performed according to the repellency index (RI) (Talukder & Howse 1993). This scale divides the lemongrass EO repellency into classes from 1 to 5 (Tab. 1). Negative values are characterized as the attracting activity.

Table 1. Repellency classes by Talukder & Howse (1993)

Class	Repellency index (RI)	Level of repellency
	<0.01	Attractive
0	>0.01 do <0.1	Neutral
I	0.1 – 20.0	Neutral
II	20.1 – 40.0	Slightly repellent
III	40.1 – 60.0	Repellent
IV	60.1 – 80.0	Strongly repellent
V	80.1-100.00	Extremely repellent

RESULTS AND DISCUSSION

Chemical analysis of *C. citratus* essential oil

The mean annual yield of lemongrass EO, obtained by the distillation of plant material under industrial conditions, was 0.85% (or 0.85 g EO/100 g of dry material), which is in accordance with the findings reported in the literature (Jimayu & Gebre 2017; Ghatas & Mohamed, 2018; Moussa 2019; Bekele et al. 2019).
The chemical composition of individual lemongrass EO components is presented in Table 2. As shown in Table 2, a total of 22 potentially active ingredients were detected and quantified. The predominant bioactive components were myrcene (31.0%), geranial (30.0%) and neral (23.6%). The results obtained are consistent with the findings of Plata-Rueda et al. (2020), who reported 13 active components of lemongrass EO, among which neral, geranial, geranyl acetate, citral, camphene and limonene were present in larger quantities. Furthermore, Brügger et al. (2019) also reported neral (31.5%), citral (26.1%), and geranyl acetate (2.27%) as the major constituents of lemongrass EO, which is congruent with the findings of Barbosa et al. (2008), Andrade et al. (2009) and Lermen et al. (2015).

Table 2. Chemical composition of lemongrass EO obtained under industrial distillation conditions

	Component	Retention indices	Retention time (min)	%
1	6-methyl-5-Hepten-2-one	987	7.384	4.1
2	Myrcene	991	7.544	31.0
3	cis-β-Ocimene	1037	9.137	0.5
4	trans-β-Ocimene	1048	9.529	0.3
5	6,7-Epoxymyrcene	1095	11.29	0.1
6	Linalool	1104	11.522	1.0
7	Perillene	1105	11.595	0.4
8	1,3,8-p-Menthatriene	1107	11.74	0.1
9	exo-Isocitral	1147	13.441	0.2
10	Citronellal	1153	13.788	0.4
11	cis-Isocitral	1164	14.277	1.3
12	Rosefuran epoxide	1176	14.774	0.1
13	trans-Isocitral	1184	15.067	1.8
14	Citronellol	1227	17.087	0.5
15	Neral	1238	17.715	23.6
16	Geraniol	1250	18.235	2.7
17	Geranial	1268	19.054	30.0

18	2-Undecanone	1295	20.007	0.2
19	Geranyl acetate	1382	23.967	0.3
20	trans-Caryophyllene	1419	25.504	0.2
21	trans-α-Bergamotene	1438	26.195	0.2
22	2-Tridecanone	1497	28.727	0.1
	Total			**99.1**

Repellent activity of *C. citratus* essential oil

In the first experimental biotest, the lemongrass EO examined was found to exhibit repellency against all the insect species considered. However, the lemongrass essential oil RI differed depending on the concentration applied (Tab. 3) and, in some cases, the exposure time (namely 1 h or 2 h). These results were confirmed in the olfactometric test conducted using a Y-tube olfactometer. As in the first test, the RI differed depending on the concentration applied, but was found independent of the exposure time.

Lower concentrations of lemongrass EO did not exhibit satisfactory repellent activity towards *P. interpunctella* larvae, regardless of the exposure period or tests applied (Tab. 3, Fig. 1). The repellent activity (Class III) of this EO was exhibited only when applied at the highest rate (0.5%), following both exposure periods of 1 and 2 hours. As there is a limited body of information on the *P. interpunctella* and *Cymbopogon* species in the literature, it can be concluded that we have made the very first report on the *C. citratus* repellency against *P. interpunctella.*

The lemongrass EO exhibited the repellent activity against *S. oryzae* when applied at a concentration of 0.2% after 1 and 2 h of treatment, as well as after 1 h of treatment with the highest concentration (0.5%) (Tab. 3, Fig. 1). The strong lemongrass EO repellency was exhibited against this species at 0.5% after 2 h of treatment. The repellency of a 0.2% solution of lemongrass EO was marked as Class IV and did not differ significantly relative to the exposure periods considered, whereas a 0.5% lemongrass EO solution was marked as Class V (i.e. extreme repellency) after both 1 and 2 h of exposure. Similar results were obtained in the Y-tube test conducted, with slight differences. After 2 h of treatment, a 0.2% lemongrass EO solution exhibited only the Class III repellent activity. Plata-Rueda et al., (2020) argued that lemongrass EO and its components such as citral and geranyl acetate have the ability to cause significant mortality and repel *S. granaries*, which is congruent with our results (although a different species of the Sitophilus genus was examined).

A.obtectus was strongly repelled by a 0.5% solution of lemongrass EO after both exposure periods considered (Class V). However, the Class IV repellent activity was observed after 1 and 2 h of exposure to a 0.2% solution of lemongrass EO. The olfactometric results obtained differ slightly. When applied at concentrations of 0.2% and 0.5%, the lemongrass EO was found to exhibit a strong repellent activity (class V), as well as after 2 h of exposure to a 0.05% solution of EO (Tab. 3, Fig. 1). As there is no available information on the repellent activity of *C. citratus* against *A. Obtectus* in the literature, the results obtained herein confirm its repellent potential for the first time ever.

Table 3. Repellency index (RI) and class of lemongrass EO in a biotest using Petri dishes

Test insect	**Concentration (%)**	**RI 1 h**	**RI 2 h**	**Repellency class (1/2 h)**
P. interpunctella	0.05	15.2	18.7	I
	0.1	26.5	31.5	II
	0.2	32.1	35.1	II
	0.5	40.7	42.7	III
S. oryaze	0.05	3.30	30.1	I/II
	0.1	19,10	34.7	I/II
	0.2	45.7	40.1	III
	0.5	50.7	60.1	III/IV
A. obtectus	0.05	35.9	57.1	III/IV
	0.1	36.1	40.7	III/IV
	0.2	**55.9**	**51.7**	**IV**
	0.5	**81.8**	**86.2**	**V**
T. castaneum	0.05	45.3	49.2	IV
	0.1	56.3	59.6	IV
	0.2	**58.3**	**61.2**	**IV/V**
	0.5	**75.4**	**76.7**	**IV**

T. castaneum adults were repelled by lemongrass EO in all the experimental treatments conducted herein. The results obtained show strong repellent activity (Class IV) at lemongrass EO concentrations of 0.05% and 0.1% and extreme repellency at lemongrass EO concentrations of 0.2% and 0.5% (Class V) (Tab. 3, Fig. 1). A number of reports in the literature support these results. The dose dependency of *C. citratus* EO repellent activity against *T. castaneum* was reported by Olivero-Verbel et al. (2010). The authors mentioned that the repellency increased with higher EO concentrations. Moreover, some authors emphasized that EOs from plants belonging to the Cymbopogon genus such as *C. martini*, *C. citratus*, and *C. flexuosus* exhibit stronger repellency against *T. castaneum* than the commercial synthetic repellent IR3535 for this insect species (Caballero-Gallardo et al., 2011; Hernandez-Lambraño et al., 2015). Đukić et al. (2016) also suggested that EO originating from different *Cymbopogon* species had differing but strongly repellent potential against *T. castaneum* adults. The authors performed olfactometric tests and found that beetles spent about half as much time in the arm of the olfactometer where the EO was applied, even at low concentrations (0.0001%), than in the control arm with no *Cymbopogon* EO. Caballero-Gallardo et al. (2011) also revealed differences in the repellent activity between Cymbopogon species (*C. flexuosus* and *C. martini*) and emphasized that they exhibit repellency even when their EO is applied at low concentrations. The dependence of the EO repellency strength on the concentration applied was described by Licciardello et al. (2013). These authors reported that *C. nardus* EO acted as a strong repellent on *T. castaneum* at rates ranging between 0.005-0.02 mL/cm^2, whereas at lower rates (0.001 mL/cm^2) the repellent activity was not recorded. Similarly, Devi et al. (2020) reported strong repellency of different Cymbopogon species EOs (*C. martini C. flexuosus* and *C. winterianus*) against *T. castaneum* adults. Moreover, a certain insecticidal effect was noted, suggesting the potential of *Cymbopogon* species as an additional control option against *T. Castaneum* (which was also confirmed herein).

Overall, the results obtained in the present study show that lemongrass EO exhibit significant repellent activity against *A. obtectus* and *T. castaneum* adults, whereas weaker repellent activity was observed against all the insects considered.

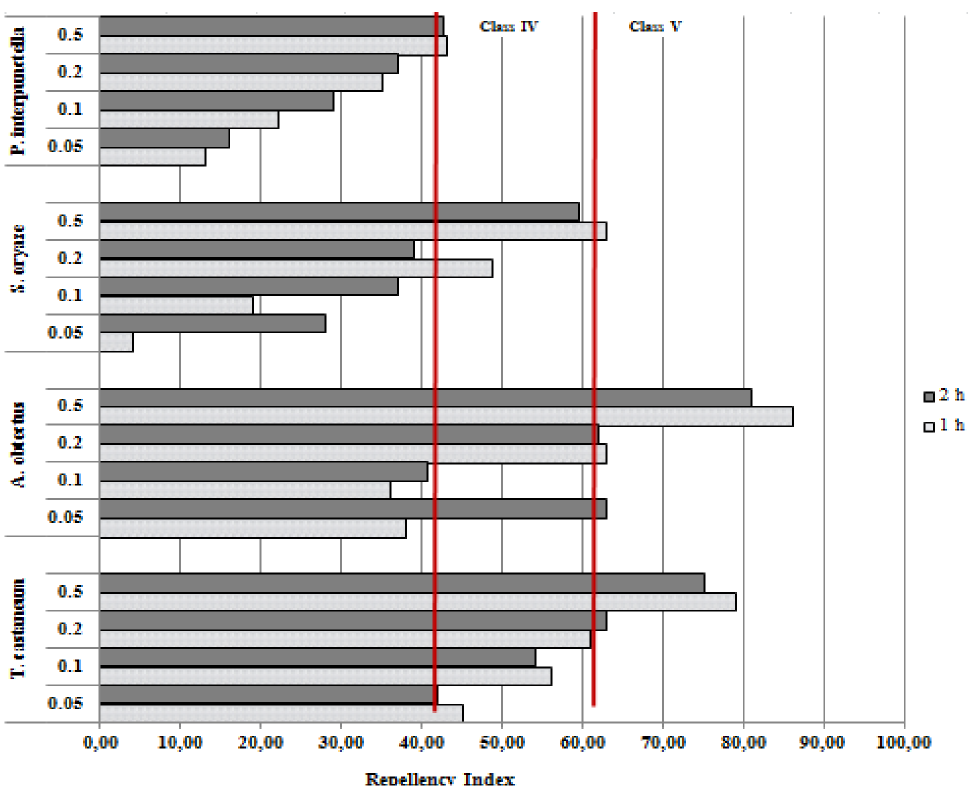

Figure 1. Repellency of lemongrass EO in an olfactory test using a Y-tube olfactometer

CONCLUSION

The results obtained indicate that lemongrass (*C. citratus*) essential oil grown under controlled conditions has the same quality as essential oil from lemongrass plants grown in their countries of origin or under favourable conditions.

Lemongrass EO was found to exhibit different dose-dependent levels (or classes) of repellent activity against major stored product pests. The repellency of lemongrass EO was not satisfactory against *P. intepunctella* larvae, regardless of the concentration applied or exposure period (marked as Class III and obtained only at the highest concentration applied (0.5%)). The extreme repellency (Class V) of lemongrass EO was recorded against *A. obtectus* and *T. castaneum* at a concentration of 0.5%, whereas strong repellency (Class IV) against *S. oryzae*, *A. obtectus* and *T. castaneum* was exhibited at lower concentrations (differing between the exposure periods considered).

The overall results suggest the good potential of lemongrass (*C. citratus*) essential oil, when grown under controlled conditions, to be used as a repellent against major stored product pests. However, further research is needed to define the insecticidal activity and properties of specific lemongrass EO bioactive compounds in order to promote *C. citratus* as a bio-rational alternative to synthetic pesticides for managing stored product pests.

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