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Research Article

TODDALIA ASIATICA (L.) LAM. ESSENTIAL OIL: A POTENTIAL NATURAL FUMIGANT AND REPELLENT AGAINST THREE COLEOPTERAN PESTS OF STORED PRODUCTS

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ABSTRACT

The essential oil of *Toddalia asiatica* was isolated from the leaves and tested for its fumigant toxicity and repellency against *Callosobruchus maculatus*, *Sitophilus oryzae* and *Tribolium castaneum*. In the toxicity studies, the LC_{50} concentration was the lowest (53.88µl) against *C. maculatus*, which showed that it was the most susceptible pest. The LC_{50} concentrations against *S. oryzae* and *T. castaneum* were 77.77 and 99.12 µl, respectively in 24h. LC_{90} concentrations of the essential oil of *T. asiatica* against *C. maculatus*, *S. oryzae* and *T. castaneum* were recorded as 95.47, 138.77 and 175.69 µl, respectively. After 1 h exposure, the repellent activity was 71% against *C. maculatus* and *S. oryzae* and 67% against *T. castaneum* at 20µl concentration compared to control. The repellent activity increased with the increase in the concentration. When the exposure period was increased to 3h the repellency was increased to 100% against *C. maculatus* and *S. oryzae* and 89.57% against *T. castaneum*. The major components identified in the essential oil of *T. asiatica* were β- Linalool (10.67%), β - Sesquiphellandrene (9.86 and 2.2%), Spathuleno (8.37%), Caryophyllene oxide (6.29%), pogostol (5.2%) and Methyl methylanthranilate (5.45%) and linalyl acetate (4.24%). The results clearly suggested that the essential oil of *T. asiatica* can be used to control *C. maculatus*, *S. oryzae* and *T. castaneum* in warehouses.

Keywords: Botanical insecticides, *Toddalia asiatica*, stored product insects, Fumigant toxicity, Repellent activity.

INTRODUCTION

Stored products meet heavy loss in both quality and quantity due to pest attack. Insect pests are major problem to stored products throughout the world. Several coleopteran insects damage the seeds, grains, pulses, cereals, products of animals and plant origin and milled products at storage sites. Globally around 10 to 40% of grain loss has been reported due to infestation by insects and other bio-agents (Papachristos and Stamopoulos, 2002). The cowpea weevil *Callosobruchus maculatus* (F.) (Coleoptera; Bruchidae) is a major pest of cowpea and other pulses. It is a problematic stored product insect in many countries especially tropical countries (Jackai and Daoust, 1986). Rice weevil *Sitophilus oryzae* (L.) is a serious pest of rice, wheat, maize and almost all cereals throughout the world. India is considered to be the native land of *S. oryzae* (Zacher, 1937). The flour beetle *Tribolium castaneum* Herbst is a problematic insect in milled products in many parts of the world particularly in the tropics (Mondal and Khalequzzaman, 2006).

Fumigation is a common method of stored product pest management. At present the fumigation is generally done by methyl bromide and phosphine. Methyl bromide has been reported for its deleterious effect on the ozone layer (Anonymous, 1993) and phosphine causes serious health problems in humans (Garry *et al*., 1989). Phosphine also causes pesticide resistance in the target pests (Zettler *et al*., 1989; Bell and Wilson, 1995; Chaudhry, 1997).

Our ancestors were using only plant products to control the stored product pests in warehouses. Plant products offer protection through repellent property and toxicity against the insect pests. Volatile compounds in plants are responsible for the repellent activity and fumigation toxicity. Plant products are safe to human beings and the environment. Toxicity and grain protecting efficacy of several volatile oils and volatile compounds against *C. maculatus, S. oryzae* and *T. castaneum* have been reported by many investigators (Keita *et al*., 2000; Kim *et al*., 2003; Negahban and Moharramipour, 2007; Mohamed and Abdelgaleil, 2008). Many plant

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essential oils are target specific and biodegradable (Isman, 2000, 2001). The U.S. EPA lists about 17 different plant essential oils as minimum risk pesticides (USEPA, 2004).

Toddalia asiatica, commonly called orange climber, is a woody liana. This plant can reach a height of 10m in forests. It is commonly distributed in the tropical regions of Africa, Asia and Madagascar. The leaves, roots and fruits of *T. asiatica* have medicinal properties and are commonly used in Ayurveda and siddha to cure malaria, stomach disorders, rheumatism and fever (Kirtikar and Basu, 1933; Chopra *et al*., 1956; Ramachandran and Nair, 1981). Toxic effects and repellent activity of *T. asiatica* essential oil have not been studied against *C. maculatus, S. oryzae* and *T. castaneum*. Hence the present study was undertaken to investigate the fumigant toxicity and repellency of these three plant essential oils against *C. maculatus, S. oryzae* and *T. castaneum*.

MATERIALS AND METHODS

Insects

T. castaneum adults were obtained from a stock culture that was continuously maintained for several generations on wheat flour at Entomology Research Institute insectary at laboratory conditions (29±1^{o}C; 60–65% R.H.; 11±0.5 h photoperiod). Adult beetles (5-7 day-old) were used for the experiments.

Plant materials and Extraction of essential oils

Fresh leaves of *T. asiatica* were collected from Poondy hills in Tamil Nadu, India. Essential oils were extracted from fresh leaves by hydro-distillation method using Clevenger-type apparatus. About 50 gm of leaves of each leaves were taken in the flask along with 500 ml water and distilled for 4 h. Anhydrous sodium sulphate was used to remove water from the extracted essential oil and the oils were stored at 4°C until use.

Treatments and concentrations

The fumigation toxicity of the essential oils was studied at five different concentrations viz., 20, 40, 80, 120 and 160µL/L. The repellent activity was studied at 5, 10, 15 and 20 µL concentrations.

Fumigation toxicity

The toxicity of *T. asiatica* essential oil on *C. maculatus, S. oryzae* and *T. castaneum* adults were tested by filter paper dip method as described by Negahban *et al*. (2007) at laboratory conditions (29±1^{o}C; 60–65% R.H.). Whatman No. 1 filter paper discs (2 cm diameter) were impregnated with different concentrations (20, 40, 80, 120 and 160 µL/L) of essential oils separately and were attached to the under surface of the screw caps of glass vials (volume 50 ml) separately. The cap was screwed tightly on the vial after the release of 10 adult beetles (5–7 days-old) along with little amount of cowpea, wheat and wheat flour as food for *C. maculatus, S. oryzae* and *T. castaneum*, respectively. For comparison a set of control, without essential oil, was maintained. Each treatment and control was replicated five times. Mortality was recorded after 4, 8, 12 and 24 h from the commencement of exposure. When no leg or antennal movements were observed, insects were considered dead. Percentage insect mortality was calculated using the Abbott's correction formula (Abbott, 1925).

Repellent activity

The repellent activity was studied at four different concentrations viz., 5, 10, 15 and 20µL using a Y-tube glass olfactometer. The stem of the Y was 20 cm long and each of the two arms was also 20 cm long. The inner diameters of the stem tube and arms were 2.5 cm. About 5µL of volatile oil was applied on a piece of filter paper (2 cm x 3 cm) and placed inside one arm tube near the opening. In the other arm of the Y a blank filter paper strip without volatile oil (control) was placed. An air current was created by an aerator through the arms and the air passed out through the stem of the Y tube. The rate of air flow was adjusted as 1.25 L/min near each arm. Twenty beetles were released into the olfactometer through the opening of the stem tube. The number of insects that moved into the control side and essential oils treated side was recorded at the end of every one hour period and the entire experiment lasted for 3 h. The experiment was replicated five times for each insect species. Other concentrations of the oil were tested by this same method. Percent repellency was calculated by the formula of Nerio *et al*. (2009):

Percent Repellency = $100 \times (C-T)/(C + T)$.

Where, C is the number of insects on the control side and T is the number of insects on the essential oil treatment side.

Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed with a SHIMADZU-QP2010 with helium as a carrier gas with a linear velocity flow on a Resteck-624 ms column (30m x 0.32 mm id, 1.8 µm film thickness). Column flow rate was 1.491 ml/min. The oven was programmed to rise to 45°C (4 min) isotherm, and then to 175 leads to 240°C at a rate of 10°C/min and 25°C/min respectively. Injector and detector temperatures were 140°C. The identification of the constituents was performed by computer library search and retention indices.

Statistical analysis

Mean values were calculated from the replication values for insecticidal and repellent experiments. The results were statistically analysed by one way analysis of variance (ANOVA) and significant differences between treatments were determined using Tukey's multiple range test at $P \leq 0.05$. Probit analysis was done to calculate Median Lethal Concentration (LC_{50}) and LC_{90} using SPSS 11.5 version software package.

RESULTS

Yield of the oils

The yield of essential oil from 50 gm leaves of *T. asiatica* was 150µL. The extraction process was repeated till sufficient quantity of the oil was obtained for the experiments.

Fumigant toxicity

Fumigant toxicity of *T. asiatica* essential oil at different concentrations and different exposure periods against three stored product pests is presented in Figure 1. Considerable differences in mortality of insects to essential oil were observed at different concentrations and period of exposures.

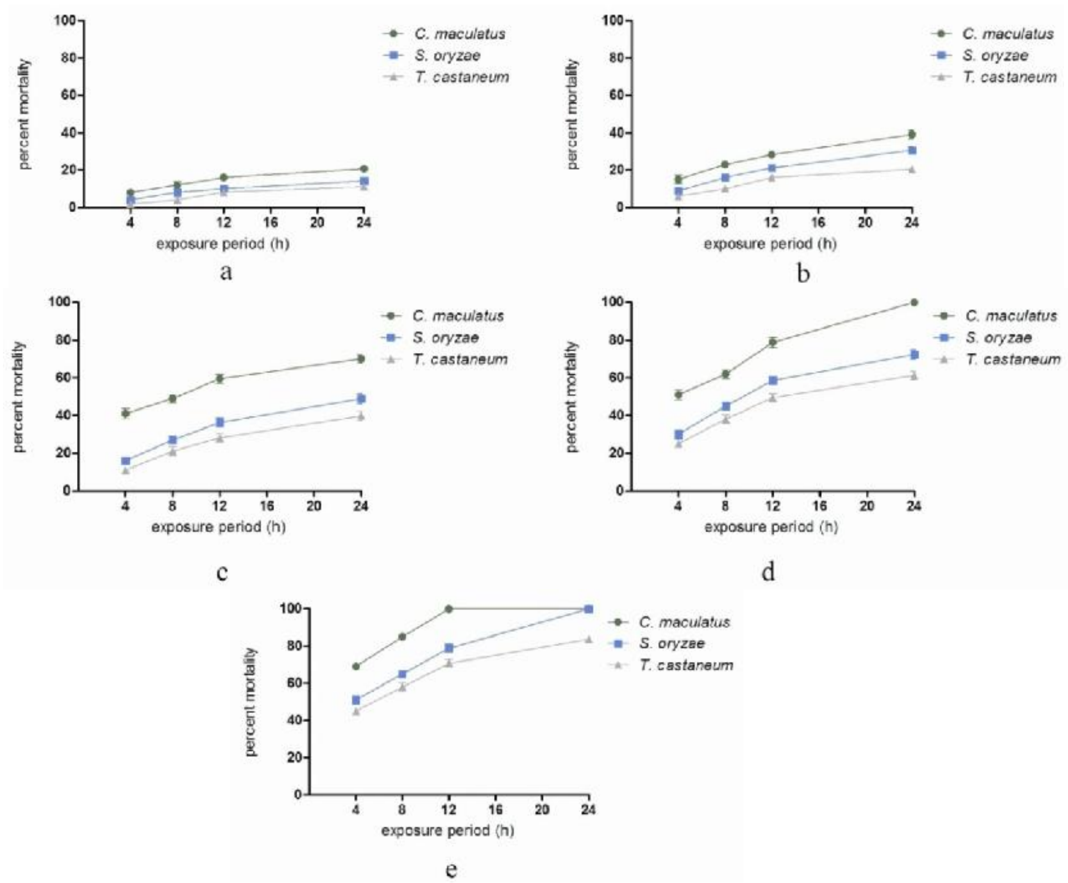

Figure 1. Mortality of *Callosobruchus maculatus*, *Sitophilus oryzae* and *Tribolium castaneum* due to fumigant toxicity of essential oil of *Toddalia asiatica* in different exposure times at 20µl/L (a), 40µl/L (b), 80µl/L (c), 120µl/L (d) and 160µl/L (e) concentrations.

At 20µL concentration, the highest percent mortality (20.67%) was observed against *C. maculatus* (Fig.1a). Mortality increased when the concentration of the oil and exposure time were increased (Fig. 1a to 1e). The highest concentration (160µL/L) of the oil presented 76.4, 68.3 and 65.5 percent mortality at the end of 24h in *C. maculatus*, *S. oryzae* and *T. castaneu*m, respectively.

The median lethal concentration (LC_{50}) and LC_{90} concentration of the oil against the three insects were calculated by Probit analysis. It was clear that *C. maculatus* was the most susceptible pest to the essential oil of *T. asiatica*, because the LC_{50} concentration of the oil was the lowest (53.88µl) against *C. maculatus* (Table 1). The LC_{50} concentrations against *S. oryzae* and *T. castaneum* were 77.77 and 99.12 µl, respectively in 24h. LC_{90} concentrations of the essential oil of *T. asiatica* against *C. maculatus*, *S. oryzae* and *T. castaneum* were recorded as 95.47, 138.77 and 175.69 µl, respectively.

Repellent activity

The essential oil of *T. asiatica* was tested for its repellent activity against the three insects using glass olfactometer. Tables 2 to 4 show the percent repellent property of *T. asiatica* essential oil at different treatment times and at different concentrations. Results clearly showed that the effect was directly proportional to the concentration of the oil and period of exposure of the insects to the oil. At the highest concentration (20 µl) the repellent activity of the oil was 100 percent against *C. maculatus* and *S. oryzae* and 89.57 percent against *T. castaneum* at the end of 3h. When the treatment was given for 1h the repellency was recorded as 71.03, 71.19 and 67.05% against *C. maculatus, S. oryzae* and *T. castaneum*, respectively.

Chemical composition of the essential oils

The chemical composition of *T. asiatica* essential oil is shown in table 5. A total of 45 components were detected in the essential oil of *T. asiatica* (Table 5) by GC-MS. The main constituents of *T. asiatica* were: Linalool (10.67%), β-Sesquiphellandrene (9.86%), Spathuleno (8.37) and Caryophyllene oxide (6.29%).

Table 1. Lethal concentrations of *T. asiatica* essential oil against *C. maculatus, S. oryzae* and *T. castaneum* adults.

Insect	LC_{50}	95% Fiducial limit		LC_{90}	95% Fiducial limit		Chi-square
		Lower	Upper		Lower	Upper	
C. maculatus	53.88	51.03	56.82	95.47	90.76	100.91	207.91
S. oryzae	77.77	73.21	82.49	138.77	131.15	147.77	319.92
T. castaneum	99.12	95.11	103.32	175.69	168.09	184.37	161.75

Table 2. Repellent activity (%) of *T. asiatica* oil against *C. maculatus*, *S. oryzae* and *T. castaneum* after 1h exposure period.

Insect	Concentration (µL/L)			
	5	10	15	20
C. maculatus	23.42 ± 3.28^{a}	51.92 ± 3.16^{a}	58.28 ± 2.35^{a}	71.03 ± 2.34^{a}
S. oryzae	21.91 ± 2.64^{a}	50.83 ± 2.13^{a}	51.50 ± 2.25^{a}	71.19 ± 2.32^{a}
T. castaneum	21.58 ± 2.35^{a}	44.56 ± 2.24^{a}	56.93 ± 2.35^{a}	67.05 ± 2.04^{a}

Values indicate mean ± SEM for five replicates; values having same alphabet in a column do not vary significantly (Tukey's HSD, p≤0.05).

Table 3. Repellent activity (%) of *T. asiatica* oil against *C. maculatus*, *S. oryzae* and *T. castaneum* after 2h exposure period.

Insects	Concentrations (µL/L)			
	5	10	15	20
C. maculatus	31.16 ± 2.23[a]	60.16 ± 3.16[a]	70.07 ± 2.33[a]	83.52 ± 2.15[a]
S. oryzae	27.97 ± 2.44[a]	57.94 ± 2.45[a]	64.54 ± 2.36[a]	80.10± 3.12[a]
T. castaneum	27.05 ± 2.53[a]	56.49 ± 3.46[a]	65.25 ± 2.52[a]	75.47 ± 2.12[a]

Values indicate mean ± SEM for five replicates; values having same alphabet in a column do not vary significantly (Tukey's HSD, $p \leq 0.05$).

Table 4. Repellent activity (%) of *T. asiatica* oil against *C. maculatus*, *S. oryzae* and *T. castaneum* after 3h exposure period

Insects	Concentrations (µL/L)			
	5	10	15	20
C. maculatus	40.26 ± 1.99[a]	79.96 ± 2.34[a]	81.37 ± 2.37[a]	100.0 ± 0.00[a]
S. oryzae	36.84 ± 2.23[a]	72.05 ± 2.16[ab]	81.31 ± 3.24[a]	100.0 ± 0.00[a]
T. castaneum	32.56 ± 2.10[a]	64.68 ± 2.68[b]	76.05 ± 2.64[a]	89.57 ± 2.51[b]

Values indicate mean ± SEM for five replicates; values having same alphabet in a column do not vary significantly (Tukey's HSD, $p \leq 0.05$).

Table 5. Composition of essential oil of *Toddalia asiatica* as analysed by GC-MS.

Peak No.	Compounds	Retention time (min)	Relative (%)
1	Allo-Ocimene	5.809	5.49
2	6-Methyl- 1- octanol	6.168	0.33
3	n- Nonanyl acetate	6.425	0.82
4	Cis-3-Hexenyl Butyrate	6.866	0.93
5	2- cyclohexen-1-one, 4-isopropyl-	7.036	0.34
6	α- Terpineol	7.218	0.90
7	n-Decyl acetate	7.296	0.70
8	linalyl acetate	7.414	4.24
9	1-(1,2,2,3- tetramethylcyclopenyl) ethanone	7.985	1.01
10	1- dodecanol	8.061	0.55
11	decyl acetate	8.349	0.92
12	5,5-Dimethyl-6-methylenebicyclo[2.2.1]hept-2-yl acetate	8.432	1.66
13	Myrtenyl acetate	8.728	0.43
14	Z-2- Octadecen-1-ol acetate	8.850	0.42
15	α–Terpinyl acetate	9.122	2.72
16	Geraniol acetate	9.422	0.54
17	β–Elemene,	9.867	0.82
18	β- Linalool	10.096	10.67
19	α–Zingiberene	10.146	0.46
20	n-Decyl acetate	10.289	2.46
21	Methyl methylanthranilate	10.454	5.45

22	Caryophyllene	10.522	2.52
23	γ– Elemene	10.568	0.86
24	β- Sesquiphellandrene	10.701	2.20
25	β - Sesquiphellandrene	10.938	9.86
26	germarca -1(10), 4(15), 5-triene, (-)-	11.299	3.84
27	δ-Guaiene	11.530	2.08
28	sesquisabinene hydrate	12.163	2.35
29	germacrene b	12.253	1.05
30	4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-	12.396	0.37
31	Caryophyllene oxide	12.606	6.29
32	humulene oxide	12.925	2.02
33	Spathuleno	13.193	8.37
34	pogostol	13.492	5.20
35	(+) spathulenol	13.620	0.66
36	α-bisabolol	13.734	1.48
37	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	14.039	1.25
38	pent-1-yn-3-ol, 3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-	14.252	1.30
39	5.β,7. β H,10.α-Eudesm-11-en-1.α-ol	14.388	0.57
40	Alloaromadendrene oxide-(1)	14.507	0.49
41	valerenol	14.955	0.18
42	dihydro-neoclovene-(i)	15.376	0.17
43	Palmitic acid	16.598	1.36
44	Methyl m-hydroxycinnamate	16.692	0.17
45	Phytol	17.912	3.47

DISCUSSION

T. asiatica essential oil showed fumigant toxicity and repellent activity against *C. maculatus*, *S. oryzae* and *T. castaneum*. Many investigators have reported repellent activity and fumigant toxicity of plant essential oils against stored product pests. Nenaah and Ibrahim (2011) have reported the insecticidal activity of *Pimpinella anisum*, *Cinnamomum camphora* essential oils against *Trogoderma granarium* and *T. castaneum.* Volatile oils of *Cymbobogan nardus*, *Mentha arvensis*, *M. piperata* and *M. spicata* recorded significant mortality in *C. maculatus* (Raja *et al*., 2001). Negahban *et al*. (2006) also reported fumigant toxicity of essential oils from various Iranian medicinal plants against *C. maculatus*, *S. oryzae* and *T. castaneum*. In the present study the activity differed between species. *C. maculatus* was the most susceptible in both fumigant toxicity and repellent activity experiments. *T. castanueum* was the most resistant insect in the present study. Bachrouch *et al*. (2010) have reported that essential oil of *Pistacia lentiscus* caused 51 and 100% mortality in the third-instar larvae and adults of *T. castaneum,* respectively at a concentration of 1023 µl/l air after 24 h of exposure. Our study clearly showed that 160 µl/l concentration of *T. asiatica* caused 65.5% mortality in *T. castaneum* adult and larvae after 24 h exposure. If the concentration will be increased to 175µl the mortality of *T. castaneum* will be 90% in 24h as evidenced from the probit analysis.

Fumigation is done for killing insects or to avoid further damage in infected commodities, whereas, repellence is a method of preventing insect attack. So, repellence is an important method for maintaining good quality stored products. Plant volatile oils are potential natural repellents against insects. Many investigators have recorded the repellent property of essential oils against coleopteran pests of stored products. Regnault-Roger (1997), Cosimi *et al*. (2009) and Nerio *et al*. (2009) have recorded the repellent activity of essential oils against stored product pests. The oils obtained from *Evodia rutaecarpa* (Liu and Ho, 1999), *Ocimum gratissimum* (Ogendo *et al*., 2008) and *Artemisia vulgaris* (Wang *et al*., 2006) were reported as repellents against *T. castaneum.* Zapata and Smagghe (2010) found that essential oils of *L. sempervirens* and *D. winteri* had fumigation toxicity and repellent activity against *T. castaneum*. Essential oil of *O. Basilicum* exhibited repellent activity against *C. maculatus* (Villalobos and Acosta, 2003). Essential oil of *O. gratissimum* showed repellent activity against *S. oryzae*, *T. castaneum*, *O. surinamensis*, *R. dominica* and *C. chinensis* (Ogendo *et al.*, 2008).

The essential oil obtained from *T. asiatica* recorded the highest repellent activity and toxicity in the present study. The biological activities of essential oils depend upon the chemical composition of the oils. The strong insecticidal and repellent activities of *T. asiatica* might be due to the presence of some major components such as β- Linalool, β-Sesquiphellandrene, Spathuleno and Caryophyllene oxide. Saxena and Sharma (1999) have reported that *T. asiatica* oil contained 12.4% linalool and had antibacterial and antifungal activities even at a dilution at 1:20. Linalool has already been reported as an insect repellent by Chapman *et al*. (1981). In the present study the oil contained 45 compounds. The major compounds including linalool might have the biological activities due to their own effect or due to synergistic effect along with other major components in the oil. Caryophyllene, a volatile compound, was already reported to be a strong fumigant and can be toxic to *Sitrophilus zeamais* (Chu *et al*., 2010). In the present study Caryophyllene has been detected in *T. asiatica* essential oil (2.52%).

T. asiatica was already reported to possess larvicidal property against mosquitoes. Borah *et al*. (2010) reported that the hexane extract of fruits of *T. asiatica* showed the highest larvicidal activity against the vector mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*. Petroleum ether extractof *T. asiatica* showed larvicidal activity against fourth instar larvae of *C. quinquefasciatus* (BinuKumari, 2010). *T. asiatica* is a medicinal plant used for different health problems in India. It has no toxicity against human beings. So the essential oil of *T. asiatica* can be used as an ecofriendly alternate in stored product pest control programmes

CONCLUSION

T. asiatica essential oil had fumigant toxicity and repellent activity against *C. maculatus, S. oryzae* and *T. castaneum* adults. The pulse beetle *C. maculatus* was the most susceptible and *T. castaneum* was the most resistant beetle to the treatment. The essential oil of *T. asiatica* can be utilized for stored product pest management in and ecofriendly way.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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