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Fumigation and repellent potential of four essential oils against major coleopteran pests of stored grain

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ABSTRACT

The essential oils extracted by hydrodistillation from the leaves of four plants, *Artemisia maritima* Linn. *Colebrookea oppositifolia* Sm. *Rabdosia rugosa* Wall. ex Benth and *Zanthoxylum armatum* DC. were analysed by mass spectroscopy to elucidate the prime components especially monoterpenes components present in these plants. Fumigation and repellent activity of these essential oils were evaluated against the adults of four major coleopteran stored-grain insect pests, *Callosobruchus analis* F. (Bruchidae), *Stegobium paniceum* L. (Anobiidae), *Tribolium castaneum* Herbst (Tenebrionidae) and *Sitophilus oryzae* L. (Curculionide). The results obtained show that all the essential oils extracted were toxic to the adult insect pests. *R. rugosa* oil was highly toxic with LC_{50} values of 2.01, 7.21, 10.9 and 18.8 µl/mL air 24 h after treatment for *C. analis*, *S. paniceum*, *S. oryzae* and *T. castaneum*, respectively. Similarly, *A. maritima* was slightly less toxic with LC_{50} values of 3.2, 9.8, 18.5 and 23.5 µl/mL air 24 h after treatment for the same four insects, respectively. *C. oppositifolia* was least efficacious with high LC_{50} values. Maximum repellency of 90 and 88% was observed for *S. paniceum* at highest concentration of 10 µl/cm^2 after 5 h exposure of *R. rugosa* and *A. maritima* oils. All four essential oils exhibited repellent efficacy at all concentrations, but especially after the shorter exposure times of 1, 3 and 5 h which decreased with increased exposure period of 24 h. *C. analis* and *S. paniceum* were more susceptible to all the four plant oils than other two insect species. Cytotoxicity studies revealed that essential oils showed no toxicity at low concentrations or a very weak toxicity at high concentrations towards BV2 cell line.

INTRODUCTION

For thousands of year's insects has been a problem associated with storing surplus dried food, with evidences from archaeological deposits (Solomon, 1965; Buckland, 1981) and written records (Yasue, 1980; Beavis, 1988; Levinson and Levinson, 1994) of the storage and infestation of cereals stretching back to 3000 BC. The global post-harvest grain losses caused by insect damage and other bio-agents range from 10 to 40% (Raja et al., 2001; Papachristos and Stamopoulos, 2002). Pulse beetle, drugstore beetle, red flour beetle and rice weevil are the major and important pests of stored commodities in the tropics. The main method of grain protection is the use of chemical agents but the continuous use of broad-spectrum pesticides and fumigants in stored grains has led to numerous problems related to environmental and human health. Thus, search for systems of grain protection that target the pest species more accurately is needed (Cox, 2004). Natural products are an excellent alternative to synthetic pesticides as plants produce secondary metabolites of insecticidal properties (Potenza et al., 2004). Plant

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extracts and essential oils have traditionally been used to kill or repel stored product insects (Tinkeu et al., 2004; Tapondjou et al., 2005; Arabi et al., 2008; Fouad et al., 2012). Essential oils are volatile, natural, complex compounds characterized by a strong odour and produced by aromatic plants. The insecticidal constituents of many essential oils are mainly monoterpenoids such as limonene, linalool, terpineol, carvacrol and myrcene (Ahn et al., 1998; Regnault-Roger and Hamraoui, 1995). It has been proved that monoterpenes are main components of plant essential oils and are biodegradable and non persistent in soil and water (Misra and Pavlostathis, 1997).

Essential oils of medicinal plants exhibit fumigant and repellent effects thus play an important role in protecting grains against insect infestations (Regnault-Roger, 1997; Isman, 2000; Bakkali et al., 2008; Batish et al., 2008). They are volatile with high insecticidal efficiency and very low persistence. The active compounds present in them are specific to particular insect groups (Huang et al., 1997) and harmless to mammals (Isman, 2000). Objective of the present study is to isolate essential oils from four species of plants, *Artemisia maritima* Linn. *Colebrookea oppositifolia* Smith, *Rabdosia rugosa* Wall. ex Benth and *Zanthoxylum armatum* DC. and were analysed by mass spectroscopy to elucidate the prime components especially monoterpenes. Fumigant and repellent activity were tested against coleopteran pests, *Callosobruchus analis* F., *Sitophilus oryzae* L., *Tribolium castaneum* Herbst and *Stegobium paniceum* L. The study was further extended for cytotoxicity of essential oils towards living cell line for their comprehensive safety evaluation.

MATERIALS AND METHODS

Extraction of essential oils

Essential oils were extracted from leaves of *A. maritima*, *C. oppositifolia*, *R. rugosa* and *Z. armatum* collected from the local areas of Shimla district of Himachal Pradesh, India. The leaves were dried in shade at room temperature (30 ± 5°C) and grounded by domestic mixer. The dried powdered material was hydro-distilled in Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample in 1:10 plant material/water volume ratio for 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (2.9% w/w) was calculated on a dry weight basis. Extracted oil was stored in a refrigerator at 4°C for further analysis.

Mass spectroscopy

Mass spectra of *A. maritima*, *C. oppositifolia*, *R. rugosa* and *Z. armatum* oil was recorded on Bruker micrOTOF Q II Mass spectrometer. The prime components of essential oils were identified by mass spectroscopy and mass spectrum of essential oil isolated from four plants has been shown in Figure 1, 2, 3 and 4.

Test insects

Laboratory cultures of *C. analis*, *S. oryzae*, *S. paniceum* and *T. castaneum* (5–10 days each) were maintained at 28±2°C and 68±2% relative humidity. Test insects of *S. oryzae*, were reared on rice kernels, *C. analis* on chickpea grains and whole meal wheat flour plus brewer's yeast (19:1) was used to rear *S. paniceum* and *T. castaneum* respectively.

Fumigant toxicity

To test the fumigant toxicity of plant essential oils on different insect pests plastic jars of 250 mL capacity with screwed metallic caps were used as exposure chambers. The essential oils of plants with different doses of 100, 300 and 500 µl, were diluted with acetone to give calculated fumigant concentrations of respectively 0.4, 1.2 and 2 µl/mL of air. The appropriate dilution of essential oil was administered in 1 ml portions on Whatman No. 1 filter paper. After allowing the solvent to evaporate for 10-15 min, the filter paper was attached to the inner surface of the screw lid of the jar using adhesive tape. At the bottom of each jar, 10 individuals along with their food source were placed and exposed to the various concentrations of oils. The insects had no contact with the diffuser and stayed at the bottom of the chamber throughout the experiment. Insect mortalities were determined and calculated after 24 h of exposure to the day of complete mortality of all insects according to the formula of Abbott (1925). Three replicates were set up for each dose and control.

Repellency tests

Repellency tests of plant essential oils were carried out according to the experimental method described by Jilani and Saxena (1990). Test solutions were prepared by dissolving 100, 300 and 500 µl of plant essential oil in 1 ml acetone. Whatman filter papers (diameter 8 cm) were cut into two equal halves one half of each dish was treated with essential oil solution as uniform as possible by using micro pipette. The other half of the filter paper was treated with acetone alone as a control. The treated and control half discs were dried to evaporate the solvent completely. Treated and untreated halves were attached to their opposites using adhesive tape and placed in Petri

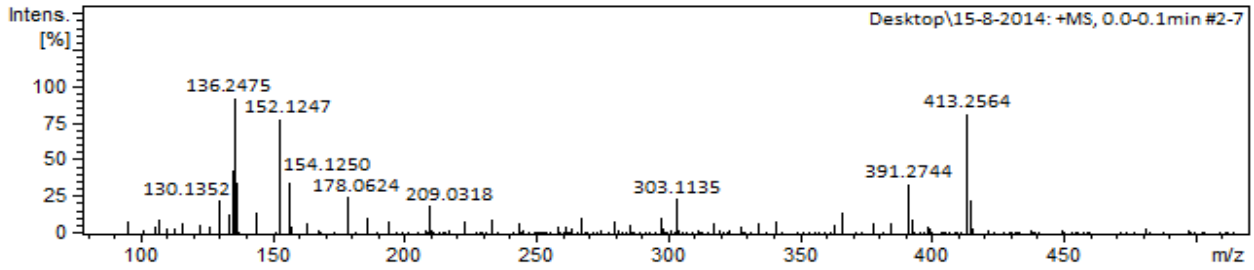

Figure 1. *A. maritima* oil.

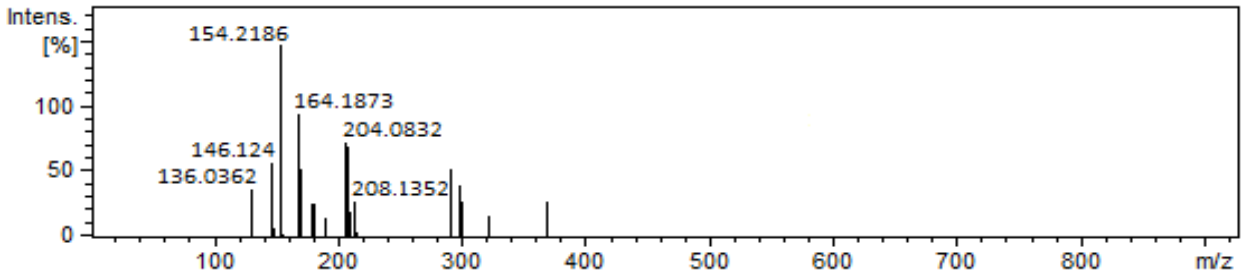

Figure 2. *C. oppositifolia* oil.

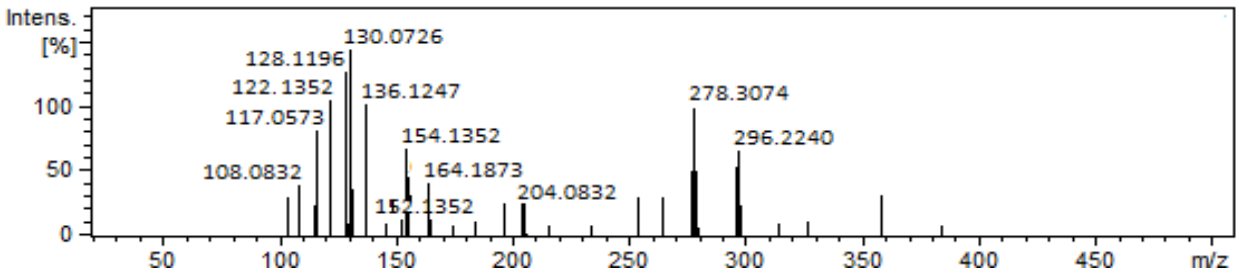

Figure 3. *R. rugosa* oil.

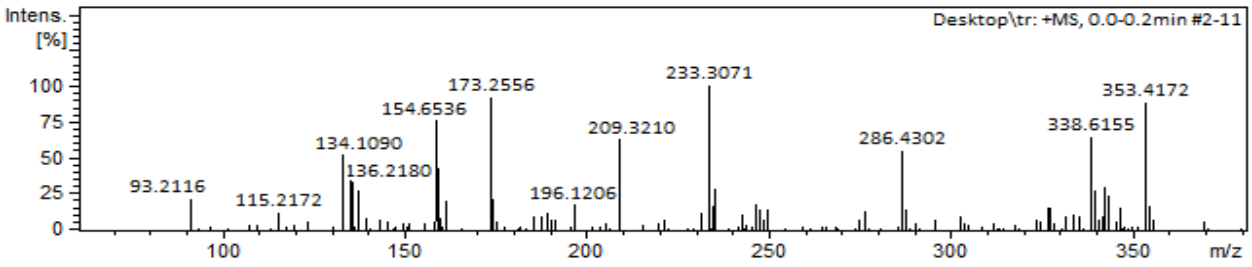

Figure 4. *Z. armatum* oil.

dishes. Twenty adult beetles of each species were released at the centre of each filter paper. The Petri dishes were then covered and sealed with parafilm. Three replications were used for each concentration. Observations on the number of insects present on both the treated and untreated halves were recorded after 1, 3, 5 and 24 h. Percentage repellency (PR) was calculated according to Nerio et al. (2009) as follows:

Table 1. Chemical constituents of the essential oil *A. maritima* and *C. oppositifolia* and parent ion peak (m/Z) values of components.

Components	Calculated	Observed
	m/Z	
A. maritima		
Alpha-pinene	136.23	136.24
Myrcene	154.24	154.12
Linalool	154.18	154.20
Camphene	136.23	136.24
Sabinene	136.19	136.24
Camphor	152.09	152.12
Methyl eugenol	178.10	178.06
1-8 cineole	154.21	154.12
Borneol	154.19	154.12
3- octanol	130.09	130.13
Thujene	136.21	136.24
C. oppositifolia		
Camphene	136.24	136.03
Limonene	136.24	136.03
Borneol	154.14	154.21
Eugenol	164.20	164.18
Caryophyllene	204.30	204.08
Germacrene	208.22	208.13
Alpha cubebene	204.35	204.08
Alpha terpineol	154.25	154.21
Beta pinene	136.24	136.03
1-8 cineole	154.24	154.21
Cumarine	146.15	146.12

Table 2. Chemical constituents of the essential oil of *R. rugosa* and *Z. armatum* and parent ion peak (m/Z) values of components.

Components	Calculated	Observed
	m/Z	
R. rugosa		
Linalool	154.25	154.13
Alpha terpineol	154.25	154.13
Geraniol	154.25	154.18
Indole	117.06	117.05
Eugenol	164.20	164.18
Vanillin	152.05	152.13
Caryophyllene	204.30	204.08
Linolenic	278.44	278.30
3- octanol	130.14	130.07
M-cresol	136.24	136.12
Phytol	296.31	296.22
Z. armatum		
Alpha-pinene	136.23	136.20
Myrcene	154.24	154.20

Table 2. Contd.

Linalool	154.23	154.20
Camphene	136.24	136.21
Sabinene	136.23	136.21
Limonene	136. 21	136.21
Geraniol	154.25	154.65
Geraniol acetate	196.15	196.12
Para-cymene	134.08	134.10
Nerol acetate	209.30	209.32

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

Nc is the number of insects on the untreated area after the exposure interval and Nt is the number of insects on the treated area after the exposure interval.

Cytotoxicity assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide] based cytotoxicity test was used to evaluate essential oils obtained from *A. maritima*, *C. oppositifolia*, *R. rugosa* and *Z. armatum* and the tests were carried out on BV2 (microglial cells). Cells were seeded in 96-well flat-bottomed microplates at a density of 5×10^4 per mL, 100 µL per well and were allowed to grow for 24 h. The essential oils dissolved in dimethyl sulfoxide (DMSO) were sterilized using a Millipore filter (pore size 0.22 µm) and were added to the culture media over a concentration range of 50-500 µg/ml. The cytotoxicity of essential oils was assessed after 24 h of exposure. The absorbance was read at 550 nm using a Muliskan PLUS plate reader (Lab system, Finland). The statistical analysis was performed using Sigma Stat 3.5.1 and Sigma Plot 11.

Statistical analyses

Tests for repellency and fumigant toxicity were performed in triplicate and data presented are mean ± SE. The means were compared by one-way ANOVA and Tukey's multiple comparison tests using software SPSS, version 11.5.

RESULTS

Components of essential oils obtained from four plants

The mass spectroscopy has been used to elucidate the components of essential oils from *A. maritima*, *C. oppositifolia*, *R. rugosa* and *Z. armatum*. The essential oils contained different terpenoid components that have been summarized in Table 1 and 2.

Fumigant toxicity

The results of space fumigation tests showed considerable difference in mortality of insect species to essential oils vapor with different concentrations and times. *C. oppositifolia* oil produced weaker fumigant activity resulting in 56, 66, 70 and 85%, mortality of *T. castaneum*, *S. oryzae*, *S. paniceum* and *C. analis*, respectively, 120 h after treatment with 2 µl/mL air while at the similar concentration *Z. armatum* oil caused 70 and 88% mortality against *S. oryzae* and C. *analis* after 120 h and 82 and 85% mortality for *T. castaneum* and *S. oryzae* after 168 h of exposure (Figure 5). *A. maritima* oil at 1.2 µl/mL air, achieved 70, 72, 78, and 100% kill for *T. castaneum*, *S. oryzae*, *S. paniceum* and *C. analis* respectively, after 120 h of treatment (Figure 6). The essential oil from *R. rugosa* even at a lowest concentration of 0.4 µl/mL air caused 100% mortality of *C. analis*, *S. paniceum* and *S. oryzae* after 120 h of treatment and for *T. castaneum* complete mortality was obtained after 168 h (Figure 7). Of the four plant oils used the essential oil from *R. rugosa* exhibited the highest activity against all the insect species followed by *A. maritima* and *Z. armatum* oil. *T. castaneum* and *S. oryzae* were the most tolerant of the four insect species tested against all treatments. Probit analysis also showed that both *S. paniceum* and *C. analis* were more susceptible to oils. *R. rugosa* oil was highly toxic with LC_{50} values of 2.01, 7.21, 10.9 and 18.8 µl/mL air 24 h after treatment for *C. analis*, *S. paniceum*, *S. oryzae*, and *T. castaneum* respectively. *A. maritima* had similarly high toxicity with LC_{50} values of 3.2, 9.8, 18.5 and 23.5 µl/mL air 24 h after treatment for the same four insects, respectively (Table 3). *C. oppositifolia* on the other hand, was less efficacious, with LC_{50} values of 4.9, 12.2, 23.4 and 32.5 µl/mL air 24 h after treatment for *C. analis*, *S. paniceum*, *S. oryzae* and *T. castaneum* respectively.

Repellent activity

The repellent action of plant essential oils was studied against the four insect pests. Results showed that *Z. armatum* oil was strongly repellent to all test insects than the other plant oils. Repellent action was highly dependent upon oil concentration and exposure time. In filter paper tests, at 10 µl/cm^2 *Z. armatum* oil showed the highest repellent activity of 92 and 88% against *C. analis* and *S. paniceum* after 5 h of time interval (Table 4). Percentage repellency was calculated as 82, 69, 58 and 50% at 6 µl/cm^2 of *R. rugosa* oil against *S. paniceum*, *C. analis*, *T. castaneum* and *S. oryzae* respectively after the exposure period of 3 h. The maximum repellency of 90 and 88% was observed for *S. paniceum* at the highest concentration of 10 µl/cm^2 after 5 h of exposure for *R. rugosa* and *A. maritima* oil (Table 5 and 6). *C. oppositifolia* oil at 10 µl/cm^2 showed 60 and 71% repellent activity against *S. oryzae* and *T. castaneum* after an exposure of 5 h *C. oppositifolia* was least effective than other oils for all the insect pests (Table 7). The four essential oils exhibited a repellent activity at all concentrations, but especially after the shorter exposure times of 1, 3 and 5 h which further decreased at increased exposure period of 24 h. Maximum repellency was observed against the four insect species after 5 h of exposure for all the treatments.

Cytotoxicity of essential oils towards BV2 cell line

Essential oils showed no toxicity at low concentrations or a very weak toxicity at high concentrations towards BV2 cell line. It was observed that growth of cell line was inhibited in a concentration dependent manner (Figure 8). At 100, 300 and 500 µg/ml of *C. oppositifolia* oil 100, 82, 65 and 60% cell survival was recorded. 85 cell survival was observed at 50 µg/ml of *Z. armatum* oil followed by 72 at 100 µg/ml and 60% at 300 µg/ml. *A. maritima* and *R. rugosa* oil showed highest inhibition of cell growth and lowest cell viability of 75 and 70% at 50 µg/ml followed by 65 and 60% (100 µg/ml), 58 and 54% (300 µg/ml) and 52% (500 µg/ml). Among all the essential oils tested *R. rugosa* and *A. maritima* oil were more effective in inhibiting the growth of cell line with IC_{50} of 223 and 244 µg/ml and showed very weak cytotoxicity followed by *Z. armatum* oil with IC_{50} of 256 µg/ml. *C. oppositifolia* oil did not show any cytotoxicity with IC_{50} >300 having IC_{50} values of 321 µg/ml.

DISCUSSION

Plant products especially essential oils having potential as insecticidal compounds are gaining tremendous importance in recent years. Monoterpenes, the chemical constituents of essential oils found in plants, are known biologically active compounds. Monoterpenoid compounds have been considered as potential pest control agents and generally, these major components determine the biological properties of the essential oils. So during the present investigation essential oils extracted from different plant materials were analysed by mass spectroscopy to elucidate the prime components especially monoterpenes of the plant oils.

The mass spectroscopy study revealed that the *A. maritima* oil is composed of a mixture of components camphene, alpha-pinene, camphor, myrcene, 1-8 cineole, borneol and linalool. Essential oil of *A. maritima* is reported to contain 1-8 cineole as the major constituent along with chrysanthenone, borneol and camphor (Jaitak et al., 2008). *C. oppositifolia* oil was composed of a mixture of components including camphene, limonene,

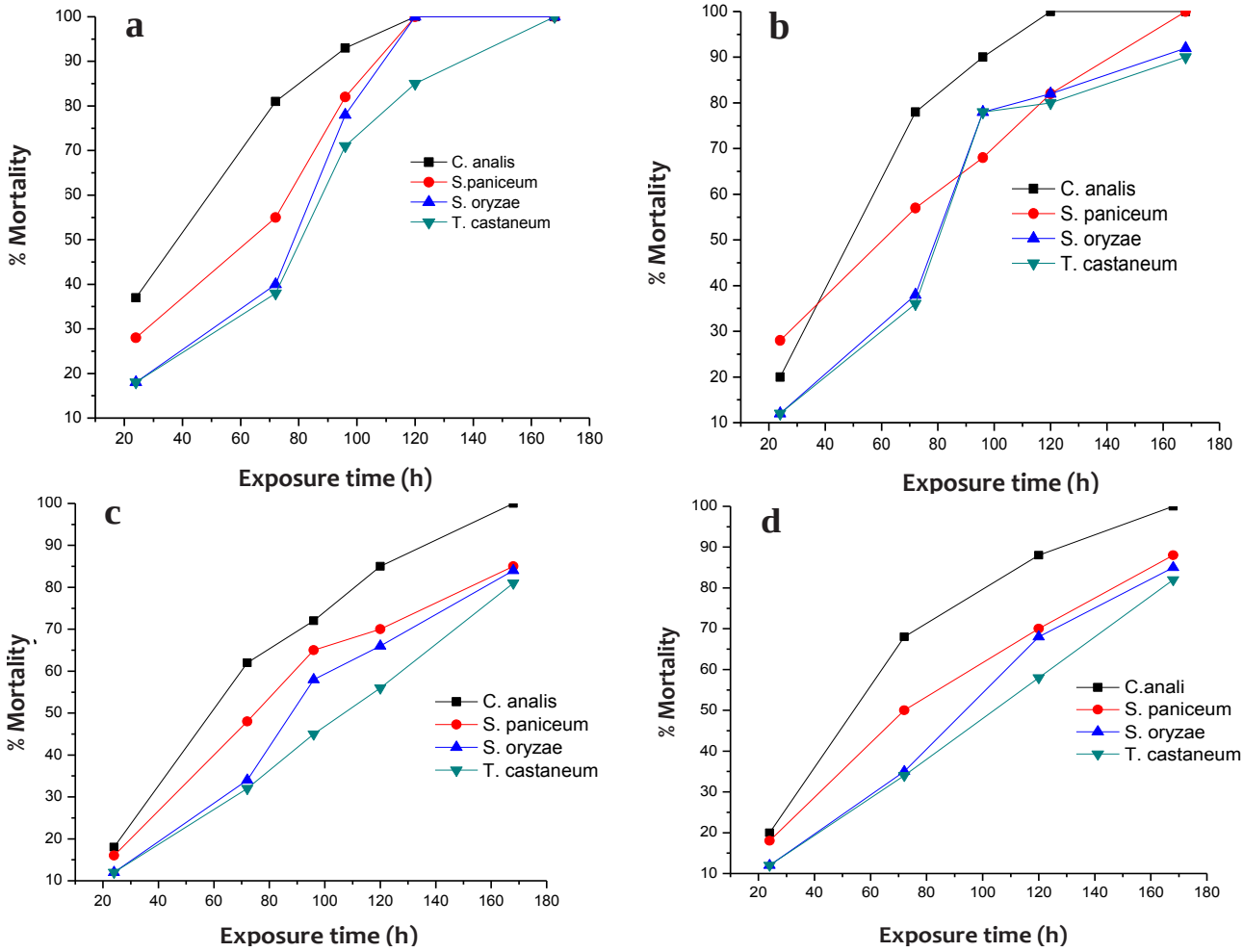

Figure 5. Percentage mortality of *C. analis, S. paniceum, S. oryzae* and *T. castaneum* exposed to 2 µl/mL essential oil of **a**) *R. rugosa*; **b**) *A. maritime*; **c**) *Z. armatum*; **d**) *C. oppositifolia* at different time intervals.

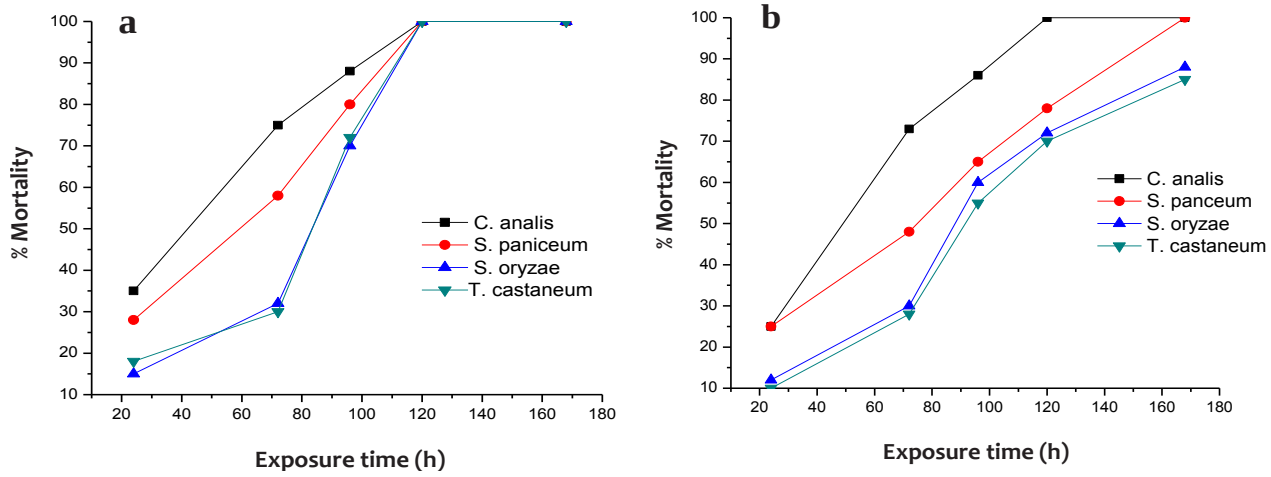

Figure 6. Percentage mortality of *C. analis, S. paniceum, S. oryzae* and *T. castaneum* exposed to 1.2 µl/mL essential oil of **a**) *R. rugosa*; **b**) *A. maritime*; **c**) *Z. armatum*; **d**) *C. oppositifolia* at different time intervals.

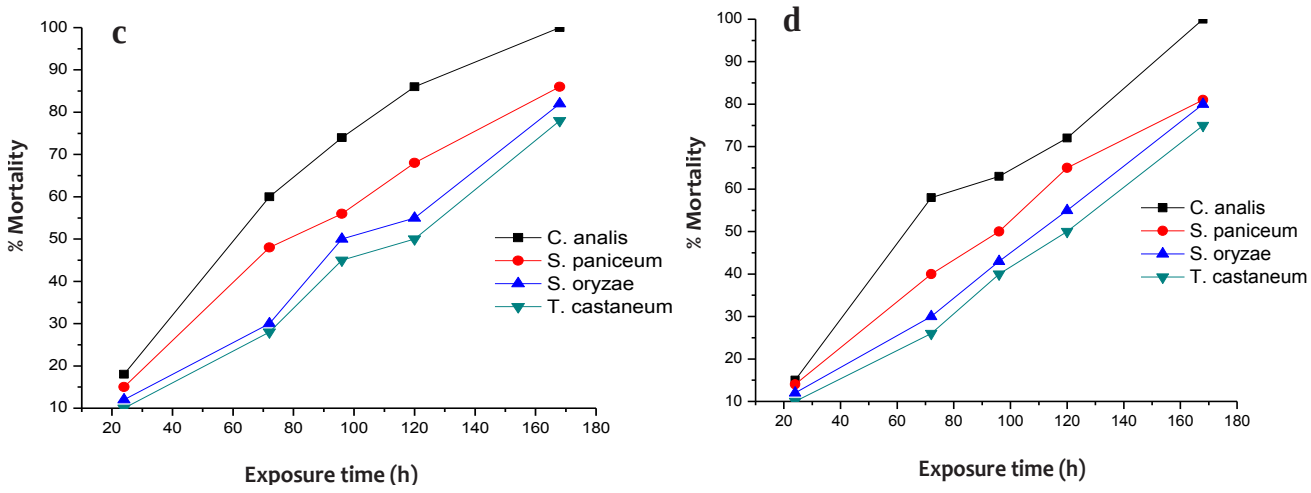

Figure 6. Contd.

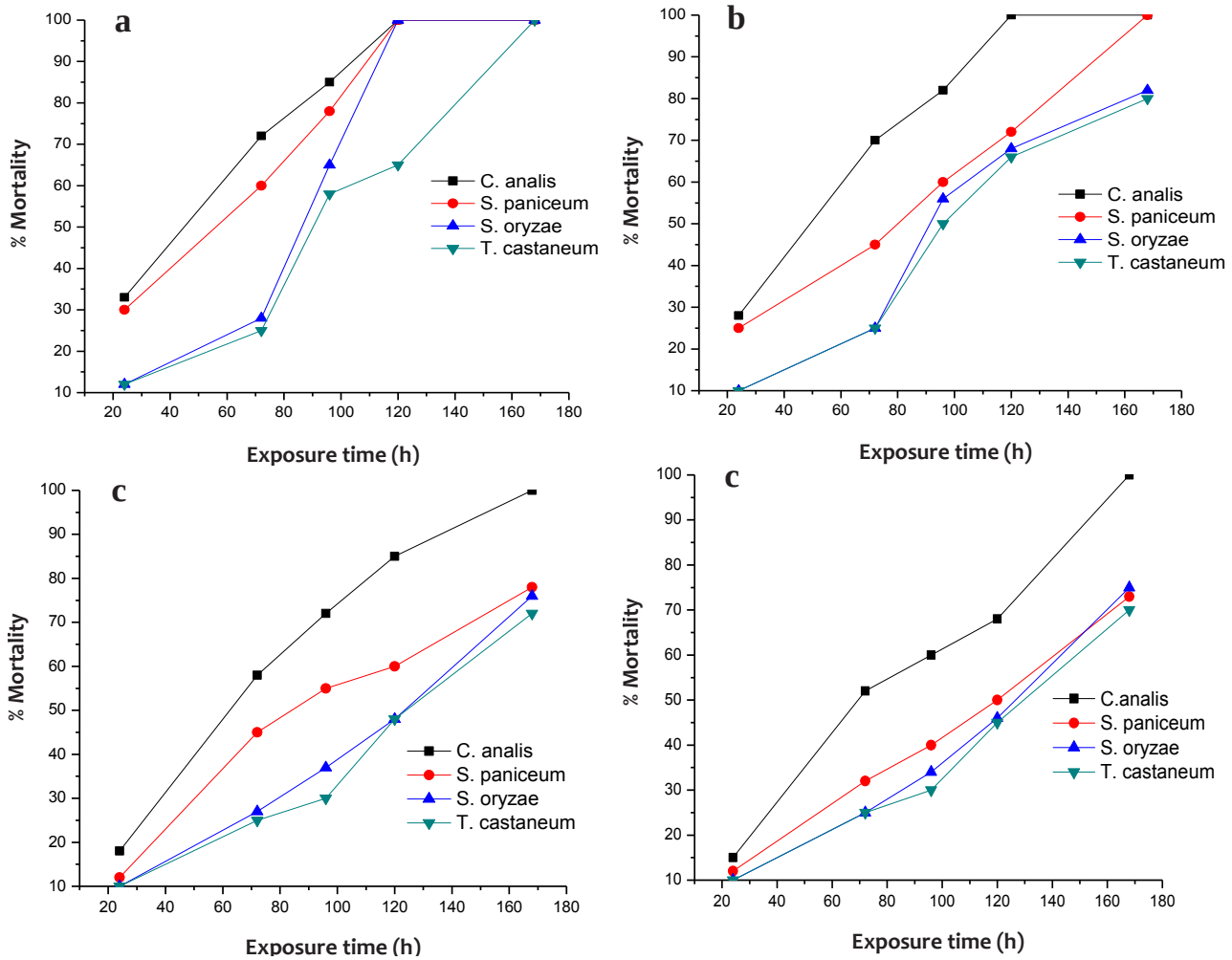

Figure 7. Percentage mortality of *C. analis, S. paniceum, S. oryzae* and *T. castaneum* exposed to 0.4 µl/ml essential oil of **a**) *R. rugosa*; **b**) *A. maritime*; **c**) *Z. armatum*; **d**) *C. oppositifolia* at different time intervals.

Table 3. LC_{50} values and 95% Fiducial Limits (FL) of plant oils against four stored grain insect pests at different exposure intervals.

LC_{50} µl/mL air	24	72	120	168
	Time (h)			
C. oppositifolia				
C. analis	4.9(1.5-12.3)	3.20(1.3-12.3)	1.98(0.43-3.98)	-
S. paniceum	12.2(7.13-226)	9.8(7.1-213)	3.80(1.2-14.3)	1.45(0.28-1.89)
S. oryzae	23.4(14.9-4.52)	10.5(6.2-250)	4.20(1.6-13.6)	2.18(0.36-3.48)
T. castaneum	32.5(16.5-235)	14.8(10.5-25.6)	10.2(7.1-230)	7.5(4.06-13.4)
Z. armatum				
C. analis	4.1(2.5-7.1)	2.3(0.5-3.23)	1.6(0.5-3.62)	-
S. paniceum	10.9(6.25-212)	6.2(4.06-11.2)	3.2(1.4-12.5)	1.12(0.26-1.98)
S. oryzae	20.5(9.0-432)	9.6(4.05-11.2)	3.96(2.1-12.6)	2.15(0.55-4.22)
T. castaneum	26.8(16.5-142)	11.5(8.3-19.2)	9.5(6.1-130)	7.1(3.2-14.5)
A. maritime				
C. analis	3.2(2.1-16.5)	1.50(0.5-4.6)	-	-
S. paniceum	9.8(6.22-251)	5.8(3.2-210)	2.10(1.2-4.22)	-
S. oryzae	18.5(8.5-50.5)	8.1(5.21-230)	3.25(1.5-13.2)	1.92(0.4-4.20)
T. castaneum	23.5(15.5-129)	10.8(7.14-230)	8.1(5.20-150)	6.2(4.03-9.52)
R. rugosa				
C. analis	2.01(0.30-4.12)	1.21(0.8-5.2)	-	-
S. paniceum	7.21(4.05-9.34)	3.15(1.8-11.8)	-	-
S. oryzae	10.9(7.19-231)	5.03(1.8-10.6)	-	-
T. castaneum	18.8(9.1-61.2)	7.23(3.15-9.35)	6.3(2.1-14.4)	-

Table 4. Percentage repellency of *Z. armatum* essential oil against four stored grain insect pests at different time intervals.

	Time (h)	Doses (µl/cm^2)		
		2	6	10
C. analis	1	60.5±1.4a	73.6±4.2b	78.2±1.2c
	3	66.1±2.1b	85.5±3.4a	88.4±1.4ab
	5	78.4±3.1ab	86.3±2.1a	92.2±2.4ab
	24	55.5±1.4bc	60.2±1.9ab	68.3±1.9bc
S. paniceum	1	57.5±1.2bc	68.4±2.4b	77.3±2.8c
	3	64.4±3.2a	72.3±1.8b	82.2±1.7a
	5	76.5±1.5ab	81.5±2.1a	88.3±2.3ab
	24	42.6±4.1cd	55.2±1.9bc	60.5±3.1b
T. castaneum	1	51.6±2.3cd	60.2±1.4ab	72.1±1.6bc
	3	60.5±1.6a	66.2±2.2b	80.5±1.2a
	5	71.5±2.2b	79.1±3.2a	82.5±2.3a
	24	35.7±1.7c	45.6±1.9c	58.1±1.6b
S. oryzae	1	48.5±2.6cd	55.5±2.9bc	68.1±1.9bc
	3	59.5±3.1a	63.4±3.1ab	75.6±2.4c
	5	60.6±4.2a	71.4±4.5b	78.6±2.8c
	24	35.4±2.8c	44.7±2.1c	55.4±1.9b

% values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

Table 5. Percentage repellency of *R. rugosa* essential oil against four stored grain insect pests at different time intervals.

	Time (h)	Doses (µl/cm^2)		
		2	6	10
S. paniceum	1	58.6±1.6a	70.3±1.4b	75.5±1.9ab
	3	62.8±2.1a	82.4±4.6a	85.6±3.4b
	5	75.5±1.9b	85.1±3.4a	90.2±2.2b
	24	35.6±4.1bc	46.2±2.6ab	62.6±2.5bc
C. analis	1	52.6±1.4ab	63.4±2.1b	72.2±1.9ab
	3	60.3±2.1a	69.5±1.8b	78.5±3.4ab
	5	72.5±3.6b	79.1±3.1a	85.6±2.5b
	24	30.6±3.1bc	42.4±4.1ab	58.5±2.8bc
T. castaneum	1	44.2±1.9	50.5±1.6ab	55.6±2.5bc
	3	52.6±2.2ab	58.1±4.1bc	70.5±2.1cd
	5	60.5±3.4a	67.5±2.2b	75.6±1.9ab
	24	20±4.5cd	25.5±1.8cd	54.4±3.6a
S. oryzae	1	38.6±1.4c	45.4±2.3ab	50.4±2.1c
	3	46.2±1.8ab	50.4±3.1c	58.4±3.1a
	5	55.6±2.3ab	60.4±4.1bc	67.2±1.8cd
	24	18.6±1.9cd	25.5±2.2cd	50.2±1.4c

% values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

Table 6. Percentage repellency of *A. maritima* essential oil against four stored grain insect pests at different time intervals.

	Time (h)	Doses (µl/cm^2)		
		2	6	10
S. paniceum	1	50.6±2.4a	68.2±1.9a	73.2±1.8b
	3	60.5±1.6a	81.5±2.2ab	83.5±2.6bc
	5	72.6±3.1ab	85.2±1.4ab	88.5±2.2a
	24	35.6±1.9cd	42.6±1.2b	60.3±3.2ab
C. analis	1	49.5±2.8b	60.6±1.6bc	72.5±1.4b
	3	56.4±3.8a	66.2±1.9a	74.5±1.1b
	5	70.5±2.3ab	76.6±2.3c	83±4.2bc
	24	28.6±1.9cd	41.5±3.1b	58.5±2.2ab
T. castaneum	1	44.4±1.6b	50.5±2.4b	53.6±4.1ab
	3	50.3±1.3a	57.2±3.6bc	70.5±2.4bc
	5	60.6±3.2c	66.1±1.7a	72.4±1.9b
	24	20.6±2.1bc	23.5±4.2cd	53.4±3.6ab
S. oryzae	1	30.5±1.9cd	42.4±1.1b	48.2±2.4c
	3	42.2±2.8cd	49.5±1.8b	55.4±2.4c
	5	48.6±3.1b	57.4±2.5bc	62.2±3.5ab
	24	18.6±1.8bc	22.6±3.1cd	50.6±2.6c

% values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

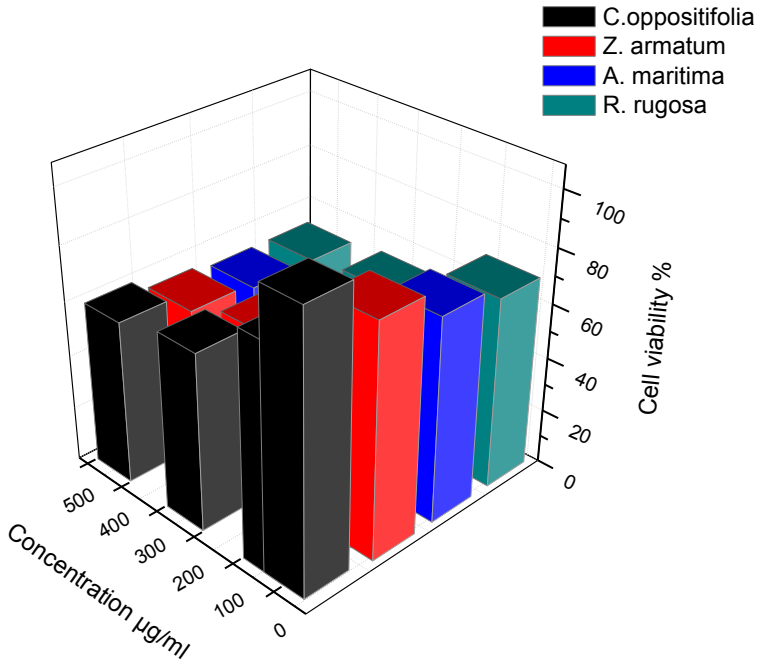

Figure 8. 3D graph represents the cell survival percentage at different concentrations of essential oils during MTT assay against BV2 (microglia) cells.

Table 7. Percentage repellency of *C. oppositifolia* essential oil against four stored grain insect pests at different time intervals.

	Time (h)	Doses (µl/cm^2)		
		2	6	10
C. analis	1	48.4±3.1ab	55.5±1.9b	68.5±2.3a
	3	56.4±1.4a	72.4±2.6a	79.1±3.4ab
	5	69.4±2.8bc	76.1±3.6a	82.6±1.6ab
	24	32.5±4.5bc	40.5±4.3b	58.4±2.1b
S. paniceum	1	45.5±1.7b	56.4±1.1b	69.2±1.8a
	3	50.5±2.5ab	62.5±3.6ab	70.5±4.1bc
	5	66.1±3.4bc	70.6±1.5a	80.6±2.1ab
	24	20.6±1.4c	38.2±4.3bc	55±1.8b
T. castaneum	1	42.4±1.8ab	48.5±3.4b	50.5±2.4cd
	3	48.4±2.2b	45.6±4.5bc	68.1±1.7a
	5	59.1±1.6a	62±3.1ab	71.4±4.3bc
	24	20.5±2.6c	20.6±1.8c	49.4±3.1c
S. oryzae	1	30.6±2.2bc	40.6±1.8bc	45.5±1.2c
	3	39.5±1.7cd	45.3±3.6bc	50.5±3.5cd
	5	42.5±3.4b	51.6±4.2ab	60.6±3.1c
	24	18.4±2.4c	20.5±2.1c	48.6±1.3c

% values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

beta-pinene, cumarine, aipha-terpineol, borneol, 1,8-cineole, eugenol, caryophyllene, alpha-cubebene and germacrene. *R. rugosa* essential oil was composed of a mixture of components including indole, 3-octanone, 3-octanol, m-cresol, vanillin, linalool, alpha-terpenol, geraniol, eugenol, caryophyllene and lenolenic. Hao et al. (2002) analyzed essential oil of *R. macrocalyx* and identified 60 components including indole, geraniol, linalool, eugenol, vanillin, caryophyllene, alpha terpeniol and linolenic acid. *Z. armatum* essential oil consisted of different components like para-cymene, alpha-pinene, limonene, sabinene, camphene, linaloool, myrcene, geraniol, geraniol acetate and nerol acetate. Similarly Barkatullah et al. (2013) identified total 34 chemical constitutent through GC-MS and linalool (53.05%), alpha-limonene (11.39%), alpha-pinene (4.08%), beta-myrcene (3.69%) and D-limonene (3.10%) were the major constituents of the *Z. armatum* essential oil. The present results show that the essential oils have varying degrees of insecticidal activity against the four stored grain insect pests studied. In the space fumigation studies oils showed strong species-specific toxicity highly dependent upon the dosage and time after treatment. The results showed higher mortality rates of C. *analis* and *S. paniceum* than *S. oryzae* and *T. castaneum* for all essential oils. *R. rugosa* and *A. maritima* oil at concentrations of 0.4 – 1.2 µl/mL of air were potent enough to achieve about 70% mortality of all the test insects within 96 h after treatment and 100% in *C*. *analis* and *S. paniceum* in 168 h after treatment. Similarly, Negahban et al. (2006) reported *A. scoparia* oil relatively more toxic to *C*. *maculatus* than *S. oryzae* and *T. castaneum.* Total mortality of all three species was achieved with the lowest concentration 37 µl/L after 24 h exposure. The cinnamon oil at a dose of 3.6 µl/mL caused 100 and 95% mortality of *C. maculatus* and *S. oryzae* respectively after 7 h of treatment and *C. maculatus* was found to be more susceptible to essential oil than *S. oryzae* (Brari and Thakur, 2015). In the present findings 80-90% mortality was observed for S. *oryzae* and *T. castaneum* after 168 h at 2 µl/mL of *Z. armatum* and *C. oppositifolia* oil. *R. rugosa* oil was highly toxic to all the insect species with LC_{50} values of 2.01, 7.21, 10.9 and 18.8 µl/ml air 24 h after treatment for *C. analis*, *S. paniceum*, *S. oryzae* and *T. castaneum* respectively. *A. maritima* had similarly high toxicity with LC_{50} values of 3.2, 9.8, 18.5 and 23.5 µl/ml air for the same four insects, respectively. In a related study *Ocimum gratissimum* oil was found to be highly toxic with LC_{50} values of 0.20, 0.50, 0.50 and 14.0 ml/l air 24 h after treatment against *C. chinensis*, *R. dominica*, *O. surinamensis* and *S. oryzae*, respectively among all the insect pests *T. castaneum* and *S. oryzae* were the most tolerant (Ogendo et al., 2008). The order of effectiveness of tested essential oils as fumigants arranged as *R. rugosa* > *A. maritima* > *Z. armatum* > *C. oppositifolia*. The essential oils exhibited strong dose and exposure time-dependent repellence against all the four insects tested. In addition to fumigant activities, the essential oils of *Z. armatum* and *R. rugosa* showed a strong repellent effect on *C. analis* and *S. paniceum* and moderate effect on S. *oryzae* and *T. castaneum.* Similar observations on the essential oils of *Schyzygium aromaticum, Aegle marmelos, Corriandrum sativum* and *Citrus reticulata* as strong repellents against *S. oryzae* and *T. castaneum* even at low concentration but more remarkable effect towards *S. oryzae* (Mishra and Tripathi, 2011). The clove oil had repellent activity on three important stored grain insect pests, *R. dominica*, *S. oryzae* and *T. castaneum* (Salvadores, 2007). *A. maritima* and *C. oppositifolia* oil also showed about 80% repellence for C. *analis* and *S. paniceum* and 60-70% for *S. oryzae* and *T. castaneum* respectively at a higher concenteration after 5 h of treatment. Similarly repellent activity of oil from *A. verlotiorum* Lamotte has been evaluated against *T. castaneum* (Novo et al., 1997) and *Artemisia saissanica* (Krasch.) Filatova against *S. granarius* (Adekenov et al., 1990). Jemâa et al. (2012) studied the repellent activity of *Laurus nobilis* essential oils from three regions and found that repellent action was highly dependent upon oil concentration and exposure time. A significant repellent activity was observed at the highest concentration mainly after 3 and 5 h of exposure. Similarly in the present investigation at higher concentration four plant oils showed significant repellent activity against all the insect pests especially after 3 and 5 h of exposure. Shukla et al. (2011) also reported that repellent behaviour of *C. chinensis* increased with the increase in the doses of essential oils of *Callistemon lanceolatus* and *Lippia* or their constituents. *C. lanceolatus* and *Lippia alba* oil showed 100 and 76% repellency, while 1,8-cineole and geranial exhibited 74.7 and 63% repellant activity. The percentage repellence was highest in *Z. armatum* > *R. rugosa* > *A. maritima* and least in *C. oppositifolia* oil for all the insect pests studied. The present work would lead to effective screening of plants having insecticidal properties, supports interest in the development of biopesticides from plants for stored products protection and the essential oil are considered as best alternatives for effective repellence against insect pests. This will also contribute to enhance the economic value of the floral diversity in general and the stored grain products in particular. As part of this investigation toxicity tests of essential oils and monoterpenes would evaluate their safe use by humans and preventing their indiscriminate use.

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