

Fumigant Toxicity and Sublethal Effects of *Artemisia khorassanica* and *Artemisia sieberi* on *Sitotroga cerealella* (Lepidoptera: Gelechiidae)

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Abstract

Fumigant toxicity and sublethal effects of essential oils from *Artemisia khorassanica* Podl. and *Artemisia sieberi* Bess were investigated against adults of *Sitotroga cerealella* Olivier. To assess the sublethal effects, adult moths were exposed to the LC₃₀ of each essential oil, and life table parameters of the surviving *S. cerealella* were studied. Higher fumigant toxicity of *A. khorassanica* (LC₅₀: 7.38 µl/liter air) than *A. sieberi* (LC₅₀: 9.26 µl/liter air) was observed against *S. cerealella*. Also, the insecticidal effects of *A. khorassanica* (LT₅₀: 9.01 h) were faster than *A. sieberi* (LT₅₀: 14.37 h). A significant extension was observed in the developmental time (egg to adult) of *S. cerealella* treated with the essential oils. In addition, fecundity of *S. cerealella* reduced by 25.29 and 35.78% following exposure to sublethal concentrations of *A. sieberi* and *A. khorassanica*, respectively. Both tested essential oils caused a significant reduction in the gross and net reproductive rates, intrinsic rate of increase (*rm*), and finite rate of increase of *S. cerealella*. The *rm* values following exposure to *A. sieberi*, *A. khorassanica*, and control were 0.098, 0.094, and 0.107 d⁻¹, respectively. The results of this study suggest that tested essential oils have a good potential to apply in integrated pest management of *S. cerealella*.

Key words: *Sitotroga cerealella*, botanical insecticide, population parameter, compositae

The Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae), is one of the most important and destructive stored product pests in the world (Shukle and Wu 2003). Feeding of *S. cerealella* larvae and producing fecal matter cause high crop damages on cereal grains (Throne and Weaver 2013).

The control of stored product insect pests is mainly based on the application of chemical insecticides, such as pyrethroids, phosphine, and dichlorvos (White and Leesch 1995, Nayak et al. 2003, Hori and Kasaishi 2005). However, the improper and indiscriminate application of these insecticides led to the long-term undesirable effects on human health, environment, and nontarget animals (Champ and Dyte 1977, Subramanyam and Hagstrum 1995, White and Leesch 1995). Because of the potential genotoxicity of phosphine and high toxicity to warm-blooded animals and potential ozone-depleting property of methyl bromide, the use of these insecticides became restricted (Dansi et al. 1984, Anonymous 1991, Bell and Wilson 1995, Meaklim 1998). Therefore, it seems wise to develop new types of safe and ecofriendly alternative management programs. Insecticide potential of many plant essential oils and their components has been investigated to be developed as new safe

fumigants. These insecticides have many advantages over synthetic insecticides viz. low mammalian toxicity, rapid degradation, low pernicious effect on environment, and local availability (Isman 2006).

The toxicity of a large number of essential oils and their constituents has been evaluated against stored product insects (Hori and Kasaishi 2005, Rajendran and Srianjini 2008; Yang et al. 2014). The *Artemisia* genus is one of the largest and most widely distributed genera of the family Compositae (Kordali et al. 2006). Earlier studies showed that the essential oils of *Artemisia* have antifeedant activity, repellency, and insecticidal effects on the insects (Tripathi et al. 2000; Negahban et al. 2006, 2007; Borzoui et al. 2016). Moreover, essential oils and their constituents could have sublethal effects on the development, fecundity, survival, and life table parameters of stored product insects (Kordali et al. 2006, Stamopoulou et al. 2007, Izakmehri et al. 2013, Borzoui et al. 2016, Nouri-Ganbalani and Borzoui 2017).

To date, no published information is available regarding the fumigant toxicity and sublethal effects of *Artemisia* species against *S. cerealella*. Therefore, the aim of this study was to assess the lethal effects of essential oils from *Artemisia khorassanica* Podl. and

Artemisia sieberi Bess against *S. cerealella* under laboratory conditions. Also, the sublethal effects of these essential oils were studied on life history and life table parameters of this pest. The results of this study could be useful in the management programs of *S. cerealella*.

Materials and Methods

Insect Rearing

The initial population of *S. cerealella* was collected from stored wheat seeds from Ardabil, Iran. The insect's population was reared on wheat seeds and maintained at $27 \pm 2^\circ\text{C}$, relative humidity of $65 \pm 5\%$, and a photoperiod of 14:10 (L:D) h. Before beginning of the experiments, the population of *S. cerealella* was reared for three generations on wheat (Gaskojen) cultivar.

Plant Materials and Extraction of Essential Oils

The essential oils of *A. khorassanica* and *A. sieberi* were extracted according to the method of Sahaf et al. (2008). The leaves of *A. khorassanica* were collected at full-flowering stage from Sabzevar region, Khorasane-Razavi (Iran), on June 2015. Also, the leaves of *A. sieberi* were collected during May 2015 from Ardabil region (Iran). The aerial parts of the plants were air-dried (at $26\text{--}28^\circ\text{C}$) in shadow for 1 wk. The dried leaves were then powdered and stored in sealed bags at 4°C until use. The ground powders were hydrodistilled using modified Clevenger-type apparatus and extracted with water for 4 h. Each hydrodistillation process was conducted using 50 g of ground powder and 600 ml of distilled water. Anhydrous sodium sulfate was used to remove water after extraction. The extracted oils were kept at 4°C and used in the experiments. The oil yield (1% w/w) was calculated on a dry weight basis.

Fumigant Toxicity Bioassay

The fumigant toxicity was studied to determine the median lethal concentrations of *A. khorassanica* and *A. sieberi* against adult of *S. cerealella*. After preliminary tests, the concentrations 12, 10.52, 9.16, 7.96, 6.96, and 6 $\mu\text{l/liter}$ air for *A. khorassanica* and *A. sieberi* were calculated to apply as appropriate concentrations against adult of *S. cerealella*. Whatman filter papers (No. 1, diameter 2.0 cm) were impregnated with different concentrations, and then they were placed on the bottom of the screw cap of a glass vial (250 ml). Then, the caps were screwed onto the bottle, each of which included 15 adults (<24-h old). All treatments were then transferred to the incubator set at $27 \pm 2^\circ\text{C}$, relative humidity of $65 \pm 5\%$, and a photoperiod of 14:10 (L:D) h for 24 h. Control insects were kept under same conditions except for exposure to the essential oils. The number of dead insects was counted 24 h after exposure to the essential oils. Each concentration was replicated three times, and 15 adults were used in each concentration and control. The bioassay experiments were repeated three times.

To assay LT_{50} values, the highest concentration (12 $\mu\text{l/liter}$ air) of *A. khorassanica* and *A. sieberi* essential oils was used against *S. cerealella* (Izadmehri et al. 2013). The LT_{50} assay was carried out in vials and conditions described above. The mortality of *S. cerealella* was checked at 6-h intervals. The control insects received all the conditions except for exposure to the essential oils. The experiments were performed in three replicates.

Effects of Essential Oils Low Concentration on Life Table Parameters

To assay the sublethal effects of *A. khorassanica* and *A. sieberi* essential oils on the life table parameters of *S. cerealella*, the LC_{30} were used because it is the mortality threshold (30%) suggested for insecticides use in integrated pest management (Desneux et al. 2006). The

number of 110 newly mated adults (<24-h old) was exposed to the LC_{30} of *A. khorassanica* (6.34 $\mu\text{l/liter}$ air) and *A. sieberi* (9.24 $\mu\text{l/liter}$ air) using the exposure system described in the section 'Fumigant toxicity bioassay' for 24 h. After exposure, 25 pairs of adults (male and female) were singly transferred into the clean oviposition containers (5 \times 10 cm [diameter by depth]). Honey solution (10%) smeared on a cotton was provided for the adults' feeding.

The oviposition containers were then inversely placed on the paper sheets (as an oviposition surface) and the paper sheets were replaced daily with new ones. The number of eggs laid was recorded every 24 h until the adult moths died, and recorded data were used for calculating life history and life table parameters. The developmental time, adult longevity, and fecundity (25 replicates) were recorded until the death of the last moth.

Data Analysis

The result of each trial was tested for curve fit using PROC GENMOD procedures (SAS Institute 2002, Robertson et al. 2007), and the data were analyzed using PROC PROBIT (SAS Institute 2002) to determine lethal concentrations (LC_{30} , LC_{50} , and LC_{90} , values) on standard and log scales with associated 95 % fiducial limits. In addition, mortality of *S. cerealella* exposed to different concentrations of the tested essential oils was analyzed by one-way analysis of variance (ANOVA). If significant differences were detected, the means were separated at $\alpha = 0.05$ by least significant difference (LSD) test. The data of LT_{50} were analyzed using SAS v. 9.2 program (PROC GLM, SAS Institute 2002) as mentioned for LC_{50} . Resulting data were analyzed based on the age-stage, two-sex life table model developed by Chi and Liu (1985) and Chi (1988). The age-stage-specific survival rate (s_{xj}) (where x = age in days and j = stage [stage 1 = egg, stage 2 = larva-pupa, stage 3 = adult female, stage 4 = adult male]) and the age-stage-specific fecundity (f_{xj}) (daily number of eggs produced per female of age x) were calculated using the iterative bisection method described by Chi and Liu (1985). Bootstrap method was used to estimate the means, variances, and SEs of the population parameters (Efron and Tibshirani 1993). The obtained data were then analyzed by one-way ANOVA followed by comparison of the means with LSD and t -test at $\alpha = 0.05$ using statistical software SAS version 9.2.

Results

Fumigant Toxicity Bioassay

The fumigant toxicity of essential oils from *A. khorassanica* and *A. sieberi* against adult of *S. cerealella* is shown in Table 1. The essential oil of *A. khorassanica* (LC_{50} : 7.38 $\mu\text{l/liter}$ air) caused higher mortality than *A. sieberi* (LC_{50} : 9.26 $\mu\text{l/liter}$ air) against adults of *S. cerealella*. The percentage mortality of *S. cerealella* exposed to different concentrations of tested essential oils is given in Table 2. As expected, the highest and lowest mortality of *S. cerealella* adults belong to the highest and lowest concentrations of *A. khorassanica* and *A. sieberi* essential oils. The percentage mortality of *S. cerealella* by different concentrations of *A. khorassanica* ($F = 38.27$; $df = 5, 17$; $P < 0.0001$) and *A. sieberi* ($F = 23.37$; $df = 5, 17$; $P < 0.0001$) essential oils was significantly different. However, the percentage mortality of *S. cerealella* by *A. khorassanica* and *A. sieberi* essential oils was not significantly different at 12 ($F = 6.12$; $df = 1, 5$; $P = 0.068$) and 10.52 ($F = 6.12$; $df = 1, 5$; $P = 0.068$) $\mu\text{l/liter}$ air concentrations (Table 2).

The LT_{50} values of *A. khorassanica* and *A. sieberi* essential oils on *S. cerealella* are listed in Table 3. The results indicated that the insecticidal effects of *A. khorassanica* oil (LT_{50} : 9.01 h) were faster than *A. sieberi* (LT_{50} : 14.37 h).

Table 1. Fumigant toxicity of *Artemisia khorassanica* and *Artemisia sieberi* essential oils against the adult stage of *Sitotroga cerealella*

Essential oil	n^a	χ^2	Slope \pm SE	Lethal concentrations ($\mu\text{L/liter air}$)		
				LC ₃₀ (95% FL)	LC ₅₀ (95% FL)	LC ₉₀ (95% FL)
<i>A. khorassanica</i>	315	31.49	4.86 \pm 0.87	5.76 (4.61–6.48)	7.38 (6.58–8.00)	13.54 (11.74–17.85)
<i>A. sieberi</i>	315	34.61	5.28 \pm 0.91	7.37 (6.50–7.98)	9.26 (8.60–10.11)	16.20 (13.72–22.27)

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 2002).

^aThe total number of adult moths used for bioassay.

Table 2. Mean (\pm SE) percentage mortality of *S. cerealella* exposed to different concentrations of *A. khorassanica* and *A. sieberi* essential oils

Concentration ($\mu\text{L/liter air}$)	Treatment	
	<i>A. khorassanica</i>	<i>A. sieberi</i>
12	91.11 \pm 2.22 aA	75.51 \pm 5.87 aA
10.52	75.56 \pm 4.44 bA	68.90 \pm 5.87 aA
9.16	64.44 \pm 2.23 cA	42.22 \pm 2.18 bB
7.96	55.56 \pm 2.14 cdA	35.56 \pm 4.19 bcB
6.96	48.89 \pm 2.24 deA	31.11 \pm 2.20 bcB
6	40.01 \pm 3.84 eA	22.22 \pm 2.21cB

Mean values in a column followed by different lowercase letters (LSD test, $P < 0.05$) and in a row followed by different uppercase letters (t -test, $P < 0.05$) are significantly different.

Effects of Essential Oils Low Concentration on Life Table Parameters

A significant increase was observed in egg incubation, larval–pupal period, and developmental time in treated insects when compared with the control. Moreover, adult life history parameters, such as female longevity, male longevity, fecundity, and oviposition period, were significantly reduced in treated insects when compared with the control (Table 4).

The age-stage-specific survival rate (s_{xj}) of *S. cerealella* exposed to tested essential oils is shown in Fig. 1. Noticeable stage overlapping was observed because of variable development rates among the treatments. The highest s_{xj} of larva–pupa, adult male, and female of *S. cerealella* was in the control insects (Fig. 1). The age-stage-specific fecundity (f_{x3}) gives daily number of offspring produced by *S. cerealella* individual at age x and stage j . As only females produce offspring, there is only a single-curve f_{x3} (i.e., the adult female is the third life stage). The maximum f_{x3} in adults treated with *A. khorassanica* and *A. sieberi* oils, and control was 9.82, 10.21, and 9.42 eggs female⁻¹ d⁻¹, respectively, that occurred at the ages of 31, 30, and 31 d, respectively (Fig. 2).

The results of sublethal effects of *A. khorassanica* and *A. sieberi* essential oils on the two-sex life table attributes of *S. cerealella* are listed in Table 5. The sublethal concentration of both tested essential oils caused a significant reduction in gross reproductive rate, net reproductive rate (R_0), intrinsic rate of increase (r_m), and finite rate of increase (λ). However, sublethal concentrations of *A. sieberi* essential oil caused significant increase in generation time (T) of *S. cerealella*.

Discussion

Plant essential oils have a high volatility and could be used as fumigants to control stored products insects. Because of low toxicity on mammals and nontarget species and no detrimental effects on

environment, plant essential oils are a suitable alternative for chemical insecticides. The essential oils of *A. khorassanica* and *A. sieberi* were rich in monoterpenoids and esters. For example, camphor and 1,8-cineole were reported as major constituents of *A. khorassanica* and *A. sieberi* essential oils (Borzoui et al. 2016, Nouri-Ganbalani and Borzoui 2017), and could be used as new natural fumigants in controlling stored product insects (Obeng-Ofori et al. 1997, 1998; Lee et al. 2004; Rozman et al. 2007; Abdelgaleil et al. 2009; Wang et al. 2009). The mortality effects of plant essential oils are highly dependent to their constituents (Ahn et al. 1998; Lee et al. 2002a,b). In the earlier studies, the high mortality effects of *Artemisia* species were reported against different stored products insects (Kordali et al. 2006, Wang et al. 2006, Negahban et al. 2007, Borzoui et al. 2016).

The results of LC₅₀ values showed higher toxicity of *A. khorassanica* essential oil than *A. sieberi*. Similar results were obtained by Borzoui et al. (2016), who found that *A. khorassanica* (LC₅₀: 9.6 $\mu\text{L/liter air}$) essential oil had more insecticidal effect than *Vitex pseudo-negundo* (Hauskn) (LC₅₀: 23.05 $\mu\text{L/liter air}$) against *Plodia interpunctella* Hübner. By comparing the LC₅₀ values, toxicity of *A. khorassanica* essential oil (LC₅₀: 7.38 $\mu\text{L/liter air}$) on *S. cerealella*, in this study, was higher than that reported for *P. interpunctella* (LC₅₀: 9.60 $\mu\text{L/liter air}$) (Borzoui et al. 2016) and *Tribolium confusum* Jacquelin du Val (LC₅₀: 22.45 $\mu\text{L/liter air}$) (Saeidi and Moharramipour 2013). Differences in the tested insect species and in the experimental conditions could explain such inconsistency. Moreover, fumigant toxicity of *A. sieberi* essential oil (LC₅₀: 9.26 $\mu\text{L/liter air}$), in this study, was higher than that reported for *Tribolium castaneum* Herbst (LC₅₀: 16.76 $\mu\text{L/liter}$) (Negahban et al. 2007) and *Trogoderma granarium* Everts (LC₅₀: 33.50 $\mu\text{L/liter}$) (Nouri-Ganbalani and Borzoui 2017), suggesting that the beetles such as *T. castaneum* and *T. granarium* were more tolerant than Lepidoptera species to *A. sieberi* essential oil.

The higher concentration of the essential oils, in this study, had much more mortality effects than lower concentration against *S. cerealella*. This finding was confirmed by Kordali et al. (2006), who found a high toxicity (80–90%) of *Artemisia* species against *Sitophilus granarius* L. (Coleoptera: Curculionidae) at a dose of 9 $\mu\text{L/liter air}$ after 48 h of exposure. Consistent with the fumigant toxicity test, the LT₅₀ evaluations revealed that the insecticidal effect of *A. khorassanica* oil was much faster than *A. sieberi*. These results are in agreement with the findings of Saeidi and Moharramipour (2013), who reported the LT₅₀ of 9.63 h for *A. khorassanica* against *T. confusum*.

Studies of sublethal effects of pesticides could provide more useful knowledge than lethal effects on bioactivity of pesticides on the insects. Demographic toxicology is an important tool for accurate assessment of the total effects of an insecticidal compound (Abedi et al. 2014). In this study, sublethal effects of *A. khorassanica* and *A. sieberi* essential oils were detected on life history and life table parameters of *S. cerealella*. In agreement with the findings of Izakmehri et al. (2013) and Borzoui et al. (2016), significant

Table 3. LT_{30} , LT_{50} and LT_{90} values of *A. khorassanica* and *A. sieberi* essential oils against the adult stage of *S. cerealella*

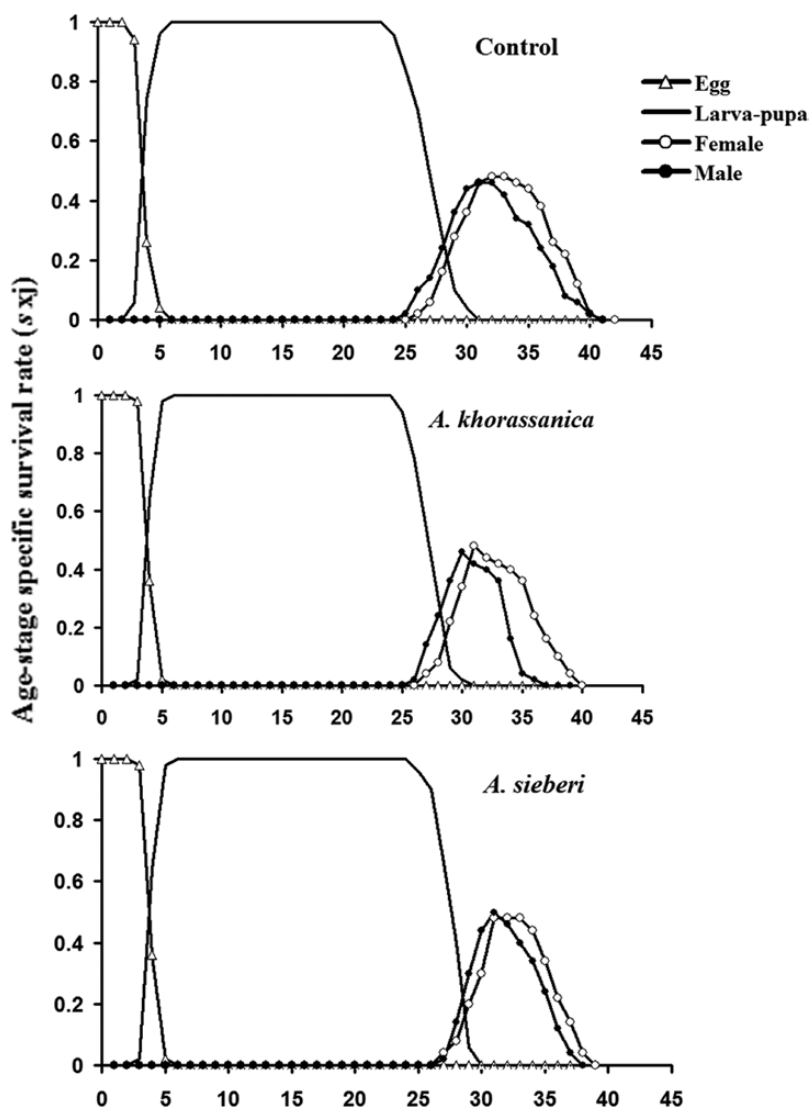
Essential oil	Concentration ($\mu\text{L/liter air}$)	χ^2	Slope \pm SE	Lethal times (h)		
				LT_{30} (95% FL)	LT_{50} (95% FL)	LT_{90} (95% FL)
<i>A. khorassanica</i>	12	65.91	3.43 ± 0.42	6.34 (4.95–7.51)	9.01 (7.63–10.28)	21.29 (18.14–26.83)
<i>A. sieberi</i>	12	45.60	3.20 ± 0.47	9.24 (7.06–10.94)	14.37 (12.32–16.80)	42.28 (31.57–72.05)

Lethal times and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 2002).

Table 4. Sublethal effects (LC_{50}) of *A. khorassanica* and *A. sieberi* essential oils on life history of *S. cerealella*

Parameter (mean \pm SE)	Treatment			Statistics of ANOVA		
	<i>A. khorassanica</i> (5.76 $\mu\text{L/liter air}$)	<i>A. Sieberi</i> (7.37 $\mu\text{L/liter air}$)	Control	F	df	P
Egg incubation (d)	4.86 ± 0.12 a	4.58 ± 0.09 b	4.24 ± 0.089 c	10.22	2, 149	<0.0001
Larval and pupal period (d)	24.24 ± 0.18 a	23.96 ± 0.15 a	23.16 ± 0.24 b	8.75	2, 149	<0.0001
Developmental time (d)	29.10 ± 0.22 a	28.54 ± 0.17 a	27.40 ± 0.25 b	16.67	2, 149	<0.0001
Female longevity (d)	6.64 ± 0.31 b	6.48 ± 0.28 b	8.4 ± 0.31 a	12.93	2, 74	<0.0001
Male longevity (d)	5.24 ± 0.22 c	6.01 ± 0.26 b	7.76 ± 0.32 a	24.04	2, 74	<0.0001
Fecundity (eggs laid)	37.48 ± 2.49 b	43.60 ± 1.95 b	58.36 ± 2.96 a	18.47	2, 74	<0.0001
Oviposition period (d)	4.01 ± 0.26 c	4.80 ± 0.24 b	6.12 ± 0.21 a	22.76	2, 74	<0.0001

Mean values in a row followed by different lowercase letters are significantly different (LSD test).

**Fig. 1.** Age-stage-specific survival rate (s_{xj}) of *Sitotroga cerealella* after exposure to LC_{50} of *Artemisia khorassanica* and *Artemisia sieberi* essential oils.

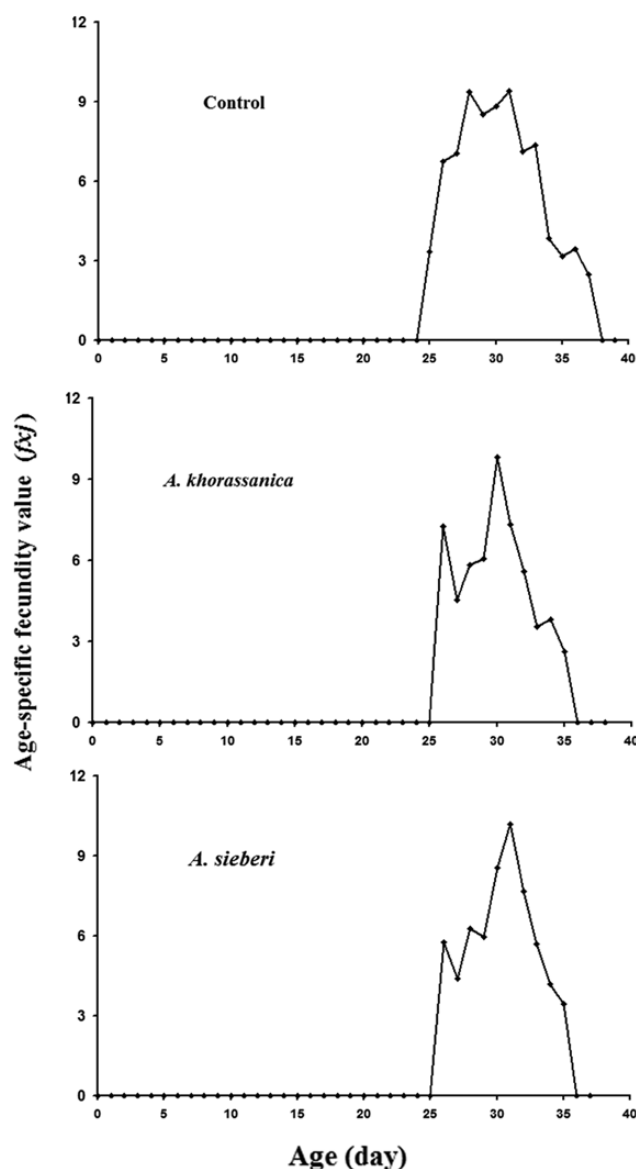


Fig. 2. Age-stage-specific fecundity (f_{xj}) of *Sitotroga cerealella* after exposure to LC_{30} of *Artemisia khorassanica* and *Artemisia sieberi* essential oils.

Table 5. Sublethal effects (LC_{30}) of *A. khorassanica* and *A. sieberi* essential oils on two-sex life table parameters of *S. cerealella*

Parameter (mean \pm SE)	Treatment			Statistics of ANOVA		
	<i>A. khorassanica</i> (5.76 μ L/liter air)	<i>A. sieberi</i> (7.37 μ L/ liter air)	Control	<i>F</i>	<i>df</i>	<i>P</i>
Gross reproductive rate (offspring)	26.22 \pm 0.172 b	26.23 \pm 0.187 b	34.36 \pm 0.241 a	539.84	2, 1499	<0.0001
Net reproductive rate (R_0) (offspring)	18.81 \pm 0.129 c	21.84 \pm 0.148 b	28.91 \pm 0.202 a	1015.34	2, 1499	<0.0001
Intrinsic rate of increase (r_m) (d^{-1})	0.094 \pm 0.0028 c	0.098 \pm 0.0022 b	0.107 \pm 0.0024 a	914.65	2, 1499	<0.0001
Finite rate of increase (λ) (d^{-1})	1.098 \pm 0.0018 c	1.103 \pm 0.0025 b	1.113 \pm 0.0014 a	918.32	2, 1499	<0.0001
Generation time (<i>T</i>) (d)	31.089 \pm 0.0149 c	31.375 \pm 0.0126 a	31.203 \pm 0.016 b	97.49	2, 1499	<0.0001

Mean values in a row followed by different lowercase letters are significantly different (LSD test).

extensions observed in the developmental time of treated *S. cerealella* could lengthen the exposing time to the essential oils before mating and producing next generation. In consistent with Borzoui et al. (2016), a significant reduction in the adult's longevity and oviposition period of *S. cerealella* treated with tested oils could decrease number of eggs laid, and eventually could negatively affect

the population of the next generation. In addition, the fecundity of *S. cerealella* treated with *A. khorassanica* and *A. sieberi* essential oils reduced by 35.78 and 25.29%, respectively. As fecundity plays an important role in population of the next generation, its reduction could suppress the population increase of the insects. These results are in agreement with those reported by Izakmehri et al. (2013),

who expressed that exposure to *Eucalyptus camaldulensis* Dehnh. and *Heracleum persicum* Desf. essential oils reduced fecundity of *Callosobruchus maculatus* (Fabricius).

The life table parameters, especially the intrinsic rate of increase (r_m), are the most useful parameters to evaluate the population growth potential of insect species (Southwood 1966, Ricklefs and Miller 2000). The lower r_m value in *S. cerealella* treated with tested essential oils than the control is mainly attributed to the lower survivorship, longer developmental time of immature stages, and lower fecundity of the pest. Reduction in this parameter can decrease the speed of population growth of *S. cerealella*. Similar results were reported by Borzoui et al. (2016) for *P. interpunctella* exposed to *A. khorassanica* and *V. pseudo-negundo* essential oils.

In conclusion, both examined essential oils showed high lethal and sublethal effects on *S. cerealella*. However, more studies are needed to improve our knowledge about human, environmental, and nontarget animals' safety of these essential oils. For the future, it is necessary to evaluate the impacts of these essential oils on *S. cerealella* under storage systems.

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