LARVICIDAL POTENTIAL OF THREE EXTRACTS FUMIGANTS OF FERULA ASSA-FOETIDA L OLEO GUM RESIN AS STORED GRAIN FUMIGANTS AGAINST TROGODERMA GRANARIUM EVERTS

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ABSTRACT

The 5th instar larvae of Trogodermagranarium Everts were exposed to various concentrations (2,4,6 and 8 $\mu\text{l}/\text{cm}^3$) of fumigant of Ferula assafoetida L gum resin in petroleum ether, diethyl and ethanol extracts. Highest mortality rates were recorded with ethanol, diethyl and petroleum ether at the lowest concentration of 2 $\mu\text{l}/\text{cm}^3$, namely, 70%,40%, and 10%, respectively, after 48 hours of exposure, with a strong repellent effect by all extracts starting with the first hours of fumigation.

KEYWORDS: *Ferula assa-foetida, Insecticidal Activity & Hormonal Disturbance*

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INTRODUCTION

Medicinal plants have traditionally been used for pest control of many field crops, and stored products(Rani 2012; Shaaya et al. 1997) are one of the most important sources of human food, which form the most important strategies for countries to maintain the stability of their people. Therefore, stored crops must be preserved from damage and harmful pests, which lead to large economic losses that are difficult to compensate. *Trogodermagranarium*Everts is considered being a stored grain pest that affects the nutritional and agricultural value of stored products because it feeds on all kinds of grains, seeds, animal food and dried fruits (Dale 1996; EPPO 2011; Honey et al. 2017). Moreover, it is considered a secondary harmful pest of stored grains and food products(Karnavar1972; Musa and Dike2009; OEPP/EPPO 1981). Larvae of *T. granarium*feed voraciously on grains and can decrease the quantity and quality of stored grains by up to 70% in a short time (Honey et al. 2017).

The use of chemical pesticides, especially methyl bromide and phosphine fumigants, is the most common method of controlling this pest in the world(Bell et al. 1985; Ogendo et al. 2004). Moreover, they can be sterilized by gamma radiation, although this method is not available in many countries in Asia or on traditional farms (Ibrahim et al. 2017; Mansour 2016). The dangerous subsequent effects of chemical insecticides on the environment, animals, plants and modification of pest resistance(Benbrook 2016; Binetti et al. 2008; Carvalho 2017)are known.

Some traditional medicinal plants have demonstrated insecticidal activity, including toxicity, repellency, antifeedant, molting disorder, prevention of egg-laying, egg-killing and forced entry to diapause for the larvae, for example:

Azadirachtaindica L, *Cinnamomumcamphora* L, *Menthapiperita*L, *Artemisia sieberi*Besser, *Ocimumbasilicum* L, *Derris elliptica* (Wall.), *Chrysanthemum cinerariifolium* and *Ferula assa-foetida*L(Zoubiriand Baaliouamer 2014; Khelfane-Goucem et al. 2016; Nerio et al. 2010; Rajendran and Sriranjini 2008;Abdelgaleil et al. 2008; Trigg and Hill

1996; Tripathi et al. 2001; Zia-Ul-Haq et al. 2012).

These spontaneous observations have been used as a guide and research has been launched since the 20th century to discover safe alternatives to preserve and conserve human agricultural wealth. The objective of this study was to test the efficiency of diethyl, ethanol, and petroleum ether extracts of *Ferula assafoetida* L oleo gum resin on the 5th instar larvae of the *Trogoderma granarium* beetle. *Ferula assafoetida* L belongs to the Apiaceae family and is widely used in Saudi Arabia (Alqasoumi 2012; Alshammari 2016) and Asia (El-Razek et al. 2001; Zia-Ul-Haq et al. 2012), commonly as a traditional medicine and spice. Other members of the Apiaceae family are also known as excellent sources of insecticidal properties (Ebadollahi 2013; Muto-Fujita et al. 2017; Tamokou et al. 2017).

METHODS

Insects

T. granarium adults and larvae were obtained from laboratory culture on wheat and oats maintained in the laboratory of College of Science and Humanities, Sattam Bin Abdul-Aziz University. One hundred pairs of 1-day-old adults were placed in a jar containing 4 kg of wheat and oats with 1g of yeast to provide additional nutrition at $28 \pm 2^\circ$ and $60 \pm 5^\circ$ relative humidity. After 10 days, the parental stocks were removed, and fresh wheat and oats were inoculated using the remains of the first culture, which contained the eggs laid. All new culture jars were closed with rubber bands.

Test Materials

Fresh (*Ferula assafoetida* L: Apiaceae) oleo gum resin or latex was obtained from the roots of plants purchased from the medicinal plant's markets in Alkharj City. These were washed and dried in the shade for six months and then ground by a high-voltage electric mill into a very fine powder.

Preparation of Extracts

To obtain the crude extraction of *Ferula assafoetida* L oleo gum resin, 100% petroleum ether, diethyl and ethanol extracts were used as a solvent and 500 g of oleo gum resin powder sample was soaked in 750 ml of each solvent and kept in the lab for 5 days with continuous manual stirring of the extract. The extract was then filtered by using a rotary evaporator.

Bioassays

Fumigation chambers consisted of small diet cups (4cm diameter x 4cm) with 10 of the 5th instar larvae introduced into each cup and covered with a nylon 60 mesh cloth in the bottom of a polyethylene cup (5.0cm diameter x 10cm). Filter paper (Whitman No.1, 10cm³) treated with each of the three previously dissolved test extracts in the solvent (100 μ l), was placed inside the polyethylene cup (containing a diet cup which has the larvae) to prevent direct contact of the larvae with the test extracts. Controls were prepared using 100 μ l of petroleum ether, diethyl, and ethanol for each test extract. Assays were conducted at the $28 \pm 2^\circ$ temperature and $60 \pm 5^\circ$ relative humidity. The exposure times were 24, 48 and 72 hours. Six replicates per treatment and 10 larvae in each replicate were used. The dead insects were counted and removed from the fumigant cup with a paintbrush.

Solutions of crude methanol extract at 2%, 4%, 6% and 8% concentrations were prepared by dissolving 0.2, 0.4, 0.6 and 0.8 μ of the crude extract in 0.8, 0.6, 0.4 and 0.2 μ of the solvent Asrar et al. (2016).

Data Analysis

Data analysis was done according to the Abbott(1925) formula. The mortality counts were corrected for control mortality. All experimental data were submitted to one-way ANOVA to determine between samples by SPSS statistical analysis (version 23) (Bluman 2007). The means were compared using the least significant difference (L.S.D) at the 0.10 level after 72 hr of exposure.

RESULTS

Table 1 compares the Fumigant toxicity of ethanol, petroleum and diethyl extract of *Ferula assa-foetida* oleo gum resin on the 5th instar larvae of *Trogoderma granarium*.

Generally, the highest toxicity was demonstrated after 72 hours of exposure to all the treatments. vapours of the ethanol extract showed high toxic activity on the larvae treated, with mortality 70% after 48 hours post -treatment at the lowest test concentration (2 $\mu\text{l}/\text{cm}^3$),

while the mortality was ranged in 10 % and 13.3% after 24 hours with concentrations 2 $\mu\text{l}/\text{cm}^3$ and 6 $\mu\text{l}/\text{cm}^3$ of the ethanol extract, In contrast, the two concentrations 6 $\mu\text{l}/\text{cm}^3$ and 8 $\mu\text{l}/\text{cm}^3$ for petroleum ether extract caused mortality from 20% to 30% after 48 hours. It is interesting that the low concentrations 2 $\mu\text{l}/\text{cm}^3$ and 4 $\mu\text{l}/\text{cm}^3$ were more toxic and caused mortality from 40% to 36.7% after the same period for the two concentrations of the petroleum ether extract, respectively. As well as concentration 6 $\mu\text{l}/\text{cm}^3$ was present mortality at 50% after 72 hours of exposed compare 43.3% mortality by the high test concentration 8 $\mu\text{l}/\text{cm}^3$.

As for the diethyl extract, the fumigants of the tested concentrations had an immediate effect on the larvae, especially the high concentrations 6 $\mu\text{l}/\text{cm}^3$ and 8 $\mu\text{l}/\text{cm}^3$ which caused a mortality of 53.3% and 46.7% respectively after 72 hours of exposure.

All of the tested extracts showed a strong direct repellent effect from the first hours of treatment, evidenced by attempted larval escape from the wheat grains provided to them in the fumigant containers.

All treatments caused shape deformities represented by complete blackening of the larval body wall.

Behavioral changes occurred in the larvae treated with all of the tested extract vapors they began to appear results and reject food in the early hours of exposure.

As the exposure time progressed, behavioral changes varied from convulsion, twisting, trouble walking and lying on the dorsal area.

In addition, some larvae exposed to diethyl extract vapor molted after 72 hours of exposure at a concentration of 8 $\mu\text{l}/\text{cm}^3$. The larvae were pupated although they had spent only 3 days as 5th instar larvae. It is likely that they died in the pupation stage, which exhibited blackening of the body wall and weakness before their pupation. It is noteworthy that no behavioral or morphological changes were observed in the untreated larvae in the control experiment. Control larvae completed the duration of the 5th instar and pupated after 7 days. This period agrees with the previous findings using conditions of $28\pm 2^\circ$ and $60\pm 5\%$ humidity with the specified period (Hagstrum and Subramanyam 2009).

DISCUSSIONS

Fumigation tests of the ethanol, diethyl, and petroleum ether extracts of *Ferula assa-foetida* oleo gum resin

showed toxic activity on the 5th instar larvae of *Trogoderma granarium* beetles from the first hours of exposure; Rafi (2002) mentioned the insecticidal property of *F. assa-foetida* oleo gum resin extract on *Tribolium castaneum*.

Peyrovi et al. (2011) confirmed the toxicity of the essential oil from *F. assa-foetida* on the adult *Ectomyeloiscera toniaecarab* moth.

There is no doubt that the chemical components of plants differ in their biological effects and resistance thereto by living organisms, whether insects, fungi, bacteria or animals (Adnan et al. 2014; Grdiša and Gršić 2013; Khan et al. 2014; Showler 2017; Tunon et al. 2006).

It is known that each of the polar alcoholic extracts dissolves precise chemical compounds (Altemimi et al. 2017). Presumably, that is the reason for the variety of effects between the three tests extracts on the treated 5th instar larvae.

Guenther 1972; Shankaranarayana et al. 1982; Khajeh et al. 2005; El-Razek et al. 2001; Karr et al. 1990; Iranshahy et al. 2011; Khan et al. 2014; Alshammari 2016; Amalraj and Gopi 2017 reported that the basic biologically active compounds of *F. assa-foetida* oleo gum resin that have insecticidal activity monoterpenes, sesquiterpenes, and oxygenated compounds: phenols, polysulfides, limonene, flavonoids, limonene, polyphenolic, and myrcene.

Celestino et al. 2013 indicated that the polysulfide compounds are responsible for the plant's strong odor and bitter taste.

The original biological nature of *F. assa-foetida* oleo gum resin is due to secondary metabolic compounds of the plant (Khajeh et al. 2005) which make it rich in natural chemical defenses known to protect the plants from natural enemies such as plant pathogens and insects (Li-Byarlay et al. 2016; War et al. 2012). Perhaps the various biological compounds are precisely the secrets of the *F. assa-foetida* oleo gum resin commonly used in traditional medicine as an antifungal (Kamble and Patil 2008), antibacterial (Shrivastava et al. 2012), insect repellent and insecticide (Kavianpour et al. 2014) and anti-cancer (Bagheri et al. 2017).

The findings that *F. assa-foetida* oleo gum resin possesses the abovementioned compounds can explain the results of this study, as according to Tunon et al. (2006) the oxygenated compounds have high repellent activities on the tick *Ixodes ricinus*.

(Lee et al. 2003; Richards 1978) confirmed the ability of monoterpenes and sesquiterpenes to transform the internal organs of susceptible insects, either through the body wall or respiratory spiracles or by influencing the air surrounding the insects and preventing the detection of food odors. As noted by Bisseleua et al. (2008), Rajendran et al. (2008), Rajendran et al. 2001, and Muto-Fujita et al. (2017), insects and their stages, such as other animals, have the ability to identify the food that suits them and can be disturbed by host odor Bisseleua et al. (2008).

Furthermore, *F. assa-foetida* oleo gum resin contains polysulfide compounds responsible for the strong offensive odor, which may be the primary reason that the larvae have been repelled from the food provided in the fumigant containers. The negative changes may have been caused by the entry of the test treatment fumigants of test extract to their internal organs.

The clarity of these effects increases with greater concentration and increased exposure time, in agreement with El-Nahal et al. (1994) who reported that the period of exposure appears to be more important than a dose of fumigant bioassays of *Acorus calamus* oil against of five stored product insect species.

This finding is not surprising as we know that *F. assa-foetida* oleo gum resin is commonly used in rice storage in the rural Indian countryside (Chaudhari 2013).

A number of researchers considered that the behavioural disorders that appear in insects or larvae treated by essential plant extracts are due to neurotoxicity inhibiting activity by lack of the receptor of the ACHE enzyme (Enan 2001) or inhibition of the neuro-hormone octopamine (Hikal et al. 2017; Jayakumar et al. 2017) or cytochrome P450 (De-Oliveira et al. 1997). However, (Chaubey 2012) considered that there was no relationship between inhibition of the ACHE enzyme and the toxic activity of the treatment and that the effect could be removed by removing the effector.

One of the strong indicators in this study which confirm the physiological effect caused by the tested extract vapors, and the diethyl extract in, particular, is the occurrence of frequent molting in addition to early pupation. These findings reflect a hormonal disturbance in the molting and juvenile hormones, which is clear in the 5th instar larvae in the early days of the treatment. A possible explanation is that the ethanol, diethyl, and petroleum ether extracts of *F. assa-foetida* gum resin play important roles in the arrest of some biochemical processes in larval development, such as the failed pupation mount followed by death, demonstrating IGRs potential. Moreover, the neurotoxic effect was evident by the confused movement.

Moreover, the antifungal property of *F. assa-foetida* oleo gum resin suggests new benefits of ethanol, diethyl, and petroleum ether extract fumigation of stored products to protect them from the high humidity and temperature problems in several regions of Asia, especially rural areas.

Sample (1992) reported that the active larvae stage, especially the last instars of most holo metabolous insects, is considered more susceptible compared to the diapause phases such as the pupa and eggs; this finding may be due to differences in the respiratory system and related to the metabolic process.

CONCLUSIONS

These studies provided valuable results for the first time on the effectiveness of ethanol, diethyl and petroleum ether extract fumigants of *F. assa-foetida* gum resin on 5th instar larvae and the feasibility of applying these extracts professionally to stored products, particularly since fumigation is the common way to control this pest. They are a valuable and safe alternative to pesticides, with their dangerous effects on humans, livestock, and the environment.

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Table 1: The Fumigant Toxicity of Ethanol, Petroleum and Diethyl Extract of *Ferula Assa-Foetida* Oleo Gum Resin on the 5th Instar Larvae of *Trogoderma Granarium* after 24, 48 and 72 Hours.

Concentration ($\mu\text{L/L cm}^3$)	Interval/Hours			*Mean $\pm$ S. E	F	95% Confidence for Mean	
	24	48	72			Lower	Upper
	%	%	%				
Day Ethyl					0.098		
0	0	0	0	0 $\pm$ 0.0		0	0
0.8	13.3	40	46.7	*10 $\pm$ 3		-3.14	21.91
0.6	13.3	33.3	53.3	*10 $\pm$ 2.64		-4.90	19.38
0.4	10	30	40	8 $\pm$ 3.46		-3.38	24.90
0.2	10	40	40	9 $\pm$ 3.05		-3.91	23.14
Ethanol					0.031		
0	0	0	0	0 $\pm$ 0.0		0	0
0.8	10	33.3	40	8.33 $\pm$ 2.72		-3.41	20.07
0.6	13.3	33.3	46.7	*9.33 $\pm$ 2.90		-3.17	21.84
0.4	13.3	40	46.7	*10 $\pm$ 3.05		-3.14	23.14
0.2	10	70	10	9 $\pm$ 6		-16.82	34.82
Table 1: Contd..							

Petroleum Ether							
0	0	0	0	0±0.0	0.354	0	0
0.8	10	30	43.3	*8.33±2.90		-4.17	20.84
0.6	13.3	30	50	*9.33±3.18		-4.35	23.01
0.4	10	20	36.7	6.67±2.33		-3.37	16.71
0.2	3.33	10	40	5.33±3.38		-9.22	19.89

*Means followed by the differ letters in each row are significantly different according to the (L.S.D) at P<0.10 after 72 hours.

*Mean percentage fumigant toxicity ± S. E for three replicates (n = 10).