Vol. 14(9), pp. 68-73, 15 June, 2019
DOI: 10.5897/SRE2019.6621
Article Number: 1E09CA961199
ISSN 1992-2248

http://www.academicjournals.org/SRE

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Full Length Research Paper

Effectiveness of using oils extracts of *Peganum harmala* and *Rhanterium epapposum* against Khapra beetle *(Coleoptera*: *Dermestidae*) and their chemical compositions

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Received 10 April, 2019; Accepted 30 May, 2019

Essential oils extracted from two medicinal plants *Peganum harmala and Rhanterium epapposum* native to the Arabian Peninsula specially Saudi Arabia, were evaluated for their larvicidal effects and emergence of Khapra beetle (*Trogoderma granarium)* adults. The oil analyzed by thin layer chromatography, the results of phytochemical analysis indicated that the presence of Alkaloids, flavonoids Triterpenes, Cumarins and Tannins in the two ethanolic extracts. The most abundant compounds were alkaloids and flavonoids. The essential oil of *P. harmala* showed toxicity against *T. granarium* third instar larvae with equivalent LD50 values of 23.5 µg/ml, when fed on treated seeds, with significant mortality rates up to 66% in three days. Whereas the toxicity effect was 49.7 µg/ml against *T. granarium* third instar, larvae contact treated surface. The essential oil of *R. epapposum* resulted into higher toxicity with LD50 values of 22.3 µg/ml with significant mortality up to 70% in three days. Comparing total mortality percentages of *R. epapposum and P. harmala* ethanoic extract on different treatments gives a good insight about their bioactivity.

Key words: Insecticidal effect, oil extract, botanical insecticide, khapra beetle.

INTRODUCTION

Rice (*Oryza sativa* L.) continues to be the main source of food calories and proteins in several countries. Rice (*Oryza sativa* L.) attacked by various arthropods same as many other stored grain products causing quantitative and qualitative losses. Furthermore, food insect contamination represents a crucial problem for food industries and for export commodities (Rajendran et al., 2002). *T. granarium* (Everts) (*Coleoptera: Dermestidae*) known as khapra beetle is one of the most famous primary insect pest of stored grains. It is mostly found in tropical and subtropical regions of Asia and Africa (Viljoen, 1990). Khapra beetle is very common in geographical areas characterized by high temperature and low humidity (Ghanem and Shamma, 2007). Infestation of the khapra beetle is often followed by colonization of secondary insect pests and fungus that

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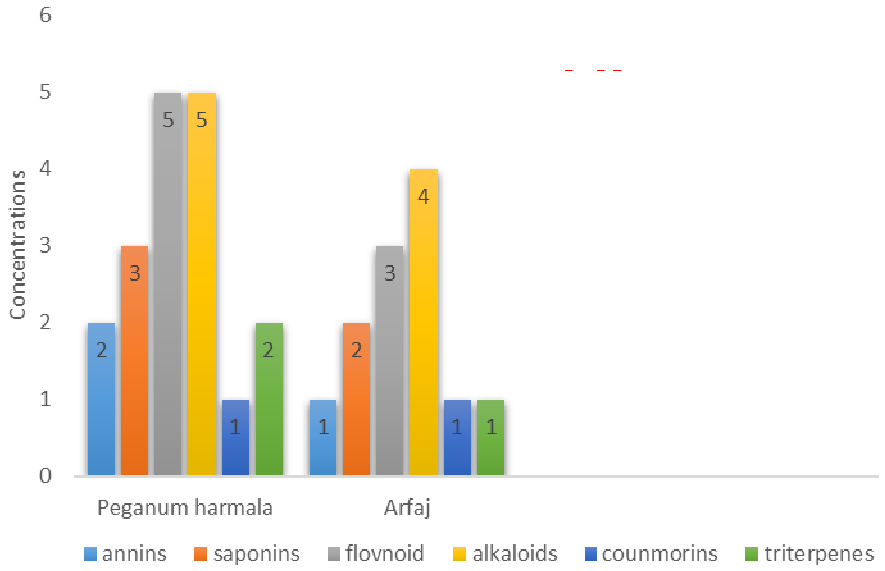

Figure 1. Phytochemical analysis of *Peganum harmala* and *Rhanterium epapposum*.

can leads to decline of quality and loss the weight of food grains (El Nadi et al., 2001). It has been reported that larvae consumed approximately 3 to 12 mg of food during their development and more food was consumed in constant darkness however, constant light accelerated development but reduced oviposition (Sohi and Upadhyay, 1986).

During the last decades, various chemicals have been widely applied as standard practice to control agricultural pests both in the field and in the postharvest processes. Generally, gaseous and liquid insecticides, mainly in phosphine and methyl bromide are commonly used for controlling the stored grain insect pest because they are cost effective (Islam et al., 2010). However, the constant use of these chemicals may cause selection of resistances to insecticides in agricultural pests, environmental pollution with negative side effects on human health and on non-target arthropods, which can ultimately leads to the disrupting of biological control (Desneux et al., 2007). Therefore, there has been increasing worldwide interest in the development of alternative and sustainable means for modern pest management strategies, including re-appraisal of plant derivate usage (Aliakbarpour et al., 2011).

All of these problems lead to encouraging more research to look for promising sources of environmentally safe and natural pesticide such as Neem, Garad, Jatropha, Fenugreek and Datura as an alternatives package of Integrated Pest Management (IPM) systems for controlling pests. The search for safe naturally occurring pesticides to control field crops and store pests has been intensified. The most promising are those derived from plants. Bio-pesticides represent one of the best alternatives to chemicals for the development of environmental-friendly and safe strategies for pest management (Copping and Menn, 2000). A number of investigations identified and screened a variety of promising chemical compounds from leaves and seeds of many botanical families, which found to be as insect feeding deterrents and growth inhibitors. These compounds have various physiological and behavioral effects on stored product insects, including toxic, repellant, and antifeeding properties (Isman, 2006). The insecticidal activity of various plant extracts and more broadly of some plant-derived substances (Botanicals) is well known and their use was maintained for thousands of years throughout all the agricultural regions of the world (Regnault-Roger et al., 2012).

MATERIAL AND METHODS

Collection of plant materials

The Arfag (*Rhanterium epapposum*) and Harmel (*Peganum harmala*) plants were collected from different locations of Majmaah area in Saudi Arabia in the early flowering period (March to April 2018) Figure 2 and 3. The fresh plant samples were air-dried in the shade for one week at environmental temperature (25 to 28°C) daytime at room until they were crisp Figure 1. The dried parts were mechanically grinded to powder by using an electric blender, and sieved through mesh size of 0.5 mm. The resulting fine powders were stored in glass jars until further uses.

Extraction of the plant oils

Samples of 50 g of Arfag and Harmel plants powder were extracted with organic solvent (Ethanol 80%) for about 72 hrs, using a soxhlet

Figure 2. The Arfag (*Rhanterium epapposum).*

Figure 3. Harmel *(Peganum harmala).*

apparatus. After extraction, the solvent removed by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remaining in the soxhlet thimble was discarded. The extracts were stored in the refrigerator under 4°C until needed for bioassay.

Phytochemical screening

The prepared organic solvent (ethanol) were subjected to phytochemical screening to tentatively identify the different chemical groups present in each Ethanoic extract, using thin layer chromatography.

Insect culture

A sample of *T. granarium* was collected from different stores in the area. The insects were kept separately together with whole rice grains in earthen pots, covered with a muslin cloth and secured with rubber bands. The insects reared to breed under laboratory conditions. To secure stock of larvae for the bioassay tests at room temperature.

Toxicity bioassay

The larva's bioassay tests of 80% ethanoic extract derived from (Arfag and Harmel powder) and control carried out. Four replications for each respective plant extract concentrations 40, 20, 10, and 5 µg/ml. Two methods of application were used.

The first method was contact toxicity using treated-filter paper bioassay by using the Impregnated filter paper described (Nenaah, 2011). Each test concentration was applied uniformly to a filter paper disc (Whatman No. 1, 9 cm diameter, 63.6 cm^2) the treated filter papers were left to dry at room temperature for 20 min. After evaporation of the solvent, each filter paper was confined to the bottom of a 9 cm diameter Petri dish.

The second method feeding toxicity by using treated seeds, each 10 g sound and clean Rice (*O. sativa* L.) seeds were treated with 1 ml of respective plant ethanolic extract concentrations, and left to dry for 30 minutes under room conditions. The treated seeds were introduced in the Petri dishes, including an ethanol as controls, where 10 third instar larval were introduced for each Petri dish containing treated-filter paper and treated seeds, while the control Petri dish were treated with ethanol only. All treatments were set up in four replicates along with control sets and arranged in a completely randomized design. After 3 days, the dead larvae were recorded and the survived larvae were kept for further 7 days to record the emerged adults. The data were statistically compared according to the completely randomized design and Duncan's Multiple Range test.

RESULTS

This experiments were conducted to check out the efficacy of two widely spread plants native to the deserts of the Arabian Peninsula specially Saudi Arabia where it is known locally as Arfag and Harmel against third instar larvae and emerged adults of *T. granarium*. The experiments were conducted using two different methods at diverse concentrations as presented in Table 1 to 4. The analysis of variance of larval mortality after 3 days and emerged adult after 10 day of feeding on treated seeds with different oil extracts concentrations of *R. epapposum* and *P. harmala* revealed highly significant variations ($P < 0.01$) Table 1. Conversely, the larval mortality from residual contact toxicity of *P. harmala* and *R. epapposum* showed known significant differences among the four different concentrations (40, 20, 10, and 5 µg/ml) compared to the untreated control (Table 2).

The feeding toxicity of larvae on residues of *R. epapposum* leaves ethanoic extract at 40 µg/ml concentration showed the best significant results 70% mortality compared with all other treatments after 7 days. While the lower concentrations 20, 10, 5 µg/ml shown 60, 50, and 40% mortality respectively. Further, the feeding toxicity of larvae from *P. harmala* ethanoic extract at 40 and 20 µg/ml concentrations gave significant mortality rates 66% and 63%, respectively, after 3 days of investigations, as compared with the control. However, the lower doses (10 µg/ml) of *P. harmala* also showed significant mortality effects 53 % compared with those of the control Table 1.

Table 1. Khapra beetle, 3rd instar larval mortality from feeding toxicity on treated Rize seeds (Method A).

Treatment	Concentration (µg/ml)	Mortality %	SD	LC50	LC90
Peganum harmala	40 µg/ml	66.7[a]	5.8	23.5	329.2
	20 µg/ml	63.3[a]	5.8		
	10 µg/ml	53.3[b]	15.3		
	5 µg/ml	30.0[c]	20		
	Control	23.3[d]	5.8		
Rhanterium epapposum	40 µg/ml	70.0[a]	0	22.3	591.7
	20 µg/ml	60.0[ab]	17.3		
	10 µg/ml	50.0[bc]	10		
	5 µg/ml	40.0[cd]	10		
	Control	23.3[d]	5.8		

Means in the same column followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan's Multiple Range test.

Table 2. Khapra beetle, 3rd instar larval mortality from contact toxicity using treated-filter paper bioassay (Method B).

Treatment	Concentration (µg/ml)	Mortality (%)	SD	LC50	LC90
Peganum harmala	40	56.7[a]	11.5	49.7	2559.8
	20	46.7[a]	25.2		
	10	53.3[a]	5.8		
	5	30.0[a]	10		
	Control	23.3[a]	5.8		
Rhanterium epapposum	40	73.3[a]	5.8	27.01	153.3
	20	56.7[a]	20.8		
	10	30.0[a]	36.1		
	5	30.0[a]	20		
	Control	23.3[a]	5.8		

Means in the same column followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan's Multiple Range test.

Table 3. Khapra beetle, emerged adults from feeding toxicity on treated Rize seeds.

Treatment	Concentration (µg/ml)	Emerged adults %	SD
Peganum harmala	40	0.0[c]	0
	20	6.7[c]	5.77
	10	6.7[c]	5.77
	5	16.7[a]	5.77
	Control	76.7[b]	5.77
Rhanterium epapposum	40	13.3[a]	5.77
	20	6.7[a]	5.77
	10	6.7[a]	5.77
	5	6.7[a]	11.55
	Control	76.7[b]	5.77

Means in the same column followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan's Multiple Range test.

Table 4. Khapra beetle, emerged adults from contact toxicity using treated-filter paper bioassay.

Treatment	Concentration (µg/ml)	Emerged adults (%)	SD
Peganum harmala	40	0.0[b]	0
	20	20.0[b]	0
	10	6.7[b]	5.77
	5	6.7[b]	5.77
	Control	55.7[a]	42.15
Rhanterium epapposum	40	26.7[a]	11.55
	20	20.0[a]	17.32
	10	20.0[a]	10
	5	23.3[a]	11.55
	Control	76.7[b]	5.77

Means in the same column followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan's Multiple Range test.

On the other hand, the emerged adults from larvae fed on *P. harmala* oil treated seed at 40 µg/ml concentration showed the best significant effect after 10 days resulting on 0% adults compared to control that have 76 % emerged adults. The emerged adults from larvae fed on *R. epapposum* oil treated seed at 40 µg/ml concentration also revealed significant effect 13% adults compared to control that have 76% emerged adults. The subsequent lower concentrations (20, 10 and 5 µg/ml) of *P. harmala* and *R. epapposum* gave lesser number of emerged adults with significant differences compared to untreated one Tables 3 and 4.

Overall, the results of testing the insecticidal effects of all ethanolic extracts of the two plants, at each four dosage rates (5, 10 and 20, 40) µg/ml, showed insecticidal activity indicating that the rate of insect mortality increases with an increase in concentrations and exposure time, conversely the number of emerged adults decreased by an increase in concentration Tables 3 and 4.

DISCUSSION

Botanical insecticides affect various insects in different ways depending on the physiological characteristics of the insect species as well as the type of the insecticidal plant. The components of various botanical insecticidal can be classified into different groups specifically; repellents, feeding deterrents, antifeedants, toxicants, growth retardants, chemosterilants and attractants (Rajashekar et al., 2012). From this study, the effects of *P. harmala and R. epapposum* ethanolic oils extracts confirmed toxic effects against larvae and the emerget adults of Khapra beetle (*T. granarium*). Since these plants showed some variable degrees of toxicity actions on the pest, hence the chances of getting lethal doses were ultimately increased with an increase in concentration. It seemed that the toxicity effects of tested plants on insects mainly through stomach action rather than contact action. Therefore, relatively longer time is required to induce their mortality effects.

The results showed that the residual contact toxicity of *P. harmala* ethanolic extract were not very effective against *T. granarium* after 10 day. These findings are in agreement with Salari et al. (2012), who reported that *P. harmala* acetonic extract was not very effective against *T. castaneum* after 3 days. While Jbilou (Jbilou et al., 2006) have reported that, the methanol extract from *P. harmala* seeds had a high insecticidal effect on *T. castaneum* after 32 days. This difference was probably due to the slow action of some plant extracts.

Considering the literature of botanical insecticides, different results were reported. In 2003, Abbassi (Abbassi et al., 2003) reported that the alkaloids extracted by ethanol from *P. harmala* leaves caused significant mortality of the desert locust, *(Schistocerca gregaria*, reduced female fecundity, as well as hatching rate when compared to the untreated control. Santos et al. (2016) found that *Tagetes erecta* and *Tagetes patula* have flavonoids compounds that can encourage and expand its use as a natural insecticide. (Pujiarti and Fentiyanti 2017) reported that *Eucalyptus deglupta* essential oils have repellent activity against *Culex quinquefasciatus* mosquito. (Santos et al., 2016) found that *T. erecta* and *T. patula* have flavonoids compounds that can encourage and expand its use as a natural insecticide.

Plant secondary natural products are natural chemicals extracted from plants and used as an excellent alternative to synthetic or chemical pesticides (Suthisut et al., 2011). The results of phytochemical analysis (Figure 1) indicated that the presence of Alkaloids, Flavonoids

Triterpenes, Cumarins and Tannins in the two ethanolic extracts of *P. harmala* and *R. epapposum*, while the Flavonoids have, the highest concentrations as compared to other components. Flavonoids are one of the most important groups of natural substances, which play an important role in the protection of plants against plant feeding insects' and herbivores (Acheuk and Doumandji-Mitiche, 2013).

Flavonoids could be useful in a pest-management strategy to protect the plant against insect pests by influencing their behavior, growth and development (Simmonds and Stevenson, 2001). The toxic effect of essential oils, other than the variability of phytochemical patterns, involves several other reasons. The entry point of the toxin, commonly, through inhalation, ingestion or absorption through the skin by insects (Regnault-Roger, 1997). Insects have shown to be very sensitive to feeding application toxicity assays with essential oils, based on the results of the feeding toxicity bioassay of larvae using *R. epapposum* and *P. harmala* ethanoic extract.

Conclusion

The *R. epapposum* and *P. harmala* ethanolic seed extract are effective and could be valuable for *T. granarium* control since it was capable of affecting the treated larvae through contact and oral toxicity. The potent insecticidal actions obtained by these plants are encouraging for additional research to incorporate such botanical extracts in integrated pest management programmes. Further evaluations needed to study bioassay of more concentrations at different exposure time, besides their phytochemical analysis through modern techniques to identified effects of each compound against *T. granarium* species and other pests.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors are grateful to the Deanship of Scientific Research at Majmaah University, Kingdom of Saudi Arabia for the financial support in carrying out this study (Project Number No. 102/38).

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