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Repellent effect of some aromatic plant extracts against granary weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae)

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Abstract
The study was conducted in the animal physiology laboratory of the Department of Biology, Ankara University. Leaves of six aromatic plants i.e. dill (*Anethum graveolens*), bay (*Laurus nobilis*), basil (*Ocimum basilicum*), fennel (*Foeniculum vulgare*), rosemary (*Rosmarinus officinalis*) and hibiscus (*Malve sylvestris*) were extracted using acetone, ethanol and water solvents. These extracts were evaluated for their repellent effect against *Sitophilus granarius* L. at 3.0, 6.0, 9.0, and 12.0 % concentrations. Three replications were used for each dose of all the plant extracts and examined in Petri dishes. Extracts of water solvent showed higher repellent effect than that of others expect ethanol extract of *R. officinalis*. Considering mean repellency rate extracts of three solvents of all six plants were in the same repellency i.e. class II expect water extract of *F. vulgare* (class III) and acetone extract of *A. graveolens* (class I). It was found that the rate of repellency increased with the increase of dose level. At 12.0 % dose level all plant extracts showed the highest repellency rate and were in repellency class III.

Keywords: aromatic plant extract, repellent, granary weevil, *Sitophilus granarius*

1. Introduction

The grain weevils (Curculionidae) are primary pests of cereal grains in storage including wheat, maize and rice. Infestation of stored grain with insects cause significant reduction in their quantity and quality. There are many insect species that infest grains in granaries especially the weevils of the genus *Sitophilus*, such as granary weevil (*Sitophilus granarius* L.), maize weevil (*Sitophilus zeamais* Motsch.) and rice weevil (*Sitophilus oryzae* L.) (Germinara *et al*., 2008; Belda and Riudavets, 2010) [7, 3]. Grain weevils are encountered in all temperate and warm temperate climates and are widely distributed in grain stores throughout Europe.

The adult granary weevil is a somewhat cylindrical beetle about two-tenths of an inch (two or three mm) long. The head is prolonged with a distinct snout extending downward from the head for a distance of about one-fourth the length of the body. The weevil is polished red brown to black with ridged wing-covers and a well-marked thorax with oval pits. Unlike the rice and maize weevils, the granary weevil cannot fly. The egg hatches in a few days into a soft, white, legless, freshly grub which feeds on the interior of the grain kernel. The grub changes to a naked white pupa and later emerges as an adult beetle.

Insects of grains in granaries are controlled mainly by direct application of contact insecticides, known as grain protectants. These compounds include many insecticides of the synthetic organophosphorus that might cause environmental pollution along with the presence of their residue in the grain, which pose health concerns to human (White and Leesch, 1995) [17]. At the same time, several insect pests have become resistant to most of commonly used synthetic organophosphorus, which someone increases the management cost. Also, the use of synthetic pesticides has resulted in toxic effects to living and non-target organisms (Askar *et al*., 2016) [2]. Thus, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants for the protection of grain products against insect infestations. There are increasing efforts to understand indigenous pest control strategies, with a view to reviving and modernizing their use (Shaaya *et al*., 1997; Belmain *et al*., 2001) [14, 4]. Plants are a rich source of novel insecticides (Dev and Koul, 1997) [6]. Plant materials with insecticidal properties have been used traditionally for generations throughout the world (Belmain *et al*., 2001) [4]. Botanical insecticides compared to synthetic ones may be safer for the environment, are generally, less expensive, easily processed and used by farmers and small industries. Since these insecticides are often active against a limited number of species, are often biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management, they could lead to the development of new classes of safer insect control agents (Kim *et al*., 2003) [8]. Many plant species, especially from tropical regions, have the potential to be used as botanical insecticide or as font of bioactive compounds (Quignard *et al*., 2003; Shaalan *et al*., 2005) [11, 13].

2. Materials and Methods

2.1 Insect species

Laboratory strain of wheat granary weevil *Sitophilus granarius* (Linneaus, 1758) was reared in the laboratory at 26 ± 2 ºC, 65 ± 5 % RH, and the natural photoperiod at Department of Biology, Faculty of Science, Ankara University, Ankara.

2.2 Rearing technique

The wheat granary weevil adults were obtained from the stock culture of the laboratory of the Plant Protection Department, Faculty of Agriculture, Ankara. In glass jars (1 L), insect of *S. granarius* was reared on wheat grains. About 300-400 unsexed adult insects were kept in 1000 ml glass jar containing 300 g of wheat grain. Weevils were allowed to oviposit for 7 d and then they were removed by sieving. The newly emerged weevils were returned to the stock cultures (Chaisaeng *et al*., 2010) [5]. After 1-2 weeks, newly emerged weevils were used for the experimental investigations. All experimental units such as stock cultures, medium cultures and vials were covered with pieces of muslin cloth fixed by rubber bands to prevent the insects from escaping and facilitate aeration (Ahmed, 1996) [1].

2.3 Plant preparation

Fresh leaves of *A. graveolens, L. nobilis, O. basilicum, F. vulgare, R. officinalis,* and *M. sylvestris* were collected kept in the shade for air-drying and then dried in the oven at 60 ºC. Dusts of dried leaves were prepared by using a grinder machine. Then the dusts were passed through a 25-mesh diameter sieve to obtain fine and uniform dust.

2.4 Extraction process

Ten grams of each unit of dust were taken in a 500 ml beaker separately and mixed with 100 ml of different solvents (acetone, ethanol and distilled water). Then the mixture was stirred for 30 min by magnetic stirrer at 6000 rpm and left stand for next 24 h. The mixture was then filtered through a fine cloth and again through filter paper (Whatman No. 1). The filtered materials were collected in round bottom flask and then condensed by evaporation of solvent in a water bath at 70 ºC, 50 ºC, and 40 ºC for water, ethanol and acetone extracts, respectively. Evaporation was done to make the volume of 10 ml and stored in a refrigerator.

2.5 Preparation of different concentration

By diluting the condensed extracts with acetone, ethanol, and distilled water, the stock solutions of plant extracts were prepared. Four different concentrations 3.0, 6.0, 9.0, and 12.0 % of each category of plant extracts were prepared by dissolving the stock solution in the respective solvent.

2.6 Repellency test

The stock extract was diluted with respective solvents to prepare 3.0, 6.0, 9.0, and 12.0 % solutions. Repellency test was conducted according to the method of Talukder and Howse (1994) [15]. Petri dishes (9 mm) were divided into two parts, treated and untreated portion. With the help of pipette, 1 ml solution of each plant extract was applied to one half of the grains. The treated half was then air-dried. Twenty *S. granarius* adults were released at the centre of each petri dish and covered. For each plant extract and each dose, three replications were used. The numbers of insect pest present on each portion of the petri dishes were counted at hourly intervals. The data were expressed as percentage repulsion (PR %) by the following formula:

$$\mathbf{PR\ (\%) = (Nc - 50) \times 2}$$

Where;
Nc: Percentage of insects present in the control half. Positive (+) values expressed repellency and negative (-) values attractency.
Data (PR %) were analysed using analysis of variance (ANOVA). Mean values were classified according to the following scale:
Class Repellency Rate

0. > 0.01 to < 0.1
I. 0.1 to 20
II. 20.1 to 40
III. 40.1 to 60
IV. 60.1 to 80
V. 80.1 to 100

(McDonald *et al*., 1970) [9].

3. Results and Discussion

The results and statistical analysis of the repellency rate of tasted aromatic plant extracts at different hours after treatment (HAT) are presented in Table 1 to 2.

Table 1: Repellency rate of different solvent extracts of different plants on *S granarius* using trated wheat grains at different hours after treatment (HAT)

Aromatic plants	Solvents	Repellency rate (%)			Mean repellency rate (%)	Repellency class
		1 HAT	2 HAT	3 HAT		
A.graveolens (Dill)	Acetone	16.31	16.22	24.33	18.95	I
	Ethanol	25.31	20.21	28.00	24.50	II
	Water	27.11	28.47	21.33	25.63	II
L. nobilis (Bay)	Acetone	28.22	16.22	23.67	22.70	II
	Ethanol	23.11	20.33	21.00	21.48	II
	Water	35.00	25.33	17.67	26.00	II
O. basilicum (Basil)	Acetone	43.56	34.67	27.00	35.07	II
	Ethanol	40.41	30.26	22.00	30.79	II
	Water	43.46	39.11	25.67	36.08	II
F. vulgare (Fennel)	Acetone	43.46	35.35	29.14	35.98	II
	Ethanol	33.27	39.11	29.14	33.84	II
	Water	49.22	39.11	32.31	40.21	III
R. officinalis (Rosemary)	Acetone	38.67	32.54	29.00	33.40	II
	Ethanol	38.17	35.61	27.11	33.63	II
	Water	32.96	32.67	27.11	30.91	II
M. sylvestris	Acetone	28.21	29.33	24.33	27.29	II

(Hibiscus)	Ethanol	27.22	27.14	24.33	26.23	II
	Water	37.37	32.67	36.11	35.38	II
Sx				5.2634	3.1267	-
Probability level				NS	NS	-

NS: Not significant

The repellency rate of acetone, ethanol and water solvents extract of six aromatic plants showed insignificant at different HAT (Table 1). But numerically the repellency rate of all the extracts was higher at one HAT then two or three HAT except few. Considering solvents used in this study, extracts of water solvent showed higher repellency rate then other extract of acetone and ethanol except *R. officinalis* plant where extract of ethanol showed higher repellency rate. The highest mean repellency rate was observed in water extract of *F. vulgare* (40.21 %) and lowest in acetone extract of *A. graveolens* (18.95 %). On the basis of mean repellency rate, it was found that extracts of acetone, ethanol and water of all six plants were in the same repellency class i.e. class II except *A. graveolens* extract of acetone (class I) and *F. vulgare* extract of water (class III). The repellency rate of different plant extracts at different dose level on *S. granarius* is presented in Table 2.

Table 2: Repellency rate of different plant extracts at different doses on *S. granarius* using treated wheat grains at different hours after treatment (HAT)

Aromatic plants	Doses (%)	Repellency rate (%)			Mean repellency rate (%)	Repellency class
		1 HAT	2 HAT	3 HAT		
A.graveolens (Dill)	3.0	6.61	2.11	12.06	6.92	I
	6.0	15.91	20.00	15.14	16.35	I
	9.0	28.17	27.97	31.31	29.15	II
	12.0	40.51	45.96	42.56	43.01	II
L. nobilis (Bay)	3.0	11.11	5.67	4.67	7.15	I
	6.0	23.01	13.96	15.04	17.33	I
	9.0	33.61	26.11	25.00	28.24	II
	12.0	41.44	41.41	33.56	38.80	II
O. basilicum (Basil)	3.0	17.01	19.96	6.96	14.64	I
	6.0	38.44	27.96	19.96	28.78	II
	9.0	50.16	41.11	30.16	40.47	III
	12.0	64.60	51.04	42.96	52.86	III
F. vulgare (Fennel)	3.0	20.11	20.00	15.17	18.42	I
	6.0	38.44	33.33	22.16	31.31	II
	9.0	49.22	43.01	30.04	40.75	III
	12.0	60.04	34.34	45.11	46.49	III
R. officinalis (Rosemary)	3.0	15.16	14.36	10.11	13.21	I
	6.0	19.11	25.06	16.61	20.26	II
	9.0	42.66	45.01	38.67	42.11	III
	12.0	57.01	44.96	39.01	46.99	III
M. sylvestris (Hibiscus)	3.0	15.16	11.33	10.12	12.20	I
	6.0	21.11	20.06	20.21	20.46	II
	9.0	33.00	31.04	33.06	32.36	II
	12.0	47.33	45.96	44.56	45.95	III
Sx 5.0117 2.9661 -						
Probability level NS NS -						

NS: Not significant

Findings showed that the rate of repellency increased with increase of dose level. At 12.0 % dose level all plants showed the highest repellency rate and were in repellency class III, but at 9.0 % dose level also *O. basilicum*, *F. vulgare*, and *R. officinalis* remained same repellency class. The highest mean repellency rate was found with 12.0 % dose level of *O. basilicum* extract (52.86 %) and the lowest rate was found with 3.0 % dose level of *A. graveolens* extract (6.92 %). There are several reports concerning the insecticidal activity of aromatic plants against stored product insects. Saljoqi *et al*., (2006) [12] tested the repellent and lethal potential of ethanol extracts of six aromatic plants against rice weevil *Sitophilus oryzae* L. They reported that extracts of bakain leaves recorded maximum repellency index with non-significance difference with habulus and mint as compared with the untreated control. Viglianco *et al*., (2008) [16] carried out investigation of some plant extracts to evaluate their repellency and feeding deterrency to control *S. oryzae* L. They used three plants for their studies. They reported hexane extract of *Solanum argentinum* with strongest repellent effects (class IV) against *S. oryzae* L. whereas, ethanol and chloroform extracts of all plants recorded moderate repellency. Naseem and Khan (2011) [10] carried out investigations on the repellency of essential oils of *Piper nigrum* and *E. camaldulensis* against *Tribolium castaneum* under laboratory conditions. *E. camaldulensis* was recorded more effective as compared to *P. nigrum* showing significantly higher repellency at all concentrations.

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