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Use of aromatic plant extracts as bio-insecticides for the control of stored-product insect, *Sitophilus Granarius*

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Abstract
Ethanol extracts of five plants materials i.e. chervil (*Anthriscus cerefolium*), bay (*Laurus nobilis*), sage (*Salvia officinalis*), fennel (*Foeniculum vulgare*) and rosemary (*Rosmarinus officinalis*) against wheat granary weevil, *Sitophilus granarius* L. were tested to determine their insecticidal properties. The results revealed that all of the tested materials with some variations had repellent and lethal effects against the pest as compared with the untreated check. Considering the % mortality of the insect as a main index, rosemary proved to be the most effective of these five plant materials, showed 60.5 % mortality, followed by sage (46.6 %) and chervil (31.4 %), while bay (19.2 %) was found less effective followed by fennel (26.4 %).

Keywords: Bio-insecticide, aromatic plant, granary weevil, *Sitophilus granarius*

1. Introduction
The wheat granary weevil, *Sitophilus granarius* Linnaeus, 1758 (Coleoptera: Curculionidae) is one of the most serious pests of stored grain, especially in temperate regions (Niewiada *et al.*, 2005) [8]. This pest is internal feeder and cause considerable loss to cereals affecting the quantity as well as quality of the grains (Ebadollahi, 2011) [4].
Synthetic insecticides have been considered the most effective and accessible means to control insect pests of stored products (Huang and Subramanyam, 2005) [7]. These chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for vertebrates especially for mammalian health (Halder *et al.*, 2010) [5].
The adverse effects of synthetic pesticides have amplified the need for effective and biodegradable pesticides. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment (Thomas *et al.*, 2002) [12]. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides (Hamza *et al.*, 2016) [6]. Among various kinds of natural substances that have received particular attention as natural agents for insect management are essential oils and extracts from aromatic plants.
Therefore, the present study was initiated to find and recommend possibly the most effective plant extracts against adults of *S. granarius* in the stored wheat grains under laboratory conditions.

2. Materials and Methods
2.1 Test insect
The wheat granary weevil, *Sitophilus granarius* L. was reared in a 1 L wide-mounted glass jars containing soft wheat grains. Mouth of the jars covered with a fine mesh cloth for ventilation and to prevent escape of the weevils. Cultures were maintained in an incubator at 27 ± 1 °C and 60 ± 5 % relative humidity (Davis and Bry, 1985). Insects used in all experiments were 1 to 7 day old adults. All experimental procedures were carried out under the same environmental conditions as the cultures. The wheat granary weevil adults were obtained from the stock culture of the laboratory of the Plant Protection Department, Faculty of Agriculture, Ankara. The life cycle can be completed in as little 30 to 40 days during the culture conditions but takes considerable longer in cooler conditions. Adult granary weevils can live up to eight months and can produce up to four generations per year.

2.2 Test plants
In this study, bio-insecticide effects of aromatic leaf extracts from chervil (*Anthriscus cerefolium* (L.) Hoffm.), bay (*Laurus nobilis* L.), sage (*Salvia officinalis* L.), fennel (*Foeniculum vulgare* Mill.) and rosemary (*Rosmarinus officinalis* L.) were tested against wheat granary weevil, *S. granarius*. Chervil, bay, sage, fennel and rosemary were obtained from a local market of Ankara.

2.3 Preparation of plant extracts
Fresh leaves of chervil, bay, sage, fennel and rosemary were washed in running water and then they were dried in the oven at 60° C to prepare fine dust. Oven dried plant materials were prepared by pulverizing the dried leaves in a magnetic stirrer. A 25-mesh diameter sieve was used to obtain fine and uniform dust and preserved them into air tight glass jar, till their use in extract preparation. At first ten grams of each dust sample were taken in a 500 ml beaker and separately mixed with 150 ml of ethanol and distilled water. Then the mixture was stirred for 30 minutes by a magnetic stirrer at 6000 rpm and left to stand for next 24 hours. The mixture was then filtered through a fine cloth and again through Whatman No. 1 filter paper. The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent in a water bath at 80 °C and 60 °C temperature for water and ethanol extracts respectively. After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labelled bottles and stored in a refrigerator until their use for insect

bioassays (Saljoqi *et al.*, 2006) [10]. The extracts were diluted to 10%. All of these extracts were used to 0.4 ml per 20g of grains.

2.4 Bioassay

For activity test, 100 ml plastic vials were used to test the extracts. The vials used for the experiment were disinfected with 70% alcohol 24 hours before inoculation. A control containing no extracts was allotted to each treatment. There was one set of two vials joined by clear plastic pipe of 1 cm diameter at an angle of 180 º for each replication. One vial of each set was provided with 30g of wheat grains, and fixed at the position A, while the other vial was kept empty, and fixed at the position B. Before filling with grains, vials A in all replications were sprayed with respective extracts, while empty vials B were not treated. Ten adults of *S. granarius* of the same age were released in vials A.

The purpose of these exercises was that either the treated vials would repel the insects and force them to move to empty vials through the plastic pipe or kill them indicating insecticidal properties of the aromatic plant extracts in both the situations. The mortality or repellency of dead and alive insects in the vial B were recorded for 15 days at an interval of 24 hours for each observation. The ones found alive in the plastic pipes were considered repelled individuals. In total there were 6 treatments including untreated control group. Each treatment was replicated 3 times. The data recorded for percent mortality and percent repellency of different treatments were subjected to statistical analysis. Means were compared by using LSD test.

3. Results

3.1 Percent repellency

Table.1 shows that the tested aromatic plant extracts played significant role on the target species by forcing it to move from treated vials to untreated vials through plastic pipe. The maximum number of repelled alive insects was recorded in the extract of rosemary leaves on days D1 and D2 which amounted to 68 % and 62 %. The total mean repellency by this treatment was determined as 23.0 till termination of the experiment. The extract from bay was found comparatively least effective on the basis of quick action but was quite effective if its total effect were taken into consideration. It gave 26 % repellency o D1, which slightly reduced after 24 h. The amount of repellency effect further lowered down at greater extent and reached at 0 level on D8, The average repellency was recorded as 9.00 %.

Table 1: Comparative repellency effects of different aromatic plant extracts on granary weevil, *Sitophilus granarius* L. infested stored wheat grain.

Aromatic plants	%Repellency / Different days of observations (D)										
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**Mean**
Chervil (*Anthriscus cerefolium*)	62 bcd	58 bcd	32 hij	10 m-r	8 n-r	4 pqr	4 pqr	2 qr	0 r	0 r	18.00 c
Bay (*Laurus nobilis*)	26 ijk	24 jkl	16 k-o	12 m-q	8 n-r	2 qr	2 qr	0 r	0 r	0 r	9.00 d
Sage (*Salvia officinalis*)	60 bcd	60 bcd	36 ghi	26 ijk	10 m-r	8 n-r	8 n-r	2 qr	2 qr	0 r	21.20 ab
Fennel (*Foeniculum vulgare*)	52 def	52 def	30 hij	12 m-q	8 n-r	8 n-r	2 qr	2 qr	2 qr	2 qr	17.00 c
Rosemary (*Rosmarinus officinalis*)	68 ab	62 bcd	50 def	26 ijk	12 m-q	8 n-r	2 qr	2 qr	0 r	0 r	23.00 a
Control	0 r	0 r	0 r	0 r	0 r	2 qr	2 qr	6 o-r	4 pqr	2 qr	1.60 e
Means	44.66 a	42.66 a	27.33 b	14.33c	7.66 de	5.33 e	3.33 ef	2.33 f	1.33 g	0.66 g	

Means with different lettrers, separately for treatments, days and their interaction are significantly different at 5% level of significance (LSD test).

The overall means of the data indicate that extracts of sage leaves gave excellent results with non-significance difference with chervil. This was followed by fennel. The least effect of repellency was recorded in case of bay.

3.2 Percent mortality

The data with respect to percent mortality of *S. granarius* observed on different days in plastic pipes and untreated empty vials are presented in Table.2. The data shows that the tested extracts had profound effect on suppression on the pests' population.

Table 2: Comparative fatal effects of different aromatic plant extracts on granary weevil, *Sitophilus granarius* L. infested stored wheat grain.

Aromatic plants	% Mortality / Different days of observations (D)										
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**Mean**
Chervil (*Anthriscus cerefolium*)	0.0 u	4.0 tu	6.0 pqr	22.0 mno	34.0 f-i	40.0 b-f	48.0 b-f	52.0 a-e	54.0aa-e	54.0 a-e	31.4 c
Bay (*Laurus nobilis*)	0.0 u	2.0 tu	12.0 q-t	16.0 p-s	20.0mno	24.0 mno	28.0 op	30.0 op	30.0 op	30.0 op	19.2 e
Sage (*Salvia officinalis*)	0.0 u	0.0 u	12.0 q-t	48.0 jkl	56.0 g-j	64.0 c-f	68.0 b-f	72.0 a-d	72.0 a-d	74.0 abc	46.6 b
Fennel (*Foeniculum vulgare*)	0.0 u	2.0 tu	14.0 p-s	20.0 mno	28.0 op	38.0 p-s	38.0 p-s	40.0 b-f	42.0 b-f	42.0 b-f	26.4 d
Rosemary	2.0	13.0	38.0	56.0	76.0	80.0	82.0	86.0	86.0	86.0	60.5

(*Rosmarinus officinalis*)	tu	p-s	p-s	k-n	e-h	ab	a	a	a	a	a
Control	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u	2.0 tu	4.0 stu	12.0 q-t	1.8 f
Means	0.3 g	3.5 g	13.6 f	27.0 e	35.6 e	41.0 d	44.0 d	47.0 c	48.0 bc	47.6 bc	

Means with different lettrers, separately for treatments, days and their interaction are significantly different at 5% level of significance (LSD test).

Maximum mortality of 86 % was found in the extracts obtained from rosemary leaves on D8 however it remained the same for rest of the observations. The least effect on pests' mortality was recorded in the commodity treated with bay ($P < 0.05$). However, all of the five treatments proved significantly better than untreated check.

The overall mean percent mortality data in Table.2 indicate that rosemary leaves gave significantly better control of *S. granarius* than all other extracts used in the experiment.

4. Discussion

The repellent property was well depicted by all the treatments soon after their application and was observed decreased with the increase in storage period of the commodities. Like fatal effects the maximum repellency was exhibited by the extracts of rosemary leaves. Chauhan *et al.* (1987) [1] on the basis of LD_{50} found that petroleum ether extracts of *Melia azadarach* were 0.0003 time less toxic than malathion. Similarly Sexena (1987) [11] discussed the antifeedant properties of *Melia azadarach* but stated that this product would require some definitions of quality control and standardization of biological properties for large scale use.

Interesting findings were reported by different scientist on *Cymbopogon citratus* but with different insect species. Devaraj and Srilatha (1993) [3] found very effective the extracts of *C. citratus* increased mortality of the maize weevil, *Sitophilus zeamais* as compared to control group. Ofuya and Okuku (1994) [9] reported the insecticidal activity of acetone extract of C. citratus against *Aphis craccivora* and caused significant nymphal mortality and inhibited reproduction. Hamza *et al.* (2016) [6] described that the examination of the volatile oils extracted from plant materials as well as bioassay for evaluating the fumigant toxicity against the granary weevil *S. granarius*. Results showed that Thuja, Eucalyptus and Peppermint oils could be applicable to the management of populations of *S. granarius*.

The effectiveness of the aromatic plant extracts against different *Sitophilus* species was generally in agreement to the findings of previous researchers, the present study cannot be strictly in line with those of previous workers due to many reasons, some of which have been mentioned above.

5. Conclusion

Aromatic plants have a range of chemicals which can be isolated and used for stored-product insects' control. These plants being medicinal would yield environmentally sound chemicals having no harmful effects on the non-target organisms.

6. References

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