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Antifeedant and repellent effects of root extracts of some aromatic plants against bean weevil *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)

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Abstract
Plant root extracts in methanol, chloroform and hexane of three widely distributed plants were evaluated for their feeding deterrency and repellency to *Acanthoscelides obtectus* Say. The plant species studied were, ginger-*Zingiber officinale* Roscoe (Zingiberaceae), garden radish-*Raphanus sativus* L. var. *sativus* (Brassicaceae) and black carrot-*Daucus carota* L. spp. *sativus* var. *atrorubens* Alef. (Apiaceae). Antifeedant potential of these root extracts was determined by the deterrence or antifeedant test using bean wafer disks. The total coefficient of deterrence (T) was determined for each root extract. Repellency tests were performed using filter-paper circles cut in halves. The root extracts were applied on each half at a concentration of 0.36 mg.cm^{-2}. Percentual repellency (PR) was determined for each root extract. The chloroform root extracts of *Z. officinale* had the strongest antifeedant effect (class ++++) followed by the methanol root extracts (class ++++) and hexane root extracts of the same species (class +++). Root extracts of *Z. officinale* had stronger antifeedant effect than *R. sativus* var. *sativus* and *D. carota* spp. *sativus* var. *atrorubens* root extracts. A moderate repellent effect of *R. sativus* var. *sativus* and *Z. officinale* root extracts on *A. obtectus* was was observed, the hexane root extracts of *R. sativus* var. *sativus* was the one with strongest repellency (class 4). Among the root extracts analysed, the strongest antifeedant effect occur with the chloroform root extracts of *Z. officinale* and the hexane root extracts of *R. sativus* var. *sativus* showed the strongest repellency effect.

Keywords: *Acanthoscelides obtectus*, antifeedant, aromatic plant extract, repellent

1. Introduction

The bean weevil *Acanthoscelides obtectus* Say, 1831 (Coleoptera: Bruchidae) is a pest of stored beans and peas throughout the United States and in many other countries. This insect belongs to the beetle family Bruchidae (commonly known as the seed weevils because the larvae develop inside the seeds of various plants). Occasionally one may take a package of dried beans or peas out of the closet only to find it infested with the bean weevil or weevils may be noticed when they try to escape through the window [1].

Dead weevils may accumulate on the sills. In the field, kidney beans, lima beans and cowpeas are attacked. In storage, all varieties of beans, peas, lentils and some seeds are attacked. The larvae feed inside the beans and when numerous, nothing but the shell remains. This reduces the food value of the beans as well as reducing the germination potential of the bean seed [1].

The adult bean weevil is oval and slightly flat. Female bean weevils are small beetles, ranging in size from 3.8 - 4.8 mm. Adult males, ranging in size from 3.1 – 4.2 mm, are smaller than females. The colour is olive brown with darker brown and grey patches on the wing covers. The elytra are shorter than the abdomen leaving a few segments exposed. The larvae is a white grub with a brown head. It is about 0.6-0.8 mm in length and pupa, 2.9 – 4.6 mm in length, are light brown [2].

Adult weevils lay eggs on the developing bean pods in the field. In 3 to 30 days the tiny grubs emerge and make their way into the seed. Here they feed until mature, and pupation occurs. When the adults are ready to emerge, they cut round holes (1/10 –inch in diameter) through the seed coat and crawl out. Indoors, in stored seed, multiple generations occur and breeding continues as long as there is any food left in the beans and the temperature is suitable for reproduction.

Aromatic plants have developed for 400 million years and have acquired effective defence mechanisms that ensure survival under rough environmental conditions and in the presence of natural enemies [3]. Besides a number of morphological protective mechanisms, plants have developed subtile chemical defence mechanisms against insects and other organisms; these defence mechanisms do not generally produce immediate death but do affect common biochemical and physiological functions [4]. Until a few decades ago, plant secondary metabolites were considered substances with no specific function, which only reflected an aspect of biodiversity [5].

Synthetic insecticides such as organophosphorus compound, pyrethroids and fumigants have been widely developed and are extensively used because of their effectiveness and easy application and storage. However, their extensive use has brought about severe disadvantages. Excessive use of insecticides causes the development of resistance on the pest populations and leads some negative impacts on human and animal health [6].

These negative effects such as resistance problem and insecticide residues in products, constitute a need to develop alternative methods and their applications in control strategies against pests of stored grains. Researchers on plant-based insecticides, as alternative pest control methods, has gained pace in recent years [3, 7, 11]. Plant extracts have a high potential for the development of agents for controlling of pests [12, 14]. These agents may inhibit pest populations in different ways such as disruption of metabolic pathways, rapid death, attractive and deterrent factor, prevention of

nutrition and ovulation and other negative effects on the insect development. Today over 2000 species of plants are known that possess some insecticidal activity. Some products derived from various plant species has been proven as toxic and repellent against pests of stored grains [3, 13, 15, 18]. Recently, plant-derived products have become more popular around the world.

In this study, antifeedant and repellent effects of root extracts of some aromatic plants such as ginger-*Zingiber officinale*, garden radish-*Raphanus sativus* var. *sativus* and black carrot-*Daucus carota* L. spp. *sativus* var. *atrorubens* on bean weevil *A. obtectus* were determined under the laboratory conditions.

2. Materials and Methods

2.1 Preparation of plant root extracts

Root extracts of the following plant species were used: Ginger-*Zingiber officinale* Roscoe (Zingiberaceae), garden radish-*Raphanus sativus* L. var. *sativus* (Brassicaceae) and black carrot-*Daucus carota* L. spp. *sativus* var. *atrorubens* Alef. (Apiaceae). Plant root material was air-dried in shade conditions and fragmented. Maceration was performed in solvents of different polarity (methanol, chloroform and hexane) for 72 h with the aim of extracting plant roots. Each extract was then concentrated to dryness using a rotary evaporator until constant weight was obtained. The extracts obtained were dissolved in the corresponding pure solvent until a 10 % (w/v) stock solution was obtained.

2.2 Test insect

A stored-product pest species, the bean weevil *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) was used for the bioassays. Weevils were reared in growth chambers at 27 ± 1 ºC and relative humidity of 65 ± 5 % with natural photoperiods. Jars covered with a fine piece of cotton cloth were used to allow the passage of air; weevils were fed on bean grains.

Adults of *A. obtectus* were placed in the rearing medium for 15 days; then they were removed and placed in a new medium to obtain a new progeny and avoid generation overlapping. The medium containing the eggs was placed in the growth chamber until adult emergence. This process was successively repeated with the aim of obtaining homogenous generations.

2.3 Deterrency test

The potential of the antifeedant effect of the extracts was determined by the feeding deterrency test described by Talukder and Howse [19]. Bean wafer disks were used as test food; the disks were saturated by dipping into either solvents only (control C) or into 10 $mg.mL^{-1}$ solution of each extract (treatment T) and were air-dried for 24 h. Bean disks were then weighed and 15 weevils were placed on them; the disks were reweighed after 7 days. Food consumption of weevils was recorded under three conditions: 1-on pure food, composed of two untreated disks (UD); 2-on food with possibility of choice between one treated (OT) and one untreated (OU) disk choice test, and 3-on food two treated (TT) disks: non-choice test.

Blank disks treated with the solvent but not offered to weevils were prepared. The experiment was arranged in a randomized complete design with three replications. Disks were dipped for two different time periods: 15 and 30 seconds. One dried, bean disks may increase in their weight as a result of water absorption from the surrounding air which was humidified for the normal growth and development of weevils. Hence, a correction procedure was applied.

Disk weight loss, which was estimated as the amount of food consumed (FC), was calculated by the formula of Serit *et al*. cited by Talukder and Howse [19]:

$$\mathbf{FC = IW_S - [(FW_S \times IW_b) / FW_b]}$$

Where

IW: Initial weight of disk after being treated with extract or solvent.

FW: Final weight of disk, subscript b: blank disk (treated with solvent and not offered to weevils), and subscript s: treated and control disks, on which weevils were released.

According to the amount of food consumed in the three different tests (UD, TT, and OT-OU) three feeding deterrent activity coefficients were calculated.

- Absolute coefficient of deterrency

$A = (UD - TT / UD + TT) \times 100$

- Relative coefficient of deterrency

$R = (C - T / C + TO) \times 100$

- Total coefficient of deterrency

$T = A + R$

The values of total coefficient of deterrency serve as an index of antifeedant activity expressed on a scale between 0 and 200. The index zero (0) stands for an inactive compound with maximum activity. An index T of 150 – 200 was designated ++++, of 100 – 150 +++, of 50 – 100 ++, and of 0 – 50 +. Mean separation of antifeedant activity of extracts were made using non-parametric Kruskall – Wallis's test ($P \leq 0.05$). Contrast analyses between plant species, solvents and time of exposure were made using the same test [20].

2.4 Repellency test

Repellency tests were conducted following the method proposed by Talukder and Howse [19, 21]. Filter paper circles of 9 cm in diameter were cut in half. Root extracts were applied on one half at a concentration of 10 $mg.mL^{-1}$. One ml of solution was uniformly applied with a pipette, in such a way as to have a treated substrate of 0.40 $mg.cm^{-2}$ root extract. The treated half-circles were air-dried until the solvent was totally evaporated. The treated and the untreated half-circles were placed contiguously on the Petri dishes and 12 adult weevils were released on each dish. Weevils present in each half circle were counted at hourly intervals for 6 h after treatment. Data were converted to express percentage repulsion (PR) using the following formula:

PR (%) = (N_c – 50) x 2, where N_c is the percentage of weevils present in the control half. Positive values (+) indicated repellency and negative values (-) attractancy. Three replications were made of each treatment. Data were analysed using a two factor completely randomized design using the different plant species and solvents as the two factors. Mean separation of repellent activity of the different root extracts and the comparisons analyses between plant species and solvents were made using Fisher's least significant difference (LSD) test ($P \leq 0.05$) [20]. Mean values were classified according to the following scale:

Class Repellency rate (%)

0 > 0.01 to < 0.1

1 0.1 to 20
2 20.1 to 40
3 40.1 to 60
4 60.1 to 80
5 80.1 to 100

3. Results and Discussion

3.1 Antifeedant test

The root extract with the strongest antifeedant effect on *A. obtectus* was the chloroform root extract of *Z. officinale*, in both treatment times (class ++++). An important antifeedant effect was also observed in the methanol root extracts of *Z. officinale* (class ++++), and with the hexane root extracts of the same species to a lesser degree (class +++) (Table 1).

The analysis of antifeedant effect of each plant, regardless of the solvent or immersion time used, shows that *Z. officinale* was significantly stronger than *R. sativus* var. *sativus* and *D. carota sativus* var. *atrorubens*. However, no significant differences were observed between the root extracts of *D. carota sativus* var. *atrorubens* and *R. sativus* var. *sativus* (Table 2). Contrasts between solvents indicate that the hexane root extracts were significantly less potent than chloroform and methanol root extracts. However, no significant differences were detected between the two latter extracts (P= 0.9214). Contrasts between immersion times indicated that they were not significantly different (P= 0.7013), suggesting that using either of them renders no difference (Table 2).

To date, numerous studies have documented the effectiveness of plant extracts but the number of studies with *A. obtectus* is very low. According to the results of a study, the contact effect of a volatile oil of Chrysanthemum on *Bemisia tabaci* (Genn.), *Tribolium castaneum* (Herbst), *A. obtectus* (Say) and *Ephestia kuehniella* (Zell.) has been investigated through in vitro bioassay methods. Rapid and devastating effects has been observed on *T. castaneum* and *E. kuehniella* larvae and *A. obtectus* adults [22].

The effects of some volatile oils extracted from lavender, eucalyptus and rosemary on A. obtectus larvae has been investigated and all extracts has been determined to be toxic (0.6 – 76 ppb LC_{50} values) depending on the insect life stage and the structure of compound. An increase on larval tolerance with the growth of larvae has also been reported [16].

Similar results of antifeedant effect on *S. oryzae* and *T. castaneum* were observed by Talukder and Howse [19, 21] with four different extracts of *Aphanamixis polystachya*, acetone extract being the most significant with a total deterrency coefficient of 159.5. Valladeres *et al.* [23] also indicated an antifeedant effect of ethanol extract of senescent leaves of *Melia azedarach* on *S. oryzae*, with antifeedant index of 100%.

Table 1: Feeding deterrent coefficients and efficacy of root extracts of three plant species on adults of *Acanthoscelides obtectus*.

Aromatic plants	Solvents	Immersion time[1]	Coefficient of deterrence[2]		Absolute Relative Total	Efficacy of root extract
Z. officinale	Methanol	15	57.36	96.84	154.20 ef	++++
		30	63.16	94.26	157.42 ef	++++
	Chloroform	15	96.96	71.29	168.25 f	++++
		30	94.91	74.47	169.38 f	++++
	Hexane	15	24.36	88.66	113.02 abcd	+++
		30	21.73	95.34	117.07 abcde	+++
R. sativus var. *sativus*	Methanol	15	26.06	79.01	105.07 abcd	+++
		30	21.11	70.17	91.28 a	++
	Chloroform	15	24.61	80.14	104.75 abcd	+++
		30	26.09	90.85	116.94 abcde	+++
	Hexane	15	56.51	73.70	130.21 bcdef	+++
		30	44.84	98.80	143.64 def	+++
D. carota sativus var. *atrorubens*	Methanol	15	47.16	94.08	141.24 def	+++
		30	49.30	96.78	146.08 ef	+++
	Chloroform	15	43.22	91.78	135.00 cdef	+++
		30	37.71	87.65	125.36 bcdef	+++
	Hexane	15	27.20	67.56	94.76 ab	++
		30	54.36	50.21	104.57 abcd	+++

Values followed by the same letter are not significantly different according to Kruskal Wallis's test (P≤ 0.05). [1] Expressed in seconds, [2] Absolute coefficient of deterrency A= (UD – TT / UD + TT) x 100, Relative coefficient of deterrency R= (C – T/C + TO) x 100, Total coefficient of deterrency T= A + R

Table 2: Probability values for Kruskal Wallis's test of total coefficients of deterrency of root extracts of three plant species on adults of *Acanthoscelides obtectus*.

Contrasts between aromatic plants	Total coefficients of deterrency[1] Mean (1)	Mean (2)	Probability
Z. officinale & *D.carota sativus* var. *atrorubens*	149.27	124.26	0.0386
D.carota sativus var. *atrorubens* & *R. sativus* var. *sativus*	124.26	112.32	0.2714
Z. officinale & *R. sativus* var. *sativus*	149.27	112.32	0.0006
Contrasts between solvents			
Chloroform & Methanol	139.91	136.87	0.9214
Methanol & Hexane	136.87	116.11	0.0314
Chloroform & Hexane	139.91	116.11	0.0271
Contrast between time period of dipping			
30 sec. & 15 sec.	133.33	130.24	0.7013

[1] Absolute coefficient of deterrency A= (UD – TT / UD + TT) x 100 Relative coefficient of deterrency R= (C –T /C + TO) x 100 Total coefficient of deterrency T= A + R

3.2 Repellency test

Among all the combinations of plant species and solvents tested, the hexane root extract of *R. sativus* var. *sativus* was the one with the strongest repellent effect on *A. obtectus* (class 4) with PR of 62%, followed by the methanol root extract of this species (class 3). Other root extracts with significant repellency activity were the chloroform root extract of *Z. officinale* (class 3) and the methanol root extract of *D. carota sativus* var. *atrorubens* (class 3) (Table 3). The percentage of repellency observed during the 5 hours after treatment (5 hat) of the test did not show a defined behaviour either between each hour after treatment. The factorial analysis indicate significative differences between plant species (P= 0.0004), solvents (P= 0.0426) and in the interaction plant species and solvents (P= 0.0006). The analysis of the repellency effect of each plant species, regardless of the solvent used, shows that the root extracts of *R. sativus* var. *sativus* and *Z. officinale* did not differ significantly stronger than *D. carota sativus* var. *atrorubens* (Table 3). Comparisons between solvents used indicate that the repellency effect of methanol root extracts was significantly higher than chloroform root extracts but was not significantly different of hexane root extracts (Table 3).

Table 3: Repellency of root extracts of three plant species in different solvents on adults of *Acanthoscelides obtectus*, using the filter paper test.

Root extracts	Solvents	Repellency (%)[1] at: 1 hat	2 hat	3 hat	4 hat	5 hat[2]	Mean(%)[1] repellency	Class repellency
Z. officinale	Methanol	24	34	24	64	20	33.20 b	2
	Chloroform	32	72	68	43	58	54.60 cde	3
	Hexane	12	32	38	38	58	35.60 bc	2
R.sativus var. *sativus*	Methanol	38	48	70	80	60	59.20 e	3
	Chloroform	8	33	16	45	35	27.40 b	2
	Hexane	14	59	69	79	89	62.00 e	4
D. carota sativus var. *atrorubens*	Methanol	38	43	32	44	64	44.20 bcd	3
	Chloroform	8	0	8	0	0	3.20 a	1
	Hexane	24	20	15	46	16	24.20 ab	2
Plant species								
Z. officinale							41.13 b	
R. sativus var. *sativus*							49.53 b	
D. carota sativus var. *atrorubens*							23.86 a	
Solvents								
Methanol							45.53 b	
Chloroform							28.40 a	
Hexane							40.60 ab	

Values followed by the same letter are not significantly different according to the Fisher's LSD test (P≤ 0.05).

[1] Percentage of repellency PR (%)= (N_C – 50) x 2, where N_C is the percentage of weevils present in the control half.

[2] Hours after treatment.

In general, the root extracts of *R. sativus* var. *sativus* and *Z. officinale* had a moderate repellency effect on *A. obtectus*. Jilani and Su [24] showed the repellent effects of extracts of three common plants in Pakistan on three stored-product pests. The powdered extracts of *Curcuma longa* had the strongest repellency effect on the three species studied.

In another study, the fumigant-repellency effects of acetone on *A. obtectus* adults has been investigated and mortality rate has been determined to be connected with the exposure time to acetone vapour [25]. Düzdemir and Yanar [26], evaluated the effects of some plant extracts used against *A. obtectus*, on bean grain yield under field conditions. Some oil extracts such as azadiractin, cypermethrin and eucalyptus were used in the research. This study, however, was not found satisfactory considering the usage of herbal preparations against *A. obtectus* instead of using synthetic chemical pesticides.

The results of the repellency bioassays indicate that the hexane root extract of *R. sativus* var. *sativus* is the one with strongest repellency effect on *A. obtectus*, with a PR of 62% (class 4). In general, the root extracts of *R. sativus* var. *sativus* and *Z. officinale* showed a moderate repellency effect on *A. obtectus*.

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