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The growth regulatory, deterrency and ovicidal activity of worm wood (*Artemisia annua* L.) on *Tribolium confusum* Duv. and identification of its chemical constituents by GC-MS

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Abstract: The chemical composition of worm wood *Artemisia annua* L. (Astraceae), a medicinal plant, was studied by gas chromatography and gas chromatography-mass spectrometry (GC-MS). About 60 compounds were identified, from which the major compounds were erythritol (50.30%), camphore (7.25%), pinocarveol (4.13%) and diethoxyethane (2.18%). The effect of crude extract on deterrency was maximal in 1000 μL/L treatment (only 4.66 ±1.05 insects were attracted to treated food) compared to control (12.6 ±0.96) The growth regulatory effect showed that 1600 μL/L treatment variously affected the weight of larvae, pupae and adult (0.48 ±0.003, 2.17 ±0.02 and 1.31 ±0.015, respectively) and finally the ovicidal effect and the number of adults appearing in F1 were similarly affected (38.5 ±1.7 and 33.75 ±2.31), comparing to control (99.25 ±1.727 and 92.5 ±1.35). The present result and previous results on this plant species indicate that it can be a good candidate for controlling stored product insects.

Keywords: *Tribolium confusum* Duv., *Artemisia annua* L., deterrency, growth regulation, ovicidal effect, GC-MS

INTRODUCTION

Stored products host a wide array of insects the damage of which may amount up to 10-40% in a non-modern storage [1]. Among them *Tribolium confusum* Duv. can be a major pest and is associated with many stored products including grain, flour, peas, beans, nuts and dried fruits, but flours appear to be the most preferred food [2]. In many storage systems, fumigation with either methyl bromide and phosphine is practiced. However, due to possible role of methyl bromide in depleting the ozone layer its use is being banned [3]. The possible development of resistant strains to phosphine, have been reported in many countries [4]. Therefore there is a need for alternative method of pest control in stored commodities. Plant extracts have been traditionally used as plant protectants and their use has been emphasized for post harvest protection of food in developing countries [5]. A large number of plant species with medicinal properties have been considered to have insecticidal, repellent and growth regulating properties [1, 6-16]. *Artemisia annua* L. (Astraceae) is widely used as a traditional medicine in many countries [17]. Species of this plant showed insecticidal, repellent or antifeedant properties [13, 18-21]. Ovicidal and insecticidal properties of *A. annua* have been worked out [22-24]. In this paper, we evaluated the potential repellent and growth regulatory activities of leaf extracts of *A. annua* against *T. confusum* and examined its chemical constituents.

MATERIALS AND METHODS

Insect cultures

Adults of confused flour beetle *T. confusum* Duv. (Col.: Tenebrionidae) were laboratory cultured, and reared on maize and wheat flour mixture (1:1) in glass jars in an incubator set at 29 ±1 °C, RH 65 ±5% and 12 D:L regime.

Plant extract

Fresh leaves of *Artemisia annua* were rinsed in distilled water and left to dry in shade. Dried leaves were ground and 5 grams of the powders were moistened with distilled water and left to stay for 3 h and after that were extracted by steam distillation for 3 h. Rate of distillation was regulated to be 1 mL/min. The extract was washed with diethyl ether 3 times (every time with 25 mL diethyl ether). The most of the diethyl ether phase was separated in a rotary evaporator and the remaining (about 10 mL) was evaporated in laboratory condition to dryness. Residual was dissolved in 2 mL of chloroform to inject to GC-mass.

Analysis of plant extract

One μL of prepared extract was injected to GC-Ms (HP Agilent 6800 N/(61530N) with CPSil5CB column (Chrompack, 100% dimethyl polysiloxane 60 m, 0.25 mm (ID), film thickness 0.25 micron). The analysis was performed under temperature programming from 100 °C (3 min) to 250 °C (5 min) with the rate of 3 °C/min. Injector temperature was 230 °C. Identification of spectra was carried out by study of their fragmentation and also by comparison with standard spectra. Area normalization was used for determination of composition percentage.

Bioassay for repellency activity

This method was based on the method of [25]. Different concentrations of the extract (100, 500, and 1000 μL/L) were prepared and 22.5 mL of each concentration was diluted in acetone and mixed with 450 mg of the flour (maize and wheat 1:1). The controls received only 22.5 mL of acetone mixed with the food. After evaporation of the solvent from the treated food (extract treated or control), 75 mg of each were transferred to Petri dishes (9 cm in diameter). The test was performed by placing one control and one treated diet with extract (containing 100, 500 and 1000 μL/L) in the experimental chamber [25] and the lid was closed. A tube 20×1.5 cm in the lid allowed us to introduce 120 adult insects of two weeks old into the chambers. Hence, all insects had equal chances for treated and control food. For each treatment, there were 6 replicates. The lid was removed after 24 h and the number of insects in each Petri dish was counted.

Bioassay on growth and ovicidal activity

This method was based on the work of [26]. For this purpose, 100 g wheat and maize flour mixture (1:1) was treated with 10 μL of acetone having 200, 400, 800, and 1600 μL/L of the extract. Each treatment was replicated 4 times with control which received acetone alone. Fifteen pairs of adult *T. confusum* were released in the Petri dishes covered with plastic sheets and kept in incubators. The insects were removed after 5 days. The number of larvae in Petri dishes were counted and weighed after 13 days for ovicidal and growth regulatory effect, the weight of pupae were measured after 25 days for growth and ultimately the number and weight of adults after 30 days for evaluation of the number of insects appearing in F1 and also for their growth.

Data analysis

Collected data were subjected to statistical analysis of variance test for significant differences in the measured parameters of the control and treated groups of insects. For all analysis of variance the Duncan's multiple range test in SAS software were used.

RESULTS

Repellency tests

The number of insects attracted to food were highest in the control sample (12.6 ±0.96) and lowest at 1000 μL/L concentration (4.66 ±1.05). The other treatments were similarly significant compared to control (Figure 1).

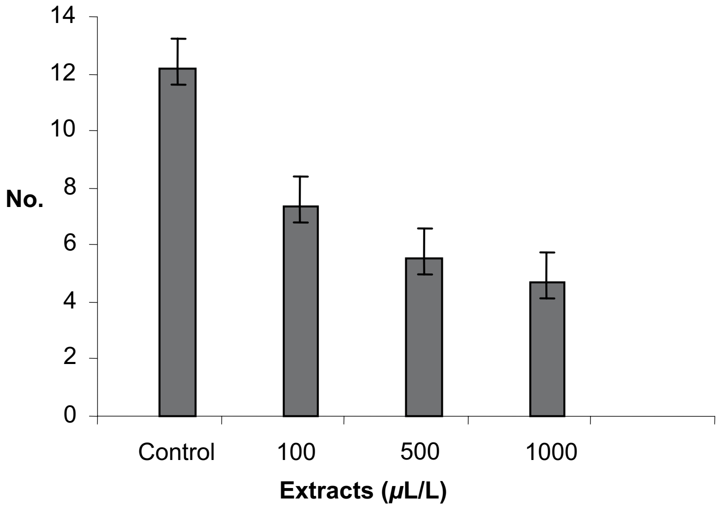

Figure 1. Number of insects attracted to different concentrations of *A. annua* extract.

Growth regulatory effect

The weight of larvae, pupae, and adults in control was the highest (0.9 ±0.002, 3.64 ±0.01 and 2.39 ±0.009, respectively) and the least weight was observed after treatment with 1600 μL/L (0.48 ±0.003, 2.17 ±0.02 and 1.31 ±0.015, respectively). Other treatments similarly showed differences with the control (Table 1).

Table 1. The weight of larva, pupa, and adult resulted from various concentrations of *A. annua* extract

Concentration [μL/L]	Larva	Pupa	Adult
Control	0.9 ±0.002a*	3.64 ±0.01a	2.3 ±0.009a
200	0.6 ±0.002b	2.97 ±0.02b	1.99 ±0.011b
400	0.57 ±0.003c	2.57 ±0.02c	1.79 ±0.012c
800	0.57 ±0.003c	2.25 ±0.02d	1.53 ±0.015d
1600	0.48 ±0.003d	2.17 ±0.02e	1.31 ±0.015e

*Means with different letters in each column are significantly different ($P<0.05$).

Ovicidal effect

The number of hatched larvae in the control sample were highest (99.25 ±1.427) while it was the least at 1600 μL/L concentration (38.5 ±1.7) Other treatments similarly showed differences from control (Figure 2).

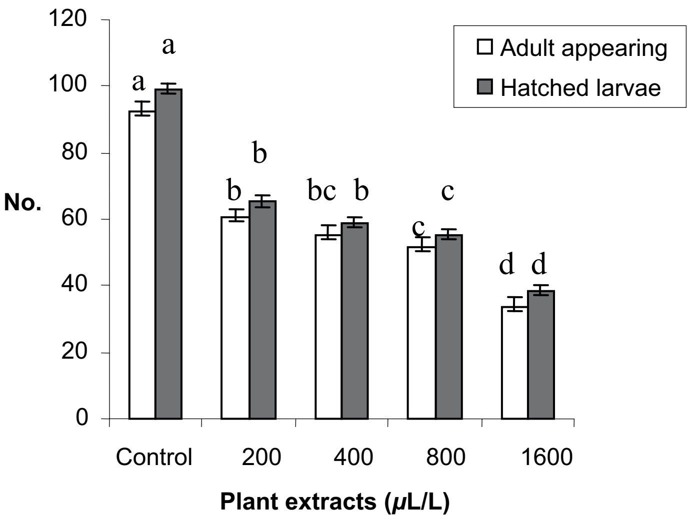

Figure 2. Number of hatched larvae and number of adults appearing in F1 generation after treatment with *A. annua* extract. Means with different letters in each group are significantly different.

Number of adults appearing in F1

The number of adults appearing in F1 was highest in the control sample (92.5 ±1.35) and lowest at 1600 μL/L concentration (33.75 ±2.31). Similarly other treatments showed differences with the control (Figure 2).

Chemical constituents of the *A. annua* extract

Table 2 depicts the compounds present in *A. annua* extract. As it is clear from the table the highest percent of the compounds present in the extract are represented by erythritol (50.33%), camphor (7.25%), cineol (1.84%), coumarin (1.70%), pinocarveol (4.13%), *p*-cymen (0.89%), furfuryl alcohol (0.93%), artemisin (0.89%) and some other compounds presented in the table.

Table 2. Chemical constituents of the extract of *A. annua*

Compound	Retention time	Composition, [%]
Benzophenone	38.85	0.63
Artemisin ($C_{15} H_{18} O_4$)	39.85	0.89
Azulene	40.22	0.40
Coumarin	28.97	1.70
Bicyclo[3.1.1]heptan-3- one,6,6-dimethyl-2- methylene	19.73	0.61
P-cymene	20.48	0.89
2-pinen-4-one	21.35	0.71
Valeraidehyde	21.58	1.09
Pinocarveol	19.23	4.13
Camphore	18.08	7.25
2- phenylethanol	17.81	1.82
Furfuryl alcohol	17.11	0.83
Erythritol	7.99	50.30
α-Butylene glycol	8.24	1.52
Benzenemethanol	14.71	1.59
Cineol (Eucalyptole)	15.19	1.84
1,2,dioxyethane	11.40	21.18

DISCUSSION

Antifeedant compounds are classified into repellents, which repel an insect without making contact with the material, suppressants which suppress biting activity after contact or deterrents, which deter an insect from further feeding after ingestion of the material [27-28]. Based on these definitions, the crude extract of *A. annua* leaves had deterrent properties.

Larval growth inhibition observed at higher concentrations of the test material in the present study is in confirmity with other studies [29]. Since the food intake is lower as expected, therefore the weight decreases. However, the plant extract did not form intermediates or other adverse effects in morphology of resultant stages. Although some studies showed this effect by some monoterpenoids on *T. confusum* [30]. The observation of reduced egg hatchability was similar to other studies where it was shown a reduction in egg viability for the cabbage webworm *Crocidolomia binontalis* Zeller, with neem (*Azadirachta indica*) seed oil treatment [31]. Similar studies have shown reduced eclosion after neem (*Azadirachta indica*) seed oil treatment [32, 33]. However, some studies [34] indicated that the plant extract (neem) did not have any effects on hatching. It may be inferred that some insect eggs may be more sensitive to plant extracts (like the present study) and some may be not so sensitive. The obvious results of reduced egg hatching was observed in lower number of adults appearing in F1 generation. This overall effect of plant extract recall analogous properties of IGRs [30, 35, 36].

Monoterpens of plant origin, are among the best known substances to have attracted attention as potential pest control agents due to their insecticidal, repellent, and/or antifeedant properties [30]. Camphor, a monoterpene which is a common constituent of some plant oils has been reported to have high repellent properties against various insects [37-39]. It was found that artimisin extracted from *A. vulgaris* can repel mosquitoes and other insects [40]. There are reports on the insecticidal activity of *P*-cymene and cineol on some insect species [41, 42]. Previous studies have shown coumarin another component of *A. annua* to be inhibitory to feeding by some herbivorous insects [43, 44]. Chemical analysis of *A. annua* (Table 1) clearly depicts the presence of a number of terpenes.

It is concluded from the results of previous and the present study that *A. annua* could be regarded as a potential insecticide, growth retardant or ovicide for control of insects [23, 24]. However further research is needed for possible application of this plant extract for stored commodities.

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