African Journal of Biotechnology Vol. 5 (10), pp. 936-940, 16 May 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Accepted 11 April, 2006

Methanol extracts from four medicinal plants, *Peganum harmala* (Zygophyllaceae), *Ajuga iva* (Labiateae*), Aristolochia baetica* (Aristolochiaceae) and *Raphanus raphanistrum* (Brassicaceae) were studied for their insecticidal effects on the stored grain pest *Tribolium castaneum* (Herbst). Response varied with plant species. Larvae growth was significantly inhibited when they were fed with extracts incorporated into the diet. Good insecticidal activity against *T. castaneum* larvae and adults was achieved with extract of *P. harmala* seeds, followed by extract of *A. iva*, *Ari. baetica* and *R. raphanistrum* aerial parts. The extracts of the four plants disrupted the developmental cycle of the insect. Extracts of *P. harmala*, *A. iva* and *Ari. baetica* inhibited F1 progeny production. These naturally occurring plant extracts could be useful for managing populations of *T. castaneum*.

Key words: *Ajuga iva*, *Aristolochia baetica*, *Peganum harmala*, *Raphanus raphanistrum*, *Tribolium castaneum*.

INTRODUCTION

Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Arnason et al., 1989). Insecticidal activity of many plants against several insect pests has been demonstrated (Jilani and Su, 1983; Isman, 2000; Carlini and Grossi-de-Sá, 2002). The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility. Yang and Tang (1988) reviewed the plants used for pest insect control and found that there is a strong connection between medicinal and pesticidal plants.

Tribolium castaneum (Herbst) is considered as a major pest of stored grains (Howe, 1965). Annual post-harvest losses resulting from insect damages, microbial deterioration and others factors are estimated to be 10-25% of worldwide production (Matthews, 1993). Control of these insects relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including development of insect strains resistant to insecticides (Zettler and Cuperus, 1990; White, 1995; Ribeiro et al., 2003), toxic residues on stored grain, toxicity to consumers and increasing costs of application. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides.

Peganum harmala L. (Zygophyllaceae), *Ajuga iva* L. (Lamiaceae), *Aristolochia baetica* L. (Aristolochiaceae) and *Raphanus raphanistrum* L. (Brassicaceae) are common plants in Morocco, and mostly in North Africa. Bellakhdar (1997) had reported that *P. harmala* and *A.*

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iva are used for the treatment of diabetes, the roots of *Ari. baetica* are used for constipation and the whole plant is applied for external use against ringworm, and *R. raphanistrum* acts against rheumatism.

The aim of our study is to evaluate the insecticidal activity of the methanol extracts from *P. harmala, A. iva, Ari. baetica* and *R. raphanistrum* against larvae and adults of *Tribolium castaneum*. We assessed the effect of different extracts on (1) weight of larvae; (2) mortality of larvae and adults; (3) larval period duration (5) adult emergence (6) progeny production (F1).

MATERIALS AND METHODS

Insects

Tribolium castaneum was obtained from laboratory cultures maintained for the last 2 years in the dark in incubators at 26 ± 1°C and 65-75% r. h. This insect was reared on wheat flour mixed with yeast (10:1, w:w). In the present study, early last instar larvae weighting 1.95 ± 0.25 mg were used. Adults of 1 week old were used for the study of plant effects on progeny production.

Plant materials

Extracts were prepared from 4 plants commonly used in traditional medicine in Morocco. Seeds of *Peganum harmala* (Zygophyllaceae) were obtained from herbal stores, while *Ajuga iva* (Lamiateae) *Aristolochia baetica* (Aristolochiaceae) and *Raphanus raphanistrum* (Brassicaceae) where collected between December and March in the Tangier Region (NW of Morocco). Plants were rinsed with distilled water, dried in an oven at 45°C for 48 h and ground to a powder with an electrical blender.

Preparation of extracts

Each plant sample (20 g) was extracted twice with 160 ml methanol using sounding apparatus for 30 min. All four extracts were stored at 4°C. For testing, extracts were evaporated to dryness and the residue was weighted and redisolved in the same solvent, at a concentration of 100 mg of crude extract/ml of methanol

Treatments

Extracts were mixed with the diet at concentration of 10%. The solvent was allowed to evaporate at 37°C for 48 h. Twenty last-instar larvae were added to each glass vial (diameter 4 cm, high 7 cm) containing treated diet. A control was prepared in the same way but extract application was omitted. Five replicates were set up for the treated and control larvae. The weight of each larvae and larval mortality were assessed every two days after treatment. Growth of surviving larvae was measured and recorded every two days up to adulthood for the following growth parameters: duration of the larval period until pupation and % of emergence. The mortality of adults, that has emergent from the treated and control larvae, is taken every 4 days.

To assess the effects of different extracts on progeny production (F1), 30 adults were added to each glass vial containing a culture medium treated as above. After 48 h, the adults were removed and the glass vials were returned to the incubator until F1 adult emergence. The F1 adults were counted and weighted. Five replicates were set up for each treatment and control.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) using Statistica Software (Statistica, 1997). Results from the progeny production and emergence rate were analysed by one-way ANOVA. The others data (weight and mortality) were analysed by two-way ANOVA, each ANOVA examined two different factors: treatment and time after treatment. Post hoc testing was carried out using the Tukey test. A significance level of 0.05 was used for all statistical tests.

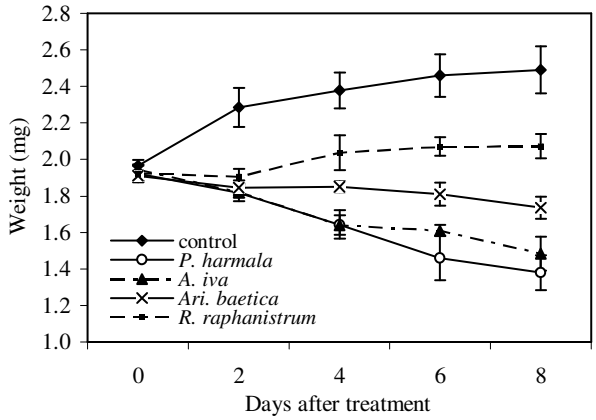

Figure 1. Effect of the extracts of different plants on weight (in mg) of *Tribolium castaneum* last instar larvae. Each point represents the mean of 100 larvae ± SD.

RESULTS

Effects on larval weight

Control larvae exhibit an increasing individual weight (26.4%) during 8 days (Figure 1); it reaches 2.49 mg. The larvae treated with the extract of *R. raphanistrum* show a slight increase of the weight (8.95%) when compared to the control. However, larval weight was reduced by extracts of *P. harmala, A. iva* and *Ari. baetica*, it reached 1.38, 1.48 and 1.74 mg, respectively, 8 days after treatment. The two way analysis of variance showed that the exposure period to the extract of all plants had a very significant effect on weight ($F = 4.19\text{-}31.59$; $df = 4$; $p < 0.01$) as did treatment ($F = 122.37\text{-}755.16$; $df = 1$; $p < 0.001$). In all cases, the relation between exposure period and treatment was very significant ($F = 11.12\text{-}73.87$; $df = 4$; $p < 0.001$)

Effects on larval mortality

Figure 2 showed that no mortality occurred in larvae fed with control diet. All treatment provoked a very highly significant effect on mortality ($F = 36.21\text{-}1092.78$; $df = 1$; $p < 0.001$). Extract of *P. harmala* caused 58% mortality

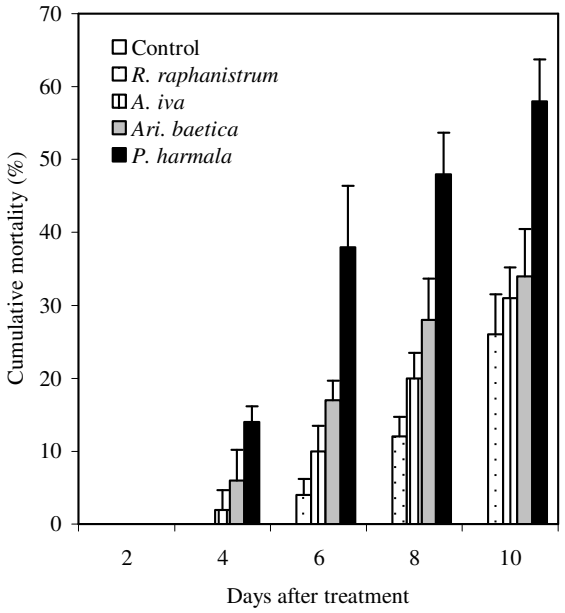

Figure 2. Effect of the extracts of different plants on cumulative mortality of *Tribolium castaneum* last instar larvae. Bars indicate standard deviation (SD) of observations.

Table 1. Effect of plant extracts on the duration of last-instar larval period, and % of emergence of *T. castaneum*[*].

Plant extract	Larval period (day)	% of emergence
Control	7.1 ± 0.28a	100 ± 0.00a
P. harmala	8.2 ± 0.40b	92.78 ± 6.62a
A. iva	6.6 ± 0.21c	98.46 ± 3.44a
Ari. baetica	7.5 ± 0.20a	98.58 ± 3.18a
R. raphanistrum	8.3 ± 0.14b	98.66 ± 3.00a

[*]Each datum represents the mean of five replicates, (n = 20).
Means within a column followed by the same letter are not significantly different (Tukey's HSD test, p < 0.05).

during the 10 days after treatment. At the same time, mortality rates for the extracts from *Ari. baetica*, *A. iva* and *R. raphanistrum* reached 34, 31 and 26%.

Effect on larval period

In control, larval duration was 7.1 days. It was the same duration when larvae were fed with diet containing extract of *Ari. baetica* (p > 0.05) (Table 1). However in treated larvae with *A. iva* the duration was 6.6 days. Tukey test showed that *A. iva* had a significant effect (p < 0.05). *R. raphanistrum* and *P. harmala* showed a very significantly longer larval period (8.3 and 8.2 days respectively) in comparison with the duration of the control larval period (p < 0.001).

Effect on emergence rate

The effect of different extracts on emergence of adults of *T. castaneum* is shown in Table 1. The emergence rate in control reaches 100%. The number of the adults emerging from the pupae treated by *P. harmala*, *A. iva* and *R. raphanistrum* was not significantly affected (P > 0.05).

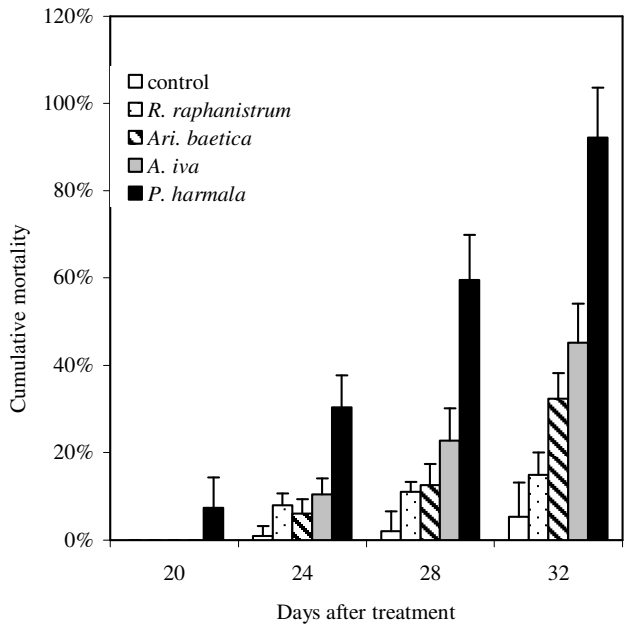

Figure 3. Effect of the extracts of different plants on cumulative mortality of adults *Tribolium castaneum*. Bars indicate standard deviation (SD) of observations.

Effects on adult mortality

The methanol extracts of *P. harmala, A. iva*, *Ari. baetica* and *R. raphanistrum* significantly affected survival of adult with 92%, 45%, 32% and 15% of mortality respectively during 32 days after treatment (Figure 3). From all extracts, a two-way ANOVA revealed that the relation between exposure period and treatment was very significant (F = 4.62-76.55; df = 4; p < 0.01).

Effect of different extracts on F1 progeny production

Progeny production of *T. castaneum* was totally suppressed with extracts of *P. harmala*, *A. iva* and *Ari. baetica* (Table 2). The number of F1 adults that emerged in the treated medium with *R. raphanistrum* was low compared with control (F = 18.26; df = 1; p < 0.01). The weights of F1 adults that emerged in treated media were not affected (F = 1.14; df = 1; P > 0.05).

Table 2. Effect of the extracts of different plants on emergence and weight of F1 adults of *T. castaneum*[*]

Plant extract	Number of F1 adults (Means ± SD[a])	Weight of F1 adults (mg) (Means ± SD[*])
Control	11.0 ± 2.0a	2.14 ± 0.05a
P. harmala	0	-
A. iva	0	-
Ari. baetica	0	-
R. raphanistrum	6.6 ± 1.14b	2.04 ± 0.11a

[*]Each datum represents the mean of five replicates, (n = 30).
[a]Means within a column followed by the same letter are not significantly different (Tukey's HSD test, $p < 0.05$).

DISCUSSION

The present work revealed the effect of four plant extracts on *T. castaneum*. Significant insecticidal activity against *T. castaneum* larvae and adults was observed with crude methanol extract from *P. harmala*, followed by extracts of *A. iva*, *Ari. baetica* and *R. raphanistrum*. The larvae were more susceptible than adults to extracts of *Ari. baetica* and *R. raphanistrum*. In contrast, adults were more susceptible than larvae to extract of *P. harmala* and *A. iva*. Methanol extracts from the studied species reduced significantly larval growth just 2 days after treatment. The most active species were *P. harmala* and *A. iva.* Moreover, extract of *P. harmala*, *R. raphanistrum*, and *A. iva* disrupted developmental cycle of larvae by prolonging or reducing the duration of the last-instar larvae. Extract of *R. raphanistrum* reduced significantly the progeny production F1, while extracts of *P. harmala*, *A. iva* and *Ari. baetica* inhibited completely F1 progeny production.

Similar observations on other plant extracts effect on several insects have been reported. For example, Sadek (2003) showed that the time of pupation of *Spodoptera littoralis* (Boisduval) of larvae increased by the extract of *Adhatoda vasica* (Nees). Jeyabalan et al. (2003) have reported that extract of *Pelargonium citrosa* (Van Leenii), prolonged the duration of larval instars and the total developmental time of *Anopheles stephensi* (Liston). Zhong et al. (2001) have also highlighted that extract from *Rhododendron molle* (G. Dorn) flowers extend the duration of development of *Pieris rapae* L.

We have shown in this work that in pupae, plant extracts have not induced any mortality. This is in agreement with other works. In fact, Bell (1978) showed that pupae may exhibit a higher tolerance to chemical agents than active stages. Papachristos and Stamopoulos (2002) have reported that larvae of *Acanthoscelides obtectus* (Say) were more susceptible than pupae to the fumigant toxicity of the essential oils from *Lavandula hybrida* (Rev), *Rosmarinus officinalis* L. and *Eucalyptus globules* (Lab). Scott et al. (2003) have reported that pupal stage of *Leptinotarsa decemlineata* (Say) was less sensitive to the *Piper nigrum* L. extracts. From the Progeny production of *T. castaneum*, emergence of adult insects from all control samples indicated that tested insects were capable of effective oviposition and that prevention of progeny emergence was exclusively due to treatment. Thus, extracts of *P. harmala*, *A. iva* and *Ari. baetica* either suppressed oviposition or killed the larvae hatching from eggs laid in the medium culture. Huang et al. (2000) have reported similar results for *T. castaneum* when the *Elletaria cardamom* (Maton) oil was applied to wheat. Huang et al. (1997) have reported that F1 progeny production of *T. castaneum* was totally suppressed by nutmeg oil.

These results suggest that there may be different compounds in extracts possessing different bioactivities. Previous works on the phytochemistry of some *Ajuga* species reported the isolation of neo-clerodane diterpenoids (Camps and Coll, 1993; Bondi et al., 2000) and phytoecdysteroids (Wessner et al., 1992). Crude ethanol extracts of *A. iva* (Simmonds and Blaney, 1992; Bellès et al., 1985) or *A. pseudoiva* (BenJannet et al., 2001) have been shown to have antifeedant activity against some Lepidoptera. On the other hand, some insects are sensitive to ingested phytoecdysteroids (Kubo et al., 1983; Tanaka and Takeda; 1993; Blackford and Dinan., 1997; Kefete et al., 2004). Thus these compounds could be responsible of some features observed in *T. castuneum*.

Some *Aristolochia* species are tested for their insecticidal activity. For example, acetone and ethanol extracts of the tubercula and several compounds isolated from *Aristolochia pubescens* L. are potential botanical insecticide agents for the control of *Anticarsia gemmatalis* L. larvae. They inhibit larval growth and induce malformed adults (Nascimento et al., 2004). *Aristolochia clematitis* L., *A. grandiflora* L. and A. *bracteata* L. are used as insect repellent against flies and maggots (Secoy and Smith, 1983), and *against* mosquitoes respectively (Zarroug et al., 1988). *A. argentina* L. showed a significant activity against *Sitophilus oryzae* L. (Broussalis et al., 1999). *Aristolochia* genus is a rich source of aristolochic acid which is unique to this genus, and of terpenoids (Wu et al., 2004). Jacobson (1982) has reported that aristolochic acid present in these species

induce sterility in *T. castaneum*.

Our results have shown that *P. harmala* posses high insecticidal activity on *T. castaneum*. Abbassi et al. (2003) have found the same effect on desert locust *Schistocerca gregaria* (Forskal). *P. harmala* is a rich source of β-carboline alkaloids as harmol, harmine and harmaline (Li, 1996; Kartal et al., 2003). These alkaloids as well as other secondary metabolites of this investigated plant may explain the toxic effect in the studied insects. The investigation on the effects of these pure molecules on *T. castaneum* is undertaken and this work is now in progress.

We can conclude that this study suggest that methanol extracts of *P. harmala* , *A. iva, Ari. baetica*, plants belonging to families taxonomically unrelated to Meliaceae, possesses toxic principles with significant insecticidal effect and could be a potential grain protectant against *T. castaneum*.

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