



Journal of
Entomology

ISSN 1812-5670



Academic
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Insecticidal Activities of Flowerheads of *Anacyclus cyrtolepidioides* Pomel Growing in Tunisia Against *Tribolium confusum* du Val

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Abstract: The effects of *Anacyclus cyrtolepidioides* flowerheads (Compositae family) on *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) adults and larvae were determined. Insecticidal activity of the flowers essential oil, four crude extracts as well as twenty one fractions deriving from solid-liquid chromatographic separation was assessed using direct contact application method. The ethylacetate crude extract and eight fractions (A₃, A₄, P₈, P₁₀, F₂, F₃, F₅ and F₇) showed a significant inhibitory effect of the test material on *Tribolium confusum* du Val growth. One hundred percent mortality of the adults was achieved twelve days after treatment using fractions A₄, P₈ and F₇, respectively. This preliminary study suggested that *A. cyrtolepidioides* may be considered as a potential source of insecticidal compounds.

Key words: Compositae, plant extracts, essential oil, insect growth inhibition, toxicity, antifeedant

INTRODUCTION

To circumvent the problems of human and animal health, pollution and disturbance of ecological balances generated by the massive use of synthesis pesticides, some alternative methods of pest management are developed. Natural pesticides have the advantage of being less phytotoxic and more degradable in the environment (Cooping and Menn, 2000). Thus, the development of new insecticides from plant extracts offers a very promising ground and especially going in the wake of the new concepts of sustainable development and the protection of natural resources. Over 2000 species of plants are known to possess some insecticidal activity (Klocke, 1989). They are still used to kill or repel insects. Traditionally, some plants belonging to the Compositae family are used as insect controllers or insecticides. For example *Flaveria bidentis* L. is used as external insecticide and *Schkurhia pinnata* (Lam) twigs are put below beds or among clothes for repelling insects particularly fleas. Essential oils and their constituents have also been shown to be a potent source of botanical pesticides (Singh and Upadhyay, 1993). Oil extracted from various parts of *Tagetes minuta* L. (Compositae) are used in the tropics as a dressing for livestock to control blowfly. The terthienyl (2,2':5',2''-terthiohene) present in the oil has been identified as an active phototoxic compound against mosquitoes. Its high level of activity makes possible its commercialisation as a mosquito larvicide (Klocke, 1989). In Tunisia, research aiming the discovery and the development of new agent for pest control based on natural products has been undertaken (Barbouche *et al.*, 2001; Ben Jannet *et al.*, 2000, 2001, 2002; Hammami *et al.*, 2006; Saïdana *et al.*, 2005, 2007; Hoalous *et al.*, 2006).

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Continuing our contribution to the biological and chemical study of Tunisian plants (Oueslati *et al.*, 2006; Bergaoui *et al.*, 2007; Hammami *et al.*, 2007; Tekaya-Karoui *et al.*, 2007; Ben Jannet and Mighri, 2007; Chaieb *et al.*, 2007). We describe here on, larval growth inhibition, toxicity and antifeedent effects of *Anacyclus cyrtolepidioides* flower heads essential oil, petroleum ether, chloroformic and ethyl acetate extracts against the major pest of stored products *Tribolium confusum* du Val (Rees, 1995; Jerraya, 2003).

MATERIALS AND METHODS

Plant Materials

Anacyclus cyrtolepidioides was collected in March 2005 at full flowering stage around Gabes city, located in the Southeast of Tunisia. Samples were identified and voucher specimens were deposited in the herbarium of Institut Supérieur Agronomique de Chott-Mariem, Université de Sousse, Sousse, Tunisia. Flowerheads were manually separated from the stems and leaves.

Preparation of Crude Extracts and Fractions

A. cyrtolepidioides fresh flowers (2000 g) were extracted with a steam distiller apparatus during 4 h. Organic solution was dried over anhydrous sodium sulphate then the solvent was removed by evaporation under reduced pressure to give an extract weighing 300 mg. Water residue was extracted three times with ethyl acetate and butanol for 96 h. The crude extracts were obtained after solvents evaporation and were indexed as follows: E₁ for ethyl acetate extract, E₂ for the butanolic extract. The ethyl acetate extract was dissolved in water then extracted successively with petroleum ether (E₃) and chloroform (E₄). The essential oil, petroleum ether (E₃) and chloroformic (E₄) extracts were simplified using silica gel column chromatography (sds, 70-200 µm/2100027) and regrouped in four fractions (from A₁ to A₄) using as eluent CH₂Cl₂; CH₂Cl₂/AcOEt, AcOEt gradients for the essential oil and in ten fractions (from P₁ to P₁₀) for E₃ and eight fractions (from F₁ to F₇) for E₄ using as eluent EP; EP/AcOEt, AcOEt gradients and stored in sealed glass vials in a refrigerator at 4-5°C prior to analysis.

Insects Cultures

Larvae (3 mm of length) and young adults (10-15 days old) of the pest *T. confusum* were obtained from same-age cultures. Insect was fed with white wheat flour and beer yeast (95:5) and incubated at a constant temperature of 30°C and 70% r.h., in darkness. Parent adults were provided by the laboratory of Entomology, High School of Horticulture and Animal production, Chott-Meriem, Sousse University, Tunisia.

Bioassay

Antifeedent, toxicity and insect growth inhibition effects of *A. cyrtolepidioides* flowers essential oil, of its crude extracts and some fractions of the chloroformic extract were evaluated. All bioassays were carried out using the method described by Bloszyk *et al.* (1995).

In fact, 5 µL from each sample of the extracts, the essential oil or the twenty one mentioned fractions were deposited separately on diet disks (1 cm diameter) weighing about 30 mg. The disks are then dried and conserved at 30°C during 24 h and weighed before being offered to larvae and young adults. Three replications of ten insects of each stage kept in 4 cm diameter glass Petri dishes were performed. A control was prepared in the same way. Control and treated disks were placed in separate Petri dishes under no choice tests. Seven days after treatment, the diet disks were reweighed.

Nutritional Indices

The feeding deterrent action was calculated as Feeding-Deterrent Index (Isman *et al.*, 1990).

$$(\text{FDI}\%) = [(C-T/C)] \times 100$$

where, C is the relative consumption rate of control disks and T is the relative consumption rate of treated disks.

The Relative Growth Rate (RGR) was calculated (Farrar *et al.*, 1989) with some modification:

$$\text{RGR} = (A-B)/B \times \text{day}$$

Where:

A = Length of alive larvae (mm) on the 12th day/number of alive larvae

B = Original length of larvae (3 mm)/original number of larvae

Mortality was determined in Petri-dishes, every four days during the essay (20 days),

$$\text{Percentage of larval mortality} = (n/N) \times 100$$

Where:

n = No. of died larvae

N = Original No. of larvae (Tapondjou *et al.*, 2005)

Statistical Analysis

Statistical comparison was performed with SPSS version 11.0. Analysis of variance (one way ANOVA), was followed by means comparison (at $p > 0.05$) and Duncan test

RESULTS

Antifeedant Activity

We tested the bioinsecticidal activity of the four extracts, essential oil and 21 fractions constituents against *T. confusum* adults and larvae (7 days old), results of the bioassay are showed in Table 1 and 2. The ethyl acetate and butanolic extracts, the crude oil and the two fractions A₂ and A₃ appeared to be attractive to *T. confusum* by presenting a negative Feeding-Deterrent Index against adults at the 7th day old.

The extracts, the essential oil and most of the fractions showed a mild phagostimulant activity against larvae especially the fractions F₂ (-283%), F₄ (-287%) and F₇ (-486%) presented a potent phagostimulants activity. Only fractions P₈ and P₁₀ seemed to be antifeedant (95 and 77%) against larvae of *T. confusum*. Those data showed how chromatographic fractionation of the crude extract can modify the specificity of the activity and allow the localisation of the antifeedant and phagostimulant activities in some fraction.

Table 1: The Feeding-Deterrent Index (%FDI) (larvae and adults) and the Relative Growth Rate (RGR) for larvae of *Tribolium confusum* caused by essential oil of *A. cyrtolepidioides*

Fractions (c = %)	% FDI (Adults)	% FDI (Larvae)	RGR (mm/mm/day)
Control	-	-	0.068±0.009c
Essential oil (A)	-135.22±4.06*b	-14.56±0.24b	0.026±0.013b
A ₁	3.96±2.75d	-13.63±4.06b	0.022±0.008b
A ₂	-143.46±9.06b	59.15±8.50d	0.033±0.019b
A ₃	-154.76±15.2a	46.56±5.00c	0.008±0.005a
A ₄	-81.40±3.44c	-84.36±3.25a	0.000±0.000a

*Each value represents mean±SD from the three different glass Petri dishes, Means followed by the same letter(s) within a column are not significantly different in Duncan's Multiple Range Test at $p > 0.05\%$

Table 2: The Feeding-Deterrent Index (% FDI) (larvae and adults) and the Relative Growth Rate (RGR) for larvae of *Tribolium confusum* caused by plant extracts and fractions of petroleum and chloroformic extracts of *A. cyrtolepidioides*

Fractions (c = %)	% FDI (Adults)	% FDI (Larvae)	RGR (mm/mm/day)
Control	-	-	0.0680±0.009c
E ₁	-103.14±2.76b	-17.98±1.73c	0.0060±0.001a
E ₂	-105.00±2.99b	-5.70±0.52c	0.0320±0.008b
E ₃	-23.08±3.5e	-38.16±0.44c	0.0200±0.008b
E ₄	-45.48±0.76d	-74.98±3.10b	0.0440±0.004b
Petroleum ether extract (E₃)			
Control	-	-	0.0680±0.009c
P ₁	0.84±0.18c	-45.38±5.11e	0.0150±0.002b
P ₂	-41.80±1.69a	-59.53±4.08d	0.0160±0.002b
P ₃	-7.01±0.08b	-69.32±0.27c	0.0150±0.005b
P ₄	-10.73±1.20b	-111.63±8.15b	0.0250±0.005b
P ₅	25.44±1.01d	-57.50±1.80d	0.0220±0.032b
P ₆	-49.61±0.97a	-60.99±0.61d	0.0200±0.002b
P ₇	-16.50±3.24b	-22.58±1.80f	0.0180±0.004b
P ₈	-4.70±0.69b	95.79±10.74g	0.0000±0.000a
P ₉	20.94±0.58d	-165.35±8.4a	0.0230±0.002b
P ₁₀	-0.86±0.16c	77.78±6.26g	0.0040±0.011a
Chloroformic extract (E₄)			
Control	-	-	0.0680±0.009e
F ₁	-14.52±1.18b	10.53±2.04c	0.0130±0.010c
F ₂	-43.29±2.51a	-283.09±15.14b	0.0070±0.010c
F ₃	26.41±3.06c	24.53±1.03c	0.0016±0.006b
F ₄	44.73±0.46c	-287.58±6.3b	0.0290±0.013d
F ₅	36.09±2.63c	-6.45±3.9b	0.0003±0.004b
F ₆	-29.44±0.60a	-48.58±0.6b	0.0220±0.007d
F ₇	-14.86±1.24b	-486.34±4.47a	0.0000±0.000a

Means followed by the same letter(s) within a column are not significantly different in Duncan's Multiple Range Test at $p > 0.05\%$

The Inhibitory Activity of the Larvae Growth

The relative growth index of crude extracts, essential oil and fractions, calculated after 12 days of experience were shown in Table 1 and 2. Larvae kept on *Anacyclus* diet disks added with butanolic extract gave no significant differences in growth length between the control and the ethyl acetate extract caused a high significant reduce of larval growth after 12 days. The difference in RGR calculated over a period of 12 days between larvae supplied with *Anacyclus cyrtolepidioides* flowerheads petroleum ether and chloroformic extracts and those kept on control (0.068) was highly significant ($p > 0.05$), whereas larvae fed on disks treated with fractions A₄, P₈ and F₇ never showed any increase in body growth (RGR = 0) and caused larval mortality. Those data revealed that those fractions have substantial toxic effects as was shown by the relatively low growth rates of larvae fed on treated diets.

Toxicity Test

Percentages of *T. confusum* mortality were calculated every four days during a twenty days essay. Figure 1 shows the results of the toxicity test. In the preliminary studies carried out to evaluate hand ling of *A. cyrtolepidioides* specie, it was found that all the crude extracts prepared from the indicated plant showed significant toxicity since the percentage of larvae mortality varied from 54% till 88% 20 days after starting the test. *A. cyrtolepidioides* ethyl acetate crude extract appeared as the most active one inducing a percentage of 88% larval mortality.

In the other hand, we noticed that percentages of larval mortality caused by petroleum ether and chloroformic extract derived fractions (P₁-P₁₀; F₁-F₇) were less than 35% during the 4th day and started to increase from the 8th day, to achieve 100% within 12 days after treatment especially for larvae fed on diet disks impregnated with P₈ and F₇, while only 25% of larvae fed on control disks died 12 days after starting the essay. This shows that P₈ and F₇ can be considered of significant activities against *T. confusum*.

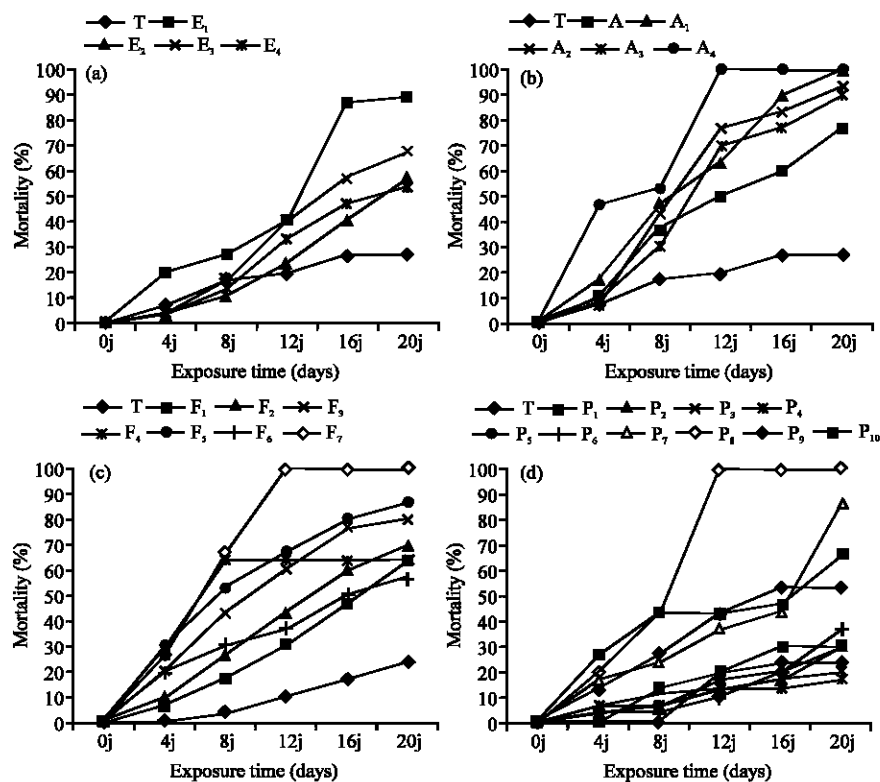


Fig. 1: Percentage mortality of *T. confusum* larvae exposed to different plant extracts, fractions of essential oil, petroleum ether and chloroformic extracts of *A. cyrtolepidioides* at concentration of 1%

Other significant toxicity effects were observed by *A. cyrtolepidioides* essential oil as well as the fractions deriving from its chromatographic separation (A_1 - A_4) within 20 days, the essential oil caused the death of 80% of *Tribolium* larvae. Fraction A_4 can be considered as the most active one inducing a percentage of 100% of larval mortality 12 days after treatment.

DISCUSSION

In this study, 4 natural extracts, essential oil and 21 fractions were tested for their bio-insecticidal activities against *T. confusum* larvae and adults. Antifeedant effect, toxicity and insect growth inhibition were followed up. Responses varied with plant material, extract type, fractions, insect stage and time exposition.

Previous studies searching for Natural products which can be useful as biologically active insecticides mentioned that the most promising botanical insect control agents are present in plants belonging to Compositae family (Jacobson, 1989). Anacycline is a natural insecticide isolated from *Anacyclus clavatus* was reported to have an inhibitory effect on *Tribolium castaneum* growth (Secoy and Smith, 1983; Pascual-Villalobos and Robledo, 1998).

Within the objective of searching Natural insecticide effective for the protection of stored food from insect infestation, we have been interested to the study of *A. cyrtolepidioides* insecticidal effects. The essential oil (A), four crude extracts (E_1 - E_4) as well as 21 fractions derived from chromatographic

separation of *Anacyclus* extracts (A₁-A₄; F₁-F₇ and P₁-P₁₀) were evaluated for their bio-insecticidal activities against *T. confusum* larvae and adults.

We noticed that *A. cyrtolepidioides* ethyl acetate crude extract was attractive against adults; it presents a high toxicity effect and shows a significant *T. confusum* larval growth inhibition when applied at 1%.

Fractions P₈ and P₁₀ deriving from the chromatographic separation of the petroleum ether crude extract presented the potent antifeedent activity (95 and 77%, respectively) and produced a significantly shorter larvae growth in comparison with the control. This suggests that this plant may contain nauseous constituents which can be responsible for antifeedent activity. On the other hand, fractions F₂, F₄ and F₇ deriving from *A. cyrtolepidioides* chloroformic extract showed a highly phagostimulant constituents reducing the growth and inducing mortality of *T. confusum* larvae. This toxic effect may be due to the reversible competitive inhibition of acetylcholinesterase by occupation of hydrophobic site of enzyme's active centre (Ryan and Byrne, 1988). Fraction A₄ deriving from *A. cyrtolepidioides* essential oil exhibited the greatest toxic effect against larvae (100% of mortality).

This research work permitted us to conclude that *Anacyclus cyrtolepidioides* specie can be considered as an interesting plant for investigation in pest control.

ACKNOWLEDGMENTS

We are grateful to Dr. F. Harzallah-Skhiri for plant identification. Laboratoire de Botanique, Institut Supérieur de Biotechnologie de Monastir-UR: Agrobiodiversity, Institut Supérieur Agronomique de Chott-Meriem, Tunisie.

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