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Chrysanthemum Methanolic Extracts as Potential Insecticidal Sources on *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae)

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Abstract: The present study describes the insecticidal activity of methanolic extracts *Chrysanthemum* species on *Tribolium confusum*. Insect responses varied according to plant species. The deterrent effect of *Chrysanthemum* methanolic extracts to stored-product insects was evaluated in multiple-choice tests. Discs were soaped with methanolic extract at 1%. Most species of tested plants have anti-feeding effect on *T. confusum* adults, except *C. macrotum* and leaves of *C. coronarium*, *C. segetum* and *C. grandiflorum* that show a phagostimuling activity. The mortality was find out after 14 and 21 days of ingestion methanolic extracts, we noted that high level mortality was detected after 3 weeks this activities was observed with *C. coronarium*, *C. macrotum*, *C. Myconis*, *C. fuscatum*, *C. paludosum*, *C. trifurcatum* and *C. grandiflorum*, but *C. segetum* had a low toxicity level. After 96 h of topical application with plant extracts at concentration of 1% on *T. confusum* adult 10-14 days showed high toxicity of *C. fuscatum* and *C. grandiflorum* flowers. These results are discussed according the presence of anti-feeding and/or toxic substances actives by consumption or topical application. The identification of these compounds and their action mode will be studied.

Key words: *Chrysanthemum*, methanolic extract, *Tribolium confusum*, toxicity, anti-feeding, topical application

INTRODUCTION

Annual post-harvest losses resulting from insect damage, microbial deterioration and other factors such as humidity, temperature, aeration and cleanliness of the bulk storage, are estimated to be 10-25% of production world wide (Mohan and Fields, 2002). However Insects are the main problem in stored grain because they reduce the quantity and the quality of grain (Madrid *et al*., 1990). The use of synthetic insecticides such as malathion, pirimiphos-méthyl, chlorpyrifos-méthyl, deltamethrin and the fumigant phosphine, are the currently products used against stored-product pests. However, these synthetic insecticide are the source of serious health and ecological problem, such development of pest strains resistance to pesticides (Riebeiro *et al*., 2003), toxicity to mammals and detection of residue in human food (Bugiho and Wilkin, 2004). These problems oriented investigators toward the research of other alternative methods like insect growth regulators and botanical insecticides.

In fact, more than 2000 plant species are a rich source of new insecticides (Klocke, 1989). The insecticidal activity of many plants derivation against several stored-product pests has been demonstrated, but, the two famous compounds are azadirachtin extract from the Indian neem tree (*Azadirachta indica* A. Juss) (Jilani and Saxena, 1990) and pyrethrum from *Chrysanthemum cinerareafolium* (Prakash and Rao, 1997).

In Tunisia, Pottier-Alapetite (1981) mentioned the existence of 13 *Chrysanthemum* species. From this species plant variability we aimed to test the insecticidal activity of flowers and leaves methanolic extracts of eight *Chrysanthemum* species on confused flour beetle. In this study we adopted two methods: Incorporation of the methanolic extract in the artificial diet and topical application on adults aged between 10 and 14 days. Then we find out the anti-feeding index and the mortality. These results are discussed with other results.

MATERIALS AND METHODS

Plant material: Eight *Chrysanthemum* species were collected in different regions of Tunisia, mainly when plants were at flowering stage. Voucher specimens are

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deposited in the herbarium of High institute of Agronomy, Chott Mariam, Sousse, Tunisia. Flowers and leaves were separated and dried at temperature 39±1°C.

Preparation of extracts: Powdered aerial parts of each plant (100 g) were extracted three times by maceration with methanol for 96 h. The extracts were taken to dryness under vacuum.

These extracts of the tested plants were dissolved in appropriate solvent at concentration of 1% and kept at 4°C.

During this study, we adopted the abbreviation (F) for flowers and (L) for leaves.

Insects cultures: *T. confusum* adults aged between 10 and 14 days were obtained from laboratory culture maintained on artificial diet of wheat flour mixed with beer yeast (95:5) at a constant temperature of 30±1°C in the dark. All experiments were carried out under the same environmental conditions as the cultures.

Bioassays

Anti-feeding studies: Flour discs were prepared from the artificial diet. Some discs are treated with methanol solution (5 μL) at concentration of 1% of methanolic extract. Other is treated with methanol alony for the controls. After evaporation of the solvent two treated discs were placed in glass vials. Ten unsexed adults aged between 10 and 14 days were added to each preweighted vial containing the discs. Insects feeding was recorded under three conditions: On pure food (control: CC), on food with possibility of choice (choice test: TC) and on food with the extracts tested (no choice test: TT). Five replicates were prepared. After, 7 days the flour discs were weighed again and insects mortality, was recorded. On the bases of the amount of food consumed in all variants the three deterrency coefficients (R = relative; A = absolute and T = total) were calculated using the formulas according to Nawrot *et al.* (1986).

$$R = C - T/C + T \times 100 \text{ (choice test)}$$

$$A = CC - TT/CC + TT \times 100 \text{ (no-choice test)}$$

- C and CC are the amount of food consumed from the control discs.
- T and TT are the amount of food consumed that had been treated with the tested extracts.

The measure of the deterrent activity of tested extracts is the total coefficient of deterrence:

$$T = A + R$$

The total coefficient of deterrence, which ranged from -200 to 200, served as the index deterrence activity. The compounds with t-values ranging from 151 to 200 are very good deterrents, those with coefficients values of 101-150 are good deterrents and those with t-values ranging from 51 to 100 were moderately active. Compounds with t-values lower than 50 are weak deterrents. Negative t-values point to attractant properties of the extract.

Toxicity by ingestion: Mortality was relived after 14 and 21 days for adult nourished on discs that was treated at 1% by flowers and leaves *Chrysanthemum* methanolic extracts for no-choice test.

Toxicity by topical application: Each extract was dissolved in acetone to obtain the concentration of 1%. One microliter of each solution was abdominally applied to adults aged between 10 and 14 days. Five replications per treatment and control were prepared. The level mortality (%) was recorded After 2, 4, 6, 24, 48, 72 and 96 h.

All theses bioassays are conducted in Entomology laboratory of High institute of Agronomy, Chott Mariam, Sousse, Tunisia, during the year of 2005-2006.

Statistical analyses: The values of deterrence coefficients and mortality were statistically analyzed by means of one-way analysis of variance ANOVA. In the cases where ANOVA results were statistically significant at p = 0.05 and DMRT test was performed.

RESULTS

Anti-feeding activity: The deterrent activity for L and F of 8 *Chrysanthemum* species obtained toward *T. confusum*, after 7 days of treatments are shown in Table 1. The Table 1 showed an antifeeding and a phagostimulant effects for the eight species. But if we compared the leaves and the flowers of each species we conclude that flowers are more antifeeding than leaves in exception of the *C. macrotum* that show a phagostimulant effect. The *C. paludosum* and *C. grandiflorum* flowers have the highest deterrence coefficients on the *T. confusum* adults that are respectively 30.5 and 36.8. However, for the same specie we can also observed an anti-feeding and phagostimuling activity of the leaves and flowers methanolic extract, it's the case of *C. coronarium*, *C. segetum* and *C. grandiflorum*, in fact, flowers show an antifeeding activity but leaves have a phagostimuling activity.

Toxicity by ingestion: The toxic propriety of methanolic extracts flower *Chrysanthemum* species evaluated against adults of *T. confusum* by ingestion of the treated discs

Table 1: Anti-feeding and toxicity activity of *Chrysanthemum* on adults of *T. confusum* in a flour disc bioassay

		Deterrence coefficients[a]			Adults toxicity	
Species	Plant part	A	R	T	14 days	21 days
Control	-	-	-	-	8 ± 4^{g}	18 ± 4^{e}
C. trifurcatum	F	8.0^{c}	7.4^{ef}	15.4^{de}	20 ± 12^{fg}	58 ± 11^{cd}
	L	15.1^{b}	11.5^{cde}	26.6^{bc}	80 ± 20^{ab}	100 ± 0^{a}
C. coronarium	F	-4.8^{d}	15.8^{bc}	11.0^{def}	92 ± 8^{a}	100 ± 0^{a}
	L	-11.6^{de}	-3.2^{g}	-14.7^{k}	22 ± 16^{fg}	800 ± 23^{ab}
C. macrotum	F	8.6^{bc}	-18.9^{i}	-10.4^{jk}	52 ± 25^{cde}	92 ± 8^{ab}
	L	-9.5^{d}	6.7^{ef}	-2.8^{ij}	36 ± 28^{def}	76 ± 29^{bc}
C. segetum	F	7.0^{c}	8.2^{cdef}	15.3^{de}	40 ± 16^{def}	56 ± 16^{d}
	L	-17.5^{e}	-9.5^{gh}	-27.0^{l}	14 ± 5^{fg}	16 ± 15^{e}
C. fuscatum	F	24.2^{a}	-11.2^{h}	12.9^{def}	76 ± 9^{abc}	100 ± 0^{a}
	L	-11.7^{de}	19.5^{b}	7.9^{efg}	80 ± 21^{ab}	96 ± 9^{ab}
C. grandiflorum	F	8.9^{bc}	27.8^{a}	36.8^{a}	100 ± 0^{a}	100 ± 0^{a}
	L	-8.5^{d}	-6.3^{gh}	-14.9^{k}	34 ± 21^{efg}	76 ± 23^{bc}
C. myconis	F	-10.5^{d}	14.6^{bcd}	4.1^{fgh}	62 ± 41^{bcd}	100 ± 0^{a}
	L	-8.9^{d}	10.3^{cdef}	1.4^{gh}	28 ± 18^{efg}	86 ± 11^{ab}
C. paludosum	F	26.9^{a}	3.6^{f}	30.5^{ab}	78 ± 8^{abc}	100 ± 0^{a}
	L	9.5^{bc}	10.0^{cdef}	19.4^{cd}	38 ± 22^{def}	92 ± 11^{ab}

[a]: A = Absolute coefficient; R = Relative coefficient; T = Total coefficient. Values followed by the same letter within a column are not significantly different at the $p<0.05$ DMRT test

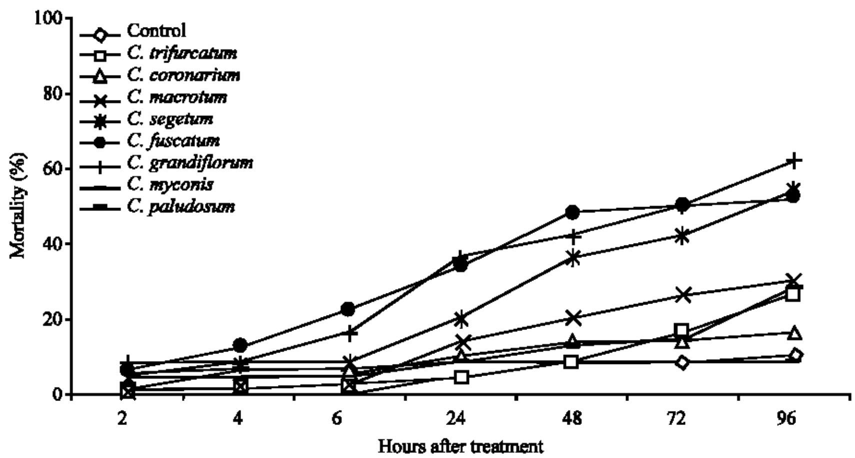

Fig. 1: Level mortality on *Tribolium* adult treated by topical application with leaves methanolic extract

flowers during three weeks showed an evolution of adults mortality level. In fact, at the first week we can't signal any mortality for any extracts. After 14 days of treatment we noted a significant ($p<0.05$) mortality for all extracts, but the highest level is caused by the F methanolic extract of *C. coronarium* (92%) and *C. grandiflorum* (100%).

After three weeks of treatment a high toxicity of five flowers methanolic extracts *Chrysanthemum*: *C. fuscatum*, *C. Myconis*, *C. paludosum*, *C. coronarium*, *C. grandiflorum* (100%), *C. macrotum* (92%) was observed.

Leaves methanolic extract are toxic, the toxicity varied between 76% of *C. grandiflorum* and *C. macrotum* and 100% of *C. trifurcatum* , but the lowest level toxicity was obtained from leaves *C. segetum* extract (16%).

In this case we noted that the mortality level increase by time, furthermore flowers methanolic extracts are more toxic than us leaves at exception of *C. trifurcatum* specie where leaves methanolic extracts (100%) are more toxic than us flowers (58%) on tribolium adult after 3 weeks of treatment.

Toxicity by topical application: The mortality level of adults treated by topical application with *Chrysanthemum* methanolic extracts is pursued after 2, 4, 6, 24, 48, 72 and 96 h (Fig. 1, 2). We noted that the greatest parts of extracts are active after 2 h in exception of *C. trifurcatum* and *C. macrotum* methanolic extract. The toxicity of treated adults increase constantly with hours, but the highest level toxicity is registered for adults treated by flower *C. grandiflorum* methanolic extract, which was 80% after 96 h. We can observe that this species is significantly more toxic than other plants.

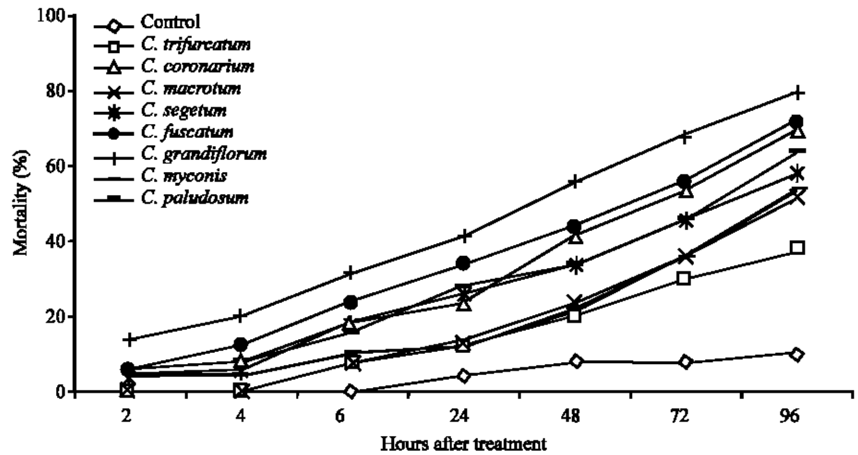

Fig. 2: Level mortality on *Tribolium* adult treated by topical application with flowers methanolic extract

DISCUSSION

The present study revealed the effect of eight *Chrysanthemum* species methanolic extracts on *T. confusum* adults. In this study, we adopted two methods: The first one studies the feeding behaviour and its consequence on tribolium survey and the second determinates the toxicity caused by topical application.

The feeding behaviour study displays that flowers and leaves methanolic extracts of 8 species have an anti-feeding and a phagostimuling activity on *T. confusum* adults. This anti-feeding activity was also observed on another pest insect such *Spodoptera littoralis* larvae treated with *C. coronarium*, *C. Myconis*, *C. segetum* flowers powders (Haouas *et al*., 2005, 2006). This effect could be attributed to alimentary factor obstruction in the central nervous system of insect which is already proved in the azidirachtin effect (Mordue and Blackwell, 1993). To intestine cytotoxic following precocenes action (Bowers, 1983) and also to the protein inhibition in digestive canal; like the effect of saponin extracted from *C. parqui* (Barbouche *et al*., 2001).

In fact, many studies have characterized several compounds having a similar effect, such as spiroketal detected in *C. segetum* (Gao *et al*., 1998), limonoid toosendanin isolated from *Melia toosedan* (Xie *et al*., 1995), terpenes and triterpenes which were respectively extracted from *Cyperrus articulatus* and *Junellia aspera* (Abubakar *et al*., 2000; Pungitore *et al*., 2005). All those compounds have showed an anti-feeding activity on different stored-pest insects.

Other works performed on synthetic compounds have showed that eugenol reduced significantly food consumption on *Sitophilus zeamais* adults at a concentration of 13.2 mg g^{-1} food (Huang *et al*., 2002). Tripathi *et al*. (2003) have reported that *d*-Limonene presents anti-feeding activity against lesser grain borer, *Rhyzopertha dominica* (F.), *S. oryzae* and red flour beetle, on adults and larvae at the concentration of 60 mg g^{-1} food.

The comparison between the toxicity resulting from the consumption of methanolic extracts and its topical application, show a relation between anti-feeding activity and the toxicity. In one hand, we noted that the more anti-feeding plants were the highest toxic on *T. confusum* adults (*C. grandiflorum* and *C. fuscatum* flowers). This relation was also observed by Xie *et al*. (1995) on three stored-product beetles with the *Melia toosedan* ethanol extract. In the other hand the toxicity revealed after 96 h of topical application on *T. confusum* adults aged between 10-14 days, showed a high level toxicity (80%) caused by *C. grandiflorum* flowers methanolic extract. This toxicity could be due to the presence of triterpenes such those observed by Pungitore *et al*. (2005). This supposes that there are at least two different compounds in the *C. grandiflorum* flowers methanolic extract.

These results suggested the presence of many compounds in different *Chrysanthemum* methanolic extracts, these compounds have an anti-feeding, phagostimuling and toxic activity. They can be distributed in each *Chrysanthemum* species or regroup in one species which is the case of *C. grandiflorum*. The isolation and identification of theses compounds will be a part of our future project.

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