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Assessment of Insecticidal Effect of *Chrysanthemum* sp. Essential Oils against *Tribolium confusum* du Val (Coleoptera, Tenebrionidae)

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ABSTRACT

As part of our vegetable heritage value we studied the insecticidal activity of essential oils extracted from four *Chrysanthemum* species against *Tribolium confusum* du Val, a stored products pest. The study of repellent activity of essential oils extracted from leaves and flowers of *Chrysanthemum* species against *T. confusum* adults show a strong repulsive effect of all species. Topical application of essential oils from *Chrysanthemum coronarium* L. and *Chrysanthemum grandiflorum* (L.) Batt. flowers on *T. confusum* pupae caused a significant insect mortality that attend 67 and 61%, respectively after 7 days of treatment. The malformation assessment of newly emerged adults issued from treated pupae showed that *C. coronarium* leaves essential oils caused the highest rate of insect malformation (10%). These results demonstrate the insecticidal activity of essential oils by pupae toxicity, by hormonal balance disruption (appearance of adored malformed) or by insect repellency. These three activities can enhance the protection of stored products against *T. confusum.* Nevertheless, further studies on other stored product insects are highly recommended to ensure maximum protection of agricultural products.

Keywords: adults, *C. coronarium*, *C. grandiflorum*, malformation, pupae, toxicity

INTRODUCTION

Insect pests are one of the major factors limiting the production and storage of agricultural products. In fact, Copping and Hewitt (1998) predict that the most effective method to limit the damage of these pests is the use of synthetic pesticides. However, the abuse use of synthetic insecticides has led to ecological problems and behavior, manifested by the appearance of residues in soil and agricultural products and the resurgence of resistant pest populations (Khambay *et al.* 1999).

Considerable efforts are employed in order to find more effective and biodegradable insecticides. Indeed, in a review of insecticidal plants, Wheeler and Isman (2001) announced that species belonging to the Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae families are the most promising ones in the fight against insects. Many reviews deal with the use of plant products against insect pests of stored products (Boussalis *et al.* 1999; Pascual-Villalobos and Fernández 1999; Abubakar *et al.* 2000; Huang *et al.* 2000; Fields *et al.* 2001; Tripathii *et al.* 2002; Isman 2006). However, only few of them deal with essential oils (Shaaya *et al.* 1997; Lee *et al.* 2001; Choi *et al.* 2006).

Tribolium confusum is one of the most serious pests in stored grain and related products (Aitken 1975; Rees 1995) and it can easily infest and damage products. This species is resistant to several traditional insecticides, which are commonly used as grain protector (Arthur 1996).

The aim of this study is to assess the insecticidal activity of essential oils extracted from four *Chrysanthemum* species on confused flour beetle, by incorporation in artificial diet and topical application on pupae. The repellent index, pupae mortality and level of new emerged and malformation adults were evaluated.

MATERIALS AND METHODS

Plant material

Chrysanthemum species were collected from four different regions of Tunisia characterized by different climates as described in **Table 1**. Plant identity was confirmed by the botanist Harzallah-Skhiri from the Laboratory of Botanic, Higher Institute of Biotechnology of Monastir, University of Monastir. Voucher specimens were deposited in the National Gene Bank of Tunisia.

Table 1 Former and new classification of the four studied *Chrysanthemum* species, sites and dates of collect, bioclimate stage of the site.

Former classification (Pottier-Alapetite 1981)	New classification (Le Floc'H *et al.* 2010)	Date of collect	Site of collect	Bioclimatic stage
Chrysanthemum grandiflorum (L.) Batt.	*Plagius grandis* (L.) Alavi & Heywood	June	Sedjenane (Western-North)	Humid
Chrysanthemum coronarium L.	*Glebionis coronaria* (L.) Spach	March	M'saken (Sahel Center)	Semi-arid
Chrysanthemum fuscatum Desf.	*Heteromera fuscata* (Desf.) Pomel	April	Gafsa (South)	Arid
Chrysanthemum trifurcatum (Desf.) Batt. et Trab.	*Chrysanthoglossum trifurcatum* (Desf.) Wilcox & *al*.	March	Sidi Bouzid (South)	Arid

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Original Research Paper

1. Essential oil extraction

Essential oils were extracted from fresh leaves and flowers by steam distillation using a Clevenger apparatus for 4 h. The essential oils were stored at 4°C until use.

Insects

Tribolium confusum pupae and adults were obtained from insect rearing in the Entomology laboratory, maintained in incubators at 30±1°C and 70-80% RH with dark. The two insect stages were fed on wheat flour mixed with yeast (10:1, w:w).

1. Repellency bioassay

Essential oils were mixed with the diet (5:95 v/w). A choice bioassay with 10 replications (dishes) offering treated and untreated diet was set up using 10 adults of *T. confusum* (10-14 days old) per dish released in the middle of a plastic Petri dish (diam. 10 cm). After 24 h (h), the number of adults present at each amount of treated or control diet was counted. Repellent index was calculated as RI = (C-T)/(C+T) × 100, with C = number of adults on control diet and T = number of adults on treated diet.

If RI > 50 the essential oil is repellent; RI < 50, essential oil is non-repellent (Pascual-Villalobos and Robledo 1999).

2. Contact toxicity by topical application

A 1% acetone solution of essential oils from each *Chrysanthemum* species was prepared and 1 µl was topically applied to the ventral area of the thoracic segments of insects using a Hamilton microsyringe. Controls were treated with the solvent alone. After treatment, insects were placed in an incubator into plastic vials containing food. Five replicates of 10 pupae each were prepared. The mortality (%) of insects was observed daily, during one week (Pungitore *et al.* 2005).

Statistical analyses

The values of repellent index, % mortality, % emergence and malformation were statistically analyzed by means of one-way analysis of variance ANOVA at significance with $P < 0.05$.

RESULTS

Repellent indices

Table 2 shows repellent indices calculated after 24 h for adults fed on diet mixed with essential oils. In all cases, we noted that values of RI exceed 50%, this result highlights the repellent effect of essential oils extracted from the four tested *Chrysanthemum* species. For all species, flowers essential oils are more repellent than those from leaves. Statistical analysis showed that essential oil obtained from *C. fuscatum* flowers had the most repellent effect on *T. confusum* adults (RI = 99.58%). Nevertheless, *C. trifurcatum* essential oils (leaves and flowers) have the lowest repellent activity on adults confused flour beetles.

Contact toxicity by topical application

Tribolium confusum pupae (less than 24 h old) were topically treated with eight C*hrysanthemum* essential oils diluted in acetone to obtain a concentration of 1%. The pupae mortality was daily followed-up during 7 days. Obtained results showed that tested essential oils have a variable effective insecticidal activity on *T. confusum* pupae (**Table 3**). In fact, from the first day to the seventh one of treatment, essential oil from *C. coronarium* and *C. grandiflorum* flowers exhibited the highest significant ($P < 0.05$) mortality on pupae. In fact, it was increased from 28 and 31% on the first day to

Table 2 Repellent index (RI) of the essential oils extracted from fresh leaves and flowers of the four studied *Chrysanthemum* species.

Species	Organ	Repellent index (RI) *
C. coronarium	Leaves	94.48 e
	Flowers	98.70 c
C. grandiflorum	Leaves	87.25 f
	Flowers	99.12 b
C. fuscatum	Leaves	95.39 d
	Flowers	99.58 a
C. trifurcatum	Leaves	78.95 h
	Flowers	79.25 g

*Means followed by the same letters are not significantly different at $P < 0.05$, determined by Duncan's multiple range test.

Table 3 Mortality (%) of treated *Tribolium confusum* pupae after topical application with essential oils from leaves and flowers of the four *Chrysanthemum* species tested at a concentration of 1%*.

Species	Organ	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Control		1 ± 0.24 b	3 ± 1.47 b	5 ± 1.14 c	7 ± 2.5 c	10 ± 4.65 c	10 ± 4.65 c	10 ± 4.65 d
C. coronarium	Flowers	28 ± 11.89 a	40 ± 15.90 a	47 ± 22.6 a	47 ± 22.6 a	52 ± 16.04 a	61 ± 23.29 a	67 ± 28.85 a
	Leaves	6 ± 2.18 b	9 ± 4.18 b	12 ± 5.7 bc	17 ± 6.85 bc	21 ± 9.61 bc	25 ± 11.94 bc	25 ± 11.94 bcd
C. fuscatum	Flowers	6 ± 2.18 b	10 ± 3.12 b	15 ± 6.8 bc	18 ± 5.7 bc	19 ± 8.94 bc	21 ± 5.63 bc	24 ± 8.22 cd
	Leaves	6 ± 2.15 b	12 ± 5.7 b	15 ± 5.96 bc	18 ± 6.54 bc	21 ± 6.12 bc	21 ± 6.12 bc	25 ± 6.52 bcd
C. grandiflorum	Flowers	31 ± 8.21a	34 ± 9.62 a	40 ± 11.72 a	42 ± 11.51a	49 ± 16.35 a	56 ± 17.24 a	61 ± 17.32 a
	Leaves	7 ± 2.7 b	10 ± 3.6 b	24 ± 6.52 b	27 ± 10.37 b	31 ± 11.93 ab	36 ± 12.31 b	41 ± 11.25 b
C. trifurcatum	Flowers	5 ± 2.30 b	9 ± 2.78 b	11 ± 4.21 bc	10 ± 3.90 c	15 ± 5.9 bc	20 ± 10.11 bc	21 ± 9.72 cd
	Leaves	10 ± 4.35 b	15 ± 6.43 b	17 ± 7.43 bc	21 ± 8.22 bc	24 ± 7.54 bc	30 ± 11.18 b	33 ± 6.12 bc

*Means followed by the same letters are not significantly different at $P < 0.05$, determined by Duncan's multiple range test.
n = 50 insects/treatment

Table 4 Emergence (%) of new adults issue from *T. confusum* pupae treated with essential oil from leaves and flowers of the four *Chrysanthemum* species at a concentration of 1%.*

Species	Organ	3 days	4 days	5 days	6 days	7 days
Control		1 ± 0.34 ab	3 ± 1.78 a	5 ± 2.33 cd	87 ± 6.89 a	93 ± 7.58 a
C. coronarium	Flowers	0 ± 0.00 b	0 ± 0.00 a	15 ± 8.54 bcd	39 ± 20.76 bc	45 ± 24.24 cd
	Leaves	0 ± 0.00 b	3 ± 1.78 a	25 ± 11.47 abc	69 ± 17.10 ab	69 ± 17.10 b
C. fuscatum	Flowers	0 ± 0.00 b	4 ± 1.56 a	18 ± 3.68 abcd	61 ± 5.23 abc	74 ± 6.52 ab
	Leaves	0 ± 0.00 b	0 ± 0.00 a	27 ± 12.56 ab	68 ± 10.56 ab	74 ± 14.75 ab
C. grandiflorum	Flowers	0 ± 0.00 b	1 ± 0.34 a	11 ± 5.61 bcd	30 ± 2.97 c	36 ± 4.18 d
	Leaves	0 ± 0.00 b	0 ± 0.00 a	3 ± 1.12 d	38 ± 6.81 bc	59 ± 8.22 bc
C. trifurcatum	Flowers	3 ± 1.78 a	7 ± 2.45 a	36 ± 16.34 a	63 ± 18.42 abc	70 ± 24.50 b
	Leaves	0 ± 0.00 b	2 ± 1.51a	22 ± 9.53 abcd	60 ± 5.31abc	61 ± 6.52 bc

*Means followed by the same letters are not significantly different at $P < 0.05$, determined by Duncan's multiple range test.
n = 50 insects/treatment

67 and 61%, respectively at the end of experiment. While, the essential oils obtained from *C. coronarium* and *C. fuscatum* leaves and *C. trifurcatum* and *C. fuscatum* flowers have a low significant effect on treated pupae.

Emergence of adults

Table 4 presents the percentage of emerged adults from pupae treated with four different *Chrysanthemum* essential oils. The results showed that the emergence of adults started at the third day of the bioassay for pupae treated with *C. trifurcatum* flowers essential oil as well as the control. However, we noted a delay (2 days) of emerged adults obtained from pupae treated with *C. coronarium* flowers and *C. fuscatum* and *C. grandiflorum* leaves essential oils. After 7 days of treatment, all survived control pupae were emerged. Concerning the treated pupae, we noted that those treated with *C. fuscatum* (flowers or leaves) essential oils give the significant percentage of insect emergence (70%). But the most active essential oil on new tribolium emergence was from *C. grandiflorum* flowers where we recorded the lowest emergence percentage (36%).

Insect malformation

Confused flour beetles pupae aged less than 24 h were treated topically with essential oils from leaves and flowers of the four tested *Chrysanthemum* species at a concentration of 1%. The highest percentage of malformation was reported for pupae treated with *C. coronarium* and *C. trifurcatum* leaves essential oils (**Fig. 1**). However, the other *Chrysanthemum* essential oils exhibit biological activity but at a lower rate (**Fig. 1**). We record the emergence of new adults with lethal aberrations (**Fig. 2A-C**). **Fig, 2A** shows the persistence of nymphal characters. In **Fig. 2B**, we noted malformation of the membranous wing that makes insect movement difficult. Moreover, **Fig. 2C** shows an adult that lost part of its left elytron while the right one is broken. It is interesting to note that all observed malformations are lethal and no adult can survive and reproduce with these anomalies.

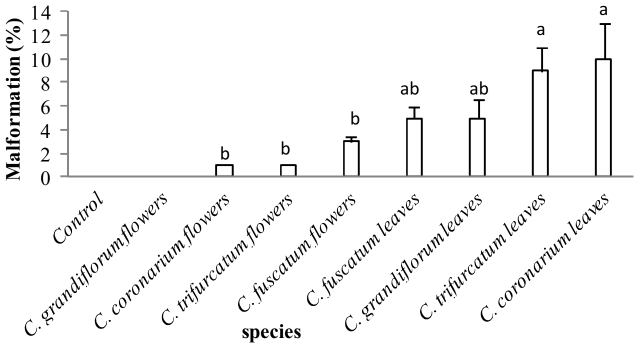

Fig. 1 Malformation (%) of new adults issue from *T. confusum* pupae treated with essential oil from leaves and flowers of the four *Chrysanthemum* species tested at a concentration of 1%. Means followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's multiple range test. n = 50 insects/treatment. Bars indicate SD.

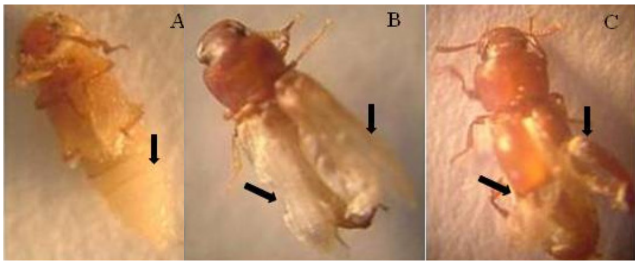

Fig. 2 Morphological aberrations observed in emerged new adults. (**A**) Persistence of nymphal characters. (**B**) Malformation of membranous wings. (**C**) Breaking elytra (Gr.x3).

DISCUSSION

The present study revealed the effect essential oils extracted from four *Chrysanthemum* species on adults and pupae of *T. confusum*. The repellent behavior study showed that all *Chrysanthemum* species oils have a repellent activity against *T. confusum* adults. This effect was previously observed for *Tribolium castaneum* (Herbst) larvae treated with acetone extract of *Artemisia barrelieri* Besser and *Stipa tenacissima* L. (Pascual-Villalobos and Robledo 1999). This activity can be attributed to the composition of tested essential oils. In fact, chemical study of essential oil obtained from *C. coronarium*, *C. fuscatum* and *C. grandiflorum* showed their richness in monoterpenes (Haouas *et al.* 2012). This relation was confirmed by Koul (2005) who demonstrated that most monoterpenes exhibit repellent activity.

In topical application of oils, monoterpenes seem to have an important role in the insecticidal activity of the essential oil (Coats *et al.* 1991; Regnault-Roger and Hamraoui 1995; Ahn *et al.* 1998; Teixeira da Silva 2004). Their high lipophilicity enables them to rapidly penetrate into insects and interfere with their physiological functions (Lee *et al.* 2002). In this study, it was demonstrated that *C. grandiflorum* and *C. coronarium* flowers essential oils have a lethal effect on *T. confusum* pupae when they are topically applied. Chemical analysis showed that *C. coronarium* and *C. grandiflorum* flowers essential oils were rich in monoterpenes (51.8 and 31.9%, respectively) (Haouas *et al.* 2012). The highest malformation of new adult issued from pupae treated with essential oils from *C. coronarium* and *C. trifurcatum* leaves could be attributed to the perturbation of hormonal balance of insect during metamorphisms. The relation between morphological perturbation and essential oil composition need to be studied.

Though these preliminary findings, they could represent the basis for further investigations on the questions raised in this study. In particular, additional research is needed to investigate the susceptibility of other stages of the insect life, such as larvae and eggs and to improve the understanding of how mono- and sesquiterpenes act on insects. Practically, in view of a possible use of the most active mono- and sesquiterpenes, it should be pointed out that longer exposure periods could be needed to bridge the tolerant phases of insect.

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