The African Journal of Plant Science and Biotechnology ©2008 Global Science Books

Three *Chrysanthemum* Flowerhead Powders in Controlling Feeding and Behaviour of *Spodoptera littoralis* (Lepidoptera:Noctuidae) (Boisduval)

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ABSTRACT

Powders prepared from *Chrysanthemum coronarium*, *C. myconis* and *C. segetum* (Asteraceae) were tested for their effectiveness on *Spodoptera littoralis* larval feeding behaviour, development and survival under laboratory conditions. The powders were incorporated into an artificial diet at three concentrations (4, 8, 16% w/w), and tested in a no-choice bioassay. Third-instar larvae were individually fed on an artificial diet added with powders of the three *Chrysanthemum* species for 10 days. Each diet disc was weighed before presenting it to the larvae, and reweighed and replaced by a newly weighed disc every 2 days throughout the trial. Each larva was also weighed every 2 days. Consumption of control and treated discs was recorded and antifeedant indices and relative growth rates (RGR) were calculated. Duration of stages, mortality and deformities were also recorded until adult emergence. A 10-days feeding period promoted the prolongation of larval instars, a potent antifeedant activity, reduction in the RGR, a moulting disruption and mortality of *S. littoralis* in a dose-dependant manner. When higher concentrations of flowerhead powders were applied the effects appeared shortly and mortality was higher especially when larvae were fed 16% of *C. myconis* powder, and a less effect was observed with *C. coronarium. Chrysanthemum* powders may contain secondary compounds with insecticidal activities causing mortality and disrupting the development of larvae and thus preventing them from causing damage to the crop.

Keywords: antifeedant activity, Asteraceae, *Chrysanthemum* species, larval instars, mortality
Abbreviations: AFI, Antifeedant index; **RGR**, relative growth rate

INTRODUCTION

Increasing public concern over environment pollution and toxicity due to the use of broad spectrum persistent synthetic insecticides whose toxic residues contaminate soils has stimulated a search for natural compounds that could replace synthetic insecticides in insect pest control (Addor 1995; Ishaaya and Horowitz 1998). During the two decades both synthetic and botanical pesticides that are biodegradable and less persistent have been identified and developed in this regard (Blaney *et al.* 1988). Molt hormones and related phytoecdysteroids, which at certain applications elicit abnormal molting associated with lethal development and reproductive derangements, have been considered as potential pest control agents (Kubo *et al.* 1983). With this in mind, many African researches have been studying a range of plant compounds that show toxicity to insects or to modify their behaviour (Ben Jannet *et al.* 2000, 2001; Chaieb *et al*. 2001a, 2001b, 2002; Haouas *et al.* 2005, 2006). In fact, more than 2000 plants species are known for their insecticidal activities (Broussalis *et al.* 1999). Among these species we can quote *Azadirachta indica* A. Juss (Meliaceae) (Weinziel and Henn 1995; Isman 1997) and *Chrysanthemum cinerariaefolium* (Asteraceae) (Burkhart and Burkhart 2002). Plants belonging to the Asteraceae family have been repeatedly reported as containing insecticidal compounds (Teixeira da Silva 2004; Teixeira da Silva *et al*. 2005). Some species from the genus *Chrysanthemeum* have been shown to have potent antifeedant activity against Lepidopterian larvae. In fact, Secoy and Smith (1983) isolated pyrethrins from dried flowers of *C. cinerariaefolium.* Polyacetylenic compounds have been isolated for the aerial parts and insect antijuvenile hormone activity has been detected from some of them (Bowers and Aregullin 1987; Sanz *et al.* 1990). From flowerheads of *C. coronarium*, sesquiterpene lactones have been isolated (El-Masry *et al.* 1984). *C. coronarium* flowerhead essential oil was active both in contact and headspace *in vitro* assays producing hyphal growth inhibition (Alvarez-Castellanos *et al*. 2001). Pottier-Alapetite (1981) reported the presence of 13 spontaneous *Chrysanthemum* species in Tunisia. In the context of ongoing interest in potential natural insect antifeedants the preliminary investigation of the antifeedant activity against the cotton leafworm *Spodoptera littoralis* larvae of flowerhead powders has been studied for three *Chrysanthemum* species (Asteraceae) growing in Tunisia.

A general idea was given about biology and behaviour of this Lepidoptera. In fact, between 2 and 5 days after emergence, females lay 1000-2000 eggs on the lower leaf surface of the host plant. Eggs were spherical, somewhat flattened, usually whitish-yellow, 0.6 mm in diameter, laid in batches and covered with hair scales from the tip of the abdomen of the female moth (OEPP/EPPO 1981). Fecundity is adversely affected by high temperature and low humidity. The eggs hatch in about 4 days in warm conditions, or up to 11-12 days in winter (Baker and Miller 1974). Larva attains 40-45 mm in length; hairless and variable in colour. They pass through six instars in 15-23 days at 25-26°C (OEPP/EPPO 1981). At lower temperatures, larvae often go through an extra instar, and maturation may take up to 3 months (Nasr 1973). The young larvae (first to third instar) feed in groups. Later, the (4^{th} to 6^{th} instar) larvae disperse and spend the day in the ground under the host plant, feeding at night and early in the morning. Pupa was 15-20

Received: 2 January, 2008. Accepted: 23 March, 2008.

Original Research Paper

mm long, red-brown. The pupal period is spent in the soil and lasts about 11-13 days at 25°C. The moths with grey-brown body, 15-20 mm long (Cayrol 1972; Mochida 1973; Brown and Dewhurst 1975) have a flight range of 1.5 km during a period of 4 h overnight, facilitating dispersion and oviposition on different hosts. Longevity of adults is about 4-10 days, being reduced by high temperature and low humidity. Thus, the life cycle can be completed in about 5 weeks (Salama and Shoukry 1972).

To the best of our knowledge no work on insecticidal activity of Tunisian *Chrysanthemum* species has been found until now. In this study, *S. littoralis* was used because it is, worldwide, an economically important pest of cotton, vegetables and ornamentals, and resistant populations cause severe problems in various regions in Tunisia. On the other hand the Lepidopterian *S. littoralis* larvae are especially interesting for studying how some plant compounds can deter feeding because they possess few taste sensilla, grouped on three organs on the cephalic capsule (Bernays and Chapman 1994; Marion-Poll and Descoins 2002). The sensilla are known to house taste neurones, which are likely to be involved in the perception of plant secondary compounds such as phytoecdysteroides (Schoonhoven 1987; Marion-Poll and Descoins 2002).

MATERIALS AND METHODS

Plant material

Three species of the genus *Chrysanthemum* L. (Asteraceae). *C. coronarium* L., *C. myconis* L. and *C. segetum* L. were collected in 2006 at the flowering stage from a natural plot in the locality of Nabeul, in the northeast coastal region of Tunisia and subsequently identified in the laboratory. Voucher specimens were deposited at the herbarium of the Higher Agronomic Institute (HAI) of Chott-Meriem, University of Sousse, Tunisia. Flowerheads were separated from each plant species, dried for one week, at room temperature and ground to 1 mm diameter particle size with a rotating-knives mill (Moulinex model). Powders were stored at 4°C and added to the *S. littoralis* larvae's artificial diet.

Insect culture

Spodoptera littoralis adults were trapped, identified by the entomologist Dr. Ben Halima-Kamel Monia, reared in Plexiglas boxes and fed with 15% (v/v) honey water solution. *Spodoptera* larvae obtained from eggs were kept on Petri dishes at 25 ± 1°C, under a 16:8 light:dark photoperiod and 70% relative humidity and reared on a maize-based artificial diet (Poitout and Bues 1974) for >30 generations. The culture has been continuously supplemented with wild moths captured in a light trap at the HAI of Chott-Meriem.

Bioassays

Artificial diet

Constituents of artificial diet were: 77.95% water, 1.83% agar, 12.84% maize semolina, 3.21% wheat germ, 3.43% beer yeast, 0.45% ascorbic acid, 0.13% benzoic acid, 0.11% nipagine (para-hydroxybenzoic acid ester) (M.W.=152.1, Biomedicals LLC., France) and 0.05% formic aldehyde.

Feeding assays

A no-choice bioassay was used to test whether the compounds could suppress or reduce feeding for a period of time. Ten larvae at the beginning of the third instar having similar weight (10-12 mg) were deprived of food for 4 h before being placed individually in Petri dishes (9 cm diameter) with artificial diet discs mixed with 0 (for controls), 4, 8, and 16% (wt/wt) of each *Chrysanthemum* species' flowerhead powder. Each disc was weighed before being presented to the larvae, and reweighed and replaced by a new weighed disc, every 2 days. The feeding trial was conducted for 10 days in 5 replicates using larvae from a different generation of the stock culture. Each larva was also weighed every 2 days for 10 days, which is the necessary period before the pupa instars.

Nutritional indices

Results presented here are therefore the outcome of pooled data of five replications. The nutritional indices were calculated as follows: Antifeedant index (AFI) was calculated, for each larvae, every 2 days until the 10^{th} day of the trial essay, from the formula: AFI=[(C-T)/ C+T))x100], with C as the consumption of control discs and T the consumption of the treated discs. A positive value for the antifeedant index indicates an antifeedant and a negative one indicate a phagostimulant effect. The relative growth rate (RGR) was calculated every 2 days over the 10-days trial as follows: RGR = final wt/initial wt/day, and expressed in mg/mg mean wt/day (Zhang *et al.* 1993).

Behavioural bioassays

Two parameters were also assessed: a) Duration of each larval instar in the control and each treatment over 10 days, by noting the date of cuticle exuviations, b) Larvae, pupae and adult mortality, were recorded until adult emergency. Larval and pupal abnormalities were also reported.

Statistical analysis

The antifeeding and nutritional indices of different treatments were compared statistically using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test by the system program SPSS 12.0 software for Windows 2003 (SPSS Inc., Chicago, IL).

RESULTS

Effects of *Chrysanthemum* flowerhead powders on feeding of *S. littoralis* larvae

A no-choice feeding bioassay was used to test whether the flowerhead powders of the three *Chrysanthemeum* species could suppress or reduce feeding for a period of time. Those powders added in the diet discs differ in their effects on the feeding behaviour of the larvae. The results of the bioassays are presented in **Table 1**. The feeding indices varied from 2.6 to 50.0. *Chrysanthemum* flowerhead powder added at 4 or 8% did not deter feeding until the 8^{th} day of the bioassay. When added at 16% and after 10 days it showed a potent antifeedant activity which reached 42.2, 47.5 and 50.0 for *C. segetum*, *C. coronarium* and *C. myconis*, respectively, which is related to a decrease in consumption probably due to anti-feeding substances present in *Chrysanthemum* species. Consumption by the 3^{rd} instar larvae of treated diet discs added with 16% of *Chrysanthemum* flowerhead powders was significantly lower than consumption of control discs or discs to which 4% or 8% of powder was added.

Effect of three *Chrysanthemum* spp. flowerhead powders on duration of larval instars

When reared on control discs, duration of the larval 3^{rd} instar varied from 3.4 ± 0.6 to 4.2 ± 0.9 days (**Table 2**). Whatever was the species and the concentration, addition of *Chrysanthemum* flowerhead powders in the diet delayed larval development. The 4^{th} instar larvae fed on 16% of *C. corornarium* or *C. myconis* flowerhead powder was the longest, 20.2 ± 1.3 and 21.5 ± 1.7 days, respectively. Duration of the 4^{th} instar for larvae nourished on 16% of *C. segetum* flowerhead powder was shorter (14.7 ± 1.4 d.). This difference in instar duration could be allotted to intrinsic composition in antifeedant substances for each *Chrysanthemum* species. The larvae nourished on an artificial diet added with *C. myconis* or *C. segetum* flowerhead powder at 16% died before reaching the stage pupa although consumption of powder at a low concentration allowed the larvae to complete the pre-pupal stage and pupate.

Table 1 Feeding indices of *S. littoralis* third instar larvae reared, along the 10 days of the trial, with artificial diet discs mixed with 4, 8, and 16% (w/w) of three *Chrysanthemum* species flowerhead powders. Powder food consumption was recorded every 2 days (2d) along the trial essays.

Flowerhead powder	Feeding index[1]				
(mg %)	2 d	4 d	6 d	8 d	10 d
C. coronarium	21.2 ± 4.6 d	2.6 ± 15 a	16.2 ± 9.3 ab	25.5 ± 9.2 abc	32.6 ± 11.7 a
	21.9 ± 3.7 d	2.9 ± 1.4 ab	21.7 ± 5.7 bcd	25.2 ± 10.8 abc	37.5 ± 11.6 ab
	27.4 ± 6.3 e	5.1 ± 1.9 bc	26.5 ± 7.2 de	43.8 ± 10.2 d	47.5 ± 12.3 bc
C. myconis	19.7 ± 4.7 cd	4.0 ± 1.7 ab	18.4 ± 8.9 abc	23.4 ± 9.3 a	37.2 ± 12.2 ab
	22.4 ± 5.4 d	6.4 ± 2.2 c	23.6 ± 6.6 cde	33.3 ± 8.0 c	34.1 ± 11.9 a
	22.2 ± 4.6 d	11.1 ± 4.3 d	27.1 ± 8.3 de	33.4 ± 9.1 c	50.0 ± 12.5 c
C. segetum	16.6 ± 3.8 bc	5.0 ± 1.8 bc	18.2 ± 5.4 abc	24.2 ± 7.7 ab	33.0 ± 10.6 a
	15.3 ± 4.4 ab	3.7 ± 1.0 ab	14.6 ± 5.4 a	27.1 ± 9.3 abc	32.0 ± 9.5 a
	11.3 ± 3.7 a	15.4 ± 4.9 e	29.7 ± 7.8 e	32.0 ± 9.1 bc	42.2 ± 11.8 abc

[1]Feeding index [(C-T)/C+T)) x100], where C and T represent the amount eaten of control and treated discs, respectively.
Values are mean ± s.e.m. (n = 30), (each value is the mean of three replicates).
Means followed in the same column by the same letter are not significantly ($P > 0.05$) different in Duncan's multiple range test.

Table 2 Mean length (in days) of the larval instars of *S. littoralis* larvae fed for 10 days on artificial diet discs mixed with 0 (control), 4, 8, and 16% (w/w) of three *Chrysanthemum* species flowerhead powders. Values are mean ± s.e.m. (n = 30).

Powder added	Larval instars		
(mg %)	3 rd	4th	5th
Control (0%)	3.4 ± 0.6 [1]a	3.7 ± 0.8 a	4.2 ± 0.9a
C. coronarium			
4	5.2 ± 0.6 c	8.2 ± 2.6 c	11.6 ± 2.6 d
8	4.6 ± 0.6 bc	7.8 ± 1.8 c	12.7 ± 1.4 de
16	9.3 ± 1.3 e	21.5 ± 1.7 f	19.4 ± 1.4 f
C. myconis			
4	4.8 ± 0.8 bc	9.1 ± 2.5 cd	12.2 ± 1.5 d
8	5.2 ± 0.8 c	10.3 ± 1.5 d	12.2 ± 1.6 d
16	9.0 ± 1.0 2e	20.2 ± 1.3 f	19.4 ± 0.9 f
C. segetum			
4	4.3 ± 0.9 b	6.3 ± 1.2 b	7.7 ± 1.6 b
8	3.5 ± 0.6 a	5.9 ± 1.4 b	9.7 ± 1.6 c
16	6.5 ± 1.1 a	14.7 ± 1.4 e	14.3 ± 1.5 e

[1]Each value is the mean of three replicates. Means followed by the same letter within columns are not significantly different according to Duncan's multiple range test at $P > 0.01$

Effect of three *Chrysanthemum* spp.'s flowerhead powders on the RGR of larvae

Independently of the *Chrysanthemum* species, the larvae of the 3rd instar fed on discs added with flowerhead powder did not exhibit more substantial growth than those offered control discs. In fact, the rate of growth, indicated by RGR, calculated for the period when insect larvae were fed on treated discs, showed a direct antifeedant effect at the concentrations tested (**Table 3**). It was more reduced when the third instar larvae were treated with 16% of *C. coronarium* powder (0.27 mg/mg/day). Relative growth rates were dose-dependant, which indicates the deleterious action of *Chrysanthemeum* flowerhead powders on insect growth.

Table 4 shows the effects of the different treatments on mortality of larvae, pupae and adults. The number of adults that emerged (from 30 treated larvae) at the end of the trial is also noted. The 5th instar larvae fed on a diet to which 4 or 8% of flowerhead powders were added showed some mortality, between 0-20%, for the three species. Independent of the species, no mortality was shown for pupae originating from larvae fed on 8% of powder. Mortality increased for pupae originating from larvae fed on 4% of *C. coronarium* or *C. segetum*, with 25% and 10%, respectively perishing. Mortality of adults increased only for those fed on 8% of *C. coronarium* (62.5%). All the 5th instar larvae died when fed on 16% of *C. myconis* flowerhead powder, whereas larvae fed with the same percent of powder from *C. segetum* or *C. coronarium* flowerheads caused 70% of larvae and 100% of pupae deaths, and none of the 30 treated insects emerged as adults. High pupal death percentage, which was typical for insects fed on 16% of *Chrysanthemum* flowerhead powder seemed to be due to defective morphogenesis in the last instar (5th one) along the period of the treatment, and some pupae showed morphological abnormalities in the pupal instar (**Fig. 1**).

DISCUSSION

The present work revealed the effect of the three *Chrysanthemum* species on *S. littoralis.* The feeding behaviour study showed that *Chrysanthemum* flowerhead powders had an antifeedant effect. This effect was previously observed for *Tribolium confusum* treated with the same *Chrysanthemum* species methanolic extract (Haouas *et al.* 2008), and for *S. littoralis* after ingestion of *Cestrum parquii* L'Herit. extract (Chaieb *et al.* 2001a). After 4 days it was also noted that the AFI was very low at the beginning of the assay

Table 3 Relative growth rates of *S. littoralis* third instar larvae reared on artificial diet added or not (control) with *C. coronarium*, *C. myconis*, *C. segetum* flowerhead powders, calculated every 2 days along 10 days. Values are mean ± s.e.m. (n = 30).

Concentration	Relative Growth Rate				
(mg%)	2 d	4 d	6 d	8 d	10 d
Control	1.0 ± 0.1[1]a	1.0 ± 0.1 a	1.3 ± 0.2 a	1.6 ± 0.5 a	2.3 ± 0.7 a
C. coronarium					
4	0.8 ± 0.1 b	0.8 ± 0.1 b	0.8 ± 0.1 bcd	0.9 ± 0.2 c	0.9 ± 0.2 cd
8	0.8 ± 0.1 bc	0.6 ± 0.1 d	0.7 ± 0.1 de	0.8 ± 0.2 cd	0.9 ± 0.2 d
16	0.7 ± 0.0 e	0.4 ± 0.0 f	0.3 ± 0.0 f	0.3 ± 0.0 e	0.3 ± 0.0 e
C. myconis					
4	0.8 ± 0.1 bcd	0.7 ± 0.1 cd	0.7 ± 0.2 cde	0.9 ± 0.2 c	0.9 ± 0.2 cd
8	0.7 ± 0.1 def	0.5 ± 0.1 e	0.6 ± 0.1 e	0.6 ± 0.1 d	0.8 ± 0.2 d
16	0.7 ± 0.1 ef	0.4 ± 0.0 f	0.3 ± 0.1 f	0.4 ± 0.1 e	0.3 ± 0.1 e
C. segetum					
4	0.8 ± 0.1 bc	0.8 ± 0.1 bc	0.8 ± 0.2 bc	1.2 ± 0.3 b	1.6 ± 0.7 b
8	0.7 ± 0.1 cde	0.6 ± 0.1 d	0.9 ± 0.1 b	0.9 ± 0.2 c	1.2 ± 0.2 c
16	0.6 ± 0.1 ef	0.4 ± 0.1 ef	0.4 ± 0.0 f	0.4 ± 0.1 e	0.4 ± 0.1 e

[1]Means followed by the same letter within a column are not significantly different according to Duncan's multiple range test at $P > 0.01$.

Table 4 Relative mortality (%) of *Spodoptera littoralis* larvae, nymphs and adults reared on artificial diet added or not with 4, 8, 16% of each *Chrysanthemum* powder species tested. Total of adults obtained (30 larvae were tested) in the end of the essay.

Concentration (mg %)	Larval mortality (5^{rd} instar) (%)	Nymphal mortality (%)	Adults mortality (%)	№ of adults emerged
Control	0	0	0	30
C. coronarium				
4	20	25	0	18
8	20	0	62.5	9
16	70	100	-	0
C. myconis				
4	20	0	0	24
8	10	0	0	27
16	100	-	-	0
C. segetum				
4	0	10	11.1	24
8	20	0	0	24
16	70	100	-	0

(-): all larvae (or nymphs) died before reaching nymph or (adult) stage.

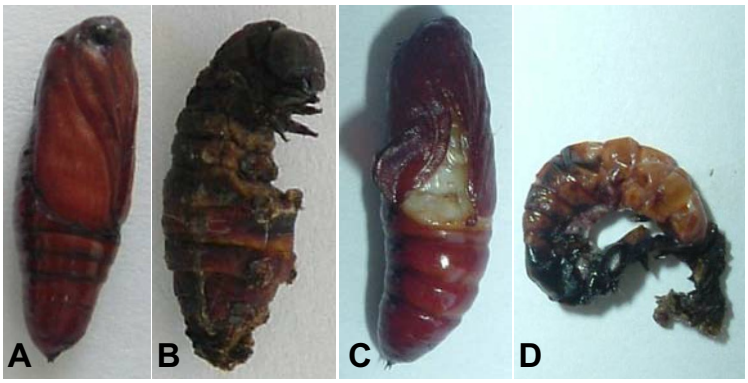

Fig. 1 State of pupae from the last (i.e. 5^{th}) instar that fed or not on *Chrysanthemum* spp. flowerhead powders added at 16% (wt/wt) in artificial diet discs. Control pupa (**A**), defective moulting of larvae, persistence of larval characters (**B**), loosening of pupa cuticule; a lethal anomaly, pupae will dried and adult will not be formed (**C**), abnormal pupa (**D**) (magnification x 5 for all figures).

which indicated a moderate phagostimulant activity, probably related to the fact that substances were neither repellents nor suppressants (Chapman 1974). Larvae at the 5^{th} instar exposed to *Chrysanthemum* flowerhead powder took longer to reach the pre-pupal stage in comparison to the control. Thirty percent of the larvae treated on *C. coronarium* or on *C. segetum* at 16% could successfully pupate but were subsequently killed at this stage whereas 100% of the 5^{th} instar larvae died when fed on *C. myconis* at 16%. Compounds present in the three *Chrysanthemum* species' flowerhead powders added at 16% were drastically lethal to the 5^{th} instar *S. littoralis* larvae. Several anomalies, relating to defective moulting, were observed (**Fig. 1A-C**). Parts of the old cuticle remained adhered to the larvae's cuticle which turned dark suggesting that some physical activity was impaired and could probably be associated with low levels of ecdysteroids and juvenile hormones (Hori *et al.* 1984). Larvae that did not moult did not complete the process properly. Perception of plant secondary chemicals may vary between the larval stages.

At a lower concentration (4-8%), 0% (powder from *C. segetum* or *C. myconis*) to 25 % (*C. coronarium*) of nymphs died before moulting. When the diet was provided in a no-choice form, the secondary metabolites present in *Chrysanthemum* powders did not deter feeding at a high proportion, but rather caused a prolongation of instars, deformities and mortality. Further studies will be undertaken to identify those compounds. Powders from *Chrysanthemum* flowerheads, especially those from *C. myconis*, were found to have a moderate antifeedant activity and conspicuous toxic effects on the larvae of *S. littoralis*. Toxicity of the powders was manifested by a high mortality, reduced growth rates and low weight gain by larvae fed on diet discs containing 16% of the powders. No larvae survived the pupation under this concentration.

Secondary plant compounds having insecticidal activities probably present in *Chrysanthemum* flowerhead powders, such as antijuvenile hormone (Bowers and Aregullin 1987), acetylenes (Sanz *et al.* 1990) or sesquiterpenes (El-Masry *et al.* 1984), were promising for the control of *S. littoralis* larvae, not only for causing mortality but also for disrupting the development of those larvae and so prevent them from causing damage to the crop.

REFERENCES

Addor RW (1995) Insecticides. In: Godfrey CRA (Ed), *Agrochemicals from Natural Products*, Marcel Dekker, New York, pp 1-63

Alvarez-Castellanos PP, Bishop CD, Pascual-Villalobos MJ (2001) Antifungal activity of the essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. *Phytochemistry* **57**, 99-102

Baker CRB, Miller GW (1974) Some effects of temperature and larval food on the development of *Spodoptera littoralis*. *Bulletin of Entomological Research* **63**, 495-511

Ben Jannet H, Harzallah-Skhiri F, Mighri Z, Simmonds MSJ, Blaney WM (2000) Response of *Spodoptera littoralis* larvae to Tunisian plant extracts and to neo-clerodane diterpenoids isolated from *Ajuga pseudoiva* leaves. *Fitoterapia* **71**, 105-112

Ben Jannet H, Harzallah-Skhiri F, Mighri Z, Simmonds MSJ Blaney WM (2001) Antifeedant activity of plant extracts and of new natural diglyceride compounds isolated from *Ajuga pseudoiva* leaves against *Spodoptera littoralis* larvae. *Industrial Crops and Products* **14**, 213-222

Bernays EA, Chapman RF (1994) *Host-Plant Selection by Phytophagous Insects*, Chapman and Hall, New York, 312 pp

Blaney WM, Simmonds MSJ, Ley SV, Jones PS (1988) Insect antifeedants: a behavioural and eclectrophysiological investigation of natural and synthetically derived clerodane diterpenoids. *Entomologia Experimentalis et Applicata* **46**, 267-274

Bowers WS, Aregullin M (1987) Discovery and identification of an antijuvenile hormone from *Chrysanthemum coronarium*. *Memórias do Instituto Oswaldo Cruz* **82 (Suppl III)**, 51-54

Broussalis AM, Ferraro GE, Martino VS, Pinzón R, Coussio JD, Alvarez CJ (1999) Argentine plants as potential source of insecticidal compounds. *Journal of Ethnopharmacology* **67**, 219-223

Brown ES, Dewhurst CF (1975) The genus *Spodoptera* in Africa and the Near East. *Bulletin of Entomological Research* **65**, 221-262

Burkhart CN, Burkhart CG (2002) Topical pyrethroids: Assessment of function and efficacy in head lice. *International Pediatrics* **17**, 209-212

Cayrol RA (1972) Famille des Noctuidae. In: Balachowsky AS (Ed) *Entomologie Appliquée à l'Agriculture* (Vol 2), Masson, Paris, France, pp 1411-1423

Chaieb I, Ben Halima-Kamel M, Ben Hamouda MH (2001a) The effect of

feeding of *Cestrum parquii* (Solonaceae) extract on various damaging Lepidoptera*: Pieris brassicae* L. (*Pieridae*) and *Spodoptera littoralis* Boisduval (Noctuidae). *53rd International Symposium on Crop Protection*, Ghent, Belgium, May 8, 2001, p 149

Chaieb I, Ben Halima-Kamel M, Ben Hamouda MH (2001b) L'impact de l'alimentation contenant du broyât de *Cestrum parquii* (Solanacées) sur des Lépidoptères dommageables. *Medicine Faculty of Landbouww, Ghent University* **66**, 479-490

Chaieb I, Ben Halima-Kamel M, Ben Hamouda MH (2002) Malformations caused by the addition of *Cestrum parquii* (Solanaceae) dry extract in the artificial diet of *Spodoptera littoralis* and *Helicoverpa armigera* (Lepidoptera, Noctuidae). *International Mediterranean Group of Pesticide Research Symposium, Sousse, Tunisia, April* 24-26, 2002, p 15

Chapman RF (1974) The chemical inhibition of feeding by phytophagous insects: A review. *Bulletin of Entomological Research* **64**, 339-363

El-Masry S, Abou-Dania AHA, Darwish FA, Abou-Karum MA, Grenz M, Bohlmann F (1984) Sesquiterpene lactones from *Chrysanthemum coronarium*. *Phytochemistry* **23**, 2953-2954

Haouas D, Ben Halima Kamel M, Harzallah-Skhiri F, Ben Hamouda MH (2005) Bioactivities of seven *Chrysanthemum* species flowers powder on *Spodoptera littoralis* (Boiduval) larvae. *Communication Applied Science, Ghent University* **70**, 799-807

Haouas D, Ben Halima-kamel M, Harzallah-Skhiri F, Ben Hamouda MH (2006) Activité bio-insecticide de l'extrait méthanolique de feuilles de huit espèces de *Chrysanthemum* sur *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae). Actes du Congrès International d'Entomologie et de Nématologie, pp 44-51

Haouas D, Ben Halima Kamel M, Harzallah-Skhiri F, Ben Hamouda MH (2008) *Chrysanthemum* methanolic extracts as potential insecticidal sources on *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae). *Journal of Agronomy* **7**, 93-97

Hori M, Hiruma K, Riddiford LM (1984) Cuticular melanization in the tobacco hornworm larva. *Insect Biochemistry* **14**, 267-274

Ishaaya I, Horowitz AR (1998) Insecticides with novel modes of action In: Ishaaya I, Degheele D (Eds) *Insecticides with Novel Modes of Action: An Overview*, Narosa Publishing House, New Delhi, pp 1-24

Isman MB (1997) Neem insecticides. *Pesticide Outlook* **8**, 32-38

Kubo I, Kluocke JA, Asano S (1983) Effects of ingested phytoecdysteroids on the growth and development of two lepidopterous larvae. *Journal of Insect Physiology* **29**, 307-316

Marion-Poll F, Descoins C (2002) Taste detection of phytoecdysteroids in larvae of *Bombyx mori*, *Spodoptera littoralis* and *Ostrinia nubilalis*. *Journal of Insect Physiology* **48**, 467-476

Mochida O (1973) Two important pests, *Spodoptera litura* and *S. littoralis* on various crops morphological discrimination of the adult, pupal and larval stages. *Applied Entomology and Zoology* **8**, 205-214

Nasr ESA (1973) Effect of temperature and relative humidity on the life cycle of the cotton leafworm, *Spodoptera littoralis*. *Bulletin de la Société Entomologique d'Egypte* **57**, 139-144

OEPP/EPPO (1981) Data sheets on quarantine organisms No. 120, *Spodoptera littoralis*. *Bulletin OEPP/EPPO Bulletin* 11 (1)

Poitout S, Bues R (1974) Elevage de chenilles de vingt huit espèces de Lépidoptères Noctuidae et de deux espèces d'*Actiidae* sur milieu artificiel simple, particularité de l'élevage selon les espèces. *Annales de Zoologie et Écologie Animale* **6**, 431-441

Pottier-Alapetite G (1981) La flore de la Tunisie, Angiosperme-Dicotylédones Gamopétales. In: Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture (Ed), *La Flore de la Tunisie*, pp 939-1007

Salama HS, Shoukry A (1972) Flight range of the moth of the cotton leaf worm *Spodoptera littoralis*. *Zeitung für Angewandte Entomologie* **71**, 181-184

Sanz JF, Falco E, Marco JA (1990) New acetylenes from *Chrysanthemum coronarium*. L. *Liebigs Annalen der Chemie* **3**, 303-305

Schoonhoven LM (1987) What makes a caterpillar eat? The sensory code underlying feeding behavior. In: Chapman RF, Bernays EA, Stoffolano JG (Eds) *Perspectives in Chemoreception and Behavior*, Springer-Verlag, New York, pp 69-97

Secoy DM, Smith AE (1983) Use of plants in control of agricultural and domestic pest. *Economic Botany* **37**, 28-57

Teixeira da Silva JA (2004) Mining the essential oils of the Anthemideae: A review. *African Journal of Biotechnology* **3**, 706-720

Teixeira da Silva JA, Yonekura L, Kaganda J, Mookdasanit J, Nhut DT, Afach G (2005) Important secondary metabolites and essential oils of species within the Anthemidae (Asteraceae). *Journal of Herbs, Spices and Medicinal Plants* **11**, 1-46

Weinziel R, Henn T (1995) Alternatives in insect management, biological and biorational approches. *North Central Region Extension Publication* **73**, 401

Zhang M, Chaudhuru SK, Kubo I (1993) Quantification of insect growth and its use in screening of naturally occurring insect control agents. *Journal of Chemical Ecology* **19**, 1109-1118