

## Insecticidal activity of some Asteraceae plant extracts against *Tribolium confusum*

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### Abstract

Sixteen aromatic plant extracts from three species belonging to the Asteraceae family, were obtained by using organic solvents of increasing polarity. They were tested for insect growth inhibition, contact toxicity and antifeedant activity against adults and larvae of confused flour beetle *Tribolium confusum* du Val (Coleoptera Tenebrionidae). Discs made of wheat were incorporated with a single dose of 1% of different extracts. The antifeedant effect, larval growth inhibition and mortality were evaluated in multiple-choice tests. Responses varied with plant-derived material extract, stage of insect and exposure time. Larval growth inhibition was significantly induced by methanolic and ethyl acetate extracts of *Mantisalca duriaei* Briq. et Cavill. and petroleum ether, chloroformic and methanolic extracts of *Rhaponticum acaule* DC. Antifeedant properties were detected in methanolic extracts of *M. duriaei* and *R. acaule*, petroleum ether and chloroformic extract of *R. acaule* and ethyl acetate extract of *M. duriaei*. *Scorzonera undulata* Vahl seemed to be, however, attractive to the flour beetle. For all extracts, mortality was higher for larvae than adults. It reached respectively 83%, 77% by using petroleum ether and methanol extracts of *R. acaule*. These results suggest that *M. duriaei* and *R. acaule* may be used in grain storage against insect pests.

**Key words:** Asteraceae, plant extracts, *Tribolium confusum*, larval growth, antifeedant activity, toxicity effect.

### Introduction

The insecticidal activity of several plants belonging to the Asteraceae family has been described in several studies. Indeed, Secoy and Smith (1983) reported that pyrethrins, isolated from the dried flowers of *Chrysanthemum cinerariaefolium* Vis. possess insecticidal properties. Oil extracted from various parts of *Tagetes minuta* L. was used in the tropics as a dressing for livestock to control blowfly. The terthienyl (2,2':5',2''-terthiophene) present in the oil has been identified as the active phototoxic compound against mosquitoes. Its high level of activity makes possible its commercialisation as a mosquito larvicide (Klocke, 1989).

Pascual-Villalobos and Robledo (1998) reported the screening for insecticidal activity of *Anacyclus clavatus* Pers., *Asteriscus maritimus* Less., *Artemisia campestris* L., *Atractylis humilis* L., *Centaurea aspera* L., *Cichorium intybus* L., *Chondrilla juncea* L., *Helianthus tuberosus* L., *Helichrysum decumbens* Batt., *Helichrysum serotinum* Boiss., *Reichardia tingitana* L., *Santolina pectinata* Lag., *Senecio lopezii* Boiss. and *Carlina corymbosa* L. against the stored grain pest *Tribolium castaneum* (Herbst). They mentioned that the most active plants inducing larval growth inhibition were *A. clavatus* and *S. lopezii*. Polar extracts of *C. intybus* and *R. tingitana* and non-polar extracts of *C. juncea* effects were 70-100% of mortality whereas repellent effects were apparently perceived in acetone extracts of *A. campestris* and *S. pectinata*. According to Dunkel and Sears (1998), *Artemisia tridentata* Nutt. had fumigant toxicity to *Rhizopertha dominica* (F.), *T. castaneum* and *Plodia interpunctella* (Hübner). Moreover, Broussalis *et al.* (1999) reported insecticidal activity in methanolic

and chloroformic extracts of *Flaveria bidentis* L. and *Tagetes erecta* L. respectively. Similar results on this latter species have been reported by Sarin (2004). The methanol extracts of *Cnicus benedictus* L. appeared to be toxic and produced an antifeedant character to the *Sitophilus littoralis* (Boisduval) larvae (Pavela, 2004).

Liu *et al.* (2006) demonstrated that the isoalantolactone, a major constituent isolated from the roots of *Inula racemosa* Hook, exhibited not only high repellence to *Sitophilus oryzae* (L.) (Coleoptera Dryophthoridae), but also strong insecticidal activities against the weevil.

Because of their pronounced resistance to insect attack, we have chosen to investigate the insecticidal activity of 3 plants belonging to Asteraceae family which has been repeatedly reported as containing insecticidal compounds (Jacobson, 1989; Torres *et al.*, 2003). This paper describes for the first time the antifeedant activity, growth inhibition and toxicity caused by plant extracts of *Mantisalca duriaei* Briq. et Cavill., *Rhaponticum acaule* DC and *Scorzonera undulata* Vahl against adults and larvae of confused flour beetle *Tribolium confusum* du Val (Coleoptera Tenebrionidae).

### Materials and methods

#### Plant material

At flowering stage, the plant material was collected in 2004 from different Tunisian regions (table 1). Voucher specimens of *Mantisalca duriaei*, Mant 2607, *Rhaponticum acaule*, Rhap 1170, and *Scorzonera undulata*, SCR 1171, were deposited in the Laboratory of Natural Substances Chemistry and Organic Synthesis, Faculty of Sciences, Monastir, Tunisia.

**Table 1.** Plants tested for insecticidal activity against *T. confusum*.

Asteraceae	Plant part	Collection date	Origin
<i>Mantisalca duriaei</i> (M)	Aerial parts	16/05/2004	Monastir
<i>Rhaponticum acaule</i> (R)	Aerial parts	18/05/2004	Chabba
<i>Scorzonera undulata</i> (SA)	Aerial parts (A.P)	12/04/2004	M'saken
<i>Scorzonera undulata</i> (SR)	Root parts (R.P)	12/04/2004	M'saken

The plant samples were air-dried for several weeks. Powdered plant tissues were extracted three times by maceration with methanol, the resultant extract was concentrated under reduced pressure. The methanol extract was extracted successively with equal volume of three organic solvents of increasing polarity (petroleum ether, chloroform and ethyl acetate). Each fraction was taken to dryness under vacuum and stored at 4 °C until tested.

### Insects

*T. confusum* is one of the most serious pests in stored grain and related products (Rees, 1995; Jerraya, 2003). It is considered a secondary pest, which can easily infest damaged or broken kernels and apart from grain, it is particularly destructive to flour and other processed grain products. This species is resistant to several traditional insecticides which are commonly used as grain protectants (Arthur, 1996; Zettler and Arthur, 1997). Furthermore, it is now established that *T. confusum* is one of the most tolerant stored-product beetle species to diatomaceous earths (Vayias and Athanassiou, 2004; Athanassiou *et al.*, 2004; 2005). The larvae and adults of the confused flour beetle which were used for the tests were taken from a culture that was kept at the entomology laboratory of High School of Horticulture and Animal Production on an artificial diet of white wheat flour and beer yeast (5% by weight) and maintained in the dark at a constant temperature of 30 °C and 70-80% R.H. Young adults (10-15 days old) and larvae (3 mm of length) were used in all bioassays (Saidana *et al.*, 2007). These bioassays were carried out under the same environmental conditions as the cultures.

### Bioassays

All the extracts were assayed for anti-insect activity using the following tests.

#### Antifeedant studies

The method described by Bloszyk *et al.* (1995) was adopted, using wafer discs made of wheat. Discs (each weighing about 30 mg and 1 cm diameter) were treated with a single dose (5 µl) at concentration of 1% of different extracts (petroleum ether: E<sub>1</sub>, chloroform: E<sub>2</sub>, ethyl acetate: E<sub>3</sub> and methanol: E<sub>4</sub>). The extracts were dissolved in the appropriate solvent. Another set of discs received only equal amounts of solvents (petroleum ether, chloroform, ethyl acetate and methanol) to serve as a control "C". The discs were left to dry and then weighed before being presented to the larvae and adults. Feeding of insects was recorded under three conditions: (1) on pure food (control: CC); (2) on food with possibility of choice (choice test: CE); (3) on food

with the tested extracts (no choice test: EE). Ten adults and larvae were introduced into each Petri dish glass (9 cm diameter) and kept at 30 °C in the dark. The loss in weight of each set of discs in these dishes (amount consumed) after 7 and 14 days was used to calculate the antifeedant index "T".

According to Bloszyk *et al.* (1995), the antifeedant coefficient provides the measure of the substance unpalatableness, which was reported as antifeedant scale. This scale infers that: (T) values from 151 to 200 as excellent, 101 to 150 as good, 51 to 100 as medium, while 1 to 50 as neutral antifeedant action. However, a negative (T) value suggests that the substance is an insect attractant.

$T = A + R$ , where  $A = CC - EE / CC + EE \times 100$  (no choice test) and  $R = C - E / C + E \times 100$  (choice test).

#### Insect growth bioassay

Larvae were removed from Petri dish glasses and measured every 4 days until the end of larval stage. Their length was recorded and compared with the control.

#### Toxicity bioassay

The number of dead adults and larvae were counted every 2 days during the bioassay. Larvae percentage and adult mortalities were calculated and compared with the control. Three replications were set up for each assay.

#### Data analysis

ANOVA and Tukey's Test were used for comparison of means (at  $p = 0.05$  and  $p = 0.01$ ), with SPSS version 10.

## Results and discussion

### Antifeedant activity

Antifeedant index (T) of *M. duriaei*, *R. acaule* and *S. undulata*, calculated after 7 and 14 days in the tested and the controlled Petri dish glasses are summarized in table 2. Potent antifeedant activity against larvae of *T. confusum* was observed in the chloroformic extract of *R. acaule*. Indeed, the antifeeding behaviour was detected within 7 days whereas *R. acaule* E<sub>1</sub> and E<sub>4</sub> showed medium antifeedant actions after fourteen days of experience. This finding is in good agreement with that of Cis *et al.* (2006) who reported that aguerin B, chlorojanerin and syringin, isolated from the chloroformic extract of *Rhaponticum pulchrum* Fisch. et Mey., were good antifeedants against *T. confusum*, *Sitophilus granarius* (L.) and *Trogoderma granarium* Everts, the most active being chlorojanerin whereas cynaropicrin and janerin were more effective against *T. confusum*. They mentioned

**Table 2.** Antifeedant index of extracts prepared from 3 plants against *T. confusum*.

Plant	Extract*	Adults				Larvae			
		7 days		14 days		7 days		14 days	
		T	antifeedant scale	T	antifeedant scale	T	antifeedant scale	T	antifeedant scale
<i>M. duriaei</i>	E <sub>1</sub>	0.12	N.A	6.50	N.A	6.91	N.A	21.6	N.A
"	E <sub>2</sub>	8.43	N.A	11.79	N.A	20.5	N.A	50.87	N.A
"	E <sub>3</sub>	0.85	N.A	11.78	N.A	23	N.A	58.62	M.A
"	E <sub>4</sub>	4.63	N.A	4.88	N.A	22.6	N.A	61.3	MA
<i>R. acaule</i>	E <sub>1</sub>	11.04	N.A	14.29	N.A	20.58	N.A	52.7	M.A
"	E <sub>2</sub>	13.11	N.A	18.14	N.A	62.7	M.A	98.4	M.A
"	E <sub>3</sub>	2.75	N.A	11.40	N.A	5.48	N.A	36.9	N.A
"	E <sub>4</sub>	2.69	N.A	6.22	N.A	21.83	N.A	59.84	M.A
<i>S. undulata</i> SR	E <sub>1</sub>	3.96	N.A	7.58	N.A	2.48	N.A	10.4	N.A
"	E <sub>2</sub>	5.99	N.A	7.23	N.A	5.32	N.A	11.4	N.A
"	E <sub>3</sub>	0.23	N.A	16.28	N.A	-2.6	A.T	-4.3	A.T
"	E <sub>4</sub>	-3.46	A.T	-6.76	A.T	-9.6	A.T	-15	A.T
<i>S. undulata</i> SA	E <sub>1</sub>	1.55	N.A	-2.65	A.T	9.6	N.A	14	N.A
"	E <sub>2</sub>	6.67	N.A	9.84	N.A	10.34	N.A	16.4	N.A
"	E <sub>3</sub>	10.25	N.A	16.42	N.A	17.41	N.A	12.4	N.A
"	E <sub>4</sub>	-3.25	A.T	-5.64	A.T	-3.6	A.T	-12.7	A.T

\* E<sub>1</sub> = Petroleum ether; E<sub>2</sub> = Chloroform; E<sub>3</sub> = Ethyl acetate; E<sub>4</sub> = Methanol.

T = antifeedant index; N.A = Neutral antifeedant; M.A = Medium antifeedant; A.T = Attractive action.

also that the highest antifeedant activity against the three insect pests was recorded for the chloroformic extract enriched in sesquiterpenes lactones. Moreover, they reported that syringin, which is an active feeding deterrent, and some structurally related phenolic glycosides are precursors of the biosynthesis of procyanidins, which serve as an important barrier to insect feeding. Antifeedant properties of 8-deoxylactucin and some other guaianolides, e.g. deacetylmaticarin, jacquinelin and 15-deoxylactucin, towards *T. confusum* were reported previously by Daniewski *et al.* (1988; 1989). Jacquinelin and 8-deoxylactucin isolated from *Picris echioides* L. appeared to be less effective antifeedants than the plant extract and the highest activity was recorded for a mixture of the two guaianolides (Daniewski *et al.*, 1989). Thus, a synergistic effect of feeding deterrents may be expected which is more beneficial to the plant than a single deterrent. Therefore, we can conclude that this activity may be attributed to the presence of the high concentration of some sesquiterpene lactones which were found to be extremely bitter and it is likely that this bitter taste acts as a deterrent to herbivores (Van Beek *et al.*, 1990; Cravotto *et al.*, 2005).

Methanolic and ethyl acetate extracts of *M. duriaei* produced also antifeedancy but they were less powerful than *R. acaule*. According to Pascual-Villalobos and Robledo (1998), *C. aspera*, which is very close to *Mantisca* species, exhibited an antifeedant activity against *T. castaneum*. This activity could be due also to the presence of guaiane-type sesquiterpene lactones which are widely distributed in the Centaureinae subtribe plants (Nowak *et al.*, 1996). There is also some evidence that the compounds are effective in preventing insect feeding in plants of the Asteracea family (Rees and Harborne, 1985; Grainge and Ahmed, 1988; Daniewski *et al.*, 1988; 1989; Passreiter and Isman, 1997). Mordue and

Blackwell (1993) reported that this action could be attributed to alimentary factor obstruction in insect central nervous system or to protein inhibition in digestive canal (Barbouche *et al.*, 2001) or also to intestine cytotoxicity by precocenes actions (Szczeapanik *et al.*, 2005).

*S. undulata* seemed to be attractive to *T. confusum*, by presenting a negative antifeedant index against both larvae and adults with methanolic extract (E<sub>4</sub>). Ethyl acetate extract (E<sub>3</sub>) obtained from the root parts of *S. undulata* and that of petroleum ether (E<sub>1</sub>) obtained from the aerial parts are found to possess attractive properties toward larvae and adults, respectively. These experimental results justify the edible use of this species as a food plant for the larvae of the nutmeg, a species of moth. It tastes sweet with very grateful flavour (Le Floch, 1983).

#### Insect growth inhibition

In this bioassay, larval lengths were recorded every 4 days (table 3). Insect growth inhibition after intake of ethyl acetate and methanolic extracts of *M. duriaei* and petroleum ether, chloroformic and methanolic extracts of *R. acaule* was more evident after 4 days. This finding strongly suggests that the plants contain nauseous constituents which are responsible for antifeedant activity and the shortness of larvae length. Needless to say that growth inhibition activity in different extracts of many species was mentioned by several authors. It appeared that methanolic and hexane extracts obtained from the aerial parts of *C. aspera* showed clear effect of growth suppression on *T. castaneum* larvae (Pascual-Villalobos and Robledo, 1998). Huang *et al.* (2002) reported that eugenol significantly reduced growth rate, food consumption and food utilisation of both adults and larvae of *T. castaneum*. In the case of methyleugenol, the growth rate and food consumption of *Sitophilus zeamais*

**Table 3.** Larval growth inhibition of *T. confusum* caused by different extracts.

	Days	Treatment	Length of larvae (mm)											
			E <sub>1</sub>			E <sub>2</sub>			E <sub>3</sub>			E <sub>4</sub>		
			Mean	SE	T	Mean	SE	T	Mean	SE	T	Mean	SE	T
<i>M. duriaei</i>	4	EE	3.4	0.2	N.S	3.5	0.2	N.S	3	0.2	*	3	0.2	**
"	"	CE	3.3	0.2	N.S	3.55	0.2	N.S	3.56	0.2	N.S	3.25	0.2	**
"	"	CC	3.33			3.7			3.36			3.75		
"	8	EE	3.8	0.3	N.S	4.2	0.3	N.S	2.9	0.3	***	2.9	0.3	***
"	"	CE	3.8	0.3	N.S	4.3	0.3	N.S	3.6	0.2	**	3.6	0.3	***
"	"	CC	3.81			4.46			4.1			4.46		
"	12	EE	4.28	0.3	N.S	4.6	0.3	*	2.8	0.4	***	2.3	0.5	***
"	"	CE	4.28	0.3	N.S	4.7	0.3	*	3.6	0.3	***	3.9	0.5	***
"	"	CC	4.48			5			4.55			4.95		
<i>R. acaule</i>	4	EE	3.2	0.2	*	3	0.2	*	3.4	0.2	N.S	3.11	0.2	**
"	"	CE	3.5	0.2	N.S	3.26	0.2	N.S	3.3	0.2	N.S	3.4	0.2	*
"	"	CC	3.7			3.33			3.36			3.75		
"	8	EE	3.8	0.3	**	3.2	0.3	**	3.9	0.2	N.S	2.7	0.3	***
"	"	CE	4	0.3	*	3.5	0.3	*	4	0.2	N.S	3.9	0.3	**
"	"	CC	4.46			3.81			4.1			4.46		
"	12	EE	4.4	0.3	**	3.2	0.3	***	4.2	0.4	*	2.5	0.3	***
"	"	CE	4.45	0.3	**	3.85	0.3	**	4.3	0.3	N.S	4	0.3	***
"	"	CC	5			4.48			4.55			4.95		
<i>S. undulata SA</i>	4	EE	3.75	0.2	*	3.01	0.1	N.S	3.2	0.2	N.S	4.1	0.1	**
"	"	CE	3.4	0.2	N.S	2.91	0.1	N.S	3.38	0.2	N.S	3.9	0.1	N.S
"	"	CC	3.33			3.13			3.36			3.75		
"	8	EE	4.3	0.3	**	4	0.3	*	4	0.2	N.S	4.7	0.2	*
"	"	CE	4	0.3	N.S	3.9	0.2	**	3.7	0.3	*	4.4	0.2	N.S
"	"	CC	3.81			4.46			4.1			4.46		
"	12	EE	4.7	0.3	N.S	4.9	0.2	N.S	4.4	0.3	N.S	5.2	0.4	*
"	"	CE	4.3	0.3	N.S	4.6	0.3	*	4.5	0.3	N.S	5	0.4	N.S
"	"	CC	4.48			5			4.55			4.95		
<i>S. undulata SR</i>	4	EE	3.23	0.14	N.S	3.13	0.1	N.S	3.7	0.12	*	4.2	0.1	**
"	"	CE	3.1	0.14	N.S	3.2	0.1	N.S	3.4	0.119	N.S	3.8	0.1	N.S
"	"	CC	3.33			3.13			3.36			3.75		
"	8	EE	3.8	0.2	N.S	3.8	0.2	**	4.4	0.134	*	4.8	0.2	*
"	"	CE	3.8	0.2	N.S	3.84	0.2	**	4.2	0.125	N.S	4.3	0.2	N.S
"	"	CC	3.81			4.46			4.1			4.46		
"	12	EE	4.2	0.3	*	4.3	0.2	**	5		**	5.4	0.4	*
"	"	CE	4.3	0.3	N.S	4.2	0.3	**	4.8		*	5.2	0.4	*
"	"	CC	4.48			5			4.55			4.95		

E<sub>1</sub> = Petroleum ether; E<sub>2</sub> = Chloroform; E<sub>3</sub> = Ethyl acetate; E<sub>4</sub> = Methanol.

EE = no choice test; CE = choice test; CC = control (pure food).

SE = standard error of the difference for mean comparison.

T = Tukey-test for comparison between treatments and the control (N.S = non significant; \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001).

Motschulsky adults and *T. castaneum* adults and larvae decreased significantly with increasing concentrations. It exhibited a more potent effect on the food consumption in the larvae of *T. castaneum* than some other benzene derivatives.

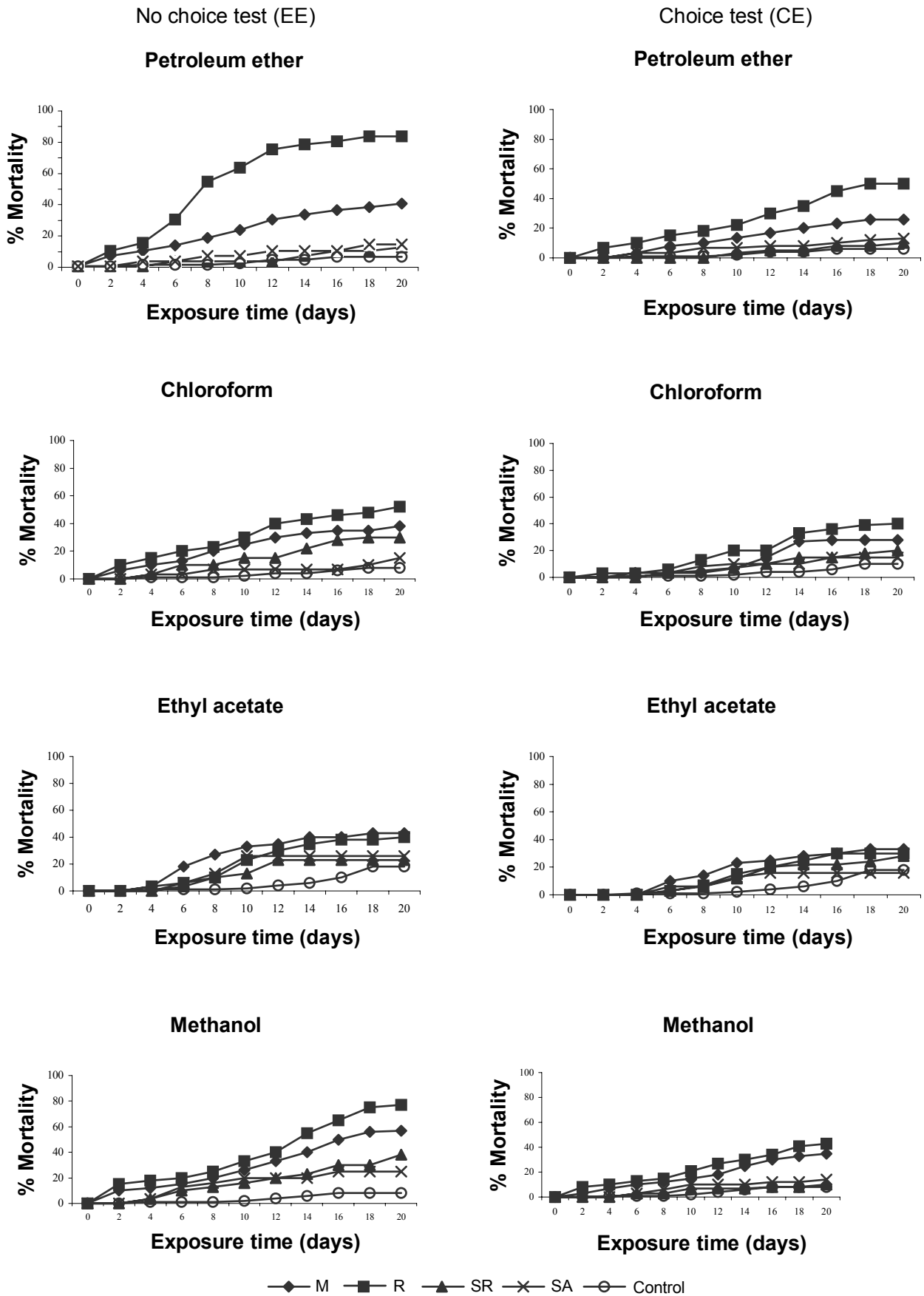
#### Toxicity

Figure 1 was a summary of results, in which the percentage mortality of *T. confusum* larvae caused by different plant extracts was represented. This preliminary experiment showed an evolution of larvae mortality level during three weeks. E<sub>1</sub> of *R. acaule* was more active because 83% of mortality in larvae was produced in comparison with the other extracts. Whereas the toxic

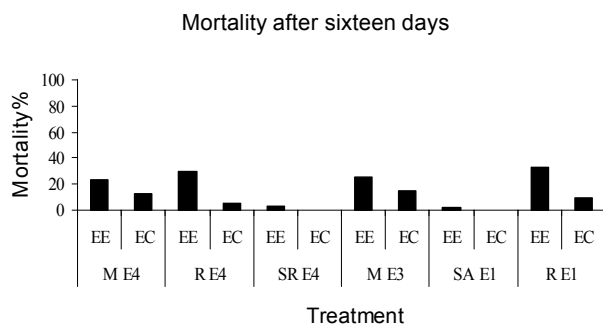
effect of methanolic extract (E<sub>4</sub>) was slower. It caused 50% of mortality at the second week after treatment.

Other significant values of larval mortality were induced by E<sub>3</sub> and E<sub>4</sub> of *M. duriaei* whereby 43% and 57% mortality was registered after three weeks of treatment.

Similar results were found with the choice test but the percentage of mortality was lesser in comparison with the no choice test. Comparing to the other extracts presenting an appreciable percentage of mortality, *R. acaule* gave the best results and appeared to be highly toxic for the confused flour beetle *T. confusum*. Higher dose rates are needed to achieve 100% mortality for larvae of this species.



**Figure 1.** Percentage mortality of *T. confusum* larvae exposed to different plant extracts at concentration of 1% (M: *M. duriaei*; R: *R. acaule*; SR: *S. undulata* root parts; SA: *S. undulata* aerial parts).



**Figure 2.** Percentage mortality of *T. confusum* adults exposed to different plant extracts at concentration of 1%.

Larvae appeared to be more sensitive than adults. These latter gave 33% of adult mortality after sixteen days when *R. acaule* E<sub>1</sub> was applied (figure 2). This toxic effect depends on several factors among which are the chemical compositions of the crude extracts. Indeed, this result can be explained by the presence of eugenol and its analogue, which is methyleugenol, identified as the main component of the volatile fraction of *R. acaule* (Boussaada *et al.*, 2008). Other authors like Ho *et al.* (1994) revealed that a hexane extract of clove flower buds, which contains mainly eugenol, demonstrated a toxic contact effect on *S. zeamais* and *T. castaneum* and reduced their fecundity. Obeng-Ofori and Reichmuth (1997) also reported that eugenol was toxic to four stored-product coleopteran species, namely, *S. zeamais*, *S. granarius* L., *T. castaneum* and *Prostephanus truncates* Horn. Moreover, isoeugenol has shown to have toxic and antifeedant activity to *S. zeamais* and *T. castaneum* (Huang *et al.*, 1997). Huang *et al.*, (2002) reported that eugenol, isoeugenol and methyleugenol exhibited similar contact toxicity to *S. zeamais* whereas the potency of these chemicals was different for *T. castaneum*. This toxic effect can be explained by the reversible competitive inhibition of acetylcholinesterase by occupation of the hydrophobic site of enzyme's active centre (Ryan and Byrne, 1988).

## Conclusion

Results of this study and earlier studies point out that some plant extracts might be useful as potent insect-control agents. *R. acaule* and *M. duriaei* species are found to have potent insecticidal activity toward *T. confusum*. They present antifeedant characteristics and affected significantly larval growth of confused flour beetle. Whereas the high toxic effect on the larvae is only exhibited by *R. acaule*. Our next approach will be targeted to concentrate efforts in a few more promising extracts to fractionate them and isolate possible active compounds.

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