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Insecticidal Activities of Tunisian Halophytic Plant Extracts against Larvae and Adults of *Tribolium confusum*

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Summary

Salt marsh plants were tested for their insecticidal activities against adults and larvae of Tribolium confusum. *Sixteen aerial part extracts of* Frankenia laevis, Statice echioides, Suaeda fructicosa *and* Tamarix boveana *were obtained using organic solvents of increasing polarity and tested for their insect growth, antifeedant and toxicity effects. Responses varied with plant material, extract type, insect stage and exposition time. Larval growth inhibition was significantly induced by chloroformic, ethyl acetate extracts of* F. laevis, S. echioides *and* T. boveana, *and petroleum ether extract of* F. laevis. *On the other hand, all extracts of* S. fructicosa *and the methanolic ones of the four plants tested didn't show any significant activity. In addition, ethyl acetate extracts of* F. laevis, S. echioides *and* T. boveana *and petroleum ether extract of* F. laevis *presented antifeedant property.* S. fructicosa *seemed to be, however, slightly attractive to the flour beetle. For all extracts, mortality was higher for larvae than adults. By using ethyl acetate extracts of* F. laevis, S. echioides *and* T. boveana, *and petroleum ether extract of* F. laevis, *mortality reached respectively 97, 87, 97 and 80%, when applied at a dose of 1%, mixed with the insect diet.*

Résumé

Activités insecticides de quelques plantes halophiles poussant en Tunisie vis-à-vis des larves et des adultes de *Tribolium confusum*

Seize extraits des parties aériennes de plantes halophiles ont été préparés avec des solvants organiques de polarité croissante. Ces extraits sont testés pour leurs activités bio-insecticides sur les larves et les adultes de Tribolium confusum *(Coleoptera : Tenebrionidae). Le pouvoir antiappétent, la toxicité et l'inhibition de la croissance de l'insecte sont suivis. Les résultats varient en fonction du matériel végétal, de la nature de l'extrait, du stade biologique du ravageur et du temps d'exposition. La croissance en longueur des larves a été significativement inhibée par les extraits au chloroforme et à l'acétate d'éthyle* de F. laevis, S. echioides *et* T. boveana *ainsi que par ceux à l'éther de pétrole de* F. laevis. *Néanmoins, tous les extraits de* S. fructicosa *ainsi que les extraits méthanoliques des quatre plantes testées ne montrent aucun effet significatif sur la croissance de Tribolium. Cependant, les extraits à l'acétate d'éthyle de* F. laevis, S. echioides *et* T. boveana, *et l'extrait à l'éther de pétrole de* F. laevis *manifestent une activité antiappétente. Quant aux différents extraits de* S. fructicosa, *ils semblent présenter un léger effet attractif sur* T. confusum. *Par ailleurs, les différents extraits utilisés montrent une toxicité plus importante chez les larves que chez les adultes de Tribolium. Les extraits à l'acétate d'éthyle de* F. laevis, S. echioides *et* T. boveana, *et l'extrait à l'éther de pétrole de* F. laevis *provoquent des mortalités larvaires respectives de 97, 87, 97 et 80%, à une dose de 1%, additionnée à l'alimentation de l'insecte.*

Introduction

The struggle against insect's proliferation for the man's survival is ancient. Intensive and uncontrolled utilization of synthetic insecticides polluted sometimes very dangerously our terrestrial and marine environment (18). Annual post-harvest losses resulting from insect damage, microbial deterioration, and other factors are estimated to be 10-25% of production worldwide, and the widespread use of synthetic insecticides has led to the development of pest strains resistant to pesticides (13).

As a rich source of bioactive chemicals, plants may provide potential alternatives to used insect-control agents. Plants can also be less toxic, readily biodegradable and of great economic interest both from the agronomic and preventive medicine points of view (16). Over 2000 species of plants are known to possess some insecticidal activity, by containing either antifeedant, repellent or insecticidal compounds that enable the crude plant material or an extracted active compound to protect stored products (7, 10).

Besides, we note that 22% of Tunisian wetlands are salty area with halophytic plants (5). This incites us to try to valorise it by looking for a possible insecticidal activity against the dangerous *Tribolium confusum* Val (Coleoptera, Tenebrionidae), in larval and adult stage, which resists to several traditional synthetic insecticides (19).

In this present study, we describe for the 1st time biological and chemical studies of some extracts of the following halophytic plants: *Frankenia laevis, Statice echioides, Suaeda fructicosa* and *Tamarix boveana.* Antifeedant properties, toxicity and larval growth inhibition were considered.

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Materials and methods

Table 1
Plants collected and tested for eventual insecticidal activity

Plants	Abbreviation	Family	Collection date	Collection region
Frankenia laevis	*F.l.*	Frankeniaceae	23-may-2004	Sebkhet Chott Mariam
Statice echioides	*S.e.*	Plombaginaceae	2-jan-2004	Sebkhet Sidi Abdelhamid
Suaeda fructicosa	S.f.	Chenopodiaceae	17-may-2004	Sebkhet El Kalbia
Tamarix boveana	*T.b.*	Tamaricaceae	17-may-2004	Sebkhet El Kalbia

1. Plant materials

The four plant species belong to several families (Table 1). Aerial parts were collected in different regions of Tunisia, mainly when plants were at flowering stage. Voucher specimens are deposited in the herbarium of Faculty of Sciences, Monastir, Tunisia.

2. Preparation of the extracts

Powdered aerial parts of each plant (500 g) were extracted three times by maceration with methanol for 96 h, then by using successively three solvents of increasing polarity: petroleum ether, chloroform and ethyl acetate. The residues obtained after solvents evaporation (at reduced pressure) were indexed as follow: E_1 for the petroleum ether extract, E_2 for the chloroformic extract, E_3 for ethyl acetate extract and E_4 for the methanolic extract.

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

3. 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. Insects were 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 laboratory of Entomology reserve, High School of Horticulture and Animal Production.

4. Bioassays

Plant extracts were tested for their antifeedant, toxic and insect growth inhibition effects. To evaluate the antifeedant property, the method described by Bloszyk *et al.* (6) was adopted, using diet discs, weighing about 20 mg and 1cm diameter. 5 µl of extracts were mixed with discs, allowed to evaporate at 30 °C during 24 h and then weighed before being offered to larvae and young adults in three replications of ten insects of each stage inside 4-cm diameter glass Petri dishes. A control was prepared in the same way but extract application was excluded. Feeding of insects was recorded under three conditions: (1) on pure food (control: CC); (2) on food with possibility of choice (choice test: EC); (3) on food with the extracts tested (no choice test: EE). The loss in weight for each set of discs (amount consumed) after 7 and 14 days was used to calculate the antifeedant index (T).

The antifeedant index provides a measure of unpalatableness of a substance, which was reported as the antifeedant scale (6). This scale infers that (T) values from 200 to 151 as excellent, 150 to 101 as good, 100 to 51 as medium, while 50 to 1 as neutral (or at most slight) antifeedant actions. On the contrary, a negative (T) suggests

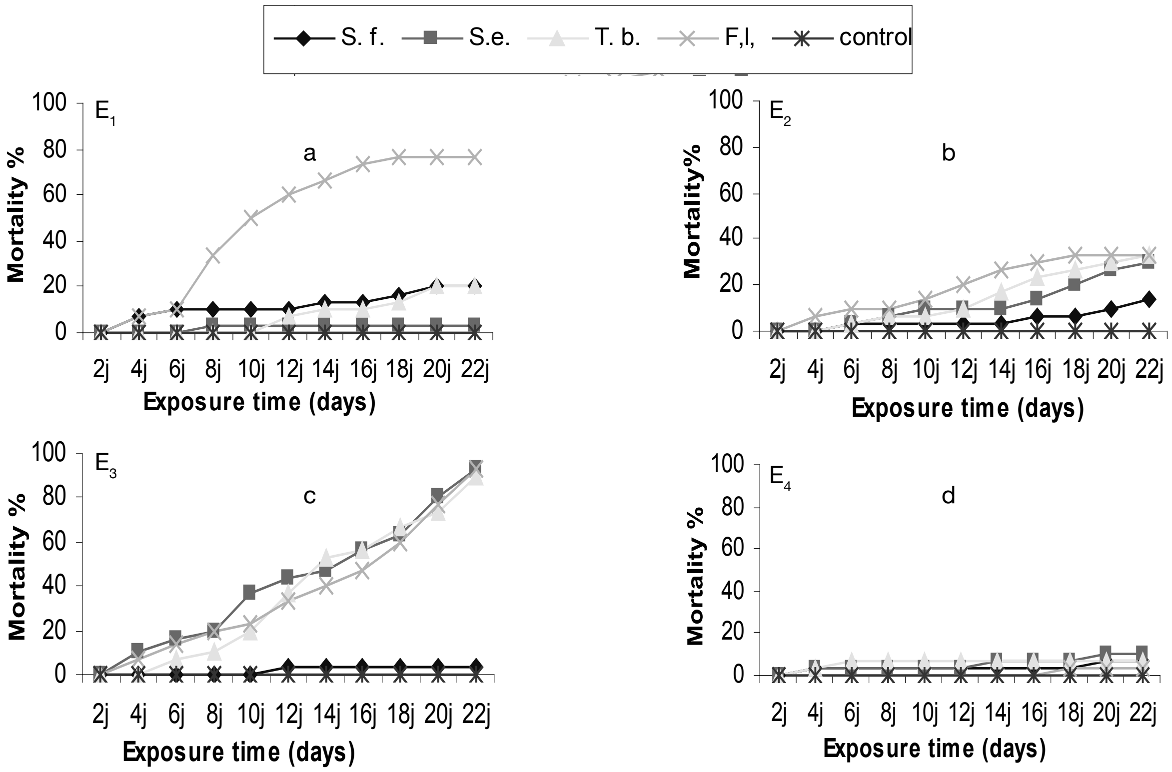

Figure 1: Mortality percentage of *T. confusum*, in larval stage, exposed with choice option to the petroleum ether extract (E_1), the chloroformic extract (E_2), ethyl acetate extract (E_3) and methanolic extract (E_4) of the four tested halophytes.

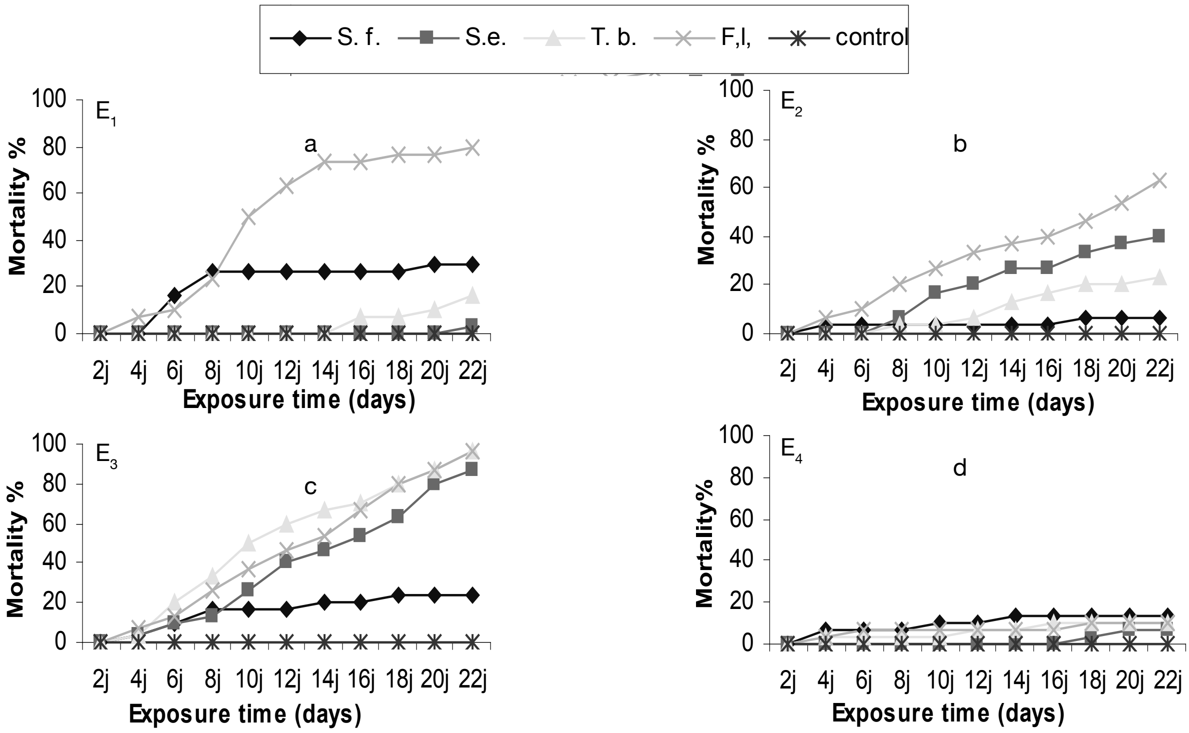

Figure 2: Mortality percentage of *T. confusum*, in larval stage, exposed without choice option to E_1, E_2, E_3 and E_4 of the four tested halophytes.

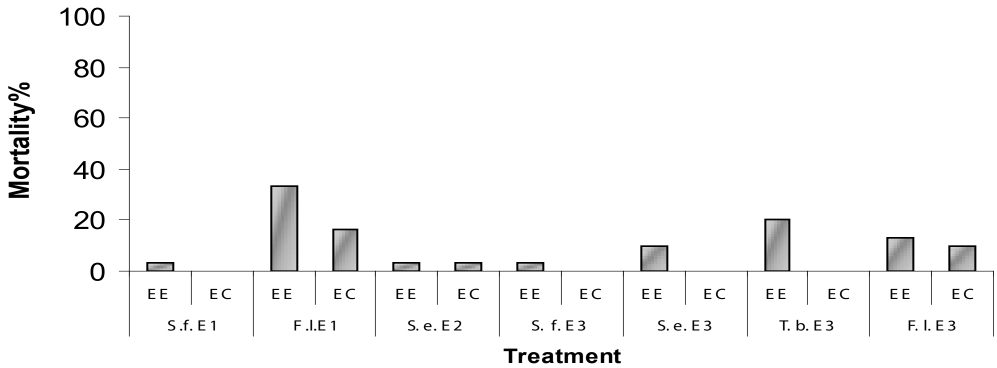

Figure 3: Mortality percentage of *T. confusum*, in adult stage exposed with and without choice option to E_1, E_2, E_3 and E_4 of the four tested halophytes and calculated after fourteen days. The cases where both EE and EC equal zero are not figured.

that the substance is an insect attractant. This attractive effect is however at most slight when (T) is contained between 0 and -50.

T= (CC-EE) +(C-E)

In the precedent described Petri dishes, mortality was determined every two days during the essay (22 days), the percentage was calculated and compared with the control.

Larval growth inhibition was obtained by measuring length growth, recorded every four days until the end of larval stage and compared with the control.

5. Statistical analysis

Statistical comparisons of larval growth were performed with SPSS version 10.0. Analyses of variance (one way ANOVA), were followed by means comparisons (at P= 0.05 and P= 0.01) and Tukey test.

Results

1. Antifeedant activity

Antifeedant index (T), calculated after 7 and 14 days in the tested and the controlled glass Petri dishes was summarized in table 2 (larvae) and table 3 (adults).

F. laevis E_1, E_3, *S. echioides* E_2, E_3 and *T. boveana* E_3 showed medium antifeedant actions after fourteen days of experience, when applied at concentration of 1%, mixed with the diet. Nevertheless, only larval period was concerned.

S. fructicosa seems to be slightly attractive to *T. confusum*, by presenting a negative antifeedant index against both larvae and adults with E_4, and only larvae with E_2 and E_3.

2. Toxicity test

E_3 of *F. laevis, S. echioides* and *T. boveana* gave the best results because, comparative to control; 97, 87 and 97% of mortality in larvae was respectively produced (Figures 1c and 2c). Other significant values of larval mortality were induced by *F. laevis* E_1 and E_2 for both choice (Figures 1a and 1b) and no choice essay (Figures 2a and 2b). Presenting high percentage of mortality with several extracts, *F. laevis* appears the most toxic halophyte for the confused flour beetle *T. confusum.*

Larvae seem to be more sensitive than adults, the mortality of which didn't exceed 33% after fourteen days, when *F. laevis* E_1 was applied (Figure 3).

3. Insect growth inhibition

Lengths of larvae exposed to different extracts of halophytic plants, are given in table 4. Although 100% of mortality was

Table 2 Antifeedant index (T), calculated after 7 and 14 days for adults of *T. confusum*

				7 days			14 days		
				%	T	AC	%	T	AC
Petroleum ether extract E_1	*S.f.*	EE		15.00	2.48	NA	36.48	9.41	NA
		EC	E	13.28			41.72		
			C	14.25			32.45		
		CC		16.51			55.15		
	S.e.	EE		10.69	6.71	NA	28.77	21.62	NA
		EC	E	14.49			40.57		
			C	15.39			35.81		
		CC		16.51			55.15		
	T.b.	EE		10.74	7.62	NA	30.66	12.65	NA
		EC	E	12.06			50.45		
			C	13.91			38.6		
		CC		16.51			55.15		
	F.l.	EE		3.53	12.9	NA	6.05	57.97	MA
		EC	E	3.18			6.00		
			C	3.15			14.88		
		CC		16.51			55.15		
Chloroformic extract E_2	*S.f.*	EE		21.34	5.31	NA	55.34	-9.6	AT
		EC	E	12.69			44.22		
			C	25.76			51.79		
		CC		13.06			38.18		
	S.e.	EE		4.53	20.52	NA	14.27	52.19	MA
		EC	E	0.68			1.27		
			C	12.15			29.56		
		CC		13.6			38.18		
	T.b.	EE		5.47	7.16	NA	13.91	23.4	NA
		EC	E	6.62			16.04		
			C	5.65			15.22		
		CC		13.60			38.18		
	F.l.	EE		4.03	9.06	NA	11.53	25.8	NA
		EC	E	5.00			13.85		
			C	4.50			13.01		
		CC		13.6			38.18		
Ethyl acetate extract E_3	*S.f.*	EE		21.08	-2.57	AT	43.15	12.66	NA
		EC	E	16.43			43.71		
			C	13.71			43.96		
		CC		21.23			55.56		
	S.e.	EE		0.35	21.86	NA	1.30	55.13	MA
		EC	E	1.98			2.86		
			C	2.96			3.73		
		CC		21.23			55.56		
	T.b.	EE		1.52	24.66	NA	3.10	53.32	MA
		EC	E	1.56			4.11		
			C	6.52			4.98		
		CC		21.23			55.56		
	F.l.	EE		1.5	18.84	NA	2.93	51.37	MA
		EC	E	2.04			3.27		
			C	0.80			2.02		
		CC		21.23			55.56		
Methanolic extract E_4	*S.f.*	EE		27.58	-14.54	AT	49.34	2.36	NA
		EC	E	13.66			34.04		
			C	13.00			46.72		
		CC		13.69			39.02		
	S.e.	EE		11.00	1.3	NA	29.08	12.68	NA
		EC	E	9.24			21.63		
			C	7.86			24.37		
		CC		13.69			39.02		
	T.b.	EE		11.48	5.48	NA	34.01	0.74	NA
		EC	E	11.02			39.02		
			C	14.29			34.75		
		CC		13.69			39.02		
	F.l.	EE		11.18	0.12	NA	28.36	1.36	NA
		EC	E	14.60			43.93		
			C	12.22			34.62		
		CC		13.69			39.02		

Table 3 Antifeedant index (T), calculated after 7 and 14 days for adults of *T. confusum*

				7 days			14 days		
				%	T	AC	%	T	AC
Petroleum ether extract E1	*S.f.*	EE		27.00	10.43	NA	43.03	15.2	NA
		EC	E	14.80			26.05		
			C	31.30			49.00		
		CC		20.94			35.28		
	S.e.	EE		15.82	3.06	NA	25.31	3.35	NA
		EC	E	15.78			26.59		
			C	13.72			19.97		
		CC		20.94			35.28		
	T. b.	EE		17.41	9.01	NA	32.85	9.25	NA
		EC	E	9.25			23.44		
			C	14.74			30.26		
		CC		20.94			35.28		
	F. l.	EE		10.68	9.28	NA	17.48	15.3	NA
		EC	E	9.75			16.62		
			C	8.77			14.10		
		CC		20.94			35.28		
Chloroformic extract E2	*S.f.*	EE		21.66	21.94	NA	39.91	16.91	NA
		EC	E	11.26			29.31		
			C	14.92			31.76		
		CC		39.95			54.37		
	S.e.	EE		13.25	3.87	NA	22.77	9.15	NA
		EC	E	36.69			43.78		
			C	13.87			21.33		
		CC		39.95			54.37		
	T. b.	EE		43.37	12.12	NA	54.34	16.30	NA
		EC	E	43.57			48.31		
			C	59.12			64.59		
		CC		39.95			54.37		
	F. l.	EE		11.51	35.4	NA	20.26	43.75	NA
		EC	E	8.29			11.59		
			C	15.25			21.24		
		CC		39.95			54.37		
Ethyl acetate extract E3	*S.f.*	EE		24.16	3.06	NA	48.73	5.73	NA
		EC	E	19.79			40.61		
			C	25.62			52.66		
		CC		21.39			42.41		
	S.e.	EE		8.33	15.9	NA	15.80	29.92	NA
		EC	E	7.54			14.52		
			C	10.37			17.85		
		CC		21.39			42.41		
	T. b.	EE		9.95	10.08	NA	18.51	16.51	NA
		EC	E	13.97			26.95		
			C	12.61			19.57		
		CC		21.39			42.41		
	F. l.	EE		18.97	2.67	NA	25.77	16.4	NA
		EC	E	8.08			16.36		
			C	8.33			16.12		
		CC		21.39			42.41		
Methanolic extract E4	*S.f.*	EE		1.03	-3.3	AT	3.40	-7	AT
		EC	E	17.70			34.03		
			C	2.5			4.00		
		CC		12.89			26.47		
	S.e.	EE		23.44	3.48	NA	33.57	2.56	NA
		EC	E	17.63			30.55		
			C	31.66			40.21		
		CC		12.89			26.47		
	T. b.	EE		13.96	0.63	NA	26.11	1.27	NA
		EC	E	13.14			22.8		
			C	14.84			23.71		
		CC		12.89			26.47		
	F. l.	EE		13.76	0.52	NA	21.63	3.4	NA
		EC	E	18.09			31.60		
			C	19.48			30.16		
		CC		12.89			26.47		

AC: antifeedant coefficient, %: consumption pourcentage, AT: attractive action, MA: medium antifeedant action, NA: neutral antifeedant action. *F.l.: Frankenia laevis, S.e.: Statice echioides, S.f.: Suaeda fructicosa and T.b.: Tamarix boveana.* CC: control, EC: choice test, E: treated disc, C: control disc, EE: no choice test.

Table 4
Larval growth inhibition of *T. confusum* caused by active halophyte extracts at concentration of 1% in diet (mm)

			4j	±SE	S	8j	±SE	S	12j	±SE	S
Petroleum ether extract E_1	EE	*S.f.*	3.64	0.7	ns	3.79	0.6	ns	4.64	0.5	ns
		S.e.	3.43	0.5	ns	3.9	0.6	ns	4.35	0.6	ns
		T.b.	3.47	0.3	ns	3.83	0.4	ns	4.35	0.6	ns
		F.l.	2.8	0.4	***	2.9	0.4	***	1.83	0.3	***
	EC	*S.f.*	3.59	0.5	ns	3.8	0.7	ns	4.5	0.5	ns
		S.e.	3.47	0.4	ns	3.94	0.6	ns	4.41	0.6	ns
		T.b.	3.73	0.4	ns	4.16	0.6	ns	4.79	0.7	ns
		F.l.	2.78	0	***	2.88	0.5	***	3.33	0.3	**
	CC		3.77	0.7		4.15	0.3		4.82	0.5	
Chloroformic extract E_2	EE	*S.f.*	3.88	0.5	ns	4.43	0.5	**	5.28	0.5	**
		S.e.	3.03	0	***	3.38	0.6	***	3.46	0.7	***
		T.b.	3.22	0.5	**	3.37	0.6	**	3.36	0.6	***
		F.l.	2.95	0.3	***	3.17	0.3	***	3.26	0.6	***
	EC	*S.f.*	3.68	0.6	ns	4.16	0.8	ns	4.91	0.8	ns
		S.e.	3.25	0.5	**	3.42	0.5	**	3.41	0.5	***
		T.b.	3.3	0.4	*	3.5	0.5	*	3.46	0.5	***
		F.l.	3.15	0.3	***	3.38	0.6	**	3.27	0.4	***
	CC		3.61	0.5		3.88	0.6		4.6	0.6	
Ethyl acetate extract E_3	EE	*S.f.*	3.65	0.5	ns	3.96	0.6	**	4.68	0.6	ns
		S.e.	2.92	0.2	***	2.92	0.3	***	3.03	0	***
		T.b.	2.9	0.3	***	2.97	0.2	***	2.92	0	***
		F.l.	3.02	0	***	3	0.5	***	3.2	0.4	***
	EC	*S.f.*	3.67	0.5	ns	4.07	0.7	*	4.5	0.8	*
		S.e.	2.89	0.2	***	3.01	0.4	***	2.83	0.5	***
		T.b.	2.83	0.3	***	2.94	0.2	***	3.41	0.4	***
		F.l.	3.05	0	***	2.97	0.3	***	3	0.3	***
	CC		3.78	0.6		4.52	0.7		5.07	0.8	
Methanolic extract E_4	EE	*S.f.*	3.69	0.5	ns	4.44	0.6	ns	4.88	0.7	ns
		S.e.	3.5	0.5	**	4.13	0.8	ns	4.3	0.6	ns
		T.b.	3.57	0.5	**	4.38	0.8	ns	4.65	0.4	ns
		F.l.	3.57	0.6	**	4.13	0.7	ns	4.3	0.5	ns
	EC	*S.f*	3.74	0.5	ns	4.39	0.5	ns	4.84	0.6	ns
		S.e.	3.62	0.5	*	4.31	0.8	ns	4.6	0.8	ns
		T.b.	3.64	0.5	ns	4.4	0.6	ns	4.53	0.5	ns
		F.l.	3.62	0.5	**	4.37	0.4	ns	4.78	0.5	ns
	CC		4.05	0.6		4.6	0.7		5.23	0.8	

S: signification of the difference between EE (or EC) and CC; ns: non significant;
*0.01< P< 0.05; **0.001< P< 0.01; ***P< 0.001. CC: control (pure food); EC: choice test (food with possibility of choice); EE: no choice test (food with the extracts tested).

not noted during the experimental period, a high significant reduction of larval growth was precociously observed with *F. laevis* E_1, E_2 and E_3, *S. echioides* E_2 and E_3 and *T. boveana* E_3 (after four days) and continued to the end of experience. Same results were found with or without choice option. By using *F. laevis* E_1, larval lengths reached only 38% of the control after 12 days with choice option.

Discussion

In the course of this present study, 16 halophytic plant extracts were tested for their bio-insecticidal activities against larvae and adults of *T. confusum*. Antifeedant effect, toxicity and insect growth inhibition were followed up. Responses varied with plant material, extract type, insect stage and exposition time.

F. laevis, S. echioides and *T. boveana* ethyl acetate extracts were more powerful inhibitor of feeding than the others. They presented high toxicity and affected significantly larval growth of the confused flour beetle, when applied at a concentration of 1%. These halophytic plants apparently look promising when they could be applied at slightly higher doses as insecticides.

High insecticidal activity of petroleum ether of *F. laevis* was also noted, which led us to deduce that *F. laevis* could be used as natural agent for pest control. In this way, only 1.6% of the survival from *Diorhabda elongate* Brullé *deserticola* (Coleoptera: Chrysomelidae) larvae to adults was averaged on three *Frankenia* plants (8). These plants seem to have real antifeedant activity, repulsive effect and almost complete sterilized action against *D. deserticola* (12), whereas, 55-67% of these insect larvae completed their development on *Tamarix* species (8).

S. fructicosa seems to be slightly attractive to *T. confusum.*

However, most *Chenopodiaceae* have been mentioned to have pesticide properties (20).

T. confusum can survive at application rates of substances which are lethal for other stored-grain beetle species (1, 2, 3, 9, 11) and slightly higher dose rates are needed to kill all larvae of this insect. Larvae seem to be more sensitive than adults (19).

The 1,8-cineole compound, isolated from essential oil of *Ocimum kenyense,* was highly toxic to *T. confusum* (14) while α-pinene and the terpineol possessed potent toxic effects to this insect (15).

F. laevis E_1, E_2 and E_3, *S. echioides* E_2 and E_3 and *T. boveana* E_3 affected significantly *T. confusum* larval growth. In this regard, an arrest in the larval stage of *T. confusum* was noted after exposure to a treatment with hydropene. This treatment caused either important morphological deformities or wing deformities and dead of emergent adults (4). The toxic effect may be attributed to reversible competitive inhibition of acetylcholinesterase by occupation of the hydrophobic site of enzyme's active centre (17).

Halophytic plants such as *F. laevis, S. echioides* and *T. boveana* seem to be interesting for investigation in pest control, then chemical researches could be done to isolate and identify their active principles. That will be a part of our future research.

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