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## Studies of Larvicidal and Adulticidal Activities of Some Halophyte Plant Extracts Against *Ceratitis capitata* (Wiedemann)

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### ABSTRACT

The aim of the study was to assess the efficacy of dichloromethane and methanol extracts of two Chenopodiaceae (*Salicornia arabica* and *Suaeda fruticosa*) and one Tamaricaceae (*Tamarix gallica*) Moroccan medicinal plants for their toxicity against the early second and third instar larvae and adults of *Ceratitis capitata*: one of the most destructive fruit pest. The activity of the extracts was performed using incorporated plant extracts into diet and determination of lethal concentration 50 value, whereas the mortality of adults was counted after treatment by feeding on cotton discs soaked by different dilutions the extract. The *C. capitata* larvae and adults were more sensitive to methanol than dichloromethane extracts. The larval mortality was observed after 24 h exposure and the highest activities on larvae and adults were found for *T. gallica* methanol extract with LC<sub>50</sub> dose of respectively 0.5% and 30 mg mL<sup>-1</sup>. The methanol extract of *T. gallica* found to be highly effective against *C. capitata* is potential to be used as an ideal ecofriendly approach for the control of this insect.

**Key words:** *Ceratitis capitata*, Chenopodiaceae, Tamaricaceae, larvicidal-adulticidal activities, bio-insecticide plant extracts

### INTRODUCTION

Worldwide crop loss without the use of pesticides is estimated to be ~70% of production and, despite its use, pre-harvest losses due to insect pest attack are ~15% of the total crop production (Krattiger, 1997). The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the world's most destructive fruit pests. Each infestation in Morocco, especially in recent years, necessitated intensive detection procedures. Once it is established, massive eradication efforts may be extremely difficult and expensive (Mazih and Debouzie, 1996). Because of its wide distribution over the world, its ability to tolerate cooler climates better than most other species of tropical fruit flies and its wide

range of hosts, it is ranked first among economically important fruit fly species. Although it may be a major pest of Citrus, it is often a more serious pest of some economically important fruits such as *Argania* tree in Morocco (Quilici, 1999).

Today, synthetic insecticides are at the forefront of insect-controlling agents. However, the environmental threat that these chemicals pose, effects on non-target organisms and the resistance of insects have all increased during the second half of last century (Wattanachai and Tintanon, 1999). Therefore, there is an urgent need to develop new insecticides for control which are more environmentally safe and also specific and biodegradable. In recent years, environment friendly and biodegradable natural insecticides of plant origin have received renewed attention as agents for vector control because they are rich in bioactive chemicals, active against a limited number of species including specific target insects, and biodegradable. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against insect larvae (Rahuman *et al.*, 2009; Mehlhorn *et al.*, 2005; Markouk *et al.*, 2000).

Morocco has a long history of traditional medicine and a large population still relies on traditional remedies for primary health care, especially in rural areas (55% of the whole population). Indeed, the geographical position of Morocco in the extreme north-west of Africa and the great diversity of its climate has favoured the development of a rich flora which is estimated at 4200 native plants and 1500 introduced species (Bellakhdar *et al.*, 1991). The aim of the present study was to investigate the bio-insecticidal activity of three plant species from Morocco: *Tamarix gallica*, *Suaeda fruticosa* and *Salicornia arabica*. *Tamarix gallica* is a tall perennial shrub tree up to 8 m, locally known as “fersig” or “tarfà”. It is abundant on the banks of the rivers in moist sandy soil with a high salt content. In the indigenous system of medicine, leave powder is used against ringworm and as anti-diarrheic. *Suaeda fruticosa* is an evergreen Shrub growing to 1 m, known as “swida” or “degmus el-bell” by the local people; it is used against prurit and for wound healing. *Salicornia arabica* is a small shrub known as “belbel” and used for the treatment of prurit and tenia (Bellakhdar, 1997).

As far as our literature survey could ascertain, no information was available on the insecticidal activities of *Chenopodiaceae* and *Tamaricaceae* species given here. Other activities have been found in the literature such as anti-protease activity (Jedinak *et al.*, 2010), antioxidant and antimicrobial activities (Ksouri *et al.*, 2009; Sultanova *et al.*, 2001). Thus, the present study was an attempt to assess the larvicidal and adulticidal activities of the dichloromethane and methanol extracts of aerial parts against larvae and adults of *Ceratitis capitata*.

## MATERIALS AND METHODS

**Plant collection:** The fresh aerial parts of *Tamarix gallica*, *Suaeda fruticosa* and *Salicornia arabica* were collected from Ouljat Oued Tensift near to Marrakesh in May 2008. They were authenticated by Prof. Abbad, plant taxonomist from the Department of Biology, Faculty of Sciences, Marrakesh. Voucher specimens respectively Mark 4227, Mark 4230 and Mark 4231 were deposited at the herbarium of the Faculty of Sciences Semlalia, Marrakesh.

**Preparation of the extracts:** The plant aerial parts were washed with tap water, shade dried at room temperature, and powdered by electrical blender. The powders were macerated with cold dichloromethane for 24 h and then successively with 90% methanol. The filtrates were obtained through a Buchner funnel with Whatman 3 MM filter paper and afterwards evaporated to dryness under reduced pressure using a rotavapor to give two extracts for each plant. The

weight of the dry extracts was recorded and the concentrates were then transferred to sample bottles and stored at +4°C until use.

The methanol active extracts (5 g) obtained of the three plants were subjected to RP18 column chromatography (50 g) for fractionation using the sequence 30, 60, 90 and 100% methanol. The fractions collected respectively from *T. gallica* (30, 60, 90 and 100 MTG), *S. fruticosa* (30 MSF, 60 MSF, 90 MSF and 100 MSF) and *S. arabica* (30, 60, 90 and 100 MSA) were tested against larvae and adults of *Ceratitis capitata*.

**Phytochemical screening of the extracts:** Phytochemical tests were carried out using standard procedures to identify the constituents of the extracts as described by Harborne (1973).

**Insect cultures:** Larvae and young adults (1-5 days old) of the pest medfly *Ceratitis capitata* were obtained from same-age cultures from National Centre of Sciences and Nuclear Technologies, Tunisia (Guerfali *et al.*, 2007).

### Bioassays

**Larvicidal assay:** The larvicidal activity was assayed according to a previously published method (Salvatore *et al.*, 2004) whereas the toxicity was evaluated by incorporating extracts into Mediterranean fruit fly *C. capitata* diet.

Adequate amounts of crude extracts were mixed thoroughly with 20 g of medium to give final concentrations of: 11, 10, 9, 8, 7, 6 and 5 mg g<sup>-1</sup> diet. Final concentrations of: 0.5, 0.2, 0.1, 0.07, 0.05, 0.02 and 0.01 mg g<sup>-1</sup> diet were used for isolated fractions.

Early second and third instar larvae were used for bioassay test. A total of 150 larvae were exposed in three replicates of 50 larvae each. Experiments were conducted for 24 h under controlled temperature and humidity (22.7±2.0°C, 64%). Both positive Fay Fanon 50 E (malathion at 0.1 mg mL<sup>-1</sup>) and negative (distilled water) control assays were carried out in the same conditions as for the extracts.

The dead larvae were counted after 24 h of exposure and the percentage of mortality was reported from the average of three replicates. Percent mortality was corrected by using Abbott's formula (Abbott, 1925).

**Adulticidal assay:** Newly emerged *C. capitata* adults selected from our adult colony were subjected to treatment by feeding on 9 cm cotton discs soaked by 20 mL of different dilutions of each extract. The concentrations tested were 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg mL<sup>-1</sup>. Reduced disks were used owing to the limited amount of dried residues of fractions.

The experiment had three replicates and each group consisted in 25 male and 25 female placed in a small cage.

**Statistical analysis:** Data on the dose-mortality effects of different extracts were subjected to computerized Probit analysis program version 1.5 for LD<sub>50</sub> values for different concentrations of each extract.

## RESULTS

The larvicidal activity of crude extracts from the Moroccan medicinal plants *Tamarix gallica*, *Suaeda fruticosa* and *Salicornia arabica* are noted and presented in Table 1 and 2. Among the

Table 1: Larvicidal activity [LC (mg g<sup>-1</sup> diet)] of crude extracts against *C. capitata* after 24 h exposition

Plant	Extract	Third instar larvae				Second instar larvae			
		LC <sub>50</sub>	LCL	UCL	±SE	LC <sub>50</sub>	LCL	UCL	±SE
<i>Tamarix gallica</i>	Methanol	5.0*	3.4	6.5	0.54	12.2*	6.0	21.0	1.25
	Dichloromethane	-				-			
<i>Suaeda fruticosa</i>	Methanol	7.1*	6.0	7.6	0.14	1.73*	10.0	29.0	1.15
	Dichloromethane	-				-			
<i>Salicornia arabica</i>	Methanol	9.0*	8.6	12.8	0.23	20.3*	14.0	35.0	3.05
	Dichloromethane	-				-			

Significant at \*p<0.05 level. LC<sub>50</sub>: Lethal concentration that kills 50% of the exposed larvae, UCL: Upper confidence limit, LCL: Lower confidence limit, -:Not active

Table 2: Adulticidal activity (LC<sub>50</sub> g mL<sup>-1</sup>) of crude extracts against adults of *C. capitata* after 24 h exposition

Plant	Extract	LC <sub>50</sub>	LCL	ULC	±SE
<i>Tamarix gallica</i>	Methanol	0.030*	0.015	0.042	0.0048
	Dichloromethane	-			
<i>Suaeda fruticosa</i>	Methanol	0.034*	0.014	0.048	0.0066
	Dichloromethane	-			
<i>Salicornia arabica</i>	Methanol	0.04*	0.018	0.045	0.0029
	Dichloromethane	-			

Significant at \*p<0.05 level. LC<sub>50</sub>: Lethal concentration that kills 50% of the exposed larvae, UCL: Upper confidence limit, LCL: Lower confidence limit, -: Not active

crude extracts tested, only methanolic extracts are active. The dichloromethane extracts of all the plants tested are inactive, pointing out that active ingredients of the extracts causing *C. capitata* mortality are mostly of polar character.

The larvicidal effect of extracts on the early second and third instar larvae of *C. capitata* was dependent on the concentration tested. Methanol extracts from *Tamarix gallica* and *Suaeda fruticosa* showed the highest toxicity to larvae when ingested through diet, inhibiting population with LC<sub>50</sub> concentrations, respectively of 5 mg g<sup>-1</sup> diet (0.5%) and 7 mg g<sup>-1</sup> diet (0.7%). These extracts showed complete mortality on *C. capitata* larvae at 1%. The methanol extract of *S. arabica* has the less activity with LC<sub>50</sub> of 0.9% (Table 1). The methanol extracts tested on the adults have very weak activities. The higher dose was obtained for *T. gallica* methanol extract (LC<sub>50</sub> = 30 mg mL<sup>-1</sup>) (Table 2).

The experiments conducted on adults of *C. capitata* of both sex revealed that females are more sensitive than males and that LC<sub>50</sub> increase with time (Fig. 1). For methanol extract of *T. gallica*, the active one, the LC<sub>50</sub> was 0.03 for females and 0.035 for males and reach 0.046 and 0.05 g mL<sup>-1</sup>, respectively.

Also the extracts obtained with dichloromethane did not cause any adverse effects. On *C. capitata* adults, almost no research on the effect of phytochemicals on adults has been conducted suggesting that adulticidal action is a potential and untapped field of research. After methanol extracts fractioning, it was found that fraction eluted with 30% methanol from *S. fruticosa* (30 MSF) was the most effective on larvae with LC<sub>50</sub> dose of 0.051 mg g<sup>-1</sup> diet (ca. 1400 fold increase) whereas fraction 60 MTG from *T. gallica* is the most active one on adults (ca. 50 fold increase) (Table 3).

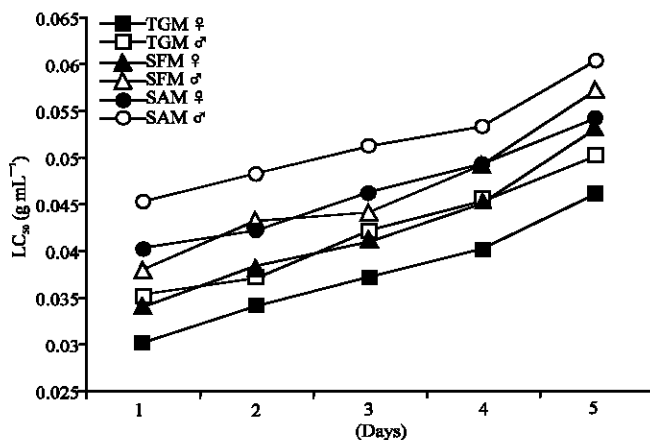


Fig. 1: Toxicity of methanol extracts of *T. gallica*, *S. fruticosa* and *S. arabica* against males and females of *C. capitata*

Table 3: Toxic activity of fractions from *Tamarix gallica* and *Suaeda fruticosa* against *C. capitata* after 24 h exposition

Extract	Larvicidal activity				Adulticidal activity			
	LC <sub>50</sub> (mg g <sup>-1</sup> diet)	LCL	UCL	±SE	LC <sub>50</sub> (mg mL <sup>-1</sup> )	LCL	UCL	±SE
30 MSF	0.051*	0.043	0.063	0.0085	-			
90 MSF	-				0.94*	0.82	1.11	0.23
60 MTG	-				0.62*	0.55	0.73	0.15
100 MTG	0.21*	0.14	0.32	0.051	-			

Significant at \*p<0.05 level. LC<sub>50</sub>: Lethal concentration that kills 50% of the exposed larvae, UCL: Upper confidence limit, LCL: Lower confidence limit, -: Not active

Table 4: Results of phytochemical screening of crude extracts of the plants tested

Plant	Extract	Yield (%)	Terpens	Flavonoids	Gallic Catechic		Saponosides	Anthocyanins	Leuco-anthocyanins	Alkaloids
					tanins	tanins				
<i>S. arabica</i>	DCM	19.00	-	+	-	++	++	-	+	++
	ME	22.10								
<i>S. fruticosa</i>	DCM	22.27	-	+	-	+++	+++	-	catechols ++	-
	ME	22.27								
<i>T. gallica</i>	DCM	11.10	++	++	+++	-	+++	-	catechols ++	+++

DCM: Dichloromethane extract; ME: Methanol extract, +++: Very high amount, ++: High amount, -: Absence

The phytochemical screening of the two extracts obtained from each plant using standard methods showed that they contain high quantities of saponins, tannins and alkaloids. *T. gallica* extracts were the most rich of these types of compounds followed by *S. fruticosa* and *S. arabica* (Table 4).

## DISCUSSION

More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes (Ahmed *et al.*, 1984). Many authors have reported the

bioinsecticide activity of extracts of plant origin (Iloba and Ekarakene, 2006; Joseph *et al.*, 2011). One of the most available plant with great value in the 21 century is the neem (*Azadirachta indica* A. Juss), whose extracts have shown considerable activity and multiple modes of action against agricultural pests, forestry insects and insects of public health (Schmutterer, 2002; Mulla and Su, 1999; Ogbuewu *et al.*, 2011). Generally the larvae seem to be more sensitive to the plant extracts than adults. In this regard, stage 3 of *C. capitata* larvae showed more lethality compared with the stage 2 for all tested extracts. This treatment caused either important pupae morphological deformities or death of emergent adults. From observation, the second instar larvae treated with the plant extracts, died slower and less than the third instar larvae because of their more tolerance to insecticides and they were able to cause more argan fruit damage.

Macedo *et al.* (2008) have reported that chitin-binding vicilin from *Erythrina velutina* seeds, a tetrameric glycoprotein, when added to diet caused strong effect on *C. capitata* larvae mortality with  $LC_{50}$  of 0.14%. The deleterious effects were associated to the binding to chitin structures present in peritrophic membrane and to gut epithelial cell. Another extract that had been mentioned to have a good efficacy is the aqueous extract from *Cestrum parqui* which showed a high toxicity to neonate larvae when ingested through diet, inhibiting pupation at a concentration above 0.6%. An  $LC_{50} = 0.9\%$  after 3 days of continuous ingestion of *C. parqui* aqueous extracts could be calculated (Zapata *et al.*, 2006). In present observation, the methanol extract of *T. gallica* aerial parts possessed higher activity than that reported by these authors. The methanol extract of *T. gallica* and *S. fruticosa* were also more efficient than TTI, a trypsin inhibitor purified from seeds of the tamarind tree (*Tamarindus indica*). Larvae mortality of *C. capitata* was 34% at the level of 4% of TTI (Araujo *et al.*, 2005).

The larvicidal activity of *T. gallica* extract is most likely due to saponosides, tannins and alkaloids (Table 4). The susceptibility of some insect species have been tested using the saponin extract of *Cestrum parqui* and shown that the extract must be incorporated in the artificial diet to obtain 100% mortality at  $10 \text{ mg g}^{-1}$  diet (Ikbal *et al.*, 2007). So far, no phytochemical work has been undertaken on *Tamarix gallica* species but recently, Orabi *et al.* (2010) have reported the isolation of three new hellinoyl-type ellagitannins and a new macrocyclic type from an aqueous acetone extract of *Tamarix nilotica*. Orfali *et al.* (2009) have reported the characterization from the same plant of a new pentacyclic triterpenoid: 3-O-trans-caffeoylisomyricadiol with potent anti-oxidant activity. Another pentacyclic triterpenoid elucidated is that reported by Sultanova *et al.* (2004) from the aerial parts of *Tamarix hispida*, namely isotamarixen and a new long-chain secondary alcohol (= laserine) isolated from the flowers of *Tamarix hampeana* (Aykaç and Akgül, 2010).

The susceptibility tests of adults of *C. capitata* to the methanol extracts of the three plants was conducted and the highest lethal activity was found for methanol extract of *T. gallica* with an  $LC_{50}$  of  $0.03 \text{ mg mL}^{-1}$ . The mortality was also recorded for five days for both males and females and it was noted that females are more sensitive than males (Fig. 1).

The failure to discover a significant new class of insecticides for the control of *C. capitata* should led many researchers back to biodiscovery studies in the search for new and economical bioinsecticides. A relatively very low number of plant derivatives have shown to be effective against the Mediterranean fly and the use of active fractions was shown to be sufficient to inhibit the emergence of the larvae. Thus, it will certainly help reduce medfly population drastically. Feeding behaviour of the adult medflies includes carbohydrates primarily from feeding on the juices of ripe fruit and honeydew and protein from decomposing fruit. On the other hand, the immature stages

develop better on diets containing higher concentration of glucose and sucrose (Demirel, 2007). Since a large variety of fruits were attacked and especially the economically important fruit of the tree *Argania spinosa* in Morocco, the discovery of plant-derived compounds that could control this insect would be of great value. Therefore, botanical insecticides should be applied as early as possible before the eggs hatch or when larvae are neonates to prevent damage under field condition. Other areas requiring attention are the mode of insecticidal action and human safety issues, as well as the best formulation to improve insecticidal potency and stability and for cost reduction.

In this study, the highly bioactive extract and fractions of *T. gallica*, which is being grown widely in most areas of the country, offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

The finding of the present investigation revealed that methanol extract *T. gallica* possesses remarkable larvicidal and adulticidal activity against *C. capitata*, the most important agricultural pest fly. Considering toxic effects of methanol extract of the plant, it is possible that the extract can be used as natural control agents. The plants is widely distributed and easy grown. Furthermore, the extraction method is simple and cost-effective and the application techniques could be relatively easily designed for on-farm use. Studies to confirm this hypothesis under field conditions and the non toxicity toward non target organisms are underway on our laboratory. Also, Further work to isolate and identify the insecticidal constituents of the crude methanol extract of *T. gallica* is needed.

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