Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(1): 17-20

Received: 07-11-2015
Accepted: 09-12-2015

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Toxicological effects of *Urginea maritima* (L.) against the red flour beetle (Coleoptera: Tenebrionidae)

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Abstract
Present study was analyzed to determine the toxicological effects of *Urginea maritime* at different concentrations (0.1, 1 and 10%) against larvae and adults of *Tribolium castaneum*. In the present study it was observed that by tropical application, leaf extract shows highest toxicity to larvae as compared to adults. LC_{50} value and LC_{90} value was found to be 27.8g/L and 55.1g/L respectively in probit analysis of leaf extract for the larvae. However the bulb extract was found to be more toxic, LC_{50} and LC_{90} values were much lesser with 16.6 and 34.4g/L respectively. The present study also determined the in vivo toxicity of leaf as well as bulb extracts on larvae and the LC_{50} value was found to be 92 and 19.3g/L respectively. Similarly in residual film toxicity, LC_{50} and LC_{90} were found to be 87.9 and 495.4g/L, respectively. However no mortality was observed in the present study in the control experiment.

Keywords: *Urginea maritima*, Toxicity, Mortality.

1. Introduction
Pest control is a major concern in agriculture and horticulture growers for underdeveloped agro-based countries [1]. Plants may provide potential alternatives to currently used insect control tools because they constitute a rich source of bioactive chemicals [2]. These plant-derived compounds are active against a limited number of species including specific target insects, biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management. Terrestrial plants produce natural substances that have demonstrated insecticidal bioactivity against several insect pests [3, 4, 5]. Neem insecticides are derived from the tropical and subtropical *Azadirachta indica*. Azadirachtin, the principal active ingredient is one of several limonoids extracted from neem seeds. Rotenone is the trade name of the insecticide derived from extracts of the tropical legumes *Derris* and *Lonchocarpus*. The main active principle is the isoflavonoid rotenone. Sabadilla is the seed extract of the Neotropical lily *Schoenocaulon officinale* which contains veratridine alkaloids which have a neurotoxic mode of action. Ryania is an extract of from the South America shrub *Ryania sp.* containing the diterpene alkaloid ryanodine, which is a contact and ingested insecticide against horticultural and ornamental crop pests. Pyrethrum is now the most important traditional botanical insecticide on the market. It is derived from the African daisy, *Chrysanthemum pyrethrum*, which produces an insecticidal oleoresin [6].
Urginea maritima (L.) Bakeries, commonly known as the white squill, is an onion-like liliaceae native to the Mediterranean Basin and well adapted to its type of climate. The plant grows through autumn to spring, and there after the leaves get dried up and the bulbs undergo through dormancy during the summer season. *Urginea maritima* (L.) is having ornamental value, it produces flowers after several years when the bulb attains s a considerable size [6, 7, 8]. It has been studied that the bulbs of *U. maritima* has some potential antifungal [9], insecticidal [10], rodenticide [11] and nematicidal activities, as well as therapeutic activity [12, 13]. However no studies were available regarding the toxicity of leaves of *U. maritima*. In order to overcome the lacunae of the information regarding the toxicity of *U. maritima,* it was therefore aimed to evaluate the insecticidal activity of methanolic extracts of leaves and bulbs of *U. maritima* on *Tribolium castaneum* (Herbst) (red floor beetle) larvae and adults under laboratory condition.

2. Materials and Methods

2.1 Plant material

In the present study the leaves and bulbs of *Urginea maritima* were collected from Chott Mariem region, Tunisia (35° 55' 06.0" N, 10° 34' 34.9 "E) in February 2015. Leaves and bulbs were, dried at 40 °C and after it, the leaves and bulbs were crushed and powdered. 100 g of powdered plant was macerated in 400ml of methanol for 24 h. The extract was filtrated through Whatman filter paper n°1 to remove peel particles. After filtration, the methanol extracts were let to evaporate at room temperature during 48 h and the remaining extract left was stored at 4 °C until use.

2.2 Insects

The red floor beetle was reared on artificial diet of semolina mixed with corn flour and beer yeast (100/50/5, w/w/w) at a constant temperature of 30±1 °C and 60-70% relative humidity in the dark. Adult insects of 7 to 10 days old and third instars larvae were used for toxicity tests under constant temperature of 30±1°C and 60-70% relative humidity.

2.3 Bioassays

In the present study three different concentrations (0.1%, 1% and 10%) of each methanolic extract of bulb and leaf were tested for its insecticidal effect.

2.3.1 Topical application bioassay

One micro-liter of each concentration of methanolic extracts was given both to 10 larvae as well as 10 adults on the abdomen, while the control received 1 µl of distilled water only (five replications each). The mortality rate was recorded at 1, 2, 3, 7, 14 and 21 days. The assessment of mortality rate was corrected for control mortality according to Schneider-Orelli's formula [14]:

$$Mc = (M_{0} - M_{e}) / (100 - M_{e}) \times 100$$

Mc: corrected mortality rate (%), Mo: mortality rate of treated adults (%), Me: mortality rate of control (%).

2.3.2 Ingestion bioassay by semolina disk

Semolina disks were prepared according to Xie [15] with modifications from Huang [16]. They were prepared by mixing 10 g semolina with 50 ml water until completely suspended. The resulting mixture was cut into small discs of 9 mm in diameter. After drying overnight at 35°C, semolina disks were dipped in each of leaf and bulb extracts at different concentrations (0.1%, 1% and 10%). Control disks were only dipped on methanol. After evaporation of the solvent, the disks were placed each one in a Petri dish containing one third instar larvae. Three replicates and 5 larvae/replicate/each concentration were used. Three weeks after, the mortality was determined.

2.3.3 Residual film bioassay

Residual film method as described by Busvine [17] was used. 500µl of methanolic extracts were applied on the bottom of Petri dishes (9 cm diameter) in such way that it made a uniform film over the Petri dishes. Only methanol was used for control plates. After solvent evaporation, 10 larvae and 10 adults were released in each Petri dish with five replications. Mortality was checked out after 1, 2, 3, 7 and 14 days of exposure.

2.4 Statistical analysis

For statistical comparison among several means, all the data on mortality was subjected to a one-way analysis of variance (ANOVA) followed by mean comparisons (at $P = 0.05$) and Student- Newman-Keuls thorough SPSS 20.0. Moreover, the mortality data was subjected to probit analysis for the determination of LC_{50} and LC_{90} values [18].

3. Results

3.1 Toxicity by topical application

During the present experiment, the toxicity of methanolic leaf and bulb extracts of *U. maritima* was tested against larvae and adults of *T. castaneum* at different concentrations. The present results showed a difference on the sensitivity of the two stage of the red flour beetle. It was observed that 10% leaf extract caused 100% mortality in larvae and 48% mortality in adults after day one. However at concentrations of (0.1% and 1%), it was observed to be more toxic on larvae than on adults (Table 1).

Similar results were also observed in the case of the bulb extract where it causes 100% mortality of *T. castaneum* larvae after day one at 10% concentration. However, adult mortality was low and didn't exceed 24% at the same concentration (Table 1).

Therefore, statistical analysis showed significant effect of leaf extract ($p \leq 0.05$) on adult mortality which reached 56% at 10% concentration since day 14 (Table 1) with LC_{50} value 92.07 g/L and LC_{90} value 159.2 g/L. But bulb extract was found more toxic to larvae (31.72% mortality at 1% concentration with 16.6 g/L and 34.4 g/L of LC_{50} and LC_{90} values respectively than leaf extract (Table 2).

Table 1: Percent mortality rate of *T. castaneum* larvae and adults treated by topical application with methanol extracts of *U. maritime* leaves and bulbs. Mortality rate was corrected using Schneider-Orelli's formula [14].

Extracts	Concentrations (%)	Insect stage	Mortality rate (%)					
			1 day	2 days	3 days	7 days	14 days	21 days
Leaf	0.1	Larvae	0	1.37	1.37	8.96	11.03	11.03
		Adults	0	0	0	2	4	4
	1	Larvae	20	17.24	17.24	17.24	19.31	19.31
		Adults	0	4	4	6	6	6
	10	Larvae	100	100	100	100	100	100
		Adults	48	52	52	54	56	56
Bulb	0.1	Larvae	0	0	4	8.96	8.96	13.10
		Adults	0	0	0	2	6	6
	1	Larvae	0	14	16	27.58	27.58	31.72
		Adults	0	2	2	2	2	2
	10	Larvae	100	100	100	100	100	100
		Adults	22	22	22	24	24	24

Table 2: Toxicity (LC_{50}, LC_{90}) by topical application of methanolic leaf and bulb extracts of *U. maritima* against larvae and adults of *T. castaneum* under laboratory conditions during 21 days.

Extracts	Stages	LC 50 (g/L)	LC90 (g/L)	Chi-square	df*	*P*
Leaf	Larvae	27.8	55.1	0.122	1	0.727
	Adults	92.07	159.2	0.006	1	0.936
Bulbs	Larvae	16.6	34.4	0.000	1	1.000
	Adultes	166.3	285.2	2.97	1	0.084

* Degree of freedom; LC: Lethal concentration.

3.2 Toxicity by ingestion

Table 3 Shows that an increasing trend was observed in mortality rate of larvae with the increasing of methanol extract concentrations. In control experiment, 0% mortality was observed. Analysis of variance revealed that there was significant difference between different concentrations of each extract. At 10% concentration, bulb extract showed highest mortality activity of 71.43% but the leaf extract was found less toxic with 53.84% larval mortality (Table 3).

Table 3: Toxicity by ingestion of methanolic leaf and bulb extracts of *U. maritima* against larvae of *T. castaneum*.

Concentrations (%)	Mortality rate (%)	
	Leaf extract	Bulb extract
0.1	25.38^{a}	35.71^{a}
1	31.00^{ab}	57.14^{ab}
10	53.84^{b}	71.43^{b}

a, b Different letters differ significantly according to the test of Student-Newman-Keuls ($p \leq 0.05$).

Moreover, Table 4 indicates lethal concentrations values of tested extracts against *T. castaneum*. LC_{50} values of leaf extract and bulb extracts were 92 g/L and 19.3 g/L, respectively in in vivo treatment.

Table 4: Toxicity by ingestion of methanolic leaf and bulb extracts of *U. maritima* against larvae of *T. castaneum*.

Extracts	LC50 (g/L)	LC90 (g/L)	Chi-Square	df*	*P*
Leaves	92	201	0,243	1	0,622
Bulbs	19,3	194,1	7,17	1	0,007

* Degree of freedom; LC: Lethal concentration.

3.3 Toxicity by residual film

Three different concentrations of each methanolic extract (leaf or bulb) were applied by the method of residual film on larvae and adults of *T. castaneum*. The results indicate that leaf extract was the most toxic to the larva of red flour beetles causing 51.72% and 41.37% larval mortality after 14 days at 10% and 0.1% concentrations, respectively (Table 5). However, no toxic effect was observed in adults.

Similar results were observed for methanolic bulb extract with a less toxicity on larvae reaching 35.71% mortality at 10% concentration and recorded no effect on adults (Table 5).

Table 5: Mortality rate of *T. castaneum* larvae and adults treated by residual film method of toxicity with methanol extracts of *U. maritima* leaves and bulbs.

Extracts	Concentrations (%)	Insect stage	Mortality rate (%)				
			1 day	2days	3days	7days	14days
Leaf	0.1	Larvae	0	20	23.33	43.33	41.38
		Adults	0	0	0	0	0
	1	Larvae	0	6.67	13.33	30	37.93
		Adults	0	0	0	0	0
	10	Larvae	16.67	23.33	23.33	50	51.72
		Adults	0	0	0	0	0
Bulb	0.1	Larvae	0	16.67	16.67	17.86	25
		Adults	0	0	0	0	0
	1	Larvae	3.33	16.67	16.67	21.43	25
		Adults	0	0	0	0	0
	10	Larvae	10	20	23.33	35.71	35.71
		Adults	0	0	0	0	0

On the basis of lethal concentrations LC_{50} and LC_{90} values the mortality % was recorded by statistical data, it was observed that among the tested materials, leaf extract showed the highest toxicity to larvae as compared to adults. The lowest LC_{50} and LC_{90} values of methanol extract of leaves of *U. maritima* against larvae of *T. castaneum* were found to be 87.9 and 495.4 g/L, respectively (Table 6). No mortality was observed in treated adults for the tested extracts.

Table 6: Toxicity by residual film method of methanolic leaf and bulb extracts of *U. maritima* against larvae of *T. castaneum*.

Extracts	LC 50 (g/L)	LC90 (g/L)	Chi-square	df*	*P*
Leaves	87.9	495.4	0.433	1	0.510
Bulbs	213.2	608.4	0.023	1	0.879

* Degree of freedom; LC: Lethal concentration.

4. Discussion

The present results revealed toxicity of methanolic extracts of leaves and bulbs of *U. maritima* against *T. castaneum*. This activity was confirmed by Hassid *et al*., [19] on *Spodoptera littoralis* larvae and adults. Adeyeye and Blum [20] had reported that this substance caused severe retardation of larval development, larval mortality resulting from exuvial ligature, death at larval-pupal ecdysis, formation of grossly deformed pupae, and nonsclerotization of the anterior ventral half of the pupal integument of the corn earworm *Helicoverpa zea*. Pascual-Villalobos and Fernandez [21] observed that ethanolic bulb extract on administration to 25-day-old larvae of *T. castaneum* caused mortality of 60% after 24h. Similar results were also found by Kotze and Dennill [22] against

Liriomyza trifolii on tomato crop. However, Civelek and Weintraub [23] reported that aqueous extract of *U. maritima* affect the percentage of parasitism of *Digliphus isaea*, *Diospyros crassinervis*, *Encarsia Formosa* and *Chrysocharis pentheus.* A great number of phenolic compounds and bufadienolides has been simultaneously analyzed and determined [24]. Pascual-Villalobos [25] reported that *U. maritima* bufadienolides induce anti-insect effects.

5. Conclusion

Our results have shown that methanolic leaf extract of *U. maritima* possesses high insecticidal activity against larvae and adults of *T. castaneum*. Our next approach will be targeted to fractionate them and isolate possible active compounds and to elucidate its more insecticidal potential.

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