Toxicity of Green Marine Algae *Ulva Lactuca* Linnaeus to The Adult Female of *Tetranychus Urticae* Koch (Acari:Tetranychidae)

Hamdy M. El-Sharabasy and Gamal A. El-Kady
Department of Plant Protection, Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt.

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Abstract: Green marine algae *Ulva lactuca* Linnaeus was extracted sequentially with different organic solvents; petroleum ether, ethanol and ethyl acetate. Each extract was tested alone, combined with xylen and xylen plus surfactant against adult females of *Tetranychus urticae* Koch. The mortality of the females was recorded for 96 hr at different concentration of each tested extract. Results revealed that Petroleum ether extract in each treatment was highly toxic against *T. urticae*. Such toxic effect was increased with time of exposure to reach its maximum after 96 hr. The addition of surfactant to all tested extracted enhancement their toxic action which was clearly observed for petroleum ether (LC_{50}= 2.6 g/ml) alone, while it was (3.1 and 1.1 g/ml) with xylen and xylen plus surfactant. Preliminary phytochemical analysis indicated that *U. lactuca* contain carbohydrates, glycosides, terpenes and steroles with a resemble amount

Keywords: *Tetranychus urticae*, green marine algae, *Ulva lactuca*, toxicity

INTRODUCTION

The two spotted spider mite, *Tetranychus urticae* Koch, has been recognized as a factor limiting the production of several crops and is controlled by biological means. For several years, chemical control of mites has been extensively practiced in Egypt to curb mite upsurge. Heavy reliance on chemical agents have had its serious drawbacks, manifested in resistance problems and high residue levels in vegetables and fruits that may hinder marketing. Several trials were carried out concerning the effect of crude extracts in higher plants against the two-spotted spider mites (Amer *et al.*, 1989 and Demetry *et al.*, 1988). In the last few decades, benefits of lower plants, particularly marine algae extended beside their nutritional value due to their protein as a fodder for grazing livestock to embrace the production of biologically active substances of pharmaceutical importance (Hamdy, 1982).

Booth (1965) pointed out the probability of controlling red spider mites, *T. telarius* (L.) in British orchards, with a seaweed extracts than with conventional fungicides and insecticides. Hamstead (1970) and Ahmed (1993) studied the effect of seaweed extracts spray on *T. urticae* and on a predaceous mite *Typhlodromus fallacis*. The present study aims to evaluate the toxic effect of different organic solvents extracts having different polarity of green marine algae *U. lactuca* against *T. urticae*.

MATERIALS AND METHODS

Organisms:

1-Mites: The phytophagous mites, *T. urticae* were obtained from a laboratory culture reared on sweet potato plants at 25±2°C and 65 ±5 % R.H.

2-Algae: Green marine algae, *U. lactuca* was collected from the Suez canal and its allied lacks. The thallus of *U. lactuca* forms an expanded sheet with a wavy margin, and has a light to deep green color. It normally grows on rocks and other substrata in the middle to low intertidal zone along the side bands of Suez canal. The thallus develops under stress into a cushion-like colony, which in this case, grows on other larger algae.

Method of extraction:

Samples *U. lactuca* were left to dry under laboratory conditions for one week. Dried materials were ground using mortar and pestle, sieved for extraction. A dried powder (40 gm) was extracted by different organic solvents successively using, Petroleum ether, Ethyl acetate and Ethanol using a Soxhlet apparatus, (solvent was added at rate of 5 ml/1mg algae). The extracts were filtered. The filtrates were evaporated till dryness in a rotary evaporator under vacuum. Each crude material obtained was weighted and re-dissolved in the suitable solvent (1g/10ml solvent), to give 10% (w/v) stock solution. Series of different concentrations were prepared and subjected to their biocidal activity against the phytophagous mites.

Toxicological studies:

1-Organic solvent alone:

The toxicity of the various extracts was assessed on the adult stage. *T. urticae* females were transferred to the lower surfaces of sweet potato leaf disks (2.5 cm in diameter) previously dipped for 20 sec. in different concentrations of each extract (1,3,4,5 and 10 g/ml). The arenas were placed with the upper surface in contact with moist cotton in Petri dishes. Ten adult females were placed on each treated disc; with 4 replicates of each concentration. A control chek with the solvent only was included. Mortality was recorded daily for 4 days after application. Corrected mortality counts according to Abbott's formula (1925) and mortality data were submitted to statistical analysis using a split plot design. The toxicity index of each extract was determined according to Sun (1950) as follows:

$$\text{Toxicity index} = \frac{\text{LC50 or LC90 of the most effective insecticide}}{\text{LC50 or LC90 of the less effective insecticide}} \times 100$$

2- Organic solvent mixed with xylene:

In this test, xylene was added to each extract and the efficacy of each mixture was assessed. Xylene was meant to improve the performance of each extract and enhance their penetration. The crude material extract was weight and re-dissolved in a mixture of xylene (1g crud extract/ 10 ml xylene), and the mixtures efficacy was assessed as described earlier. The control check was dipped in xylene only.

3- Organic solvent mixed with xylene and surfactant:

Xylene and surfactant were added to each extract and the efficacy of each mixture was assessed. The addition of xylene and surfactant was meant to improve the performance of each extract and enhance their penetration. The crude material extract was weight and re-dissolved in a mixture of xylene (1g crud extract / 9 ml xylene/ 1ml surfactant), and the mixtures efficacy was assessed as described earlier. The control check was dipped in mixture of xylene and surfactant.

Chemical study of dried green marine algae *U. lactuca*:

U. lactuca was collected and dried for one week at room temperature then ground using mortar pestles to complete some tests to determine, carbohydrates and glycosides, flavonoids, tannins, sterols & terpenoids, chlorides & sulfates and resins. Also, determine moisture, ash and crude fiber content. All procedures were based on those of Habib *et al.*, 1987.

RESULTS AND DISCSSION

Effects of *Ulva lactuca* extracts on the adult female of *Tetranychus urticae*:

The toxicity of three *U. lactuca* extracts (ethanol, ethyl acetate and petroleum ether) against adult females of *T. urticae* after 24, 48, 72 and 96 hrs are shown in Table (1). Results obtained showed that after 24 hrs of treatment, petroleum ether extract was the most effective at the LC_{50} level, followed by ethanol and ethyl acetate extracts; (The LC_{50} were 31.4, 42.6 and 58.8 g/ml respectively). The same trend was found after 48, 72 and 96 hrs of application. Data showed that the toxicity increased by increasing time of exposure and the mortality was increased steadily and maximum mortality was reached 96 hrs after treatment (80 % mortality at the highest concentration 10 g/ml). the two spotted spider mite, *T. urticae* was highly susceptible to even low concentration (1g/ml) of each extracts. Statistical analysis revealed that no significant differences in the toxicity of three extracts. This finding is in agreement with the reports of Booth (1965) and Hamstead (1970) and Barakat *et al.* (1984) who obtained better control of the spider mite *T. urtcae* with the seaweed than with convention fungicides. The present results showed that petroleum ether extract was the most effective because it's the least polar solvents used to extract the seaweed and the components extracted by petroleum ether were the least water soluble compounds. Amer *et al.* (1989) mentioned that petroleum ether extract of *Abrus precatorius* seeds was very efficient extracts in causing high mortality between adult females of *T. urticae*. Amer *et al.* (1991) reported that petroleum ether extract of green marine algae *Codium sp.* was the most effective against the adult females.

Effects of *U. lactucae* extracts mixed with xylene against adult females of *T. urticae*:

Toxicity data in Table (1) revealed that each of the tested extract when mixed with xylene gave a low mortality. Nevertheless, data showed that petroleum ether extract was the most effective against adult females followed by ethanol and ethyl acetate extracts. The LC_{50} values after 24 hr were (38.4, 85.1 and 112.2 g/ml) respectively, while after 96 hr were (3.1,5.7 and 14.7 g/ml) respectively. Data showed that the toxicity of each extract was increased as the time of exposure increased. In this try, we found that xylene may be reduced the toxicity and number of mortality at all concentrations and at all time of exposure.

Effects of *U. lactucae* extracts mixed with ylene and surfactant against adult females of *T. urticae*:

Results in Table (1) indicated that after 24, 48, 72 and 96 hrs. of application, petroleum ether extract was the most effective on the adult females at the LC_{50} level followed by ethanol and ethyl acetate extracts. Data showed that mortality increased as the time of exposure was increased and petroleum ether extract still ranked the first, followed by ethanol and ethyl acetate. The percentages mortality after 96 hrs were 82.5, 62.5 and 57.5 % for petroleum ether, ethanol and ethyl acetate, respectively, and *T. urticae* was highly susceptible to even low concentration (1g/ml) of any tested extracts.

Data have indicated that xylene and surfactant mixture has increased the mortality percentages at all concentrations of all extracts, throughout the different exposure tests. This finding is in agreement with the reports of Ishaaya *et al.* (1986) who used the mineral oil or surfactant to increase efficacy of some insecticides against *Bemisia tabasi*. Neumann *et al.* (1987) reported that vegetable oil-surfactant mixtures used as an adjuvant in the field trials increased the efficacy of some insecticides against a large number of insects. Farag *et al.*, (1993) and Salem (1996) mentioned that surfactant increased mortality of *Spodoptera exigua* at LC_{25} and LC_{50} and mentioned that surfactant used as carriers and adjuvant to improve pesticides performance.

As *U. lactuca* extracts appeared to be more toxic to the spider mite *T. urticae*, thus, could be used as a selective natural product in integrated control of spider mite. Moreover, the abundant availability of seaweeds from coastlines of Egypt would make it of economical use in mite control programs. Therefore, it could be recommended for use in IPM programs against the fore mentioned phytophagous mite.

Results in Table (2) indicated that the toxicity indexes of each group of toxicant at LC_{50} levels were 63.4, 49.1 %, 54.4, 21.1 and 61.1 and 35.5 % for the solvent extracts alone, solvents with xylene and solvent, xylene and surfactant, respectively. Whereas the Petroleum ether extracts for each group was the most potent extract 100 %.

Table (1): Toxicity of different organic solvent extracts of *U. lactuca* against *T. urticae.*

Extract	24 hr			48 hr			72 hr			96 hr		
	LC_{50} g/ml	LC_{90} g/ml	Slope	LC_{50} g/ml	LC_{90} g/ml	Slope	LC_{50} g/ml	LC_{90} g/ml	Slope	LC_{50} g/ml	LC_{90} g/ml	Slope
Solvent alone												
P. ether	31.4	257	1	10.2	204	0.58	3.5	467	1.06	2.6	177	1.47
Ethanol	42.6	269	0.71	12.3	467	0.84	8	870	0.69	4.1	575	0.6
E. acetate	58.8	371	0.71	16.1	800	0.11	8	1288	1.2	5.3	1698	0.52
Solvent with xylen												
P. ether	38.4	316.2	0.67	25.1	181.9	0.69	10.3	432.8	0.33	3.1	63.1	0.39
Ethanol	85.1	257	0.86	38	109.6	0.64	20	460.5	0.69	5.7	79.4	0.52
E. acetate	112.2	257	0.94	50.2	368	1.2	27	875	0.98	14.7	79.4	0.58
Solvent, xylen and surfactant												
P. ether	10.7	109	0.5	6.2	660	0.69	1.2	87.1	0.57	1.1	30.1	0.81
Ethanol	20.6	338	0.52	20.8	741	041	4.1	500	0.12	1.8	117	0.26
E. acetate	25	162	0.37	40.9	794	0.28	6.1	107	0.6	3.1	100	0.41

Table(2): Toxicity index of different organic solvent extracts of *U. lactuca* against *T. urticae* after 96hr.

Extract	Solvent alone		Solvent with Xylen		Solvent, Xylen and Surfactant	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
P. ether	100	100	100	100	100	100
Ethanol	63.4	30.8	54.4	79.5	61.1	25.7
E. acetate	49.1	10.4	21.1	79.5	35.5	26.9

Preliminary phytochemical analysis of *Ulva lactuca* :

Data presented in Table (3) showed that the examined green algae *U. lactuca* gave negative for flavonoids, saponins and tannins. While, positive results were found for carbohydrates and /or glycosides, sterols and /or terpenes, chlorides and /or sulfate and resins. All the previous component which act as toxicants is still not known and needs fractionation and isolation. The determination of chemical constituent; (Table 4) showed that the moisture content for this algae was 12.7% and the level of ash content was found to be 25.5% while the crude fiber content was 0.41%. These finding are in agreement with those of El-Tawil and Khalil (1983) and Demetry *et al.* (1988).

Table (3) : Preliminary phytochemical analysis of *U. lactuca*

No.	Test	Result
1	Carbohydrates and /or glycosides	+ve
2	Flavonoids	-ve
3	Saponins	-ve
4	Terpenes	-ve
5	Sterols and /or terpenes	+ve
6	Chlorides and /or sulfate	+ve
7	Resins	-ve

Table (4): Constituent of dry *U. lactuca*

No.	Test	%
1	moisture content	12.7
2	ash content	25.5
3	crude fiber content	0.41

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سمية الطحلب البحرى الأخضر *Ulva Lactuca* Linnaeus على الإناث الكاملة لـ *Tetranychus Urticae* Koch (Acari:Tetranychidae)

حمدى محمود الشرباصي- جمال عبد الستار القاضي
قسم وقاية النبات- كلية الزراعة- جامعة قناة السويس- ٤١٥٢٢- الإسماعيلية- مصر

تم إستخلاص الطحلب الأخضر *Ulva Lactuca* Linnaeus بإستخدام مجموعة مختلفة من المذيبات العضوية هى الإثير البترولي، الإيثانول وأسيتات الإيثيل. تم إختبار كل مستخلص على حده بالزيلين والزيلين مضافا إليه مادة سطحية ضد الإناث الكاملة لـ *Tetranychus Urticae* Koch. تم تسجيل نسب الموت للإناث بعد ٩٦ ساعة على تركيزات مختلفة لكل مستخلص تم إختباره. أشارت النتائج إلى أن الإثير البترولي عند كل معاملة كان أعلى سمية، وتزيد السمية مع زيادة وقت التعريض حتى تصل إلى أقصى معدل بعد ٩٦ ساعة. إضافة المادة السطحية على كل مستخلص تم إختباره حسنت من التأثير السام وكان ذلك واضحاً عند إضافته إلى الإثير البترولي حيث كانت قيمة LC_{50}= ٢,٦ جرام/مل عندما تم إختباره بمفرده بينما كانت ٣,١، ١,١ جرام/مل بعد إضافة الزيلين والزيلين مضافا إليه المادة السطحية. تم عمل تحليل مبدئي للمكونات الكيميائية للطحلب وإتضح أنه يحتوى على كربوهيدرات، جليكوسيدات، التربينات والإستيرودات