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TOXICITY OF SOME MICROALGAE TO THE CITRUS BROWN MITE *EUTETRANYCHUS ORIENTALIS* (KLEIN)

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Abstract

The citrus brown mite *Eutetranychus orientalis* is a common spider mite, which is a harmful pest in citrus orchards in Egypt. The toxic activities of some microalgae isolated from Egyptian soils and water are studied against *E. orientalis*. The toxicity of whole cultures (culturing broth and cell) of seven algal strains was tested against *E. orientalis*. Three strains out of the tested seven strains showed higher toxicity with mortality percentage reached up to 99 % after 72 h recorded against adult individuals of *E. orientalis* with a strain of *Chlorella minutissima*. The toxic activity was also tested against immature individuals and eggs of *E. orientalis*. The highest mortality percent against immature individuals was observed with *C. oocystoids*. Eggs were not affected with any of the tested algal strains. When cells and culturing broth were tested separately the activity was higher in culturing broth which means that this activity is due to some extracellular metabolites secreted by algae in their culturing medium. When the organic solvent n-butanol was used to extract the active constituents from the whole cultures (culturing broth and cell), the activity was found to be higher in aqueous phase compared to the butanolic phase.

Key words: Biocontrol, *Eutetranychus orientalis*, algal toxicity, microalgae, *Chlorella*.

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1. Introduction

Eutetranychus orientalis is a serious pest attacks some economically important crops and causes significant damage to fruit trees in addition to horticultural and ornamental plants (Rasmy, 1978; Dhooria, 1985; Gupta, 1985; Sangeetha and Ramani, 2011). It is a polyphagous organism feeding on over 200 species from 60 families (EFSA PLH Panel, 2013). It is recorded in many countries worldwide. In India *E. orientalis* was noted as a major pest in many areas (Dhooria and Butani, 1984). Heavy infestations on citrus plants resulted in defoliation and branches even dieback (Yesilayer and Cobanoglu, 2010). Risks associated with the use of synthetic pesticides have led to the growth of an environmental movement seeking sustainable alternatives in pest control. Evidence of this includes the growth of organic agriculture products. Among the alternatives for pest control, biopesticides had a global market value of approximately US$1 billion in 2010. This market is expected to grow to US$3.3 billion in 2014 (Lehr, 2010). Biopesticides encompass a large number of technologies, from microbials to botanicals.

Microalgae are group of microorganisms that are naturally present in the soil. Their ecological positions at the base of most food webs and their essential roles in nutrient cycling and oxygen production are critical to all ecosystems. Many reports showed that, algae are rich in natural products such as antibiotics (Bloor and England, 1989; Issa, 1999; Fathi and Al- Kahtani, 2010), Antitumor (Tanaka *et al.*, 1984; Morimoto *et al.*, 1995; Suzuki *et al.*, 1999; Teneva *et al.*, 2013) and antiviral (Ohta *et al.*, 1998; Priyadarshani and Rath, 2012; Abdo *et al.*, 2012). Also many studies confirmed their ability to control pathogenic fungi (Martin, 1995; Abo-Shady *et al.*, 2007; Abedin and Taha, 2008). Although, there are many reports for antimicrobial, antitumor, and antiviral activity of algae, there are few studies about its acaricidal activity (Abdel-Aziz and Abdel-Raouf, 2002; Ibraheem and Abdel-Aziz, 2002). Therefore, the present study aims to investigate the effect of acaricidal activity of seven algal strains, isolated from some Egyptian water and soils, against the serious pest *E. orientalis*. The toxic effects of the whole culture (containing the culturing broth and the algal cells), the separated algal cells and culturing broth, and the butanolic and the aqueous phases were studied against this pest.

2. Materials and Methods

2.1. Tested algae

The seven microalgal strains used in this study were provided from Algae and Plant Physiology Laboratory, Faculty of Science, Al-Azhar University, Assiut, Egypt, and identified according to the following references (Prescott, 1987; Boney, 1983; Komarek and Fott, 1983; Canter-Lund and Lund, 1995). These algae are listed in Table (1).

Table (1): List of algal strains used in this study and their isolation places

Strain number*	Name	Source of isolate	Place of isolation
1	*Chlorella acuminate*	Water	Assuit
2	*Chlorella botryoides*	Water	Sohag
3	*Chlorella minutissima* AS1	Soil	Assuit
4	*Chlorella minutissima* QW1	Water	Qena
5	*Chlorella oocystoids*	Water	Sohag
6	*Chlorella minutissima* AW1	Water	Assuit
7	*Scenedesmus sp.*	Water	Sohag

***** Strain numbers mentioned in this table are used in all figures instead of their algal strains

2.2. Culturing of algae

The medium BG11 (Stanier *et al*., 1971) was used for culturing of algae. Algal strains were cultured in 500 ml conical flasks containing 200 ml of BG11 medium under static conditions with continuous illumination about 3500 lux for 7 days to study the effect of acaricidal activity of these algal strains on the serious pest *E. orientalis*.

2.2. Preparation of algae for treatment

2.2.1. Whole cell culture

At the beginning of the stationary phase, the whole cultures were adjusted to fixed optical density (0.7) at 760 nm (Védrine *et al*., 2002) using sterilized distilled water. The whole cultures including cells and their culturing broth were applied to *E. orientalis*

2.2.2. Separated cells and culturing broth

Algal cells were separated from the culturing broth by centrifugation at 5000 rpm for 10 min. The separated algal cells were then, resuspended in an amount of distilled water equal to that of their original culture. The supernatant (culturing broth) as well as the algal suspension were separately used directly for the application.

2.2.3. Butanolic extract

Algae were grown as mentioned above and at the beginning of the stationary phase; equal volume of n-butanol was added to the whole algal culture and then transferred to separating funnel shacked for 10 min and left till complete separation to two phases, aqueous phase and butanolic phase. Each of these two phases were collected separately from the separating funnel and tested for its activity against *E. orientalis*.

2.3. Acaricidal studies:

Phytophagous citrus brown mite *E. orientalis* Klein was collected from infected citrus trees and identified according to Krantz (1970) to study the toxic effects of mentioned algal strains against it.

2.4. Rearing of mites:

A pure culture of *E. orientalis* was carried out on pots diameter 25 cm cultivated with kidney beans *Phaseolus vulgaris* in sunny place.

2.5. Toxicity test:

To evaluate the algal activity on the adult stages of the mite, twenty newly emerged adult females were transferred on kidney beans leaf discs (4 cm diameter). Two leaf discs were kept on a moist cotton pad in each Petri-dish (15 cm diameter) (each disc was considered as a replicate) and continuously moistened during the experiment. Each dish was replicated with 4 replicates. The disc surface carrying the adult females of the same age was sprayed separately with algal strains preparations (mentioned above) using a manual atomizer. The untreated control was sprayed by BG11 medium only. The dishes were kept in incubator at 30ºC and 70 $\pm$ 5% relative humidity. Mortality percent was calculated after 24, 48 and 72 hrs of treatments according to Abbott's formula (Abbott, 1925).

2.6. Ovicidal action:

For studying the algal activities on eggs, twenty adult females of *E. orientalis* Klein were allowed to lay eggs on upper surface of kidney beans leave discs (4cm diameter). The discs were placed in Petri-dishes (each disc was considered as a replicated). The adult females were removed after 24 hrs and the deposited eggs were counted. The disc surfaces carrying the eggs were sprayed separately with the tested algal suspension using a manual atomizer. The treated eggs and untreated control were kept under the previously mentioned incubator conditions. After five days of treatments, the numbers of non-hatching eggs were counted and the mortality percentages were corrected according Abbott's formula (Abbott, 1925).

2.7. Response for repellency effect:

This experiment was carried out according to Meisner *et al.* (1970). Kidney beans leaves were marked into three equal regions, one of the two marginal sides was coated with single algal suspension and other one was coated with BG11 medium. The adult females of *E. orientalis* were transferred to the middle region of the experimental leaves. Distribution percent of the females was calculated after 24, 48 and 72 hrs after treatment.

3. Results

3.1. Effect of the whole algal culture on adult *E. orientalis* individuals

The whole algal cultures, including cells and their culturing broth, were applied to adult individuals of *E. orientalis* and the mortality rates were determined. The mortality rates were measured after 24, 48, and 72 h. The results were shown in Fig. (1) indicated that, three algal strains showed higher rates of mortality after 72 h. The highest activity (99.25%) was achieved with *Chlorella oocystoides* (strain no. 5), followed by *C. minutissima* AW1 (97 %) and *C. acuminatae* (strain no. 1) (95.1%). Other algal cultures showed medium activities varied from 64% with *C. minutissima* AS1, to 69.7% with *C. minutissima* QW1.

3.2. Effect of the whole algal culture on immature *E. orientalis* individuals

Generally, the activity of algal cultures on the immature individuals was lower than that on adult individuals (Fig. 2). The highest mortality rate (94%) was recorded with strain no. 5 (*C. oocystoids*) which achieved the highest activity (99.25%) also against adult individuals. The activity pattern is not the same against adult and immature individuals. For example algal strain no. 1 (*C. acuminatae*) showed higher activity against adults and lower activity against immature individuals, while algal strain no. 2 (*C. botryoids*) showed lower activity against adults and higher activity against immature.

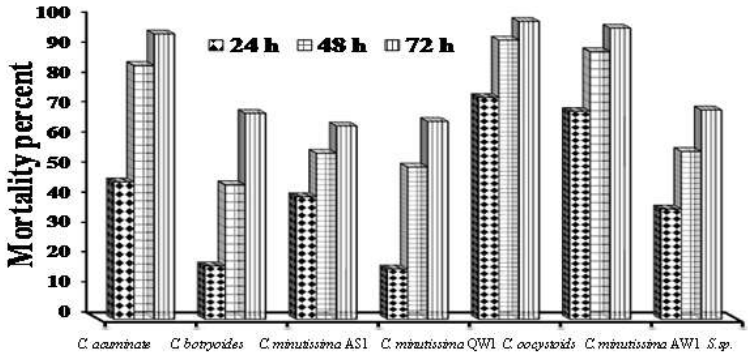

Figure (1) Toxic effects of whole cultures of different algal strains on mortality percent of adult *E. orientalis* individuals after 24, 48, and 72 h.

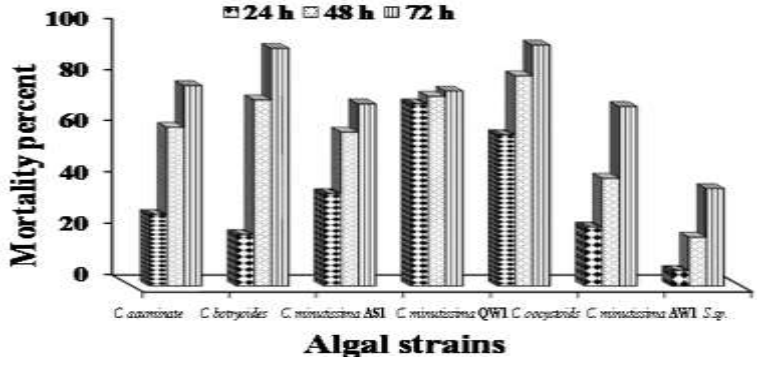

Figure (2) Toxic effects of whole cultures of different algal strains on mortality percent of immature *E. orientalis* individuals after 24, 48, and 72 h.

3.3. Effect of the whole algal culture on eggs of *E. orientalis*

The effect of whole algal culture of each algal strain was investigated against eggs of *E. orientalis* (Fig. 3). All algal strains were not effective and the highest activity (7.5%) was recorded with algal strain no. 1 while the lowest activity (1.3%) was found with algal strain no. 7. These results suggest that using of algae against eggs of *E. orientalis* is inappropriate for biocontroling this pest.

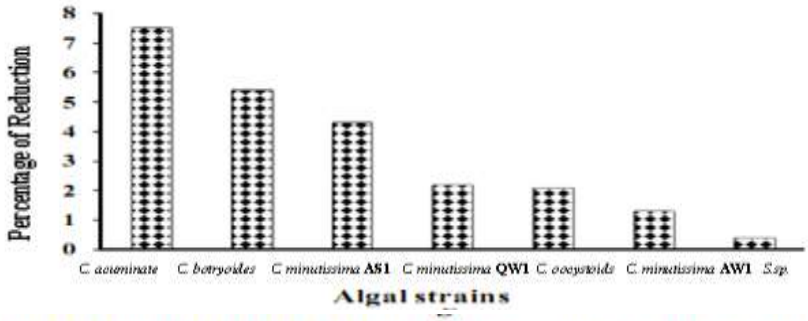

Figure (3) Toxic effects of whole cultures of different algal strains on hatching percent of eggs of *E. Orientalis* after 5 days.

3.4 Effect of separated algal cells and culturing broth on *E. orientalis*

Some algal strains (1, 5 and 6) represented high activity against adult and individual *E. orientalis* were chosen to determine whether the toxic activity of these strains is due to extra- or intra-cellular metabolites produced by cells. Cells and broth were separated by centrifugation (as described in materials and methods), then cells were resuspended in distilled water and treatments were done with cells suspension and culturing broth separately. Results were shown in Fig. (4) indicated that, the toxic activity of culturing broths was higher than that of the cell suspensions with small difference. For example, the mortality rate of culturing broth of algal strain no. 6 (*C. minutissima*) was 87.6% while that of its cell suspension was 78.3%.

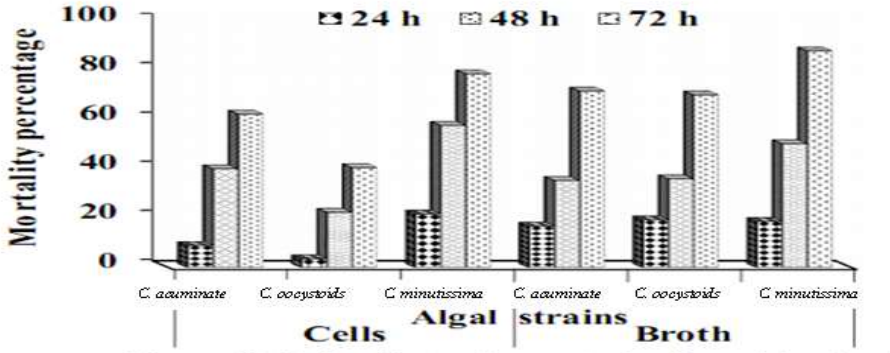

Figure (4) Toxic effects of separated cells and broth of different algal strains on mortality percent of adult *E. orientalis* individuals after 24, 48, and 72 h.

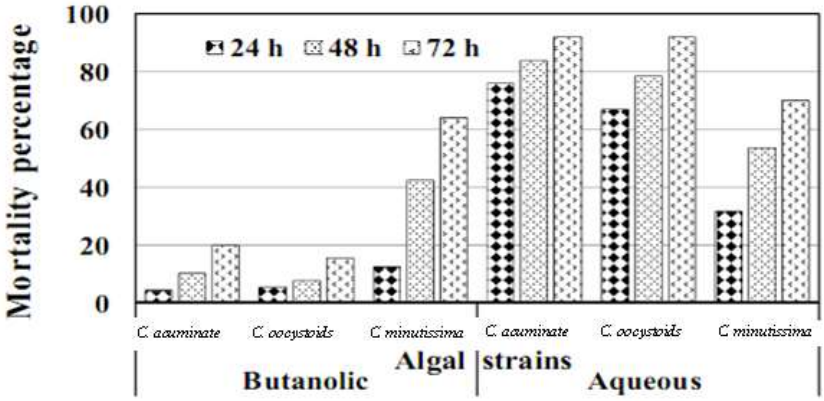

Figure (5) Toxic effects of butanolic phase and aqueous phase of different algal strains 1, 5, and 6 on mortality percent of adult *E. orientalis* individuals after 24, 48, and 72 h.

3.5. Effect of aqueous and butanolic extracts of algal strains on *E. orientalis*

Butanol was used to extract the active constituents from culturing broth as described in materials and methods section. Both aqueous and butanolic phases were tested. The results were illustrated in Fig. (5) cleared that, the toxic activity looks concentrated in aqueous phase, while butanolic phase showed low activity. The highest activity was measured with aqueous phases of both strains no. 1 (*C. acuminatae*) and no. 5 (*C. oocystoids*) (91.96%) compared to 20% and 16% with their butanolic phases, respectively. Butanol itself showed toxic activity against *E. orientalis* and to overcome this problem we dried the butanolic phases using rotary evaporator at 35ºC and redissolved it in distilled water, then used for treatment.

4. Discussion

The toxic activities of algal strains vary from species to another and they are also varied even at the strain level. Different *C. minutissima* strains isolated from different samples (water and soil) and from different localities (Assiut and Sohag) showed different activities (Results section). Variation of the properties and activities of different strains of the same species were recorded in many reports (Vizcaino *et al.,* 2005 and Morris *et al*., 2007). Our results showed higher mortality rates reached up to 99% against adult individual and 94 % against immature individuals of *E. orientalis*; however the activity against eggs were very low. These results are higher compared to that obtained by Abdel-Aziz and Abdel-Raouf (2002), when they used the metabolites of *Dunaliella* sp. against *Tetranychus urticae*. Their results showed upto 41.6% mortality percent, while in our case it reached upto 99%. Variation in activity toward adult and immature individuals may be due to different toxicity mechanism against adult and immature individuals. As

well as, using different algal strains and different pest may be the reason of different results. Also, Refaat *et al.* (2002) demonstrated that the mortality percentage of *E. orientalis* was higher than that of *T. urticae* when they were treated with essential oils of *Ocimum basilicum* and *Lavandula officinalis*, which means that *E. orientalis* is more sensitive than *T. urticae*.

Many studies of different researchers were done on the toxicity of different plant extracts as miticides in integrated pest management programs (Amer *et al*., 2000) while, the other studies conducted on the effect of algal extracts on mites are rare. Most of studies were concerned with the effects of algae on insects. Angerilli and Beirne (1974) and Dhillon *et al*. (1982) found that, the free floating unicellular green alga *Chlorella ellipsoidae* produces some substances which affect the development and immature stages of mosquitoes. Similar results were obtained by Amonkar, (1969) and Nassar *et al*. (1999) as they found that, some blue green and green algae produce substances that inhibited larval development and delayed the survival and development of the adult females of mosquitoes. All these studies are in accordance with our results as they prove the toxicity of some algae to some insects which is a group of animals related to mites (both are belonging to Arthropods).

Our results showed that, the toxic activity of algae is due to extracellular substances. However some of these compounds couldn't be completely separated from cells by centrifugation. These non-separated compounds may be the reason of the toxicity of cell suspension. These results are similar to that obtained by Abdel-Aziz and Abdel-Raouf, (2002) and Ibraheem and Abdel-Aziz (2002). The active substances remain in the aqueous phase after extraction with butanol; this may facilitate the utilization of these algal liquid cultures for treating *E. orientalis* and lower the costs of extraction and preparation.

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سمية بعض الطحالب المجهرية لحلم الحمضيات *البنى*

Euteranychus orientalis (Klien)

تعتبر الدراسات التي تهدف إلى استخدام الطحالب لمكافحة الطفيليات الحيوانية التي تسبب أمراضًا للنبات نادرة. ولقد تم في هذا البحث اختبار التأثيرات السمية لبعض أنواع الطحالب المصرية على حلم الحمضيات البني. ولقد تمت دراسة هذه التأثيرات باستخدام المزارع الطحلبية السائلة والتي تحوى الخلايا الطحلبية وبيئة النمو على الأفراد اليافعة والأفراد غير المكتملة النضج وكذلك البيض. ولقد أظهرت النتائج نسب وفاة وصلت إلى حوالي 99% من الأفراد البالغة و94% في الأفراد غير مكتملة النضج ولم تظهر إلا تغيرات طفيفة على معدلات فقس البيض. وعلى مستوى آخر فلقد تمت دراسة تأثير الخلايا الطحلبية منفصلة وكذلك بيئة نموها منفصلة ، بعد أن تم فصلهم بالطرد المركزي، وأظهرت النتائج أن سمية بيئة النمو أعلى من سمية الخلايا مما يدل على أن المواد الفعالة تتواجد خارج الخلية. وأوضحت النتائج أيضا أن المواد الفعالة أكثر ارتباطا بالماء من ارتباطها بالبيوتانول مما يسهل استخدامها لمكافحة حلم الحمضيات البني حيث يمكن استخدام بيئة نمو الطحلب دون استخدام أي مذيبات عالية التكلفة أو غير آمنة بيئيا.