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Assessing the Effectiveness of Various Microalgal Species as Green Control Against Citrus Orchard Infestations of *Eutetranychus orientalis* (Acari: Tetranychidae)

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ABSTRACT

The citrus brown mite, known as *Eutetranychus orientalis*, is a frequent spider mite that poses a threat to citrus farms in Egypt. Therefore, we chose this species as most common and the most numerous among the species that were surveyed on citrus trees. The goal of this study was to evaluate the effect of five microalgae with four treatments against *E. orientalis* and the mortality rates were determined and measured after different periods under laboratory and field conditions. The obtained results showed that all algal treatments exhibited high toxic action against *E. orientalis* after 72 hours of application. The culturing broth only (T3), with algae treatment of *Pseudanabaena limnetica* had the highest mortality rate (100%) after 72hr, followed by *Chlorella vulgaris*, *Chlorococcum sp*., *Scenedesmus obliquus*, and *Synechocystis aquatilis* (96.7%) under field conditions. In addition, the same treatment showed high toxic effects against *E. orientalis* after 72 hours and a week of application under field conditions, where the greatest reduction rates are found in *P. limnetica* and *S. aquatilis* (83.29 & 85.08%) and (83.77 & 85.92%) after 72 hr and a week of application, respectively followed by *Chlorococcum* sp. and *S. obliquus* (82.19 & 85.64%) and (82.33 & 86.73%). The lowest reduction (76.30 & 85.31%) were observed in *C. vulgaris* after 72 hours and a week. The above mentioned results indicated the possibility of controlling the citrus brown mite, *E. orientalis* on citrus navel trees by using the culturing broth of microalgae as eco-friendly biopesticides instead of traditional synthetic pesticides.

Keywords: Biocontrol, microalgae, toxicity effect, citrus brown mite, citrus.

1. Introduction

Eutetranychus orientalis is a dangerous pest that damages fruit trees, horticultural plants, decorative plants, and several commercially significant crops (Rasmy 1978; Dhooria 1985; Gupta 1985; Sangeetha and Ramani 2011). It consumes more than 200 species from 60 families, making it a polyphagous organism (EFSA Panel on Plant Health 2013). Numerous nations around the world have recorded it. In various regions of India, *E. orientalis* was identified as a significant pest (Dhooria and Butani 1984). According to (Yesilayer and Cobanoglu 2010), severe infestations on citrus plants caused defoliation and even dieback in certain branches. Because of the dangers of using synthetic pesticides, there is now a growing environmental movement looking for sustainable pest control methods. In 2010, the global biopesticide market was worth about $1 billion USD. According to (Lehr 2010), this market is anticipated to reach US$3.3 billion in 2014. Microbials and botanicals are just two of the many technologies that make up biopesticides. Alternative approaches are required to effectively manage mite infestations Baran Imani *et al*. (2020). A marine alga is a highly promising non-insecticide pest management strategy. According to Asimakis *et al*. (2022), algae can reduce crop pests by acting as insecticides.A type of microorganism found naturally in soil is called microalgae. All ecosystems depend on their ecological locations at the base of the majority of food webs as well as their vital roles in the creation of oxygen and the cycling of nutrients. Numerous studies have demonstrated that algae are abundant in natural products, including antiviral (Ohta *et al*. 1998; Priyadarshani and Rath 2012; Abdo *et al*., 2012), antitumor (Tanaka *et al*., 1984; Morimoto *et al*., 1995; Suzuki *et al*., 1999; Teneva

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et al., 2013), and antibiotics (Bloor and England, 1989; Issa, 1999; Fathi and Al-Kahtani 2010). Additionally, numerous investigations have validated their ability to manage pathogenic fungi (Martin 1995; Abo-Shady *et al*., 2007; Abedin and Taha 2008). Rania *et al*. (2025) found that marine algae worked better on *T. urticae* stages than veggie agro culture. Therefore, the purpose of this study is to determine how successful five different species of algae are at combating *E. orientalis*. In lab and field conditions, the toxic effects of the whole cell culture, the separated cells and culturing broth, the culturing broth alone, and the butanolic extract were evaluated against this pest on navel orange.

2. Materials and Methods

2.1. Tested Algae:

2.1.1. Experimental organism and culture media for microalgae:

The microalgae strains were obtained from Algae Laboratory, Department of Botany and Microbiology, Faculty of Science, Menoufia University. The five microalgae strains included *Chlorella vulgaris*, *Pseudanabaena limnetica*, *Scenedesmus obliquus*, *Synechocystis aquatilis* and *Chlorococcum* spp.

The microscopic image of algae as shown in (Fig 1)and were cultured in Bold's Basal Medium (BBM), the optimum medium for microalgae cultivation (Nichols and Bold 1965),These composition of Bolds Basal medium was listed in (Table 1).

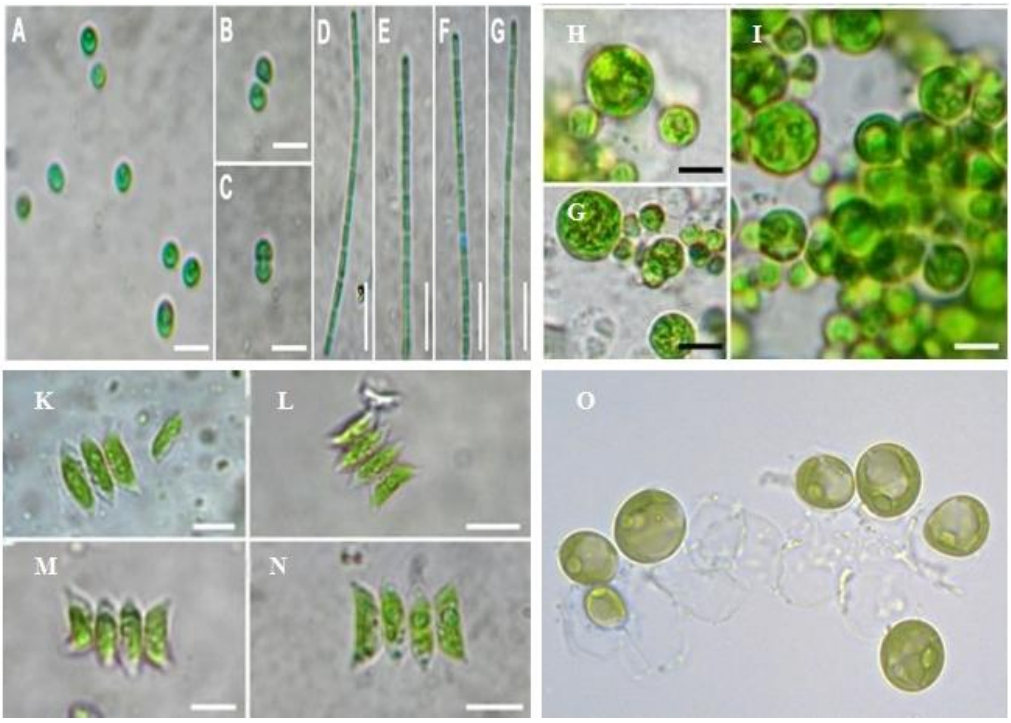

Fig. 1: The light microscopy micrographs of the cyanobacterial and green algal isolated in the present study. **A–C**: *Synechocystis aquatilis*, **D–G**: *Pseudanabaena limnetica*, **H–J**: *Chlorococcum* sp., **K–N**: *Scenedesmus obliquus,* **O**: *Chlorella vulgaris* Scale bars: 10 µm.

- 10 ml of solution (I,6) and 1ml of solution (7,8,9) was added to liter of distilled water.
- pH was adjusted at 7.5.
- The medium was autoclaved at 121°C, under 1.5 atmospheric pressure for 30 min.

2.1.2. Culture Techniques for Microalgae

Erlenmeyer flasks (1000 ml) containing 700 ml of medium were sterilized in an autoclave at 121°C under 1.5 atmospheric pressure for 30 minutes. After cooling, the flasks were inoculated with 70 ml of pre-cultured organisms and incubated under continuous fluorescent light at an intensity of 35 E/m²/s,

with a pH of 7.5, and at a temperature of 30 ± 2°C. To enhance the growth of the microalgae, a mixture of 97% air and 3% CO_2 was used for aeration. During a 20-day incubation period, biomass production was collected at the end of the incubation.

Two field experiments (The normal and water stress treatments) were conducted at Gimeaza Research Station, Field Crops Research Institute, Agricultural Research Center during two successive seasons of 2019/2020 and 2020/2021 using three local quinoa cultivars namely; Quinoa 1, Rainbow and the American cultivar. The three quinoa cultivars were different response for drought stress tolerance where (Quinoa 1 was high tolerance, followed by the cultivar rainbow and the American cultivar was classified as moderate to sensitive). The quinoa cultivars were sowing under normal and water stress treatments in a randomized complete block design with three replicates for each experiment in both growing seasons. Under normal irrigation, the first irrigate at agriculture time, then irrigation every ten days and prevention of irrigation two weeks before harvest. For water stress treatment, the first irrigate at agriculture time, then irrigation every twenty one days, and prevention of irrigation two weeks before harvest. The recommended agricultural practices of growing quinoa were applied in both growing seasons. The physical and chemical analysis of Gimeaza soil during the two growing seasons is presented in Table (1).

Table 1: Composition of Bolds Basal medium was prepared as follows:

Stock solution (SL)	Component	Stock solution g/L	ml/Liter	Solution (SL7) (Trace elements) 1ml/L	g/L	Solution (SL8) (EDTA stock)	g/100ml
SL1	$NaNO_3$	25	10.0	$MnCl_2.7H_2O$	1.44	EDTA (disodium salt)	5.0
SL2	KH_2PO_4	17.5	10.0	$ZnSO_4.7H_2O$	8.82	KOH	3.1
SL3	$CaCl_2.2H2O$	2.5	10.0	$CuSO_4.5H_2O$	1.57	Solution (SL9) (Fe solution)	g/L
SL4	$MgSO_4$ $7H_2$	7.5	10.0	$Co(NO_3)_2.6H_2O$	0. 49	$FeSO_4.7H_2O$	4.98
SL5	NaCl	2.5	10.0	$Na_2MoO_45H_2O$	0.71	Conc.H_2SO_4	1.0ml
SL6	H_3BO_3	5.7	10.0	$MnCl_2.7H_2O$	1.44		

1.3. Preparation of algae for treatment:

-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.*

- 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, re-suspended 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.

- 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 into 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*.

1. Toxicological studies

2. Studies of acaricidal:

Phytophagous citrus brown mite *Eutetranychus 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.

2.1. Rearing of mites:

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

2.2. Laboratory Experiment:

2.2.1. Toxicity test of five microalgae strains against *Eutetranychus orientalis* under laboratory conditions:

To evaluate the algal activity on the adult stages of the mite, ten newly emerged adult females were transferred on Round citrus leaf discs. One leaf disc was kept on a moist cotton pad in each Petri-dish (15 cm diameter) and continuously moistened during the experiment. Each dish was replicated with three 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 BBM medium only. The Petri-dishes were kept in incubator at 30ºC and 70 ± 5% relative humidity. Mortality percent was calculated after 24, 48 and 72 hrs of treatments according to Abbott's formula (Abbott 1925).

2.3. Field Experiment:

2.3.1. Toxicity test of five microalgae strains against *Eutetranychus orientalis* under field conditions:

Under field conditions, five microalgae strains were applied as spray treatments, each compound was represented by three Navel orange trees (spaces were left between transactions in all directions). In addition, three trees of Navel orange variety were sprayed with water and left without any applications, which served as control. The applications were done in the beginning of the growth season of citrus (15 March, 2022), leaf samples were taken before treatments and 12 hr., 24 hr., 48 hr.,72 hr., 1 week and 2 weeks after applications. Numbers of citrus brown mite *E. orientalis* was counted, then the reduction percentages was computed for all tested microalgae strains using Henderson and Tilton (1955) equation.

Results

1. Harmful effects of certain microalgae on the citrus brown mite, *Eutetranychus orientalis* under laboratory conditions:

The citrus brown mite, known as *E. orientalis*, is a frequent spider mite that poses a threat to citrus farms in Egypt. Therefore, we chose this species of the most common and the most numerous among the species that were surveyed on citrus trees specifically at Alshohadaa district in Menoufia governorate. The toxic activities of some microalgae isolated from water are studied against *E. orientalis*. The toxicity of whole cell culture (T1), separated cells and culturing broth (T2), culturing broth (T3) and butanolic extract (T4) of five algal types were applied to adult individuals of *E. orientalis* and the mortality rates were determined under laboratory conditions. The mortality rates were measured after 6, 12, 24, 48 and 72 h.

1.1 Impact of the whole cell culture (T1), on adult individuals of *E. orientalis*:

The whole algal cultures, including only cells of algae was applied to *E. orientalis* adult and the mortality percentages were determined under lab conditions. The mortality rates were measured after 6, 12, 24, 48 and 72 hr. The data were given in Tables (2 and 3) and Fig. (2) indicated that five algal species showed higher rates of mortality after 72 h for all treatments. The highest mortality rate (96.7%) was achieved with *C. vulgaris* (T1), followed by *S. obliquus* (T1) (93.3%) and *S. aquatilis* (T1) (86.7%). Other algal cultures showed medium mortality rates varied from 73.3% with *P. limnetica* to 70.0% with *Chlorococcum* sp. Types.

1.2. Impact of separated algal cells and culturing broth (T2), on *E. orientalis*:

As for, the whole algal cultures, including separated cells and culturing broth, were applied on *E. orientalis* adult and the mortality percentages were determined and the highest mortality (96.7%) was recorded with *P. limnetica*, *Chlorococcum* sp. and *S. aquatilis* followed by *C. vulgaris* (90.0%) while the lowest mortality (73.3%) was found with *S. obliquus*.

1.3. Impact of culturing broth only (T3), on adult individuals of *E. orientalis*:

All algal treatments exhibited high toxic action against mature *E. orientalis* after 72 hours of application under lab conditions, as indicated by the results in Tables 2 and 3. *P. limnetica* had the highest mortality rate (100%) after 72hr, followed by *C. vulgaris*, *Chlorococcum* sp., *S. obliquus*, and *S. aquatilis* (96.7%). In comparison to the other treatments, this one was thought to be the most successful in combating brown citrus mites under lab conditions.

1.4. Impact of Butanolic extract (T4), on adult individuals of *E. orientalis*:

As stated in the materials and methods section, butanol was utilized to extract the active ingredients from the culturing broth. Tables 2 and 3 presented the data, which demonstrated that the butanolic extract's hazardous activity had low mortality rates. *P. limnetica* and *Chlorococcum* sp. had the lowest mortality rates (50 and 63.3%, respectively), whereas *C. vulgaris* had the greatest mortality rate (93.3%), followed by *S. obliquus* and *S. aquatilis* (70.0%).

Table 2: Toxic effects of different microalgae species against *Eutetranychus orientalis* adults under laboratory conditions

Algae species	Treatments	Mean number of mites					Overall mean
		6 hr	12hr	24 hr	48 hr	72 hr	
C. vulgaris	***T1**	9.67	7.67	4.33	2.33	0.33	4.87
	***T2**	9.33	8.67	5.00	1.33	1.00	5.07
	***T3**	8.00	2.67	1.67	1.00	0.33	2.73
	***T4**	9.67	9.00	8.67	3.67	0.67	6.34
P. limnetica	**T1**	9.00	8.33	7.33	6.00	2.67	6.67
	T2	9.00	5.00	1.67	0.67	0.33	3.33
	T3	7.33	3.00	1.67	0.67	0.00	2.53
	T4	9.67	9.33	9.00	8.33	5.00	8.27
Chlorococcum sp.	**T1**	9.33	9.00	8.67	7.00	3.00	7.40
	T2	8.67	7.67	5.67	4.00	0.33	5.27
	T3	7.33	2.67	1.67	0.67	0.33	2.53
	T4	9.33	9.33	8.00	7.00	3.67	7.47
S. obliquus	**T1**	8.33	4.33	2.33	1.67	0.67	3.47
	T2	8.67	8.67	6.33	6.00	2.67	6.47
	T3	8.33	2.33	2.00	1.33	0.33	2.86
	T4	9.67	9.33	7.67	5.67	3.00	7.07
S. aquatilis	**T1**	8.00	5.67	2.67	2.33	1.33	4.00
	T2	9.00	8.00	7.33	5.33	0.33	5.99
	T3	7.67	3.00	2.00	1.00	0.33	2.80
	T4	9.33	9.00	8.67	5.67	3.00	7.13
Control	**-**	10	10	10	10	10	10

***T1**= Whole cell culture, ***T2**=Separated cells and Culturing broth, ***T3**=Culturing broth, ***T4**= Butanolic extract.

Table 3: Mortality rates of *Eutetranychus orientalis* adults at different treatments of algae species under laboratory conditions.

Algae species	Treatments	Mortality percentage after					Rank	Overall mean mortality
		6 hr	12 hr	24 hr	48 hr	72 hr		
C. vulgaris	*T1	3.3	23.3	56.7	76.7	96.7	2	51.34
	*T2	6.7	13.3	50.0	86.7	90.0	4	49.34
	*T3	20.0	73.3	83.3	90.0	96.7	2	72.66
	*T4	3.3	10.0	13.3	63.3	93.3	3	36.64
P. limnetica	T1	10.0	16.7	26.7	40.0	73.3	6	33.34
	T2	10.0	50.0	83.3	93.3	96.7	2	66.66
	T3	26.7	70.0	83.3	93.3	100	1	74.66
	T4	3.3	6.7	10.0	16.7	50.0	9	17.34
Chlorococcum sp.	T1	6.7	10.0	13.3	30.0	70.0	7	26.00
	T2	13.3	23.3	43.3	60.0	96.7	2	47.32
	T3	26.7	73.3	83.3	93.3	96.7	2	74.66
	T4	6.7	6.7	20.0	30.0	63.3	8	25.34
S. obliquus	T1	16.7	56.7	76.7	83.3	93.3	3	65.34
	T2	13.3	13.3	36.7	40.0	73.3	6	35.32
	T3	16.7	76.7	80.0	86.7	96.7	2	71.36
	T4	3.3	6.7	23.3	43.3	70.0	7	29.32
S. aquatilis	T1	20.0	43.3	73.3	76.7	86.7	5	60.00
	T2	10.0	20.0	26.7	46.7	96.7	2	40.02
	T3	23.3	70.0	80.0	90.0	96.7	2	72.00
	T4	6.7	10.0	13.3	43.3	70.0	7	28.66

***T1**=Whole cell culture, ***T2**=Separated cells and Culturing broth, ***T3**=Culturing broth, ***T4**= Butanolic extract.

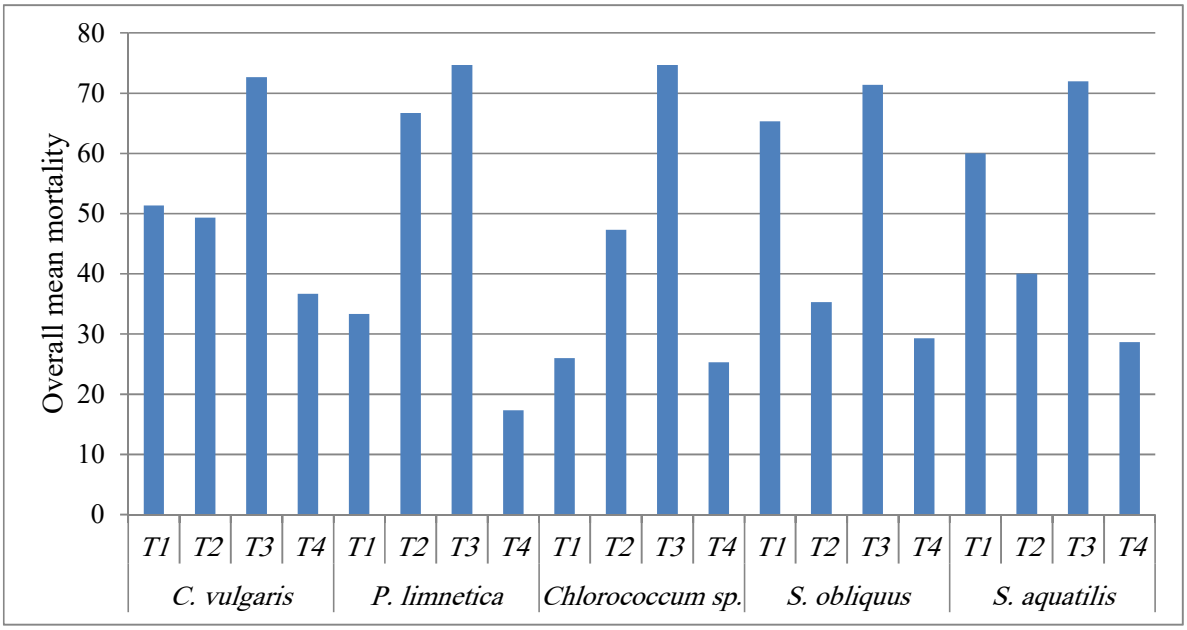

Fig. 2: Overall mean Mortality of *Eutetranychus orientalis* adults at different treatments of algae species under laboratory conditions

T1= Whole cell culture, T2= Separated cells and Culturing broth, T3= Culturing broth, T4= Butanolic extract.

2. Harmful effects of certain microalgae on the citrus brown mite, *Eutetranychus orientalis* under field conditions:

Citrus farms in Egypt are at risk from the common spider mite known as the citrus brown mite, *E. orientalis*. It seriously damages citrus trees as well as ornamental and horticultural plants (Rasmy 1978; Dhooria 1985; Gupta 1985; Sangeetha and Ramani 2011). More than 200 species from 60 families are consumed by this polyphagous organism (EFSA PLH Panel 2013). It is recorded in many countries worldwide. *E. orientalis* was identified as a significant pest in numerous regions of India (Dhooria and Butani 1984). Heavy infestations on citrus plants resulted in defoliation and branches even die back (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 such using algae.

Field experiments were conducted to confirm and validate the results obtained in the laboratory, the five algae used in the study were applied in the field to control the brown citrus mite on citrus trees of the Navel variety specifically at Alshohadaa region in Menoufia governorate. This was done based on the results of the algae treatments that were obtained in the laboratory and that produced positive results for the five algae used in the study.

Therefore, the toxicity of whole cell culture (T1), separated cells and culturing broth (T2), culturing broth (T3) and butanolic extract (T4) of five algal types were applied against *E. orientalis* and the reduction percentages were determined after 12, 24, 48, 72 h, one week and 2 weeks under field conditions.

2.1. Impact of the whole cell culture (T1), against *E. orientalis* on navel orange:

The reduction percentages on navel orange were determined using the complete algal cultures, which included only the algae cells, against *E. orientalis* after 12, 24, 48, 72 hours, one week, and two weeks of application. The results in Tables (4 and 5) and Fig. (3) show that after 72 hours and one week of application, higher rates of reduction were seen for all treatments. As for, *S. obliquus* exhibited the largest decline rate (77.35 and 81.81%) after 72 hours and a week, respectively, followed by *C. vulgaris* and *S. aquatilis* (72.84 & 81.19%) and (72.14 & 76.55%). For other algal cultures, the medium reduction rates were 49.37 and 55.96 % for *P. limnetica* and 53.44 and 60.26 % for *Chlorococcum* sp.

2.2. Impact of separated algal cells and culturing broth (T2), against *E. orientalis* on navel orange:

After applying the whole algal cultures, which included separated cells and culturing broth to *E. orientalis*, the reduction percentages were calculated. Both *S. obliquus* and *S. aquatilis* showed the highest reductions (79.49 & 84.75%) and 79.75 & 83.60%), respectively, followed by *C. vulgaris* (71.69 & 77.77%), while *Chlorococcum* sp. showed the lowest reductions (61.56 & 68.30%).

2.3. Impact of culturing broth only (T3), against *E. orientalis* on navel orange:

The results in Tables (4 and 5) show that all algae treatments showed high toxic effect against *E. orientalis* after 72 hours and a week of application under field conditions. The greatest reduction rates are found in *P. limnetica* and *S. aquatilis* (83.29 & 85.08%) and (83.77 & 85.92%) after 72 hr and a week of application, respectively followed by *Chlorococcum* sp. and *S. obliquus* (82.19 & 85.64%) and (82.33 & 86.73%). The lowest reduction (76.30 & 85.31%) were observed in *C. vulgaris* after 72 hours and a week.

2.4. Impact of Butanolic extract (T4), against *E. orientalis* on navel orange:

Tables (4 and 5) presented the data, which demonstrated that the butanolic extract's hazardous activity had low reduction rates. *S. aquatilis* had the greatest reduction rates (53.73 & 60.01%), followed by *C. vulgaris*, *P. limnetica* and *S. obliquus* (43.19 & 52.93%), (42.10 &53.93%) and (44.62 & 51.93%), respectively. whereas *Chlorococcum* sp. had the lowest reduction (36.97 & 44.48%).

Table 4: Effect of the algae Treatments on Population of *Eutetranychus orientalis* infested Navel orange variety under field conditions.

Algae species	Treatments	pre-treatment	Mean number of mite/10 leaves Post-treatment						Average
			12 hr	24 hr	48 hr	72 hr	1 week	2 weeks	
C. vulgaris	*T1	49.0	47.33	36.33	26.33	15.67	11.67	23.00	26.7
	*T2	45.0	.4267	39.67	26.33	15.00	12.67	31.33	27.9
	*T3	43.0	.3967	30.00	.1567	12.00	08.00	22.67	21.3
	*T4	50.3	50.00	40.33	35.00	33.67	30.00	42.00	38.5
P. limnetica	T1	52.0	51.33	50.00	42.00	31.00	29.00	41.00	40.7
	T2	47.3	45.00	.4367	30.00	19.67	15.67	28.67	30.4
	T3	42.3	40.00	.3133	.1067	08.33	08.00	21.33	19.9
	T4	44.0	44.00	33.00	28.33	30.00	25.67	38.00	33.1
Chlorococcum sp.	T1	51.6	.5033	49.00	40.00	28.33	26.00	33.67	37.8
	T2	45.6	44.33	41.67	31.00	.2067	18.33	29.67	30.9
	T3	47.6	45.00	35.00	18.00	10.00	08.67	20.00	22.7
	T4	42.6	41.67	30.33	22.00	31.67	30.00	44.00	33.0
S. obliquus	T1	55.0	52.33	.4167	25.00	14.67	12.67	24.00	28.3
	T2	48.3	45.00	.3233	.2033	11.67	09.33	21.33	23.3
	T3	41.6	38.00	28.00	10.33	08.67	07.00	16.00	18.0
	T4	53.6	.5267	44.00	35.67	35.00	32.67	45.67	40.9
S. aquatilis	T1	42.6	.4067	.2867	24.67	14.00	12.67	22.33	23.8
	T2	43.3	42.00	30.33	29.67	10.33	09.00	26.00	24.5
	T3	52.3	49.33	35.00	16.33	10.00	09.33	24.00	23.9
	T4	52.0	.5167	38.67	30.00	28.33	26.33	39.67	35.7
Control		56.3	55.33	57.00	61.67	66.33	71.33	74.67	64.3

***T1**=Whole cell culture, ***T2**=Separated cells and Culturing broth, ***T3**=Culturing broth, ***T4**= Butanolic extract.

Table 5: Reduction percentage of *Eutetranychus orientalis* Population by using five different Algae species under field conditions.

Algae species	Treatments	Reduction percentages of *E. orientalis* Population after						Average
		12hr	24 hr	48 hr	72 hr	1 week	2 weeks	
C. vulgaris	T1	1.66	26.73	50.92	72.84	81.19	64.59	49.66
	T2	3.46	12.88	46.56	71.69	77.77	47.48	43.31
	T3	6.08	31.05	66.71	76.30	85.31	60.33	54.30
	T4	1.14	20.81	36.48	43.19	52.93	37.05	31.93
P. limnetica	T1	0.50	4.98	26.22	49.37	55.96	40.52	29.59
	T2	3.20	8.82	42.10	64.71	73.85	54.30	41.16
	T3	3.80	26.86	76.98	83.29	85.08	61.99	56.33
	T4	1.81	25.88	41.19	42.10	53.93	34.85	33.29
Chlorococcum sp.	T1	0.83	6.28	29.29	53.44	60.26	50.84	33.49
	T2	1.18	9.83	37.99	61.56	68.30	50.99	38.31
	T3	3.90	27.44	65.51	82.19	85.64	68.35	55.51
	T4	0.58	29.76	52.91	36.97	44.48	22.21	31.15
S. obliquus	T1	3.13	25.13	58.48	77.35	81.81	67.08	52.16
	T2	5.21	33.89	61.58	79.49	84.75	66.71	55.27
	T3	7.16	33.60	77.36	82.33	86.73	71.03	59.70
	T4	0.09	18.98	39.29	44.62	51.93	35.81	31.79
S. aquatilis	T1	2.96	33.60	47.19	72.14	76.55	60.52	48.83
	T2	1.32	30.83	37.45	79.75	83.60	54.73	47.95
	T3	4.03	33.90	71.50	83.77	85.92	65.40	57.42
	T4	1.16	26.51	47.30	53.73	60.01	42.45	38.53

***T1**=Whole cell culture, ***T2**=Separated cells and Culturing broth, ***T3**=Culturing broth, ***T4**= Butanolic extract.

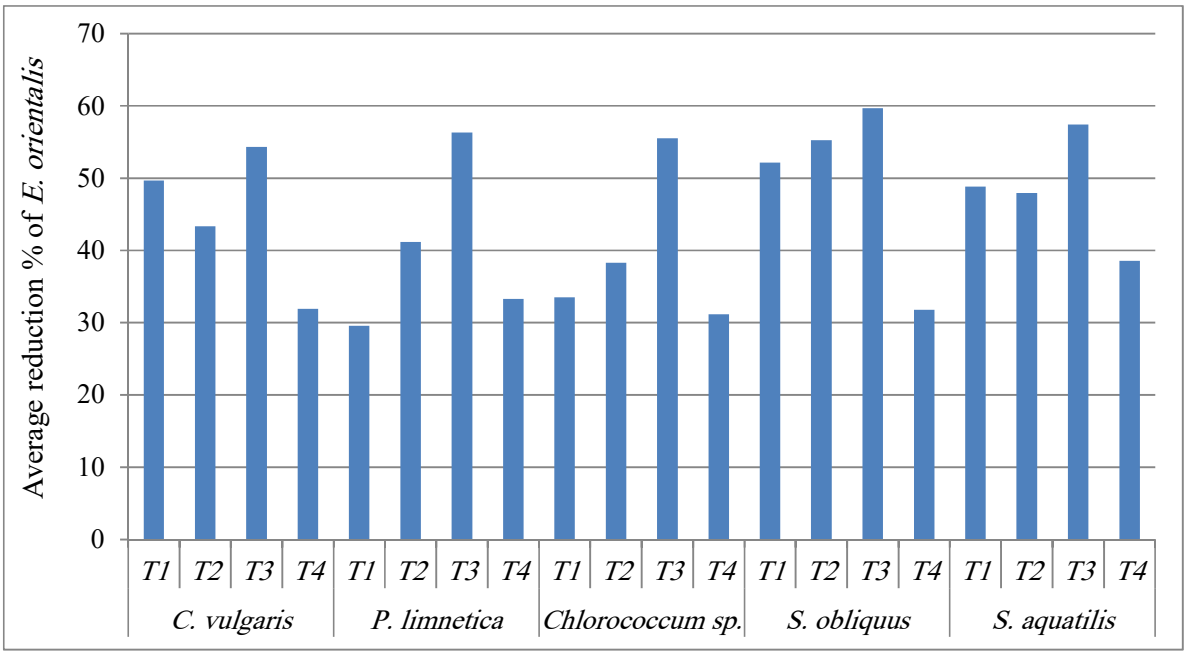

Fig. 3: Average Reduction percentage of *Eutetranychus orientalis* Population by using five different Algae species under field conditions
T1= Whole cell culture, T2= Separated cells and Culturing broth, T3= Culturing broth, T4= Butanolic extract

4. Discussion

Discussing the foregoing results cleared that the toxic activities of algal types vary from species to another and they are also varied even at the treatment level. Variation of the properties and activities of different Algae were recorded in many reports (Vizcaino *et al*., 2005 and Morris *et al*., 2007). Our results showed higher mortality rates reached up to 100% against *E. orientalis* adult. These results are higher compared to that obtained by (Abdel-Aziz and Abdel-Raouf, 2002), when they used the metabolites of *Dunaliella* sp. against *Tetranychusurticae*. Their results showed up to 41.6% mortality percent, while in our case it reached upto100%. Variation in activity toward adult individuals maybe due to different toxicity mechanism against adult individuals. As well as, using different algal species and different pest may be the reason of different results. 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 greenalgae *Chlorella ellipsoidae* produces some substances which affect the development and immature stages of mosquitoes. All these studies are in agreement 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).

The obtained 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. According to Rania *et al*. (2025) the marine alga formulation may be useful as an environmentally friendly bioactive component for the integrated crop management of *T. urticae* stages.

From all the aforementioned results laboratory and field studies, we may conclude that the evidence presented indicates that micro-algae can serve as bio-pesticides instead of traditional synthetic pesticides. They effectively manage pathogens and pests (mite and insect) without affecting the yield of crops. This approach offers an economical way to naturally combat pests. The ultimate decision of the people to avoid the use of chemical or synthetic pesticides has led to the promotion of eco-friendly micro-algal pesticides. These can be utilized in organic farming, which does not rely on chemical

substances. Therefore, micro-algae play a key role in producing compounds that have anti-micropes, Acaricidal, insecticidal, herbicidal, and larvicidal properties. The secondary metabolites generated by micro-algae can serve as excellent bio-pesticides to protect agricultural crops. However, further studies are necessary to fully investigate the pesticidal properties of micro-algae and their metabolites. So, production of bio-pesticides with micro-algae will be able to replace the chemical pesticides and promotes the sustainable agriculture and protective environment.

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