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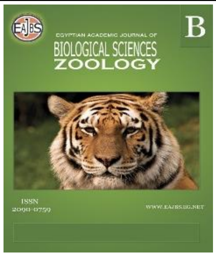

Acaricidal Impacts of Two Algae on *Tetranychus urticae* Koch (Tetranychidae) in Laboratory and Semi-Field Conditions

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ABSTRACT

Tetranychus urticae Koch (Acari: Tetranychidae) is the most common mite in greenhouses and on field-grown crops, causing significant damage to a variety of crops. Traditional agricultural practices, such as the use of synthetic pesticides, cause a variety of difficulties, including environmental contamination, pest resistance, and adverse impacts. As a result, these detrimental consequences have highlighted the need for new efficient, safe, and environmentally friendly natural alternatives to synthetic pesticides. Algal products serve an important role as chemical alternatives in agricultural applications. The current study is to bioassay biocide effects of two commercial algal products (marine algae and veggie) as natural and safe bio-pesticides on *T. urticae* under laboratory and semi-field conditions. The findings showed that mortality increased as concentration and time increased. A marine alga is more efficient than veggie, after 7days of exposure to *T. urticae* adult females, LC_{50} values were 0.429 and1.369 g/l, respectively. Also, the LC_{50} values for marine algae and veggie for treated eggs of *T. urticae* were 1.69 g/l and 2.57 g/l, respectively under laboratory conditions. The reduction percentage of *T. urticae* immature stage after 14 days of application with marine algae and veggie were 89.81 and 88.67%, respectively.

INTRODUCTION

Tetranychus urticae Koch is one of the most important pests that cause losses to many horticulture, ornamental and agronomic crops. The mite is a major pest of many crops, including field crops, fruits, vegetables, ornamentals and others. Conventional synthetic acaricides are usually used repeatedly to manage mites. Their efficacy has been limited, nevertheless, by mite resistance that has developed as a result of their ongoing use. Concerns over chemical residues in food and harmful environmental impacts are also raised by the use of these substances. Hence, there is a need for alternative methods to control mite infestations efficiently Imani Baran *et al.* (2020).

The development of agro-ecological systems using biopesticides is an eco-friendly choice that contributes to the reduction of agrochemical use Costa *et al.* (2019). Numerous studies have documented that pesticidal or repellent properties of algal extracts control many arthropods and other agricultural pests and have an impact on the health of humans and animals Ali *et al.* (2013). Terrestrial plants lack the inherent active compounds found in algae. Numerous research conducted in recent years have documented a variety of active compounds derived from algae that exhibit a wide spectrum of biological activities, such as neuroprotective, antibacterial, antiviral, antioxidant, and anticancer properties. These biological effects are caused by bioactive substances that are present in algae, including fatty acids, polysaccharides, polyphenols, and pigments Singh *et al.* (2018). One of the most promising non-insecticide pest management strategies is the use of marine algae. By functioning as pesticides, algae can lessen crop pests Asimakis *et al.* (2022). According to

Cheung *et al.* (2014), it might function as a biopesticide. Moreover, Asimakis *et al.* (2022) pointed to the increasing human numbers as necessary to produce more food. Algae have products that may serve humans in different biotechnological areas. The potential of algal metabolites had bio-pesticides. Because they are safer for both humans and the environment and frequently have fewer aftereffects than traditional pesticides, biotoxins are an essential class of crop protectants (Copping and Menn ,2000). This study's goal is to assess the toxicity of various algal formulations against *T. urticae*.

MATERIALS AND METHODS

Culture of *Tetranychus urticae*:

The stock colony of *T. urticae* was collected from cucumber plants (*Cucumis sativus* L.) that were infested. They were reared in a plastic tray (23 × 20 × 70 cm). Adult mites were moved to a clean castor bean leaf, lower side up, and set on cotton pads that had been moistened and were sitting on sponges in the foam plate (15 × 20 cm). At room temperature, the colonies were reared in a laboratory environment. Water was added when needed. Mites were housed in a laboratory at 25 °C with appropriate moisture levels at 70% RH. The same environmental conditions were used for the cultures used for all bioassays Mahmoud (2016).

Source of Algae:

Two commercial algae formulations were investigated under laboratory conditions and semi- field conditions to select the most effective samples on the biotic potential of *T. urticae*. The commercial algae that were used marine algae and veggie Agro culture. Vert Company, Egypt.

Experimental Design:

In an experimental foam plate (15 x 20 cm), a castor bean leaf disc (3.0 cm in diameter) was turned over and set on wet cotton pads resting on sponge. Water was added as needed to maintain the culture's health and prevent the mites from fleeing. There were 40 experimental foam dishes overall, with 10 copies for each of the three treatments and a control.

Laboratory Experiment:

Treatment Adult Females of *T. urticae*:

Twenty adult female individuals of *T. urticae* were positioned on the underside of a freshly disc of castor beans *Ricinus communis* L. leaves. Three concentrations (1, 2 and 3 g/l) in each treatment and 10 replicates of each concentration were given a prior treatment with a fine camel hairbrush. The leaf discs were subjected to one of the previous procedures. In every test, a control consisting of two drops of Triton X-100 and pure water was employed. Mortality was calculated using a binocular microscope at 3-, 5-, and 7-days post treatments and corrected using Abbott's method (1925), and Finney's (1971) estimates of the LC_{50}, LC_{90}, and slope values were used. The castor bean discs were maintained at room temperature (25±2°C, 70±5% RH). When a mite was probed with a fine camel brush and its body or appendages did not move, it was deemed dead (Elhalawany and Dewidar, 2017; Elhalawany *et al.*, 2019).

Treatment Immature Stages of *T. urticae*:

To assess the efficacy of marine algae and Veggie on immature stages of *T. urticae* they were treated with three concentrations of algae products (1, 2, and 3 g/l). Each set was replicated 10 times. Seven days following treatment, the LC_{50} values for immature stages were assessed. Mortality was calculated using a binocular microscope at 3-, 5-, and 7-days post-treatments and corrected using Abbott's method (1925), and Finney's (1971) estimates of the LC_{50}, LC_{90}, and slope values were used.

Treatment Eggs of *T. urticae*:

Castor beans leaf discs served as the ovipositor's substrate. Ten mite females were placed on each of the six leaf discs used for each treatment, and after laying eggs for 24 hours, the females were taken out. After that, six replicates of leaf discs (20 eggs/replicate) were used per concentration (1, 2, and 3 g/l). Eggs were sprayed by a glass atomizer in each concentration for each alga and other in distilled water (control). Eggs were maintained at room temperature under laboratory conditions for seven days till hatching. The quantity of eggs that hatched and those that did not was noted. Corrected mortality counts according to Abbott’s formula (1925) and LC_{50}, LC_{90} and slope values were estimated According to

Finney (1971).

Semi Field Experiment:

Cucumber plants were grown in pots under open field at Qaha Station, Plant Protection Research Institute, Qalubia governorate. Each pot contains three plants, after two weeks of planting; the plants were infested with 40 adults of *T. urticae* per each pot. After two weeks the concentrations of the two algae suspensions were sprayed, and control (untreated) was sprayed with distilled water. Twenty leaves were chosen randomly from each treatment, and the number of immature mite stages was counted before spraying and after three, five, seven, and fourteen days of application using the aid of a stereomicroscope. The reduction in the percentage of the spider mite was estimated using Henderson and Tilton (1955) equation.

Statistical Analysis:

Probit analysis (Finney, 1971) was applied to the data from each dose-response bioassay in order to determine the LC_{50} and LC_{90} values using Ldp line software. The reduction percentage of *T. urticae* moving stages was analyzed by one-way ANOVA, and means were compared by using the LSD test at $\alpha = 0.05$ in the SAS Program version 9.1.3 (SAS Institute, 2003).

RESULTS

Toxicity Effect of Two Algae Products for Adult Females of *T. urticae*:

Results in Figure (1), proved that, the LC_{50} values of marin algae and veggie suspensions that control the adult females of *T. urticae* after 7 days of treatment were 0.429 and 1.369 g/l, respectively. And the corresponding LC_{90} values were 17.303 and 21.092 g/l respectively.

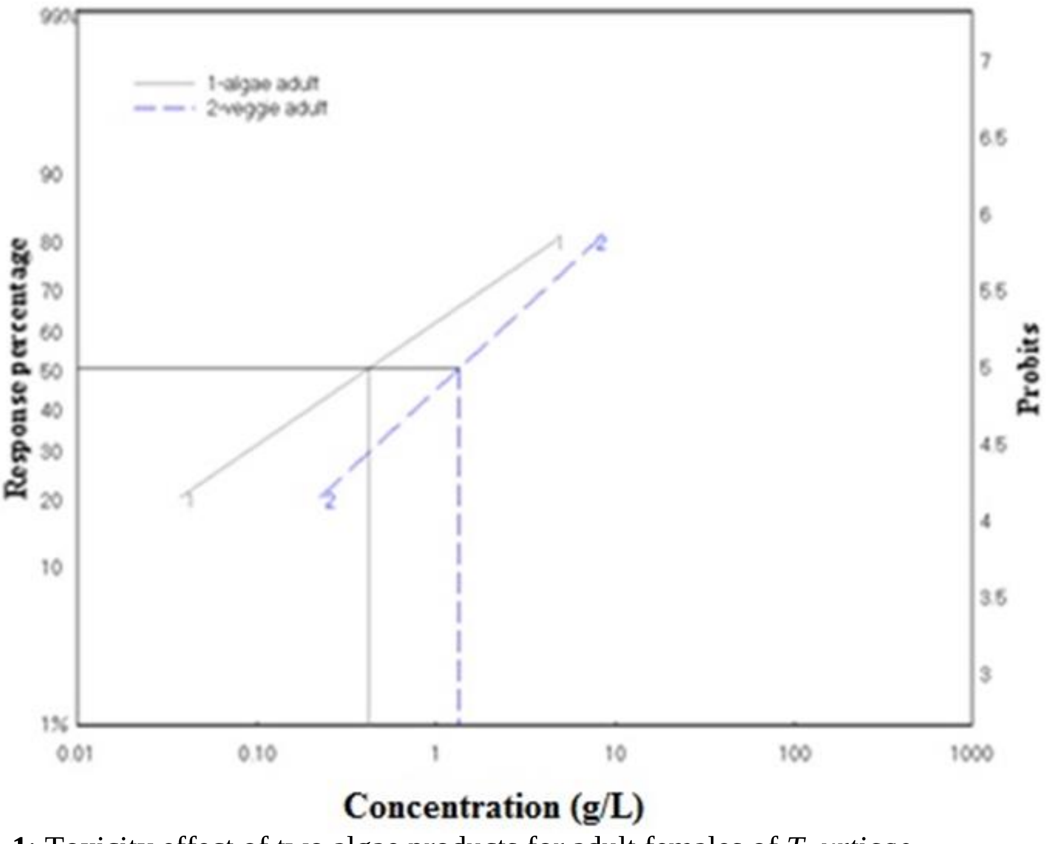

Fig. 1: Toxicity effect of two algae products for adult females of *T. urticae*.

Toxicity Effect of Two Algae Products for Immature Stage of *T. urticae*:

Results in Figure (2), indicated that, the LC_{50} values of Marin algae and Veggie suspensions against the immature stage of *T. urticae* after7 days of treatment were 0.491 and 0.509 g/l, respectively.

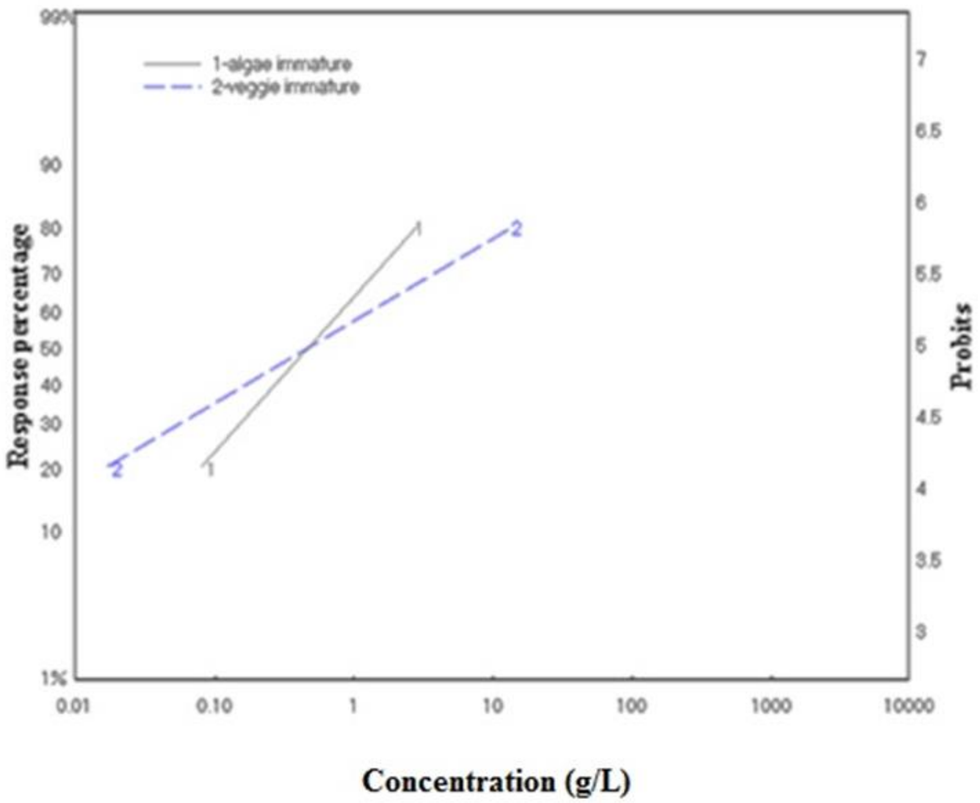

Fig. 2: Toxicity effect of two algae products for immature stage of *T. urticae*.

Toxicity Effect of Two Algae Products on Eggs of *T. urticae* After 7days:

The effects of two algae at various concentrations (1, 2, and 3 g/l) of aqueous suspensions were tested to evaluate their toxic impacts after 7 days against eggs of *T. urticae* and the obtained results were shown in (Fig. 3). The two algae products tested had toxic impacts against the eggs of *T. urticae*. Marine algae was the most potent algae tested eggs (LC_{50}= 1.69 g/l) and Veggie (LC_{50}= 2.57 g/l) of *T. urticae*, respectively.

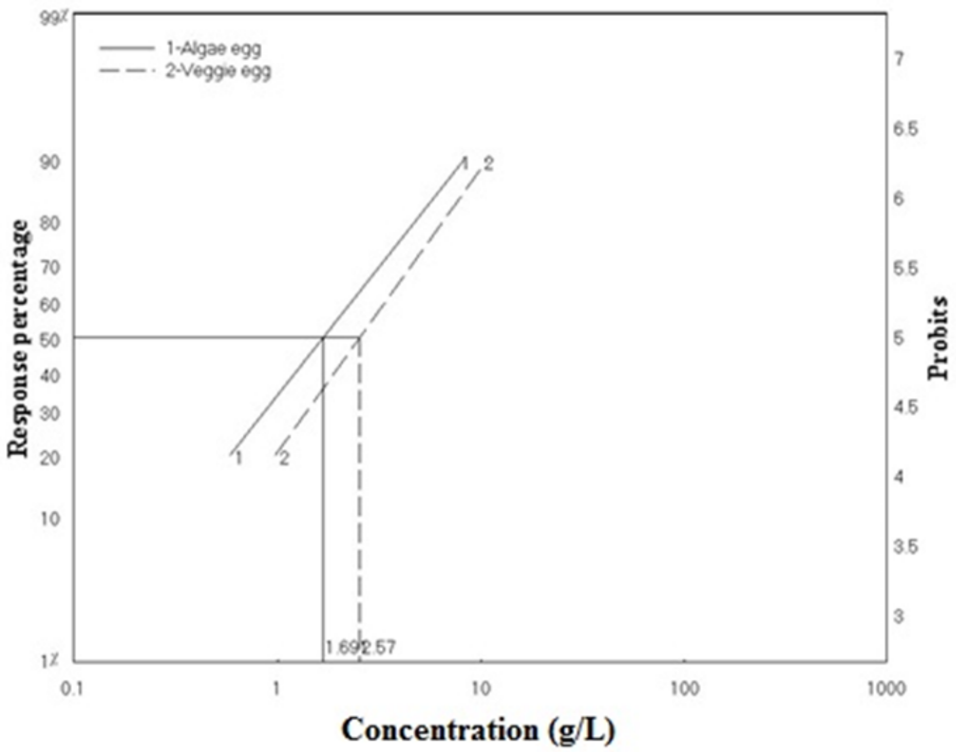

Fig. 3: Toxicity effect of two algae products on eggs of *T. urticae* after 7days in laboratory.

Semi-Field Experiment:

The reduction percentages of *T. urticae* adults were assessed after the two algae were sprayed with three different concentrations on cucumber plants under semi-field conditions, and the control was sprayed with distilled water. The data in Table (1), demonstrated that there was a significant difference between the two algae suspensions in number of adults after application. Results indicated that the reduction percentage of *T. urticae* adults on cucumber leaves after spray increased gradually.

Table 1. Reduction percentages (mean ± SD) of *Tetranychus urticae* adults caused by three concentrations of marine algae and veggie on cucumber under semi-field conditions.

Treatments	levels	Reduction % ± SD of *T. urticae* after			
		3 days	5 days	7 days	14 days
Alage	1mg/l	31.81±4.45 c	43.55±1.94 de	57.88±0.11 c	67.78±1.35 c
	2mg/l	43.28±2.45 ab	54.25±2.24 b	62.90±0.93 b	71.33±0.94 b
	3mg/l	47.78±3.85 a	58.01±2.92 a	66.69±2.55 a	74.15±2.46 a
Veggie	1mg/l	28.68±2.34 c	39.40±3.53 e	50.19±1.74 d	61.70±0.10 d
	2mg/l	39.17±1.57 b	45.33±0.74 cd	56.39±1.41 c	67.12±2.04 c
	3mg/l	43.40±3.44 ab	47.57±1.92 c	60.88±0.22 b	68.86±1.42 bc
F-value		16.26	31.02	47.36	21.34
P-value		<.0001	<.0001	<.0001	<.0001
LSD 0.05		5.64	3.56	2.55	2.80

mean ± SD have the same letters within the same column not significantly different at $p < 0.05$ according to LSD test.

The data given in Table (2), showed that the immature stages of *T. urticae* were affected by the tested two algae suspensions. This study used an alternative pesticide using three concentrations for each alga. The findings showed that either concentration or exposure duration increased the reduction percentage of immature stages.

Table 2. Reduction percentages (mean ± SD) of *Tetranychus urticae* immature stages caused by three concentrations of marine algae and veggie on cucumber under semi-field conditions.

Treatments	levels	Reduction % ± SD of *T. urticae* after			
		3 days	5 days	7 days	14 days
Alage	1mg/l	45.33±0.90 bc	59.29±0.68 d	69.92±0.55 c	81.54±1.09 c
	2mg/l	56.93±5.26 ab	67.56±2.13 b	78.53±0.30 b	85.49±0.53 b
	3mg/l	61.92±3.38 a	72.84±0.84 a	85.11±1.28 a	89.81±0.19 a
Veggie	1mg/l	39.89±3.40 d	53.19±2.03 e	66.10±2.40 d	79.28±0.51 d
	2mg/l	50.93±4.26 bc	60.20±2.61 d	77.29±0.95 b	85.46±2.11 b
	3mg/l	58.33±1.34 a	64.29±1.15 c	83.17±1.31 a	88.67±1.10 a
F-value		17.88	47.49	95.31	39.70
P-value		<.0001	<.0001	<.0001	<.0001
LSD 0.05		6.13	3.07	2.33	1.97

mean ± SD have the same letters within the same column not significantly different at $p < 0.05$ according to LSD test.

DISCUSSION

This study investigated that marine alga was more effective on *T. urticae* stages than veggie. Results showed that mites' mortality was increased with increasing concentrations and periods of time after application. Ali and Sallam (2023) indicated that algae are an important part of agriculture systems and that they have been used as biocontrol agents in integrated crop management, especially under modern climatic changes. Irfana and Purushothaman (2020) explained that there are a lot of micro-algae species able to produce metabolites with pesticidal activities. Therefore, microalgae may be utilized to produce biopesticides, which would take the place of chemical pesticides. These biological effects are caused by the bioactive substances that are present in algae, including fatty acids, polysaccharides, polyphenols, and pigments. Singh *et al.* (2018) mentioned that lectins are widely spread and can be found in plants, animals, and microorganisms. Singh *et al.* (2015) demonstrated that red algae, are known to be a potent source of lectins with unique properties. The lectin produced by *Gracilaria ornate*, It has been discovered that one kind of red algae possesses acaricidal properties. Veronico and Melillo (2021) indicated that the extracts and compounds from micro- and macro-algae are also effective in controlling plant-parasitic nematodes that causes annual loss of 10–25% of worldwide yield. Pasdaran *et al.* (2016) demonstrated that all three volatile oils of three algae showed 55-90% mortality of *Oryzeaphilus mercator* (Fauvel) and 60-80% mortality of *Tribolium castaneum* (Herbst 1797) at a dose of 12 μl/l air after 48h of exposure. Leite *et al.* (2005) found that exposure of this Lectin to female cattle ticks (*Boophilus microplus*) Canestrini significantly reduced tick weight after the Oviposition period, egg mass weight, hatching period, and mean larvae survival time. Hankins and Hockey (1990) indicated that the counts made on August 9 before the treatments were applied, showed on average 68% more mites on the rows that were treated with the seaweed extract than on the control rows. Four days later there were only 31% more mites on the seaweed treated plants (after two sprays) and by ten days (three sprays) there were 37 % fewer mites. The counts on August 24 and August 29 showed that the number of mites was reduced; the percentages of reductions were 62% and 54%, respectively.

Conclusions

Nowadays there is a growing demand for the application of plant and natural products as acaricides due to very low health risks, biodiversity conservation, an increasing shift in consumer demand for safer food, and finally, the current trend towards increasing organic farming under modern climatic changes. In conclusion, this work revealed that the Marine alga formulation could be considered as having potential effects as an eco-friendly bioactive component for the integrated crop management of *T. urticae* stages.

Declarations:

Ethical Approval: Not applicable

Competing interests: The authors declare that there is no conflict of interest.

Author's Contributions: RHM conducted the experiments, analyzed the results, prepared the figures, and contributed to the manuscript drafting. ZMM was responsible for the preparation and characterization of mite stages. MHI had revised the manuscript and designed the study. All authors reviewed and approved the final manuscript and have provided their consent for publication.

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Availability of Data and Materials: The data utilized and analysed during this study are available upon reasonable request from the corresponding author.

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ARABIC SUMMARY

التأثيرات القاتلة لنوعين من الطحالب على العنكبوت الأحمر العادي في ظروف المعمل وشبه الحقل

رانيا حسن محمود*، زينب محجوب مصطفى، منال حسيني إبراهيم
قسم بحوث القطن والمحاصيل الحقلية، معهد بحوث وقاية النبات، مركز البحوث الزراعية، الدقي، الجيزة، مصر.

يعتبر أكاروس العنكبوت ذو البقعتين أكثر الأكار وسات النباتية في البيوت المحمية والمحاصيل المزروعة في الحقول، مما يتسبب في أضرار جسيمة لمجموعة متنوعة من المحاصيل. تتسبب الممارسات الزراعية التقليدية، مثل استخدام المبيدات الحشرية المصنعة، في مجموعة متنوعة من الصعوبات، بما في ذلك التلوث البيئي ومقاومة الآفات والآثار الضارة. ونتيجة لذلك، أبرزت هذه العواقب الضارة الحاجة إلى بدائل طبيعية جديدة فعالة وآمنة وصديقة للبيئة للمبيدات الحشرية المصنعة. تلعب منتجات الطحالب دورًا مهمًا كبدائل كيميائية في التطبيقات الزراعية. أجريت الدراسة الحالية لاختبار التأثيرات الحيوية لمنتجين تجاريين من الطحالب (الطحالب البحرية والخضراء) كمبيدين حيويين طبيعيين وآمنين على العنكبوت ذو البقعتين في ظل ظروف المختبر وشبه الحقل. وأظهرت النتائج أن معدل الوفيات ارتفع مع زيادة التركيز والوقت، وكانت الطحالب البحرية أكثر كفاءة من الخضراء، فبعد 7 أيام من التعرض للإناث البالغة من العنكبوت ذو البقعتين، كانت قيم الجرعة النصف قاتلة 0.429 و1.369 جم / لتر، على التوالي. كما كانت قيم الجرعة النصف قاتلة للطحالب البحرية والطحالب الخضراء لبيض العنكبوت ذو البقعتين المعالج 1.69 جم / لتر و2.57 جم / لتر، على التوالي في ظل ظروف المختبر. وكانت نسبة الانخفاض في مرحلة العنكبوت ذو البقعتين غير الكاملة بعد 14 يومًا من التطبيق بالطحالب البحرية والخضراء 89.81 و88.67٪، على التوالي.

الكلمات المفتاحية: الطحالب، التأثير الحيوي، الطبيعي، المبيدات الحشرية، أكاروس العنكبوت ذو البقعتين.