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EFFECT OF THE INSECTICIDE ABAMECTIN ON THE METABOLIC ACTIVITY OF *CHLORELLA VULGARIS* BEYERINCK

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Abstract

This investigation studied the effects of Abamectin on the growth and some physiological activities of *Chlorella vulgaris*. The results showed that growth parameters decreased with increase in Abamectin concentrations. All the applied treatments of Abamectin strongly reduced the total sugars contents, total protein and total amino acids of *Chlorella vulgaris*. The drop was more prominent and highly significant at higher dose. The data also showed that Abamectin treatment suppressed the activity of acid phosphatase, alkaline phosphatase, GOT and GPT of *Chlorella vulgaris*.

Introduction

Today environmental problems are multiple and complex, especially those arising from the disposal of identification and the assessment of the toxicity of such substances like pesticides. Pesticides differ from most industrial organic chemicals in that they are brought into the environment with the explicit intention of exerting toxic effects on one or more target organisms. Unfortunately, their toxicity is usually not limited to the location where they are applied. They reach other locations and environmental compartments through various physical transport processes, adversely affecting organisms that happen to be present (Deneer, 2000; Jianyi *et al*., 2002; Kamel *et al*., 2007).

Algae composing the primary producer level are of initial importance in providing the energy that sustains invertebrates and fish in most aquatic ecosystems. The action of toxic substances on algae is therefore not only important for the organisms themselves, but also for other links in the food chain. Algal toxicity tests are increasingly being used in bioassay test batteries for environmental management of chemical discharges and it has been observed in several studies that for a large variety of chemical substances. Algal tests are relatively sensitive bioassay tools (Seguin *et al*., 2001; Ma and Liang, 2001; Ma *et al*., 2002; Mostafa and Helling, 2002; Friesen Pankratz *et al*., 2003; Fathi, 2003).

Recently, a number of acaricides were registered at the Ministry of Agriculture in Saudi Arabia to control the mite Oligonychus afrasiaticus, which

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infest dates and causes severe damage. Among these acaricides, abamectin, flufenoxuron and amitraz are widely applied on dates to control the mite infestation. Due to the large amount of dates consumed by Saudi residents (an average of 10 dates daily per person), the search for safe pesticides with negligible residual deposits has always been preferred (Kamel *et al*., 2007).

The avermectins are a family of macrocyclic lactones, produced by the soil organism *Streptomyces avermitilis*, which were discovered in the mid-1970's as a direct result of a screening effort for natural products with anthelmintic properties. Insecticide Abamectin belongs to the family avermectins. It contains at least 80% avermectin B1a and not more than 20% avermectin B1b. Abamectin acts by stimulating the release of c-aminobutyric acid thus causing paralysis (Turner and Shaeffer, 1989). It is used to control motile stages of mites and some other insects on fruits and vegetables and has limited plant systemic activity. Abamectin is highly unstable to light and has been shown to photodegrade rapidly on plant and soil surfaces and in water following agricultural applications. Abamectin was also found to be degraded readily by soil microorganisms (Lasota and Dybas, 1990).

Abamectin, widely used as a veterinary anthelmintic, medicine against a variety of animal parasites and insects, can runoff from the sites of application and becomes an aquatic pollutant (Tatjana and Nevenka, 2006). The aim of this study was to identify the toxicity of abamectin on the growth and some physiological activities of the green alga *Chlorella vulgari*s.

Material and methods

Organism and culture condition

Chlorella vulgaris Beyerinck was isolated from Al-Asfar Lake, Al-Hassa, Saudi Arabia. Isolation and purification was made by dilution and plating technique. The alga was grown in 250-mL flasks containing 100 mL Kuhl's medium (1962), and incubated in an illuminated incubator (Precision, USA) at 22°C, and irradiance at 150µmol $m^{-2}s^{-1}$, provided by cool white fluorescent lamps set on 14:10 h photoperiod. All cultures were shaken twice daily to prevent cells from clumping. Sterile technique was used at all times.

Treatments

Abamectin (1.8% EC) Arab Industrial Company, Dammam, Saudi Arabia was used in this study. The main structural characteristics and structural formula of Abamectin were presented in (Table 1 and Figure 1). A standard stock solution of Abamectin was prepared by dissolving Abamectin in acetone. Appropriate volumes of the standard stock solution were added to culture flasks (250-mL). Medium was then added and flasks were left for one hour to obtain aqueous solutions of 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mM. Preliminary experiments were carried out to determine the suitable range of pesticide concentrations. All cultures (three per treatment) received identical inocula and were incubated under

the prescribed growth conditions. At the end of the incubation period the cultures were harvested and washed several times by distilled water to measure the various parameters. The calculated values are the mean of triplicates and the standard deviation was less than 50% of these mean values.

Table (1): The main structural characteristics of abamectin.

Common name	abamectin (BSI, draft E-ISO, ANSI); abamectine ((*f*) draft F-ISO
Chemical Name	avermectin B1
Appearance	Abamectin is a colorless to yellowish crystalline powder
Molecular Weight	873.11
Water Solubility	Insoluble
Solubility in Other Solvents	v.s. in acetone, methanol, toluene, chloroform, and ethanol.
Melting Point	150-155 ^{o}C
Vapor Pressure	Negligible
Composition	A mixture containing ³80% avermectin B_{1a} (i) and £20% avermectin B_{1b} (ii).
Chemical Class	Insecticide/miticide
Acute toxicity	Abamectin is highly toxic to insects and may be highly toxic to mammals as well
Effects on birds	Abamectin is practically nontoxic to birds
Effects on aquatic organisms	Abamectin is highly toxic to fish and extremely toxic to aquatic invertebrates

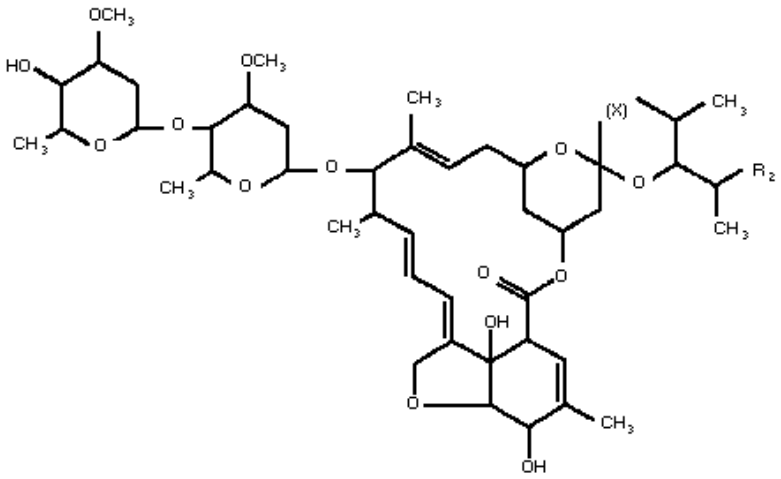

Figure (1): Structural formula of Abamectin

Growth

Growth was measured in terms of cell number using a Haematocytometer, which was used for calculation of growth rate (Nichols 1973). Chlorophyll a was estimated according to Metzner *et al*. (1965).

Photosynthetic oxygen evolution

Photosynthetic oxygen evolution was measured at 27 ^{o}C by bubble counting method Using Cobra3-Basic-Unit (Phywe, GmbH and Co. KG). Prior to the experiment the algae were resuspended in the free incubation L Kuhl's medium and the oxygen bubble counting was measured after the addition of Abamectin concentrations.

Biochemical analysis

The anthrone method (Roe, 1955) was applied for total carbohydrate estimation using fresh material and glucose as a standard. Total amino acid content was determined according to Moore and Stein (1948). Total protein was measured according to Lowry *et al*. (1951).

Algal extraction for enzyme assay

Fresh algal samples were instantly ground immediately after the experimental period with a known volume of distilled water and little of pure acid washed sand. Samples were then centrifuge at 10,000rpm for 15 minutes, made up to a known volume and frozen. The methods of Bergmeyer (1974) were adopted for the estimation of both glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT). Both acid and alkaline phosphatases were estimated in the same extract by estimating the liberated phosphorous colorimetrically at 700 nm by the sulphite metol method (Burton and Riley, 1954).

Statistics

Results were tested by one-way analysis of variance (ANOVA). ANOVA effects and treatments differences were considered significant when $P< 0.05$.

Results and Discussion

Pesticides play an important role in agricultural practices. Increase in the use of pesticides has elicited extensive research into pesticide effects on non-target organisms such as algae. Therefore, their potential effects on the aquatic primary producers are particularly important, and have to be studied in ecotoxicological experiments (Berard, 1996; Ma and Chen, 2005).

Results illustrated in Figure (2) clearly revealed that the growth rate (μ) of *Chlorella vulgaris* irrespective of some minor fluctuations, decreased with increased Abamectin concentrations in the culture medium; the drop was more prominent and highly significant at the higher than lower doses. A similar inhibitory effect on cell number by different pesticides were reported, with

Chlamydomonas (Cain and Cain, 1984); Sce*nedesmus obliqnus* and *Chlorella pyrenoidosa* (Ma *et al*., 2002); *Chlorella kesslerei* and *Anabaena inaequalis* (Mostafa and Helling, 2002); *Scenedesmus bijuga* (Fathi, 2003); *Tolypothrix scytonemoides* (Rajendran *et al*., 2007). Figure (2) further shows that the Chl.a content of Chlorella *vulgaris* decreased continuously with increasing Abamectin concentration. This reduction was significant at the highest concentration (1.0 mM). These findings are in agreement with several previously published data (Hammouda, 1994; Seguin et al., 2001; Mostafa and Helling, 2002; Fathi, 2003; Rajendran *et al*., 2007). Reduction of chlorophyll and phycobiliprotein contents in cyanobacteria by insecticide treatment has been reported earlier (Marco and Orus, 1993). Growth and photosynthetic pigments, i.e., chlorophyll-*a*, carotenoids and phycocyanin were adversely affected by pesticides treatment and the inhibition was found to be dose dependent (Prasad, *et al*., 2005; Rajendran *et al*., 2007).

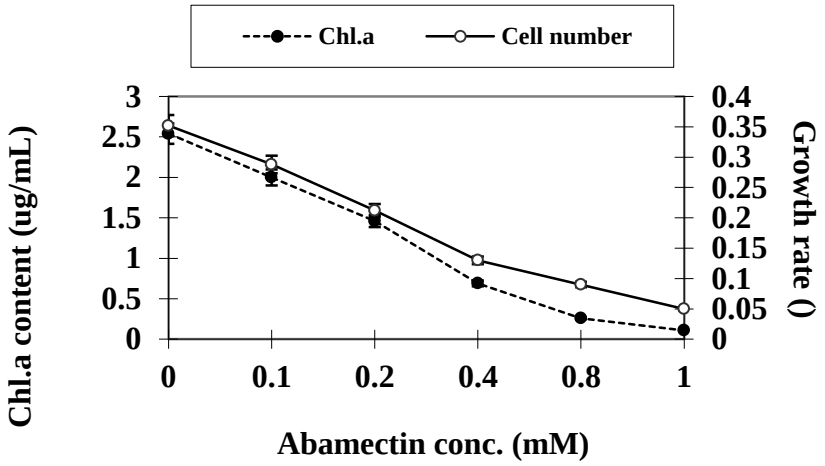

Figure (2): Effect of Abamectin on Chlorophyll *a* and Growth rate (μ) of *Chlorella vulgaris* Beyerinck at various concentration after 7 days growth period. Vertical bars indicate SE, n=3.

The interference of Abamectin with growth and Chl. *a* was further clarified by testing the effect of the insecticide on photosynthetic oxygen evolution, which indicate a remarkable reduction in the value by increasing the concentration of the insecticide (Fig. 3). The highest inhibitory effect of Abamectin on photosynthetic oxygen evolution (about 95% inhibition) was detected at the concentration of 1.00 mM. Pesticide induced reduction in photosynthetic oxygen evolution might be due to inhibition of PS II activity as a result of damage to the thylakoid lamellar membrane or by disruption of water

splitting machinery of the photosynthetic apparatus (Singh and. Tiwari, 1988). Marco *et al*. (1990) reported that the photosynthetic activity in *Anabaena* PCC 7119 was unaffected by trichlorfon treatment, however a slight effect in *Gloeocapsa* sp. was detected after 24h of exposure.

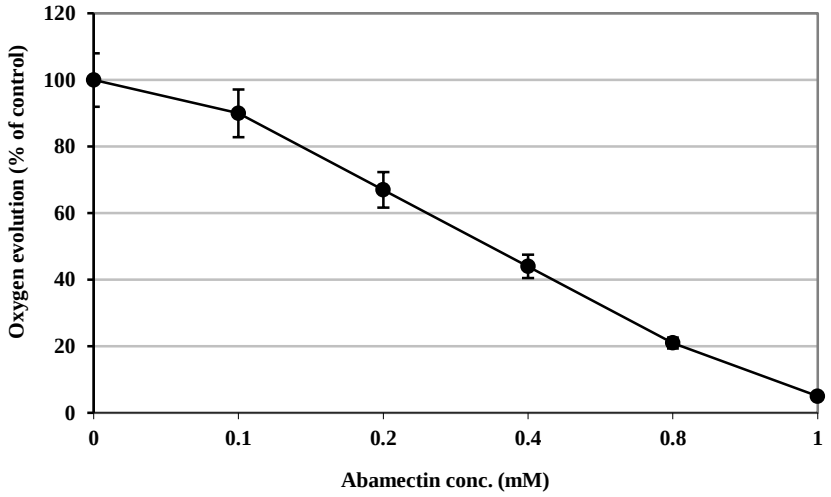

Figure (3): Effect of different concentrations of Abamectin on photosynthetic oxygen evolution *of Chlorella vulgaris* Beyerinck. Vertical bars indicate SE, n=3

Data presented in Table (2) show that the total sugars contents of *Chlorella vulgaris* increased following treatment with the lower concentrations of Abamectin. However the amounts of these fractions appeared to be significantly decreased at the highest concentration of the tested insecticide. The inhibitory effect of relatively high concentrations of Abamectin on total sugars production in the present study might be due to retardation in the rate of CO_2 photoassimilation (Mansour *et al*., 1993). Similarly, Mansour *et al*. (1993) reported that relatively high concentration (3.5 ppm) of butachlor significantly decreased the levels of carbohydrate fraction of Nostoc kihlmani. However, Eladel *et al*. (1999) showed that no consistent dose-dependent of thiobencarb changes occurred in total carbohydrate of *Protosiphon botryoides*.

Results in Table (2) showed that applying Abamectin to *Chlorella* suppressed the total protein content in comparison to control values. This effect is more pronounced at the higher doses. Eladel *et al*. (1999) reported that at 3 mgL^{-1} of thiobencarb, the protein content of *Protosiphon botryoides* decreased. Although the modes (s) of action of pesticides are not well understood, they seem to inhibit fatty acids and protein synthesis (Tomlin, 1994; Eladel *et al*.,

1999), or due to the denaturation of proteins induced by them (Bueno *et al*., 2004, Rajendran *et al*., 2007).

Table (2): Effect of Abamectin on some physiological characteristics of *Chlorella vulgaris* Beyerinck at various concentrations after 7 days growth period. Results of one-way ANOVA comparison of treatments to controls indicate *P< 0.05; **P < 0.01; ***P <0.001.

Conc. (mM)	**Total protein [% dry. mass]**	Total sugars [%dry. mass]	**Total amino acids [%dry. mass]**
0.0	37.61 ± 0.10	72.22 ± 0.30	28.26 ± 0.15
0.1	33.52 ± 0.15	44.32 ± 0.25	26.54 ± 0.10
0.2	28.65 ± 0.10	36.62 ± 0.10	18.00 ± 0.00
0.4	20.44 ± 0.20	30.33* ± 0.20	24.44 ± 0.00
0.8	12.81** ± 0.11	20.62*** ± 0.20	16.21** ± 0.00
1.0	12.00** ± 0.10	18.64** ± 0.20	7.66** ± 0.00
One-Way ANOVA	***	**	**

All the values are mean of three replicates [n = 3] ± SE

The results show successive increase in total amino acids content as Abamectin concentration increased between 0.1and 0.4 mM, and the maximum value appeared at 0.2 mM (Table 2). Concentrations higher than 0.4 mM of Abamectin were inhibitory. Higher concentrations of pesticides were inhibitory to total amino acids of *Nostoc muscorum*. In addition, the amino acids produced in algal cells differed quantitatively and qualitatively according to the type of alga and conditions of cultivation (El-Ayouty and Ezzat, 1991; Fathi, 2003). Soliman *et al*. (1994) reported that the synthesis of some major amino acids depends on the provision of carbon skeleton from TCA cycle, which can be indirectly affected by the herbicide.

Data presented in Figure (4) show that Abamectin treatment suppressed the activity of acid and alkaline phosphatase of *Chlorella vulgaris*. Moreover, the highest doses of Abamectin were more suppressive to the activity of both enzymes. Regarding too GOT and GPT enzymes, the lower doses of Abamectin (0.1 and 0.2 mM) decreased the activity of these enzymes. Above 0.8 mM of Abamectin the enzyme activity was significantly inhibited (Fig. 5). Mansour *et al*., (1993) reported that GOT and GPT activities of some Cyanophyta were markedly enhanced by different concentrations of butachlor, oxadiazon or thiobencarb. Soliman *et al*. (1994) revealed that malate dehydrogenase of *Nostoc kihlmani* and *Anabaena oscillarioides* increased significantly in response to treatment with low and moderate doses of pesticides, while inhibited with higher doses. Similarly, glutamine synthetase activity was suppressed by Propanil in *N. muscorum* (Singh and Tiwari, 1988; Rajendran *et al*., 2007).

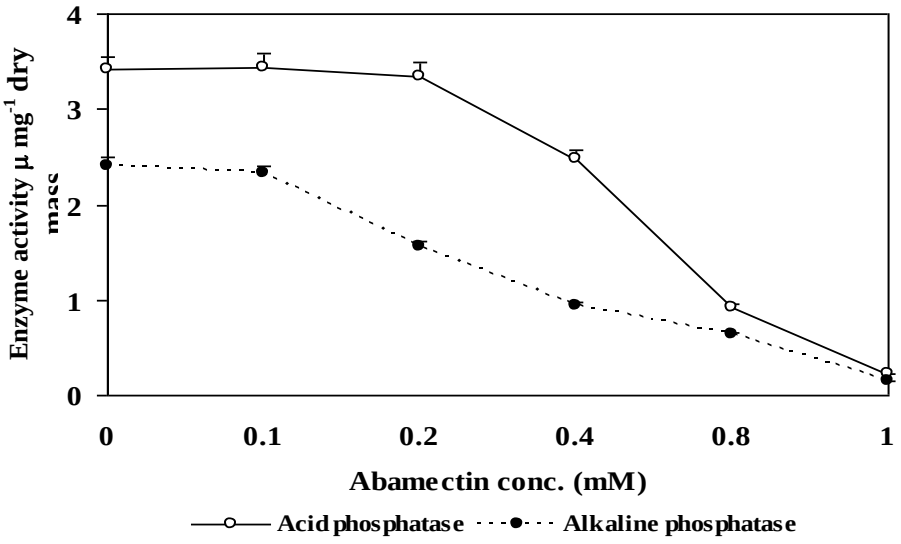

Figure (4): Effect of different concentrations of Abamectin on acid and alkaline phosphatase activity in *Chlorella vulgaris* Beyerinck at various concentrations after 7 days growth period. Vertical bars indicate SE, n=3

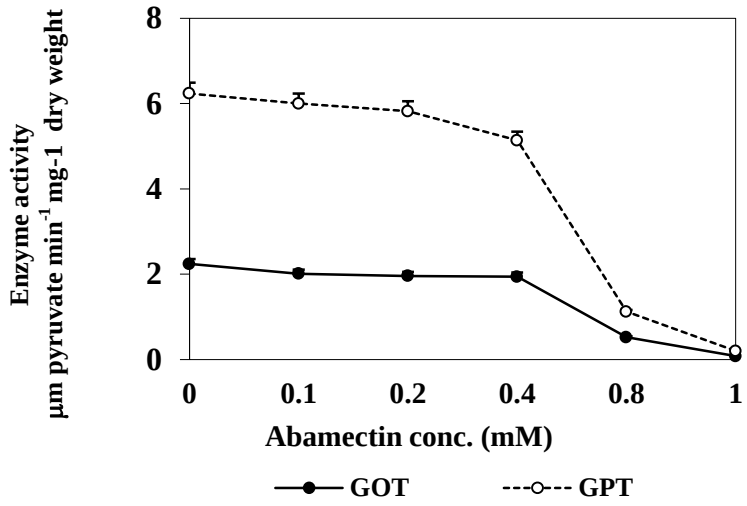

Figure (5): Effect of different concentrations of Abamectin on GOT and GPT activity in *Chlorella vulgaris* Beyerinck at various concentrations after 7 days growth period. Vertical bars indicate SE, n=3

On the other hand, glutamine synthetase activity was enhanced in *N. calcicola* in the presence of Bavistin, Phosphomidon, and Rogar (Anand and Subramanian, 1997).

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تأثير المبيد الحشري اباميكتين على النشاط الايضي لطحلب الكلوريلا فولجارس

عادل أحمد فتحي ومحمد عبد الوهاب الفريدان

قسم الأحياء – كلية العلوم – جامعة الملك فيصل
المملكة العربية السعودية

من أهم مصادر تلوث الأوساط المائية في السنين الأخيرة هي المبيدات بجميع أنواعها سواء كانت حشرية أو عشبية أو فطرية. ومن هذا المنطلق فأن هذا البحث يهدف إلى دراسة تأثير أحد المبيدات الحشرية الشائعة الاستخدام في المملكة العربية السعودية والذي يمكن أن يتسلل إلى البيئات المائية عن طريق الصرف الزراعي وغسيل التربة وهو مبيد أباميكتين. تم في هذا البحث دراسة تأثير المبيد الحشري اباميكتين على النمو وبعض الانشطه الفسيولوجية لطحلب الكلوريلا فولجارس. وقد أظهرت النتائج انخفاض معدلات النمو مع زيادة تركيز المبيد. كما أظهرت النتائج التأثير المثبط للأبامكتين على السكريات والبروتينات والأحماض الأمينية لطحلب الكلوريلا ، وقد كان الانخفاض أكثر بروزا ووضوحا عند التركيزات العالية. كما أظهرت النتائج أيضا التأثير المثبط للمبيد الحشري اباميكتين على بعض النشاطات الأنزيمية لطحلب الكلوريلا.