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EFFECTS OF SOME ORGANOPHOSPHOROUS INSECTICIDES ON FRESH WATER ALGAE UNDER LAB CONDITIONS

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ABSTRACT

The current search is a continuation of a series studies, designed to investigate the effects of insecticides on the viability and activity of soil and water microorganisms, from results can be noted the deep effects of these types of pollutants on one of important organisms (algae), by reduction in growth rate reach to 68.18% in *Anabeana* sp. treated with 250 ppm of malathion, and 75.4% rate in *Chlorococcum* sp at 250 ppm of diazinon, while *Chlorella vulgaris* gave high inhibition rate at concentration of diamethoate 250 ppm at 72 hrs exposure time 63.3% with significant increasing at p≤ 0.05. as well as chlorophyll concentration was reduced significantly due to treated with different concentration of organophosphourus insecticides, highest inhibition rate recorded in *Anabeana* sp. 86.7% at 250 ppm of malathion at 72 hours exposure time. Also can be noted paleness, swelling and aggregation in treated cells.

KEYWORDS : Green Algae, Insecticides, Organophosphorous, Cell Count, Chlorophyll

It is quite obvious that the use of organophosphorous insecticides has brought impressive economical benefits, in particular, through the percentage of applied this kinds of insecticides is often reported to be deposited on non-target areas, contamination of soil and water is inevitable. Under this background, frequent assessments on the impacts to non-target organisms are of prime importance. However, the vast majority of the present investigations dealing with the impact of organophosphorous on aquatic organisms and mechanism involved in degradation are based on laboratory bioassays (Debenest T. et al., 2010).

Furthermore, toxicity studies are often targeted on individual strains, because, such findings assist in assessing the direct impacts of herbicide on the organisms of concern. However, generation of remediation strategies for contaminated waters, merely based on such findings would not be advisable. Thus, field studies conducted in natural environments are encouraged as they could come up with much broader understanding as to how fresh water algae cope with organophosphorous toxicity. The important of algae comes from their role in atmospheric oxygen production, nitrogen fixation, basis of most aquatic food webs, help to purify water by absorbing nutrients and heavy metals from streams and rivers (Haleem A. M. et al., 2012). Algae can be valuable indicators of environmental quality. Many are sensitive to changes in pH, in nutrient levels or in temperature (Al-Saadi H. A. et al., 2000 ; Al-Janabi Z. Z., 2012). Monitoring species abundance and composition can be useful to identify changes in water quality caused by changes in surrounding land use. Several parameters were measured to investigate the effects of pesticides and other pollutants on algal activity, algal biomass one of these parameters, measured by evaluating chlorophyll pigment concentrations, using either classical spectrophotometry or liquid chromatography. The measurement of chlorophyll a is one of the most widely used parameters to assess effects of pesticides on algae growth. Numerous authors have shown that exposing algae, to concentrations of pesticides that ranges from 10 to 1000 µg/L produced a decrease in chlorophyll a concentration Stauber J. L., 1997 and Stein J. R., 1973. Likewise evaluating of cell density can be give good idea about pesticide effects on algal viability and development. In the present study three insecticides (malathion, diazinon and dimethoate) were applied separately at 50, 100 and 250 ppm on three Iraqi fresh water algae (*Clorella vulgaris*, *Chlorococcum* sp. and *Anabeana* sp.). Chlorophyll concentration, cell count were determined in both treated and control group.

MATERIALS AND METHODS

Algae Samples

Algae samples were obtained from bio-indication lab/ Environmental research center/ University of technology at stationary phase, included two species unicellular green algae (Clorella *vulgaris*, *Chlorococcum* sp.) in addition to one species of cyanobacteria (*Anabeana* sp.).

All isolations of algae cultured and maintenance

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with sterile synthetic media at 25ºC these media including (mg/L in double distilled water) (Glucose 30, $NaNO_3$ 40, $MgCl_2.6H_2O$ 12.2, $CaCl_2.2H_2O$ 4.41, $MgSO_4.7H_2O$ 14.7, K_2HPO_4 1.04, $NaHCO_3$ 15, H_3BO_3 0.185, $MnCl_2.4H_2O$ 0.416, $ZnCl_2$ 0.00327, $CoCl_2$. $6H_2O$ 0.00143, $CuCl_2.2H_2O$ 12×10-6, $NaMoO_4.2H_2O$ 0.00726, $FeCl_2.6H_2O$ 0.160, $Na_2EDTA.2H_2O$ 0.3) (Stein J. R., 1973).

Algal Growth Measurement

Growth density of studied algae was measured by

Cell count determination

Cell count was determined by using counting chambers for well-mixed suspension algal growth (Stanier et al., 1986).

Chlorophyll concentration identified

A five milliliters of growth suspension was harvested by centrifugation at 4000 rpm for 5 minutes, the

Table 1 : Effects of Cell Number of Examined Algae Exposed to Three Type Insecticides

Malathion									
Concentration PPM	*Chlorella vulgaris* × 10^5 cell/ml			*Chlorococcum sp.* × 10^5 cell/ml			*Anabeana sp.* × 10^5 cell/ml		
	24	48	72	24	48	72	24	48	72
Control	1.4±0.11 a	1.4±0.32 a	1.5±0.13 a	0.9±0.12 a	0.72±0.22 a	0.61±0.02 a	1.1±0.22 a	1.1±0.56 a	0.71±0.32 a
50	1.2±0.21b	0.9±0.23 b	0.76±0.15 b	0.88±0.13 b	0.56±0.25 b	0.46±0.05 b	0.98±0.02 b	0.81±0.05 b	0.66±0.11 b
100	0.96±0.12c	0.73±0.11 c	0.65±0.17 c	0.76±0.02 c	0.43±0.19 c	0.35±0.18 c	0.87±0.21 c	071±0.2 c	0.42±0.21 c
250	0.72±0.32 d	0.55±0.10 d	0.48±0.21 d	0.42±0.07 d	0.25±0.09 d	0.28±0.02 d	0.54±0.27 d	0.35±0.15 d	0.26±0.34 d
Diazinon									
Concentration PPM	*Chlorella vulgaris* × 10^5 cell/ml			*Chlorococcum sp.* × 10^5 cell/ml			*Anabeana sp.* × 10^5 cell/ml		
Control	1.4±0.11 a	1.4±0.32 a	1.5±0.13 a	0.9±0.12 a	0.72±0.22 a	0.61±0.02 a	1.1±0.22 a	1.1±0.56 a	0.71±0.32 a
50	1.4±0.55 b	1.2±0.11 b	1.3±0.76 b	0.78±0.11 b	0.66±0.15 b	0.54±0.15 b	0.92±0.05 b	0.88±0.16 b	0.86±0.45 b
100	1.1±0.23 c	0.89±0.45 c	0.87±0.02 c	0.66±0.43 c	0.64±0.23 c	0.27±0.02 c	0.84±0.13 c	0.65±0.14 c	0.54±0.03 c
250	0.85±0.12 d	0.76±0.21 d	0.67±0.23 d	0.51±0.17 d	0.43±0.26 d	0.15±0.01 d	0.69±0.08 d	0.44±0.18 d	0.36±0.14 d
Dimethoate									
Concentration PPM	*Chlorella vulgaris* × 10^5 cell/ml			*Chlorococcum sp.* × 10^5 cell/ml			*Anabeana sp.* × 10^5 cell/ml		
Control	1.4±0.11	1.4±0.32	1.5±0.13	0.9±0.12	0.72±0.22	0.68±0.32	1.1±0.22	1.1±0.56	0.71±0.32
50	1.1±0.32 b	0.91±0.12 b	0.81±0.25 b	0.88±0.13 b	0.71±0.13 b	0.54±0.21 b	0.81±0.11 b	0.76±0.03 b	0.62±0.15 b
100	0.99±0.33 c	0.87±0.15 c	0.72±0.11 c	0.76±0.02 c	0.53±0.31 c	0.42±0.19 c	0.66±0.01 c	0.64±0.21	0.47±0.07 c
250	0.76±0.04d	0.76±0.17 d	0.55±0.29 d	0.61±0.09 d	0.46±0.07 d	0.36±0.17 d	0.43±0.01 d	0.42±0.25 d	0.45±0.33 d

Each number refer M±SD for triplicates.

Different litters refer to significant differences at $p \leq 0.05$.

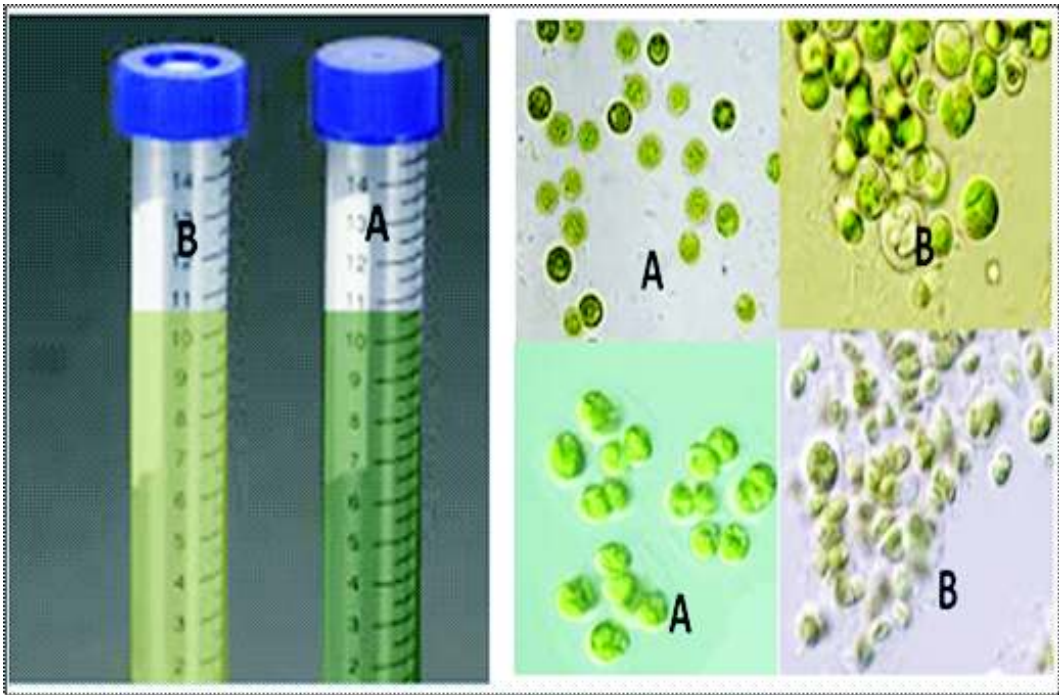

Figure (1): (A) Untreated *Chlorella vulgaris* Tube, (B) Treated *Chlorella vulgaris* Tube, (A) Above Untreated *Chlorella vulgaris* Cells, (B) Above Exposed *Chlorella vulgaris* Cells to 250 ppm Malathion (A) Below Unexposed *Chlorococcum* sp. Cells, (B) Below Exposed *Chlorococcum* sp. Cells to 250 ppm Malathion.

resulting pellets was extracted with 5 ml of 90% acetone at 4 ºC overnight, absorbance of the supernatant was examined by spectrophotometer at 665 nm (Striekland and Parsons, 1972).

Pesticide Solutions Prepare and Experimental Design

Three commercial 40% insecticide solutions (Malathion, Diazinon and Dimethoate) (Agro-chinaCo., China) were used to study their effects on fresh water algae communities. 1000 ppm stock solution of each insecticide were prepared and kept in dark cold conditions until use. 10ml media putted in screw cap universal tube, three concentrations of each insecticide were added separately (50, 100, 250) ppm for (24, 48 and 72) hrs with three replications of each treatment, tubes without added considered as control, 1 ml of algal suspension was added to each tube with final cell number reach to 105 cell/ml.

Statistical Analyses

Complete randomized designed was followed in this study, all data was analyzed by using SPSS software program version 16 by using ANOVAI test and multiple comparisons at ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

Cell Count

From results show in table 1 can be noted the effects of insecticides (Malathion, Diazinon and Dimethoate) on fresh water algae, by reduction in cell count constantly exposure, the highest inhibition rate in malathion was observed in *Anabeana* sp. in concentration 250 ppm at 48 hrs exposure time reach to 68.18%, while diazinon insecticide records greatest inhibition rate in *Chlorococcum* sp 75.4% at 250 ppm and 72 hrs exposure time, *Chlorella vulgaris* gave high inhibition rate 63.3% at concentration of diamethoate 250 ppm at 72 hrs exposure time with significant increasing at $p \leq 0.05$. In Iraq, insecticides used without rationalization leading to leaking large quantities to soil and water (Haleem et al., 2013), causes disruption in the ecosystems and redistribution of micro flora in these environments, cell count of fresh water algae suffered from deep affected this due to DNA damage or abnormal migration of the chromosomes during cell division, more over insecticides may causes deformation of cell wall. Actually the present results are agreed with

Table 2 : Chlorophyll Concentration (mg/l) in Three Fresh Water Algae Exposed to Three Insecticides

Malathion									
Concentration PPM	*Chlorella vulgaris*			*Chlorococcum sp.*			*Anabeana sp*.		
	24	48	72	24	48	72	24	48	72
Control	125±12.7a	145±22.4 a	177±12.6 a	112±21.6 a	132±14.8 a	144±17.9 a	115±14.56 a	146±27.3 a	158±11.6 a
50	98±11.6 b	95±21.7 b	88±12.8 b	96±10.8 b	83±4.5 b	76±11.5 b	87±2.2 b	76±6.4 b	61±5.5 b
100	75±14.5 c	77±6.5 c	67±4.7 c	76±18.5 c	56±3.8 c	45±2.3 c	65±5.7 c	54±8.9 c	42±4.7 c
250	56±18.3 d	51±1.6 d	43±8.3 d	63±2.8 d	43±11.6 d	32±14.9 d	44±9.4 d	34±3.3 d	21±2.4 d
Diazinon									
Concentration PPM	*Chlorella vulgaris*			*Chlorococcum sp.*			*Anabeana sp*		
Control	125±2.7 a	145±2.4 a	177±9.6 a	112±11.6 a	132±14.8 a	144±17.9 a	115±14.56 a	146±27.3 a	158±11.6 a
50	112±8.6 b	95±3.9 b	77±2.8 b	103±8.7 b	86±7.5 b	77±3.1 b	109±8.4 b	91±3.9 b	82±6.9 b
100	91±8.9 c	83±8.4 c	65±10.2 c	89±4.6 c	73±3.2 c	61±1.9 c	92±6.9 c	81±2.1 c	67±5.3 c
Dimethoate									
Concentration PPM	*Chlorella vulgaris*			*Chlorococcum sp.*			*Anabeana sp*.		
Control	125±12.7 a	145±22.4 a	177±12.6 a	112±21.6 a	132±14.8 a	144±17.9 a	115±14.56 a	146±27.3a	158±11.6 a
50	110±7.8 b	79±6.5 b	61±7.5 b	91±2.2 b	82±8.9 b	61±4.2 b	87±8.9 b	86±2.8 b	66±7.8 b
100	75±11.2 c	65±5.4 c	43±2.7 c	76±8.1 c	71±6.6 c	51±3.5 c	78±6.5 c	75±7.1 c	51±16.7 c
250	66±4.9 d	51±6.2 d	31±5.1 d	51±6.5 d	43±2.8 d	33±1.7 d	61±5.6 d	54±1.5 d	42±11.4 d

Each number refer M±SD for triplicates.

Different litters refer to significant differences at $p \leq 0.05$

findings of (Hussain, 2013) when testing the growth rate of unicellular fresh water algae *Senedesmus quadricuda* exposed to three herbicides Igran, Oxyflufen and dawn -up inhibited toreach 54.6%, 67.3% and 44.1% respectively, beside increasing in treated cells diameter, that may refer to failure of these cells to complete the cell division, this lead reduction in biomass and cell count.

Chlorophyll Concentration

As we now all algae are autotrophic organisms play an important role in food chain, oxygen supplies, nitrogen fixation, in addition to their ability to treat the environment from some pollutants. In the same time these organisms affected by many contaminants such as pesticides, chlorophyll one of more affected molecule, because of the photosynthesis is the most important physiological process in algae (Tang et al., 1997; Ma J., 2002 and Stauber J. L., 1995). From table (2) greater inhibition rate of chlorophyll was observed in *Anabeana* sp. in con. 250 ppm at 72 hrs exposure time in malathion insecticides 86.7%, in the same time diazinon and diamethoate record high inhibition rate in *Chlorella vulgaris* at 250 ppm 63.2 and 82.4 respectively table. Figure

(1) shows influence of malathion at 250 ppm on the viability and chlorophyll concentration of two examined unicellular green algae, from picture can be noted paleness of treated cells beside aggregation and swelling.

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