International Journal of Advanced Research in Biological Sciences ISSN: 2348-8069 www.ijarbs.com Coden: IJARQG(USA)

Research Article



SOI: http://s-o-i.org/1.15/ijarbs-2016-3-1-16

A study of P/R Ratio value of BGA using Sevin as a pesticide

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Abstract

SEVIN 50% W.D.P. based on carbaryl. 1. Naphthylmethyl carbamate, is a broad spectrum pesticide for control of pests on fruits, vegetables, forage, cotton and other crops, as well as poultry and pets. In order to know the extent of toxicity five different concentrations of the toxicant (Sevin) are taken – LC_0 (2.13 (ml. Γ^1)), LC_{10} (2.54 ml. Γ^1), LC_{50} (3.01 ml. Γ^1), LC_{90} (3.25(ml. Γ^1), LC_{100} (3.35 ml. Γ^1). Uni -algal, axenic culture of *Anabaena cylindrica*, Lemm. was inoculated and the survival percentage was determined. Three lethal concentrations (LC_{10} , LC_{50} , LC_{90}) were chosen to look into the differential effects of different concentrations of the pesticide (Sevin) on the BGA. P / R ratio value significantly increased with the increase in exposure period. The value increased from 1.08 to 7.44 within 15 days of exposure. High ratio values indicate stress in the exposed system.

Keywords: Sevin, Anabaena Cylindrica, P/R Ratio, photosynthetic efficiency.

Introduction

The introduction of synthetic insecticides carbamates in 1970s contributed greatly to pest control and agricultural output. The rampant use of these chemicals, under the adage, "if little is good, a lot more will be better" has played havoc with human and other life forms (Aktar et al, 2010). A closely related group of insecticides are the carbamate esters first discovered by the Geigy Company in Switzerland in 1947, although the most generally effective member of the group carbonyl or Sevin (N - methyl a naphthylcarbamate) was not introduced until nearly a decade later. This assumes increasing importance as a possible replacement for DDT. In 1943, Templeman and Sexton working for Imperial Chemical Industries in England independently discovered the herbicidal activity of the Phenoxyacetic acids. Two well known examples are 2 - methyl - 4- chloro (MCPA) and 2,4 dichloro (2,4-D) phenoxyacetic acid. Warren (1998) also drew attention to the spectacular increases in crop

yields in the United States in the twentieth century. Webster et al. (1999) stated that "considerable economic losses" would be suffered without pesticide use and quantified the significant increases in yield and economic margin that result from pesticide use. There is now overwhelming evidence that some of these chemicals do pose a potential risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993; Igbedioh, 1991: Jeyaratnam, 1981). No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, though a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990). The world-wide susceptibility to deaths and chronic diseases due to pesticide poisoning number about 1 million per vear (Environews Forum, 1999). The possible role of BGA in the productivity of rice has been demonstrated by

the extensive work of Singh (1961). Rodgers *et al.*, (1979) reported that the biofertilizers can be a good substitute for synthetic nitrogen fertilizers in terms of net productivity and Venkataraman (1972) reported that biofertilizers can maintain the fertility level of the crop fields. This project was designed to evaluate the eco-toxicological effects on photosynthetic and respiratory activity.

Materials and Methods

Test Organism: Anabaena cylindrica, Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae. It shows three different types of cells viz. vegetative cells, heterocysts and akinetes. The spores and vegetative cells are always cylindrical in shape. The vegetative cells fix CO_2 and evolve O_2 where as heterocysts are unable to fix CO_2 or evolve O_2 but can fix nitrogen under aerobic condition (Stewart, 1976). The akinetes are perennating spores that develop between vegetative cells and heterocysts and obtain fixed carbon and nitrogen from them.

Selection of Toxicant: SEVIN 50% W.D.P. based on carbaryl. 1. Naphthylmethyl carbamate, is a broad spectrum pesticide for control of pests on fruits, vegetables, forage, cotton and other crops, as well as poultry and pets. It is relatively free from handling hazards and may be applied in the immediate preharvest period without concern for excessive residues. SEVIN 50% W.D.P. has a low mammalian toxicity. It is generally regarded as one of the safer insecticides Sevin 50% W.D.P. is normally non-phytotoxic Sevin 50% W.D.P. is compatible with most of the pesticides, except those of alkaline nature.

Selection of Concentration of Pesticide and Duration: The selected concetrations were 2.13 ml/L,2.54ml/L, 3.01 ml/L,3.25 ml/L ,3.35ml/L and exposure were 0,3,6.9,12 and 15 days. After exposure the alga were allowed to recover in normal condition in three consecutive periods of 5 days upto 15 days.

Oxygen and Carbon dioxide Evolution Measurements: The oxygen evolution and Carbon dioxide evolution was determined manometrically at 26° C and 2400 ± 200 Lux light intensity in a photo-

Warburg's apparatus (New Paul, India) following the procedure of Hannan & Potouillet (1972) and Oser (1965). The algal culture suspension was centrifuged and the residue was suspended inside the Warburg's flask with 3 ml of substrate solution (nutrient solution and different concentration of the toxicants). The central well was kept blank for the entire period of experimentation for oxygen evolution measurements. Triplicate manometers were used simultaneously with adequate thermobarometers for necessary corrections. The flasks were acclimatized with the temperature and light intensity for 15 minutes with normal shaking of (72-84 strikes/minute) of the apparatus. Then the oxygen evolution was recorded after air tightening the Warburg's flask with normal shaking. The evolution of oxygen was therefore recorded by a fall indicated in the closed arm of the manometer. The readings were multiplied by flask constant (a factor, determined earlier for each Warburg flask) and the data were expressed in terms of ml of oxygen evolved hr $^{-1}$ g $^{-1}$ dry weight of the algal tissue, after necessary thermobarometric corrections, which indicate the net value of O_2 evolved minus the oxygen consumed during respiration.

Carbon dioxide Evolution Measurements: The same procedure adopted for oxygen evolution measurement was followed (Hannan & Potouillet, 1972) and Oser (1965) except that the central well of the Warburg's flask contained a Wick Whatman Filter Paper soaked in 10% KOH solution to absorb the carbon dioxide produced during the experiment. The procedure followed by Sahu (1987) and Rath (1991) was adopted.

Results and Discussion

A graded series of concentrations of the pesticide, Sevin was prepared in different experimental conical flasks. The dilutions were made with the nutrient medium. Unialgal, axenic culture of *Anabaena cylindrica*, Lemm. was inoculated and the survival percentage was determined. Table - A describes the toxicity values of the pesticide, Sevin on *Anabaena cylindrica*. The following concentrations were selected for further detailed studies pertaining to the effects of Sevin on the blue-green alga of the bluegreen alga.

Table- I: Showing deduced lethal concentration values after 15 days of exposure from the toxicity testing data.

Lethal Concentration (LC)	Pesticide Concentration(ml. l ⁻¹)	Percent Survival (PS)
LC_0	2.13	PS ₁₀₀
$LC_{10}(A)$	2.54	PS ₉₀
LC ₅₀ (B)	3.01	PS ₅₀
$LC_{90}(C)$	3.25	PS_{10}
LC_{100}	3.35	PS_0

In all figures, LC_{10} , LC_{50} and LC_{90} were expressed as A, B & C respectively and 'Con.' Stands for control. Table- A indicated that with the increase in concentration of the toxicant (Sevin) the survival percent decreased significantly showing a negative correlation. The above three lethal concentrations were chosen to study the differential effects of different concentrations of the pesticide (Sevin) on the blue-g.

Effect of sub-lethal concentrations of the pesticide, Sevin on the oxygen evolution of Anabaena cylindrica at different days of exposure and recovery has been presented in Fig. 1. and 2. Control set showed a normal and steady increase in O_2 evolution with the increase in exposure period. The oxygen evolution increased from 272.5+ 16.2 to 378.4+24.5 µl of O₂ evolved $hr^{-1}g^{-1}$ dry weight within 15 days of exposure. At 2.54 ml 1^{-1} Sevin concentration, a significant increase in oxygen evolution over the control values up to 15th day of exposure was marked. The oxygen evolution increased from 272.5+ 16.2 to 377.8 + 19.3 μ l of O₂ evolved hr⁻¹ g⁻¹ dry weight within 15 days of exposure. When the exposed alga was transferred to toxicant free medium for recovery studies, instead of showing any further increase the oxygen evolution decreased significantly, when compared to the respective control value. On 15th day of recovery, the value decreased from 491.4 + 13.9 to $358.5 + 12.2 \mu$ l of O_2 evolved hr⁻¹ g⁻¹ dry weight. The data showed a positive (significant) correlation (r=0.996; p<0.001 in

control and at conc. A, r=0.989; p≤0.001) value with the increase in exposure period (Fig.1). At 3.01 ml 1⁻¹ and 3.25 ml 1⁻¹ of Sevin concentration, a significant decrease in oxygen evolution was marked, when compared to the control value. At LC₉₀ (3.25 ml 1⁻¹), drastic decline in oxygen evolution was marked, the value decreased from 378.4±24.5 to 88.6±11.8 µl of O₂ evolved hr⁻¹ g⁻¹ dry weight. The values in all exposure periods were less than the inoculated value at '0' day (Fig.2)

No significant recovery was observed at higher concentrations of the toxicant. Fig. 2 represented the percent change (increase/decrease) in oxygen evolution, over the control value. At 2.54 ml 1^{-1} , highest percent increase (2.8%) was marked on 9th day of exposure. In recovery studies, the values showed a decrease, when compared to control value and the maximum (-27.04%) was marked on 15th day of recovery (Fig. 3). At LC₅₀ ($3.01 \text{ ml } 1^{-1}$) ,0.7% increase was recorded on 3rd day of exposure, then the oxygen evolution decreased significantly and a maximum of 32.8% decrease was recorded on 15th day of exposure. When the exposed alga was transferred to pesticide free growth medium, no recovery was marked, rather further depletion in oxygen evolution was marked. On 15th day of recovery 54.9% decrease was recorded and at LC_{90} (3.25 ml⁻¹), the percent decrease being the highest (72.08%) on 15th day of exposure.

Table -II: Correlation co-efficient (r) between days of exposure and different parameters of study of the algae,	
exposed to three different concentrations of the pesticide and control. (NS = Not significant).	

Concentration of the insecticide ml l ⁻¹	Photosynth- etic rate	Percent change in photosynthet-ic rate	Respiration rate.	Percent decrease in respiration rate.
Control (0.0)	0.996		0.966	
$P \leq$	0.001		0.01	
А				
$(2.5 \text{ml } 1^{-1})$	0.989	- 0. 493	0.958	0.905
$P \leq$	0.001	N S	0.01	0.05
В				
$(3.0 \text{ml } 1^{-1})$	0.981	- 0. 786	-0.751	0.966
$P \leq$	0.001	0.05	NS	0.05
С				
$(3.25 \text{ml } 1^{-1})$	- 0.992	- 0.995	- 0.983	0.983
$P \leq$	0.001	0.05	0.01	0.01

No significant recovery was observed in any of the three experimental sets. During recovery period, instead of showing any recovery, the depletion was significant. In conc. 'A', 27.04%; in conc. 'B' 54.9%

and in conc. 'C' 92.14% decrease in oxygen evolution was marked on 15th day of recovery. The correlation coefficient analysis between days of exposure and photosynthetic rate indicated the existence of significant positive correlation in control (r = 0.996,

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 $p \le 0.001$), in conc. A (r = 0.989, $p \le 0.001$) and a negative but significant (r = 0.981, $p \le 0.001$; r = -0.985, $p \le 0.001$) correlation was marked in conc. B and conc. C, respectively (Table - II). The percent change in photosynthetic rate in conc. A and conc. B showed a non-significant negative correlation with exposure period and in conc. C, a negative significant (r = -0.995, p \leq 0.001) correlation was marked (Table - II) with exposure period. The ANOVA test indicated the existence of significant difference between rows and non-significant difference.





Fig.4 and 5 represented the changes in respiration rate (μ l of CO₂ evolved hr⁻¹ 50 ml culture) of control and Sevin exposed Anabaena cylindrica at different days of exposure and recovery. In the control set the CO_2 evolution increased from $160.2 \pm 6.5 \ \mu l$ of CO₂ evolved hr⁻¹ 50 ml culture (0 day) to 232.4 ± 12.4 µl of CO₂ evolved hr⁻¹ 50 ml culture, within 15 days of exposure. In concentration A ($2.54 \text{ ml } 1^{-1}$) of Sevin, the respiration rate decreased up to 202.4 + 11.4 µl of CO₂ evolved hr⁻¹ 50 ml culture within 15 days of exposure, showing a positive (r=0.966, p<0.01)correlation (Table - II), but this value was far less than the control value. A maximum of 12.9% decrease was recorded on 15th day of exposure .When the exposed alga was transferred to toxicant free medium, the value further depleted when compared to control value on 15th day of recovery and a maximum of 18.5% decrease was recorded on 10th day of recovery and 18.2% decrease was recorded on 15th day of recovery. This indicated no recovery altogether. In conc. 'B' (3.01 ml 1⁻¹) of Sevin, the exposed alga, Anabaena showed further decrease in CO₂ evolution and the value was 161.2 ± 8 . μ l of CO₂ evolved hr⁻¹ 50 ml culture on 15^{th} day of exposure. Here, the CO₂ evolution rate increased and then decreased indicating

a non-significant negative (r = -0.751, p = NS)correlation with the increase in exposure period (Fig. 4). A maximum of 30.63% decrease was recorded on 15th day of exposure and 28.56% decrease was recorded on 5th day of recovery and 34.24% decrease over the control value was recorded on 15th day recovery, indicating partial damage caused to the metabolic system by the pesticide, Sevin (Fig. 5). In concentration C, $(3.25 \text{ ml } 1^{-1})$, the CO₂ evolution rate drastically declined from 160.2 + 6.5 to 11.9 + 2.1 µl of CO₂ evolved hr⁻¹ 50 ml culture , within 15 days of exposure. All the values were significantly less than the respective control values and these exposed values were interestingly much less than the inoculated value (Fig. 3 &4). A maximum of 94.87% decrease in CO₂ evolution rate was observed on 15th day of exposure (Fig.6). When the exposed algae were transferred to pesticide free medium for recovery studies, no recovery was marked. Rather, the values further depleted and maximum of 14.01%, 28.56% and 96.27% was recorded on 5th day of recovery. On 15th day of recovery, little increment was observed in conc. A & B. No significant recovery in respiration rate was marked. The percent changes in respiration rate during recovery period have been explained in Fig.6.





The correlation coefficient analysis between days of exposure and respiration rate indicated the existence of positive correlation in control (r = 0.966, $p \le 0.01$), in conc. A (r = 0.958, $p \le 0.01$) and in conc. B a nonsignificant correlation (r=-0.751, $p\le NS$), where as a negative significant correlation (r=-0.983, $p\le 0.01$) was marked in concentration C. The percent decrease in respiration rate showed all positive significant correlations with the days of exposure (Table-III). The ANOVA test indicated the existence of significant difference between the rows and non-significant difference between columns. Fig 7 and 8 indicates the changes in P / R ratio in control and pesticide

exposed blue-green alga. The ratio value increased from 1.08 to 1.62 in control on 15^{th} day of exposure. In conc. A., the ratio values were more than the control values. The value increased from 1.08 to 1.86. In conc. B., the ratio value increased significantly from 1.08 to 1.93 on 9th day of exposure. Afterwards, at higher exposure periods, the ratio value decreased to 1.57. In case of conc. C., the P / R ratio value significantly increased with the increase in exposure period. The value increased from 1.08 to 7.44 within 15 days of exposure. High ratio values indicate stress in the exposed system (Fig. 7 and 8).



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Conclusion

Insignificant increase in photosynthetic rate at concentration A and significant depletion in oxygen evolution rate at concentration B and C was marked. A maximum of 74.6% decrease on 15th day of exposure in concentration C and 96.46% decrease in 15th day of recovery was marked. The photosynthetic rate increased with the increase in exposure period in the control set. In case of conc. A, the photosynthetic rate significantly increased over the control value up to 12th day of exposure and on 15th day of exposure, no change was marked. When the exposed alga was transferred to toxicant free medium, significant decrease in the photosynthetic rate was marked. However, in Conc. B & C, significant decrease in the photosynthetic rate was observed. Significant lower values were observed in conc. C, where the obtained values were less then the inoculation value. No recovery was marked in exposed cultures, during recovery period. Significant depletion in respiration rate was marked in all the exposed cultures, when compared to the control values. A maximum of 94.9% decrease and 97.8% decrease was recorded on 15th day of exposure and 15th day of recovery in conc. C of the toxicant, respectively. The P/R ratio value increased with the increase in Sevin concentration at all exposure periods, when compared to the respective control values pesticide, Sevin on the blue-green alga, Anabaena cylindrica, Lemm.

Acknowledgments

The authors acknowledge the service rendered by the P.G. Dept. of Botany and Bio -technology, Khallikote University, Berhampur and the Environmental Research Unit P.G. Dept. of Botany for completion of

the research on time. We also thank the people and the institutions who are directly or indirectly involved incompletion of the arduous and painstaking assignment.

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<u>How to cite this article:</u> Rasmita Padhy and A.K.Panigrahi. (2016). A study of P/R Ratio value of BGA using Sevin as a pesticide. Int. J. Adv. Res. Biol. Sci. 3(1): 118-125.