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Impact of 2,4-D and Carbaryl on Growth, Pigment production and Nitrogen fixation of *Anabena*

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Abstract: *Pesticides a class of new synthetic chemicals comprise herbicides, molluscicides, raticides, nematocides and insecticides have been constantly used in the fields to increase the crop production. Constant use of these chemicals tend to have a negative impact on soil microflora and the " Beneficial nitrogen fixing organisms" face a challenge for survival in the soil habitat as they are exposed to these pesticides. Cyanobacteria are the most dominat primary producers amongst the photosynthetic microorganisms in the paddy field ecosystems by virtue of fixing both nitrogen as well as carbon dioxide. Anabaena is a nitrogen fixing, heterocystous cyanobacterium used in paddy cultivation. Anabaena was exposed to different concentrations of 2,4 – D and carbaryl and mixture of both for a period of 18 days.. Anabaena exhibited a higher growth rate at low and high concentrations of 2,4 –D whereas it exhibited a lower growth rate in the presence of carbaryl and mixture of both. Since a drastic change was observed in growth rate, pigment and total nitrogen content in the presence of carbaryl and mixed pesticide solution, individual and combined effect of the above exerts a toxic effect on Anabaena.*

Key words: *Anabaena*, 2.4 –D , carbaryl, Nitrogen, chlorophyll

1. Introduction:

The Earths atmosphere contains elemental nitrogen as its major component. Among the biological nitrogen fixation *Azolla- Anabaena* symbiosis appear significant and promising for exploitation in agriculture and forestery. In rice ecosystems, photoautotrophic blue green algae (BGA) and heterotrophic photosphere bacteria play a joint role in meeting the nitrogen needs for lowland rice cultivation.

Cyanobacterial, a unique group of Gram negative bacteria evolved 2 x 10^9 years ago (Kannan 1999), are atypical among the prokaryotes due to the profession of specific cells called "Heterocysts" for nitrogen fixation (Fay *et al.*, 1968). They are both unicellular filamentous heterocystous as well as non-heterocystous photoautotrophic diazothrophs. They are ubiquitous in distribution. (Venkataraman 1977).

Herbicides are often reported to induce alterations in growth pattern of BGA. The commonly used herbicide 2, 4- D to kill weeds has more effect on the aerobic microbes. The impact of herbicides is directly linked to growth, growth parameters, pigment content and physiological characteristics such as nitrogen fixation (Patel and Rao 1999). Carbaryl, a carbamate insecticide is used widely in rice culture to control insect pests. It is generally used as a foliar spray (Rajagopal, B.S. and Rao V. R. 1984).

In this study, *Anabaena* isolated from cultivated rice field was analyzed for its response pattern against different concentrations of 2, 4 –D , carbaryl and a mixture of the above two. This response was analysed on the basis of the change in specific growth rate, pigment concentrations and total nitrogen content.

2. Materials & Methods:

2.1Growth pattern analysis of the *Anabaena*

To anlalyze the growth pattern of cyanobacterial isolate, freshly grown algal mat was taken from the 15-20 days old cultures. Growth was measured turbidimetrically at 760nm.

2.2Impact of pesticides in the growth of *Anaabena:* (Rath and Adhikary 1994)

To record the effect of pesticides on cyanobacteria, changes in the specific growth rate of the *Anabaena* was monitored. At expontential growth phase, the cyanobacterial cultures were centrifuged at 3000rpm for 10 minutes. After centrifugation, the supernatant was discarded and the pellet was transferred to a sterile homogenizer and was gently homogenized. From this, 0.1ml of the cell suspension was inoculated with 9.9ml of sterile BG11 medium incorporated with herbicide, insecticide and both herbicide and insectide combination at various concentrations [20ppm, 40ppm, 60ppm, 80ppm and 100ppm]. The inoculated tubes were incubated under 12hrs light/dark cycles at 28°C ± 2°C up to 15 days.

Growth was measured at 3days intervals from the day of inoculation. The inoculated tubes of each concentration with control were taken. Growth of

the cyanobacterial culture with or without pesticide treatment was expressed as specific growth rate constant that was calculated with or without pesticide treatment was expressed as specific growth rate constant that was calculated using the formula

$$K = \log (N_1/N_0) (3.222/ t)$$

Where K is specific growth rate constant

N_1 = Cell concentration at the end of the experiment period

N_0 = Cell concentration at the beginning of the experiment

t = days

2.3 Estimation of pigment content of the *Anabaena:*

In order to understand the impact of herbicide and insecticide individually and together on, *Anabaena*, chlorophyll and carotene content of the isolate as taken as a parameter as this would indicate the positive and negative response of the test organism to the chemical. This was carried out by adopting the methodology of Jayaraman (1996).

2.4 Estimation of Total cell Nitrogen by Micro-Kjeldhal method.

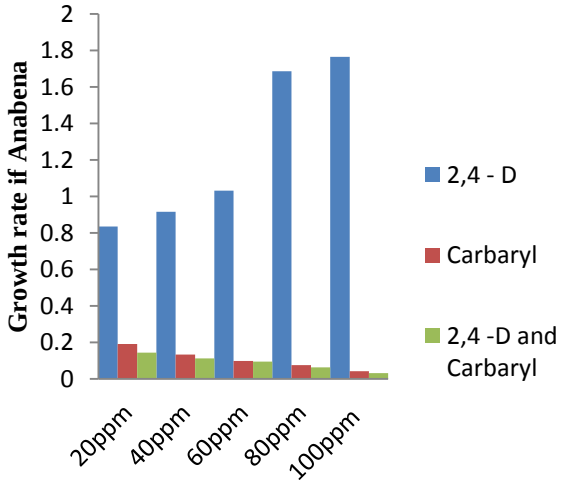
Total nitrogen content was determined by modified Micro-Kjeldhal method {Nesslerization} of Umbriet *et al.*, (1972).

3. Result and Discussion:

Anabaena is a nitrogen fixing, heterocystous cyanobacterium that is ubiquitous in paddy fields forming a major constituent of the algal biotypes in nine out of fifteen states in India and is also a component of the algal biofertilizer increasingly used in paddy cultivation. The effect of different pesticides including herbicides, insecticides on growth, nitrogen fixation and ammonia excretion in *Anabaena* was reported earlier (Kannaiyan *et al.*, 1981; Padhy *et al.*, 2001; Adhikary *et al.*, 1984.)

In this background, the effect of 2, 4-D carbaryl and their mixture on growth was evaluated. (Fig 3.1.). *Anabaena*, the most popular organism with wide spread application as biofertilizer when exposed to different concentration of 2, 4 –D exhibited a synchronized changes in the growth pattern. A slight reduction in specific growth rate was observed till 12 days of incubation. After this, there was an increase in specific growth rate was found to be directly proportional to the herbicides concentration. This observation clearly indicated that the test BGA can utilize 2, 4 –D as a nutrient in the event of exhaustation of the nutrients provided in the basal medium. Degradation of pesticides by cyanobacteria is not new as many species of BGA were observed to degrade and tolerate a wide variety of pesticides. Rath and Adhikary (1994) have reported several observations on pesticide resistant and degrading nature of *Anabaena* when exposed to different concentration of furadan (Carbofuran).

Fig : 3. 1 Impact of pesticides in the growth of *Anabaena* (18 days)

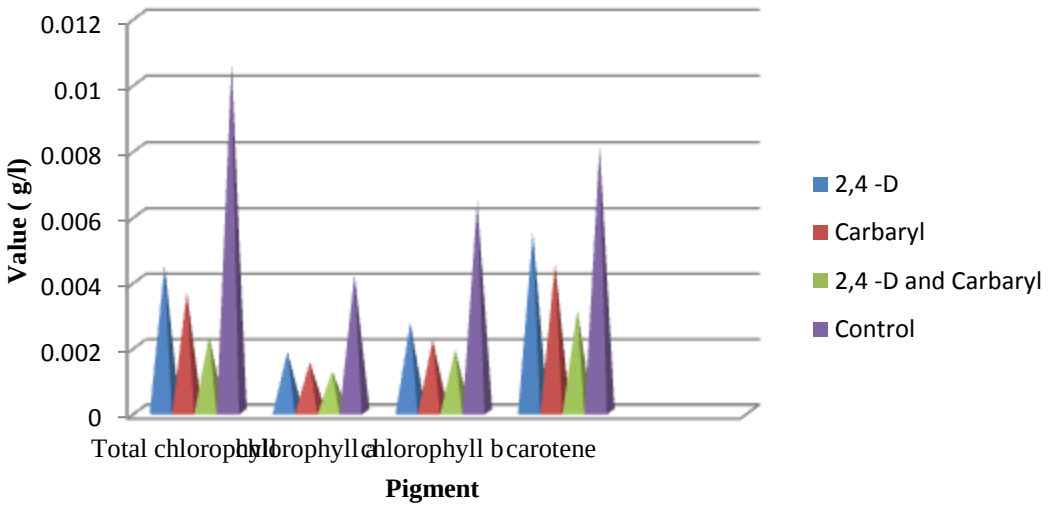


Growth of *Anabaena* was severely affected when exposed to different concentrations of carbaryl lower concentration increased the growth rate while higher concentrations reduced the growth rate. This clearly indicates that carbaryl at lower concentrations may or may not have a stimulatory effect on growth but at higher concentrations, will either suppress the growth or inhibit the growth completely. Adhikary *et al.*, 1984 have reported that lower and higher concentrations of carbaryl have a stimulatory and inhibitory effect on *Anabaena* respectively.

The combined effect of 2, 4 –D and carbaryl on growth rate was observed to be more than their individual effect. Chinnaswamy *et al.*, 1983 reported that the growth promoting activity of *Anabaena flos-aquae* was suppressed by the combined effect of herbicide and insecticide whereas; such activity was not affected by their individual effect.

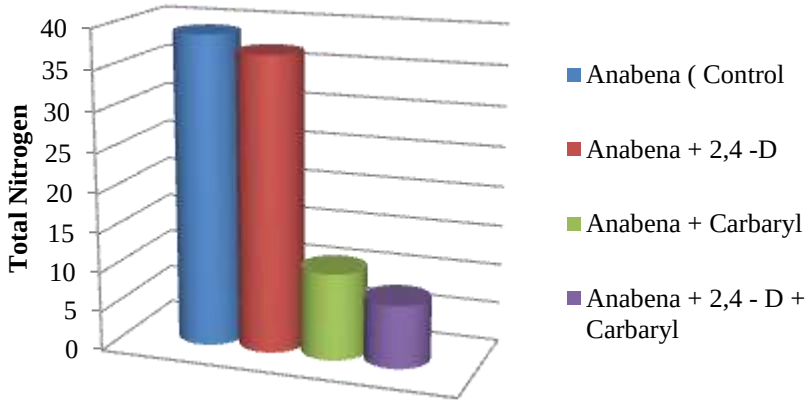
Exposure of *Anabaena* to 2, 4-D has resulted a significant change in the concentration and composition of their photosynthetic pigments (Fig 3.2). More significant reduction was observed in the total chlorophyll. When *Anabaena* was exposed to carbaryl and the mixture of 2, 4-d and carbaryl, a remarkable reduction was observed in total chlorophyll, chlorophyll ‘a’ and ‘b’ and carotene. Changes in pigment concentration in *Anabaena* in response to change in growth media was reported by Mohanan and Sharma (1986). Awasthi (1998) realizing the sensitivity of pigment system has suggested the use of chlorophyll content as index to access the impact of pollutants on BGA.

Fig : 3. 2Impact of pesticide on pigment content of *Anabaena*



A reduction in the total nitrogen was observed in the presence of pesticides.(Fig 3.3). In comparison with the control, a slight reduction was observed in the presence of 2,4 –D and a remarkable reduction was observed in the case of carbaryl and a mixture of 2,4 –D and carbaryl. The results observed by De Silva *et al.*, 1975 indicated that some pesticidal compounds can increase the growth rate as well as severally limit the nitrogen fixing capacity of *Anabaena, Nostoc*.

Fig : 3.3 Effect of 2,4 -D and Carbaryl separately and synergistically on Nitrogen fixation of *Anabaena*



The present investigation clearly opens up that the 2, 4 –D degrading potential of *Anabaena*, and toxicity of carbaryl on the same. A probable mechanism of resistance to 2, 4-D might be the presence of sheath outside the cellwall of *Anabaena.* (Rath and Adhikary 1994). But this was not applicable to carbaryl as the growth rate, pigment and total nitrogen content decreased with increasing concentration of carbaryl. The potency of *Anabaena* as biofertilizer will not be lost even after the treatment with 2,4 –D. A probable mechanism of resistance of *Anabaena* to 2, 4- D might be due to the presence of a sheath outside the cell wall eventhough there existed a light change in chlorophyll and total nitrogen content. Hence 2, 4 – D is a better choice of pesticide compared to carbaryl.

4. Conclusion:

Anabaena, a lab stock procured from CAS in botany was exposed to different concentration of 2, 4 –D and carbaryl and a mixture of both for a period of 18 days. Samples were withdrawn periodically and the specific growth rate was recorded photometrically. *Anabaena* exhibited a higher growth rate at low and high concentrations of 2, 4-D whereas it exhibited a lower growth rate in the presence of carbaryl and mixture of 2, 4- D and carbaryl. A slight reduction was observed in the pigment and total nitrogen content of *Anabaena*

when exposed to 2, 4 –D whereas a drastic reduction was observed in the presence of carbaryl and mixture of both.

2, 4 – D at higher concentration has comparatively lesser effect and enhances the growth of *Anabaena* than carbaryl and mixed (2, 4-D + Carbaryl). A probable mechanism of resistance of *Anabaena* to 2,4 –D might be due to the presence of a sheath outside the cell wall eventhough there existed a light change in chlorophyll and total nitrogen content. Since a drastic change was observed in growth rate, pigment and total nitrogen content in the presence of carbaryl and mixed pesticide solution, individual and combined effect of the above exerts a toxic effect on *Anabaena*. So, 2, 4 – D is a safer pesticide than carbaryl.

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