Comparative Assay on Effect of Carbamate, Organophosphate and Organochlorine Pesticides on the Nitrogen-fixing *Anabaena* Species

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Abstract

The total nitrogen fixed by the species of genus *Anabaena* viz. *A. circinalis* and *A. orientalis*was estimated by conventional Micro- kjeldahl method after 28 days of harvesting at each ppm concentration of carbamates, Furadan, Sevin, organophosphate, Rogor and organochlorine, Endotafpesticides along with the untreated control.The pragmatic results indicated a progressive decrease in the total nitrogen content of both the tested *Anabaena* species with increasing concentrations of the pesticides. However, nitrogen fixation of *A. circinalis* and *A. orientalis* also increased at the lower doses of pesticides viz. 10 ppm of carbamate, Furadan and Sevin; 5 ppm of organophosphate, Rogor and organochlorine, Endotaf. In the laboratory cultures, the carbamate pesticides Furadan and Sevin were less toxic than organophosphate, Rogor and organochlorine, Endotaf to the tested *Anabaena* species. It was concluded that higher doses of pesticides application i.e. more than 20 ppm of Furadan and Sevin and even 10 ppm of Rogor and Endotaf, adversely affected the occurrence and survivability of *Anabaena* species in the laboratory culture which are responsible for nitrogen fixation. Organochlorine, Endotaf was found to be highly toxic to both the tested *Anabaena* species than the carbamates, Furadan, Sevin and organophosphate, Rogor pesticides in sequence.

KEYWORDS: Furadan, Sevin, Rogor, Endotaf, Total nitrogen, *Anabaena.*

INTRODUCTION

Blue-green algae are unique prokaryotic organisms having the capacity to perform mutually compatible functions like nitrogen fixation and photosynthesis (Tiwari *et al*., 2005). The beneficial effect of blue-green algae is due to the fixing of atmospheric nitrogen and addition of some enzymes and growth promoting hormones to the soil (Satapathy and Adhikari,1993 and Singh *et al*., 2000). It has been reported that the part of nitrogen requirements for the crops (25- 35 %) could be met by algalisation under different agroclimatic conditions (Ghose and Saha, 1997; Rai *et al*., 2000). *Anabaena, Nostoc, Calothrix, Hapalosiphon*and *Aulosira* are foremost nitrogen fixing cyanobacteria occurred in various agro-practices areas. Such forms hold potential for maize, rice, mungbean, tomato and sugarcane and wheat crops by fixing nitrogen (Gafur and Parvin, 2008).

In agriculture, introduction of fertilizer responsive crop varieties has necessitated the use of enormous amounts of pesticides during production and storage. Variety of pesticides like organochlorines, organophosphates, carbamates and synthetic pyrethroids are now in use. Many chlorinated and organophosphorus pesticides, which are not readily soluble in water, are emulsified. These are dispersed in water as fine particles that are attracted to surfaces (Kapoor and Arora, 2000). This affinity results in their accumulation through adsorption on to the surface of living

organisms (Satyandra Kumar *et al.*, 2000; Das, 2008). These agrochemicals also damage wide variety of beneficial microorganisms because of their long persistence in the environment (Rajendran*et al*., 2006; Islam *et al*., 2007). The effect of individual agrochemical on individual species is extremely variable, but generally detrimental.

The present condition of environmental complications that has been generated due to indiscriminate and extreme use of pesticide applications envisioned to control the pests occurred on the agricultural crops. Blue-green algae are of great agricultural significance and having capability to adopt, survive, establish and colonize the soil in reasonable time. Such studies are beneficial in aware the farmers to implement better farm management practices that in order will reduce the chemical fertilizer input and problem of environmental deprivation.

MATERIALS AND METHODS

Four commercial grade pesticides as two carbamate pesticides, Furadan and Sevin (Union Carbide Ltd.) and one each of organophosphate and organochlorine, Rogor and Endotaf respectively (Rallis India Ltd.) were used in the present investigation. The effect of Furadan (carbofuran, 3% G), Sevin (carbaryl, 50%), Rogor (dimethoate, 30%) and Endotaf (endosulfan, 35%) belonging to carbamate, organophosphate and organochlorine group was studied on the nitrogen fixation of *Anabaena circinalis* and *Anabaena orientalis*in the laboratory cultures. These pesticides are generally used to control sucking, lepidopterous and nematode pests and mites that occurred in maize, wheat, sugarcane, cotton, onion, vegetable and oil yielding crops.

The carbamate and organophosphate pesticides are used as contact and stomach action pre- emergence systemic pesticides while, Endotaf attacks on central nervous system and make interference in α-amino butyric acid receptor activity of the pest. The pesticide application rates recommended to control various crop pests of this region are 0.75 kg/ha and 1.0 kg/ha for carbaryl and carbofuran (Sevin and Furadan) and 0.5 and 0.7 liter/ha for endosulfan (Endotaf) and dimethoate (Rogor), respectively which will provide a range of 5- 10 ppm in the agricultural crop field.

The effect of four commonly used pesticides viz. Furadan, Sevin, Rogor and Endotaf was studied on nitrogen fixation efficiency of *Anabaena circinalis* and *Anabaena orientalis*in experiments with 2.5, 5, 10, 20, 50, 100, 250 and 500 ppm concentrations of each pesticide in the 50 ml of nitrogen free BG-11 medium (Rippka*et al.*, 1979). Total nitrogen fixed by *Anabaena* species was estimated by conventional Micro- kjeldahl method (Jackson, 1958) in the laboratory cultures after 28 days of harvesting at each concentration of four pesticides. Experiments were conducted by inoculating equal amounts of actively growing unialgal isolates into cotton stoppered conical flasks.

RESULTS

The pragmatic results depicted in the Table- 1 indicated a progressive decrease in the total nitrogen fixedby*Anabaena circinalis* and *Anabaena orientalis* with increasing concentrations of the pesticides. Increased total nitrogen content over the untreated control was recorded in both the species of genus *Anabaena*with carbamate pesticides, Furadan and Sevinupto 10 ppm dose level. Whereas in the presence of 20 ppm concentration of carbamate pesticides, total nitrogen content was consistently decreased with the increasing concentrations of pesticides. At the higher dose level i.e. 500 ppm with Furadan, in *Anabaena circinalis* 80.6% decrease in total nitrogen content was observed while in *Anabaena orientalis* 80.8% decrease was noted than

the control. On the other hand, with Sevin at 500 ppm concentration, 78.6% and 73.3% decrease in total nitrogen contentwas recorded in *Anabaena circinalis* and *Anabaena orientalis*, respectively. (Fig. 1 and 2)

Moreover, with Rogor and Endotaf pesticides upto 5 ppm dose level, enhancement in total nitrogen content was observed than the control in both the species of genus *Anabaena* viz. *A. circinalis* and *A. orientalis*. Whereas in the presence of 10 ppm concentration of both the pesticides, total nitrogen content was regularly declined with the increasing concentrations of pesticides. At 250 ppm of Rogor, 85.8% (*A. circinalis*) and 83.7% (*A. orientalis*) reduction in the nitrogen fixation was observed than the control. Whereas with Endotaf at 100 ppm dose level, 82.5% (*A. circinalis*) and 82.9% (*A. orientalis*) decrease was recorded. Simultaneously further increase in pesticides dose level, growth and nitrogen fixation was ceased at 500 ppm of Rogor and 250 ppm of Endotaf in both the tested species of genus *Anabaena*. (Fig. 1 and 2)

The results obtained during the present investigation revealed that in laboratory cultures, the carbamate pesticides Furadan and Sevin were less toxic than organophosphate, Rogor and organochlorine, Endotafin sequence to the tested *Anabaena* species. Further, a progressive decline in the nitrogen fixation of both *Anabaena* species occurs with increasing concentrations of pesticides. Among the four different pesticide treatments,

DISCUSSION

The reduction in total nitrogen content of the pesticide-adapted *Anabaena* strains may occur due to the inhibition of some stage(s) during the process of nitrogen fixation in the presence of higher concentrations of pesticides. Further stimulatory effect of Furadan, Sevin at lower concentrations on nitrogen fixation by *Anabaena* speciesunder culture conditions may be due to the presence of nutrients in media that minimizes the toxicity of carbofuran (Kar and Singh, 1978 a; Sharama and Gaur, 1981).DaSilva *et al.* (1975) reported a stimulatory effect on nitrogen fixation in most studied blue-green algal forms while a general pattern of gradual decline occurred with increasing dose level of eight widely hitherto used pesticides. Increased nitrogen fixation in the presence of doses of Furadan (Kar and Singh, 1978 b), 2, 4- D (Das and Singh, 1977),Sevin (Adhikary*et al.*, 1984), Rogor(Pattnaik and Prakash Rao, 1982) and Endosulfan (Adhikary, 1989)by the blue-green algae was reported by the earlier workers.

Similarly, Grant *et al*. (1983) noted a 3 and 10-fold increase in the nitrogen fixing capability of rice field cyanobacteria when Perthane was used at lower concentrations. Shivaprakasha and Shivappa Shetty (1986) and Shivaram and Shivappa Shetty (1988) observed that nitrogenfixation by the isolates of *Anabaena variabilis*, *Calothrix* sp., *Cylindrospermummusicola*, *Hapalosiphonwelwitschii*, *Nostoc*sps. were favoured at lower concentrations of the pesticides Rogor, Dithane M-45, and 2,4- D sodium salt in pure culture whereas higher concentrations gave an adverse effect. Satyandra Kumar *et al.* (2000) found that, Endosulfan at 12 ppm proved lethal for *Anabaena* sp. while extreme fragmentation and subsequent death occurred in *Spirulina platensis* at 8 ppm. Kiran *et al.* (2006) foundlower concentrations of Monocrotophos and Butachlor caused stimulation of growth and nitrogen fixation and partial or complete inhibition at higher concentrationsin *Anabaena* sp. and *Westiellopsisprolifica*. Islam *et al*. (2007) revealed that, nitrogen fixation in *Nostoc* and *Anabaena* isolates was increased after the application of Furadan at concentration up to 50 ppm. The blue-green algal isolates were more

tolerant to Furadan and the effects of Furadan were stimulatory at field dose on the growth and nitrogen fixation. Das (2008) reported a pesticide concentration dependent reduction in nitrogen content of the *Anabaena variabilis*, *Nostocmuscorum*, *Calothrixparietina*and*Westiellopsisprolifica* with organophosphate pesticide, Rogor.

SUMMARY AND CONCLUSION

From the experimental results, in general it was seen that higher doses of pesticides application i.e. more than 20 ppm of Furadan and Sevin and even 10 ppm of Rogor and Endotaf, adversely affected the occurrence and survivability of *Anabaena* species in the laboratory culture which are responsible for nitrogen fixation. Endotaf was found to be highly toxic to both the tested *Anabaena* species than the Furadan, Sevin and Rogor pesticides in sequence. Further it was concluded that at the recommended doses of field application, all the studied pesticides had no deleterious effect on nitrogen fixation of *Anabaena* species.

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Table- 1: Total nitrogen (%) fixed by *Anabaena* species at different concentrations of Furadan,Sevin, Rogor and Endotaf pesticides. (Harvested after 28 days of incubation)

Concentrations of pesticides (ppm)	**Total Nitrogen (%) Fixed**	
	Anabaena circinalis	***Anabaena orientalis***
0.00 (Control)	**5.28%**	**4.80%**
Furadan2.5	5.46 (+3.4)	4.86 (+1.2)
5	5.39 (+2.0)	5.10 (+6.2)
10	5.35 (+1.3)	4.94 (+2.9)
20	4.24 (-19.7)	3.84 (-20.0)
50	3.76 (-28.7)	2.95 (-38.5)
100	2.89 (-45.2)	2.29 (-52.3)
250	1.34 (-74.6)	1.37 (-71.4)
500	1.02 (-80.6)	0.92 (-80.8)
Sevin2.5	5.37 (+1.7)	4.87 (+1.4)
5	5.51 (+4.3)	4.96 (+3.3)
10	5.45 (+3.2)	4.92 (+2.5)
20	5.10 (-3.4)	4.10 (-14.5)
50	4.12 (-21.9)	3.72 (-22.5)
100	3.27 (-38.0)	3.05 (-36.4)
250	2.58 (-51.1)	2.32 (-51.6)
500	1.16 (-78.0)	1.28 (-73.3)
Rogor2.5	5.40 (+2.2)	5.18 (+7.8)
5	5.32 (+5.3)	4.92 (+2.5)
10	4.56 (-13.6)	4.18 (-12.9)
20	3.38 (-35.9)	3.52 (-26.6)
50	2.72 (-48.4)	2.94 (-38.7)
100	1.87 (-64.5)	1.36 (-71.6)
250	0.75 (-85.8)	0.78 (-83.7)
500	--	--
Endotaf2.5	5.44 (+3.0)	5.00 (+4.1)
5	5.36 (+1.5)	4.22 (-12.1)
10	4.14 (-21.6)	3.37 (-29.8)
20	3.00 (-43.1)	2.58 (-40.2)
50	1.80 (-65.9)	1.76 (-63.3)
100	0.92 (-82.5)	0.82 (-82.9)
250	--	--
500	--	--

Values represents mean of three replicates; figures in parenthesis show percent increase (+) or decrease (-) as compared to total nitrogen content (%) in the untreated control.

Fig. 1: Total nitrogen (%) fixed by ***Anabaena circinalis***at different concentrations (ppm) of Furadan, Sevin, Rogor and Endotaf pesticides.

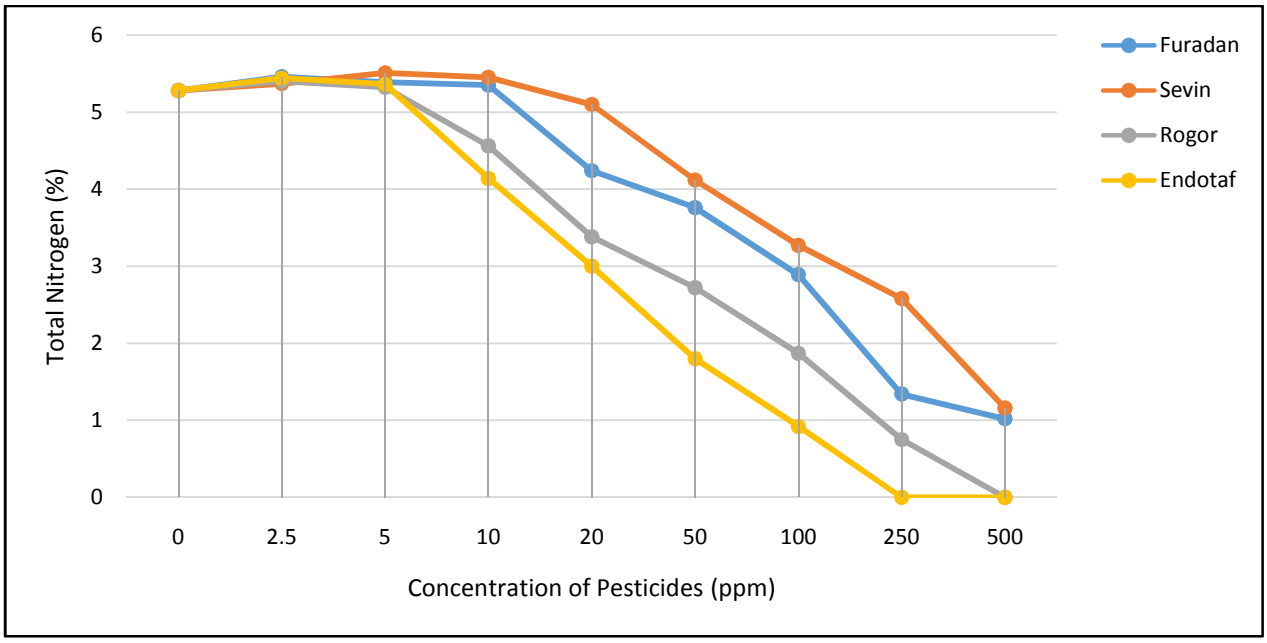

Fig. 2: Total nitrogen (%) fixed by ***Anabaenaorientalis***at different concentrations (ppm) of Furadan, Sevin, Rogor and Endotaf pesticides