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Short Communication

Effect of carbamate, organophosphate and organochlorine pesticides on nitrogen fixation of cyanobacteria *Nostocpaludosum*: a quantitative assay

Ganesh S. Shinde
Department of Botany, K.J. Somaiya College of Arts, Commerce and Science, Kopargaon (MS), India
gsshindebhumi@gmail.com

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Abstract

During the present investigation, four commonly used commercial grade pesticides Furadan and Sevin belongs to carbamate and Rogor and Endotaf from organophosphate and organochlorine group, respectively, were used to study their effect on the nitrogen fixation of commonly occurring cyanobacteria (blue-green alga), nostocpaludosum. Total nitrogen (%) fixed by the tested alga at each concentration (ppm) of studied pesticides was estimated by conventional Micro- kjeldahl method. The results obtained in the laboratory cultures indicates that, total nitrogen content fixed was progressively decrease with the increase in concentrations of the Furadan, Sevin, Rogor and Endotaf pesticides by tested Nostocpaludosum. However, at lower doses of the pesticides viz. 2.5 ppm to 10 ppm of Furadan, Sevin and 2.5 ppm to 5 ppm Rogor and 2.5 ppm of Endotaf, total nitrogen fixed by the tested alga increased over the control. While at higher dose level i.e. 250 ppm Furadan, Sevin, Rogor and 100 ppm Endotaf, the Nostocpaludosum showed 76.5%, 81.4%, 88.2% and 94.8% decline in total nitrogen content than the control respectively. In general, it was seen that at higher doses of pessticides application i.e. more than 10 ppm Furdan, Sevin, Rogor and Endotaf adversely affected the nitrogen fixation efficiency of Nostocpaludosum in the laboratory cultures. Further it was concluded that, indiscriminate use of studied pesticides had deleterious effect on survival, nitrogen fixation of Nostocpaludosum and also at the recommended doses of pesticides had no adverse effects under various crop fields.

Keywords: *Nostocpaludosum*, furadan, sevin, rogor and endotaf pesticides, nitrogen fixation.

Introduction

Cyanobacteria (Blue-green algae) are unique in reducing the atmospheric nitrogen by the process of "Biological nitrogen fixation"[1]. The cyanobacteria contain nitrogenase and fix atmospheric nitrogen for which these are used as biofertilizer to maintain and improve soil status[2]. The observation in this regard showed that *Nostoc*, *Calothrix, Hapalosiphon, Aulosira* and *Anabaena* were dominant nitrogen fixing cyanobacteria encountered in various agro-practices areas of Kopargaon tahsil, Maharashtra state. Such forms hold promise for crops such as maize, rice, mungbean, tomato and sugarcane[3] and wheat[4] by fixing nitrogen. The potential impact of these microorganisms on agriculture through their use as biofertilizers, soil conditioner, plant growth regulators and soil health ameliorators has been well recognized[5,6].

In agriculture, introduction of fertilizer responsive crop varieties has necessitated the use of enormous amounts of pesticides during production and storage. In India, about 125 pesticides are registered and newer compounds are being added to the list to overcome the problem of acquired resistance. In 1980, about 40,000 formulations were commercially available with over 2000 new ones being added to the market each year[7]. These are dispersed in water as fine particles that are attracted to surfaces. This affinity results in their accumulation through adsorption on to the surface of living organisms[8].

Variety of pesticides like organochlorines, organophosphates, carbamates and pyrethroids are now in use. Their effect could be inhibitory, selective or even stimulatory depending on the type, biological property and concentration of pesticides and the algal strains[9].

The present data suggest that cyano bacterial strains used in biofertilizers have capacity to tolerate the pesticide levels recommended for field applications. Several researchers have reported the toxicity of a variety of fungicides, insecticides, herbicides and other pesticides to unialgal cultures of blue-green algae[10-22].

Blue-green algae are eco- friendly biofertilizers as well as having ability to adopt, survive, establish and colonize the soil in reasonable time. Such investigations are useful in awakening the farmers to adopt better farm management practices that in turn will reduce the chemical fertilizer input and problem of environmental degradation due to excessive use of pesticides.

By considering all these issues along with societal responsibilities the present investigation was carried on effect of commonly used pesticides viz. carbamate, Furadan, Sevin, organophosphate, Rogor and organochlorine, Endotaf on the nitrogen fixation efficiency of commonly occurring Cyanobacteria, *Nostocpaludosum* isolated from agro-practices areas of Kopargaon tahsil, Maharashtra state.

Materials and methods

The soil samples were collected from agro-practices areas of Kopargaon tahsil as per the procedure given by Singh R.N.[23]. Modified nitrogen free Fogg's[24,25] and BG-11[26] liquid medium were used for isolation and pure culture of blue-green algae from the collected soil samples. Unialgal isolates were grown and replicated in conical flasks containing 50 ml nitrogen free Fogg's medium and incubated for 28 days at 28±2^{0}C under 16/8 hrs. light/ dark cycles with 2-5 K Lux light intensity from white fluorescent tubes. Solid agar media were also prepared by adding 2% agar-agar powder in the above mentioned liquid media. These media were sterilized and used to prepare plates and slants to obtain unialgal cultures. Blue-green algal growth appeared in the plates and tubes were isolated and purified by repeated subculturing in liquid medium followed by dilution and streak plate method on solid BG-11 medium. Taxonomic identification and characterization was done by following the monographs and keys given by Prescott G.W.[27], Desikachary T.V.[28]. From the raised unialgal cultures, a commonly occurring Cyanobacteria *Nostocpaludosum* is used for the present study.

The effect of commonly used four commercial grade pesticides viz. Carbamate, Furadan, Sevin, organophosphate, Rogor and Organochlorine, Endotaf was studied on nitrogen fixation efficiency of *Nostocpaludosum* in experiments with 2.5, 5, 10, 20, 50, 100, 250 and 500 ppm concentrations of each pesticide in the 50ml of nitrogen free BG-11 medium. Experiments were conducted in triplicate sets by inoculating equal amounts of actively growing unialgal isolate *Nostocpaludosum* into cotton stoppered conical flasks. Total nitrogen fixed by the alga after 28 days of harvesting at each concentration of studied pesticides was estimated by conventional Micro- kjeldahl method[29] in the laboratory cultures.

Results and discussion

The pragmatic results regarding nitrogen fixation of Cyanobacteria, *Nostocpaludosum* at various concentrations of studied pesticides in laboratory cultures were depicted in Table-1 and was proved statistically significant. A progressive decrease in the total nitrogen fixed was seen with increasing concentrations of the pesticides concentrations in the tested blue-green alga. However at the lower doses of pesticides viz. 10 ppm of carbamate, Furadan, Sevin; organophosphate, Rogor and 2.5 ppm of organochlorine, Endotaf, the total nitrogen fixation was also increased in *Nostocpaludosum*.

Table-1: Effect of different concentrations of Furadan, Sevin, Rogor and Endotaf pesticides on total nitrogen (%) fixed by *Nostocpaludosum*. (Harvested after 28 days of incubation).

Conc. of pesticides (ppm)	0.00 (Control)	2.5	5	10	20	50	100	250	500
Furadan	4.09	4.28 (+4.6)	4.49 (+9.7)	4.15 (+1.4)	2.98 (-27.1)	2.34 (-42.7)	1.78 (-56.4)	0.96 (-76.5)	--
Sevin	4.09	4.38 (+7.0)	4.22 (+3.1)	4.18 (+2.2)	3.10 (-24.2)	2.72 (-33.5)	1.46 (-64.3)	0.76 (-81.4)	--
Rogor	4.09	4.18 (+2.2)	4.25 (+3.9)	3.57 (-12.7)	2.76 (-32.5)	1.68 (-58.9)	1.12 (-72.6)	0.48 (-88.2)	--
Endotaf	4.09	4.37 (+6.8)	4.19 (-2.4)	2.92 (-28.6)	2.24 (-45.2)	1.15 (-71.8)	0.21 (-94.8)	--	--

Values represents mean of three replicates; figures in parenthesis () show percent increase (+) or decrease (-) relative to the nitrogen fixation in the control.

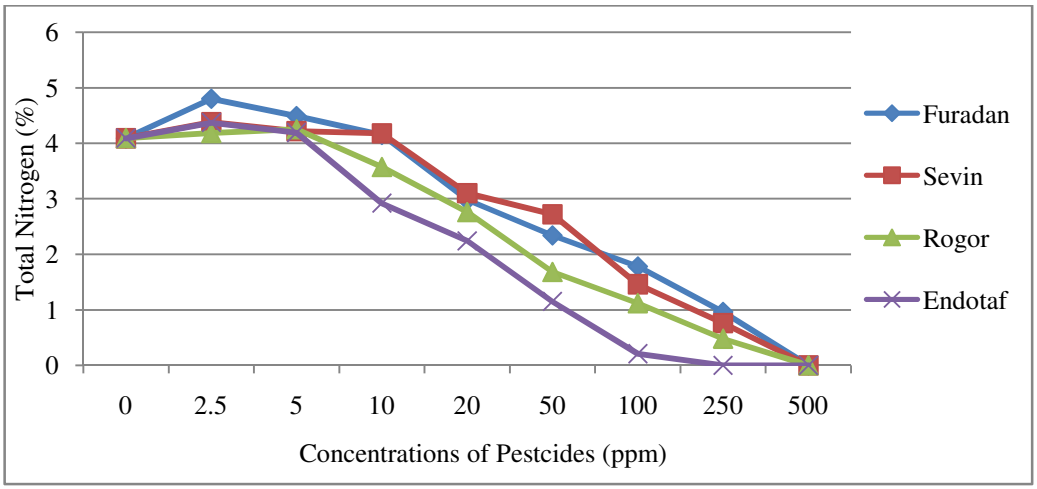

Figure-1: Effect of Furadan, Sevin, Rogor and Endotaf pesticides on total nitrogen fixation (%)by *Nostocpaludosum.*

While at 20 ppm dose level of carbamate and organophosphate pesticides, total nitrogen content was consistently decreased with the further increasing concentrations of pesticides. At the higher dose level 250 ppm with Furadan, Sevin and Rogor, *Nostocpaludosum* showed 76.5%, 81.4% and 88.2% decrease in total nitrogen content respectively over the untreated control, where as at 500 ppm dose level growth and nitrogen fixation was ceased. On the other hand, with Endotaf even at 100 ppm concentration, 94.8% decrease in total nitrogen content was noted and further increase in pesticide dose level (at 250 ppm) the growth and nitrogen fixation was terminated in Cyanobacteria, *Nostocpaludosum*.

The results obtained during the present investigation revealed that, in the laboratory cultures organophosphate, Rogor and organochlorine, Endotaf pesticides were found to highly toxic as compared to carbamate pesticides Furadan and Sevin to the tested blue-green alga. Further, a progressive decline in the total nitrogen content fixed by the blue-green alga occurs with the increase in pesticides concentrations level. Among the studied four different pesticide treatments, Endotaf was found to be highly toxic to the *Nostocpaludosum* than the other studied pesticides.

This reduction in total nitrogen content (%) of the pesticide-adapted strain of *Nostocpaludosum* may exhibited due to the inhibition of some stages during the process of nitrogen fixation in presence of higher concentration levels of pesticides. Further stimulatory effect of Furadan, Sevin at lower concentrations on nitrogen fixation by *Nostocpaludosum* under culture conditions may be due to the presence of nutrients in media that minimizes the toxicity of carbofuran[30]. These views are coincides with the reports of earlier workers; Furadan[31]; Sevin[32]; organo-chlorine[33]; Monocrotophos and Butachlor[34] and Rogor[22].

Conclusion

From the pragmatic results, in general it was seen that higher levels of pesticides application i.e. more than 20 ppm of Furadan and Sevin, and even 10 ppm of Rogor and Endotaf, adversely affected the survivability and total nitrogen content fixed by Cyanobacteria *Nostocpaludosum*in the laboratory culture, which are responsible for nitrogen fixation. Further it was concluded that the recommended doses of field application of studied pesticides had no deleterious effect on nitrogen fixation of blue-green alga[35]. Caution should be taken to determine the appropriate dosage of these agro-pesticides application before using them into the crop fields. Since, it is essential to screen efficient cyano bacterial strains those are capable of growing and fixing nitrogen at higher rate even in presence of recommended doses of the agrochemicals including pesticides, before their inoculation into the field.

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