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Differential response of cyanobacteria to sevin (carbaryl, 50%) and endotaf (endosulfan, 35%) pesticides: A qualitative and quantitative assay

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Abstract
In the present research endeavor, a total of 19 cyanobacterial taxa appeared in the composite soils in the control flasks belonging to 14 genera and 06 families. Their taxonomical characterization was made by using standard literature following Desikachary after 30 days of incubation. Stock solutions of Sevin (carbaryl, 50%) and Endotaf (endosulfan, 35%) pesticides were freshly prepared and added to the BG-11 culture media to obtain the 100, 250, 500 and 1000 ppm concentrations. The occurrence of cyanobacteria remained almost unaffected even in the presence of Sevin pesticides at 100 ppm dose in the test soil. However, their quantitative occurrence was decreased to a considerable extent. With increase in the dose level, proportionate decrease in their occurrence was noted. At 500 ppm dose level, most of the unicellular and filamentous forms of the cyanobacteria could not grow in the test soils except the cyanobacterial species *Gloeocapsa kuetzingiana*, *Lyngbya polysiphoniae*, *Scytonema subtile* and *Calothrix javanica*. The effect of organochlorine pesticides, Endotaf on the occurrence of cyanobacterial forms were quite specific. These cyanobacterial species were almost completely eliminated from the soils in presence of 500 ppm concentration of Endotaf. Only *Lyngbya polysiphoniae* and *Calothrix javanica* tolerated upto 500 ppm of Rogor. However, few filaments of *Lyngbya polysiphoniae* appeared in the soils supplemented with 500 ppm of Endotaf. Further increase in pesticides concentration at 1000 ppm with Rogor and Endotaf none of the cyanobacterial species were able to grow. The obtained results showed that, the organochlorine pesticide Endotaf was more toxic than the carbamate, Sevin to the cyanobacteria. From the obtained results, it was also seen that with higher doses of pesticide application in the crop fields i.e. more than 100 ppm of Sevin and even at 100 ppm of Endotaf, qualitative and quantitative occurrence of heterocystous and non- heterocystous cyanobacteria decreased considerably.

Keywords: sevin, endotaf, cyanobacteria, qualitative and quantitative occurrence

Introduction

Cyanobacteria are unique in reducing the atmospheric nitrogen by the process of Biological nitrogen fixation [1]. The cyanobacteria contain enzyme nitrogenase and can able to fix atmospheric nitrogen for which these are used as biofertilizer to maintain and improve soil status [2]. Cyanobacteria have potential impact on agriculture through their use as biofertilizers, soil conditioner, plant growth regulators and soil health ameliorators.

Introduction of fertilizer responsive crop varieties has necessitated the use of enormous amounts of pesticides during production and storage. These cyanobacterial forms used in biofertilizers are capable of tolerating pesticide levels recommended for field applications and the effect of individual agrochemical on individual species is extremely variable, but generally harmful [3]. The present situation of environmental problems that has been created due to indiscriminate and excessive use of pesticides and variety of agrochemicals envisioned to control the pests in various crops. 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. This affinity results in their accumulation through adsorption on to the surface of living organisms [4], [5], [6]. These agrochemicals also damage wide variety of beneficial microorganisms because of their long persistence in the environment [7], [8]. Therefore, pesticides used in routine applications in crop fields have important ecological effects in addition to those usually anticipated. To address these issues along with social responsibilities, the present investigation was carried out to study the influence of Sevin and Endotaf pesticides on qualitative and quantitative occurrence of soil cyanobacteria in the laboratory cultures.

Materials and Methods

Commonly used pesticides Sevin (carbaryl, 50%) and Endotaf (endosulfan, 35%) belonging to carbamate and organochlorine group were used for studying its effect on survivability of soil cyanobacteria. 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 of this region.

The carbamates are used as contact and stomach action pre-emergence systemic pesticides that make disturbance in the cholin esterage reversible activity while organochlorine, Endotaf attacks on central nervous system and make interference in α-amino butyric acid receptor activity of the pest. The recommended pesticide dose to control various crop pests are 0.75 kg/ha for carbaryl, Sevin and 0.5 liter/ha for endosulfan, Endotaf that will offer a range of 5- 10 ppm in the

crop field.

Stock solutions of these pesticides were prepared freshly in the sterilized media and added to the BG-11 [9] culture media to obtain the desired concentrations (100, 250, 500 and 1000 ppm). The pH of all the media was adjusted to 7.5. Ten gram proportions of air dried soil was taken in sterilized 50 ml of the BG-11 medium (with different concentrations of various pesticides). After addition of nutrient solution. the cultures were agitated to ensure uniform distribution of the pesticides. For each concentration, triplicates were set up and incubated at 28 ± 2^0 C under 16/ 8 hours light/ dark cycles with 2- 5 K Lux light intensity from white fluorescent tubes.

The cyanobacterial forms appeared in the culture flask after 30 days of incubation were identified using standard monographs of [10], [11], [12]. The survival percentage of the cyanobacteria was calculated in the laboratory cultures on the basis of the number of species present in the respective treatments by taking survival in the control flask as 100%.

Results

A total of 19 cyanobacterial forms appeared in the composite soils of the control flasks which belongs to 14 genera and 06 families. Their taxonomical characterization was made by using standard and relevant literature given by [11]. These isolated species belonged to three orders *viz*. Chroococcales, Nostocales and Stigonematales. The order Chroococcales represented by 3 genera and 3 species, which holds *Chroococcus minutus, Gloeocapsa kuetzingiana* and *Aphanothece pallida*. The order Nostocales signified by 9 genera, among which family Oscillatoriaceae consists of 3 genera and 4 species *viz*. *Oscillatoria okeni, O. animalis, Phormidium fragile* and *Lyngbya polysiphoniae*. The family Nostocaceae denoted by 4 genera and 8 species, which are *Cylindrospermum musicola, Nostoc punctiforme, N. paludosum, N. Linckia, N. calcicola, Anabaena oryzae, A. fertilissima* and *Aulosira aenigmatica*. The family Scytonemataceae and Rivulariaceae shows single genus each with its species *viz*. *Scytonema subtile* and *Calothrix javanica* respectively. While the order Stigonematales characterized by 2 genera with its species, *Hapalosiphon welwitschii* and *Westiellopsis prolifica* belonging to single family Stigonemataceae.

Among the 19 cyanobacterial species appeared in the control flask, 07 species were non- heterocystous and 12 were heterocystous. *Nostoc punctiforme, Anabaena fertilissima, Hapalosiphon welwitschii* and *Westiellopsis prolifica* were present abundantly in the test soils, have been reported to fix atmospheric nitrogen in pure culture [13] and thus signifying the richness of beneficial cyanobacteria in the soils of the study area.

The results revealed that, the occurrence of cyanobacteria were remained almost unaffected even in the presence of Sevin at 100 ppm dose level in the test soil. However, their quantitative occurrence was decreased to a considerable extent (Table- 1b). Further increase in the pesticide dose level, proportionate decrease in their occurrence was noted.

At 500 ppm dose level of the carbamate pesticides Sevin, most of the unicellular and filamentous forms of the cyanobacteria could not grow in the test soils. To the contrary, *Gloeocapsa kuetzingiana, Lyngbya polysiphoniae, Scytonema subtile* and *Calothrix javanica*, which possess well-defined sheath around their cells or trichomes were found exceptional to this. While, none of the other cyanobacterial taxa tested could tolerate 1000 ppm concentration of the carbamate, Sevin. (Table- 1a)

The effect of the organochlorine, Endotaf was quite specific on the occurrence of cyanobacterial forms. Even at 100 ppm of Endotaf, both qualitative and quantitative occurrence of heterocystous and non- heterocystous cyanobacteria was decreased considerably. These cyanobacterial species were almost completely eliminated from the soils in presence of 500 ppm concentration of Endotaf. With further increase in the pesticide concentration, almost all the cyanobacterial forms did not grow in the culture. However, few filaments of *Lyngbya polysiphoniae* appeared in the soils supplemented with 500 ppm of Endotaf. Further increase in pesticides concentration at 1000 ppm of Endotaf, none of the cyanobacterial species could grow. (Table- 1a)

The results regarding the occurrence and survival percentage of cyanobacteria in the culture indicated that, soil cyanobacteria show variable resistance to pesticide treatments. The survival percentage of the cyanobacteria was reduced upto 50% at 500 ppm of carbamate pesticide, Sevin. Whereas, 5.26% survivability was observed at 500 ppm dose level of Endotaf. (Table- 1a and b)

Table 1a: Qualitative occurrence of cyanobacteria in the presence of Sevin and Endotaf pesticides at the end of 30 days.

Sr. No.	Cyanobacterial species	Control	Sevin (ppm)				Endotaf (ppm)			
			100	100	250	500	1000	250	500	1000
1.	*Chroococcus minutus* (Kuetz.) Nag.	+	+	+	-	-	-	+	+	-
2.	*Gloeocapsa kuetzingiana* Nag.	+	+	+	+	-	-	+	+	+
3.	*Aphanothece pallida* (Kuetz.) Rabenh.	+	+	-	-	-	-	+	-	-
4.	*Oscillatoria okeni* Ag. ex Gomont	+	+	+	-	-	-	+	+	-
5.	*O. animalis* Ag. ex Gomont	+	+	+	-	-	-	+	+	-
6.	*Phormidium fragile* (Meneghini) Gomont	+	+	+	-	-	-	-	-	-
7.	*Lyngbya polysiphoniae* Fremy	+	+	+	+	+	-	+	+	+
8.	*Cylindrospermum musicola* Kuetz. ex Born.et Flah.	+	-	-	-	-	-	+	-	-
9.	*Nostoc punctiforme* (Kuetz.) Hariot.	+	+	-	-	-	-	+	-	-
10.	*N. paludosum* Kuetzing ex Born. et Flah.	+	+	+	-	-	-	+	+	-
11.	*N. linkia* (Roth) Bornet ex Born. et Flah.	+	+	+	-	-	-	+	-	-
12.	*N. calcicola* Brebisson ex Born. et Flah.	+	+	+	-	-	-	+	-	-
13.	*Anabaena oryzae* Fritsch	+	+	-	-	-	-	-	-	-
14.	*A. fertilissima* Rao, C. B.	+	+	-	-	-	-	+	-	-

15.	*Aulosira aenigmatica* Fremy	+	+	+	-	-	-	+	-	-
16.	*Scytonema subtile* Mobius	+	+	+	-	-	-	+	+	-
17.	*Calothrix javanica* de Wilde.	+	+	+	-	-	-	+	+	-
18.	*Hapalosiphon welwitschii* W. et G. S. West	+	-	-	-	-	-	-	-	-
19.	*Westiellopsis prolifica* Janet	+	+	+	-	-	-	+	+	-
Total number of cyanobacterial species appeared		19	17	16	13	02	01	00	09	02
Percentage (%) survival		100	89.47	84.21	68.42	10.52	5.26	00	47.36	10.52

(+ Present, - absent)

Table 1b: Quantitative occurrence and survival percentage of cyanobacterial species in the culture at different concentrations of pesticides.

Pesticide	Concentration (ppm)	Cyanobacteria			
		Heterocystous		Non- heterocystous	
		Total number of species	Percentage survival	Total number of species	Percentage survival
Control	Without Pesticide	16	100	03	100
Sevin	100	14	87.5	03	100
	250	13	81.2	03	100
	500	07	43.7	02	66.6
	1000	01	6.2	01	33.3
Endotaf	100	11	68.7	02	66.6
	250	01	6.2	01	33.3
	500	01	6.2	00	00
	1000	00	00	00	00

Discussion

The pragmatic results showed that, the organochlorine pesticide Endotaf was more toxic than the carbamate, Sevin to the tested cyanobacteria. [14] found that, survivability and nitrogen fixation of *Westiellopsis prolifica* was reduced to 72 and 93 percent, respectively in the presence of 100 ppm of Endotaf and concluded that Endotaf was more toxic than the Furadan, Sevin and Rogor pesticides. [15] recorded the tolerance level of Carbendazim was 500 ppm for *Aulosira fertilissima* and Captafol was tolerated at 100, 500 and 1000 ppm by *Calothrix*, *Westiellopsis* and *Nostoc* species, respectively. Similarly, [16] reported that Endosulfan was found to be more effective in reducing the survivability of *Chlorella vulgaris* and *Anabaena doliolum* than dimethoate, Rogor. According to [17], organochlorine compounds alter cell membrane permeability and integrity and interfere with synthesis of DNA, RNA and proteins and can inhibit enzyme activity and photosynthesis. In this view organochlorine pesticides were found to be hazardous due to their toxicity and longer persistence in the environment. [18] reported *Anabaena* sp. was found to be the most susceptible while *Westiellopsis prolifica* as the most tolerant strain to the Monocrotophos and Butachlor pesticides. The glutamine synthetase activity and release of free amino acids in the culture medium of cyanobacteria were also reduced in presence of pesticides.

[19] found that, low concentrations of Dichlone stimulated growth of *Nostoc calcicola*, *Nostoc muscorum* and *Anabaena cylindrica* cyanobacteria, while decrease in growth was observed at higher concentrations. Similarly, [20] reported the 1000 ppm of two organophosphate pesticides, Dimeton and Rogor as lethal for *Nostoc linckia* and *Anacystis nidulans*. [6] observed that Endosulfan at 12 ppm proved lethal for *Anabaena* sp. while extreme fragmentation and subsequent death occurred in *Spirulina platensis* at 8 ppm. Among the organisms, *Anabaena* sp. was more tolerant of Endosulfan than *S. platensis*. Likewise, [3] studied comparative tolerance of Isoproturon, Saturn and BHC on a heterocystous cyanobacterium *Cylindrospermum majus* and concluded that the level of tolerance was upto 300 µg/ml for Isoproturon and Saturn, while it was 0.5 µg/ml for BHC. [5] found *Calothrix parietina* was comparatively more tolerant, while *Anabaena variabilis* was more sensitive to an organophosphate pesticide, Rogor (dimethoate 30 EC). However, *Nostoc muscorum* that does not possess thick slime layer tolerated relatively higher pesticide doses. [21] revealed the average acute toxicity of the carbamate insecticides to the cyanobacteria and the green algae in the descending order of carbaryl > carbofuran, Propoxur, Metolcarb > carbosulfan. They found wide variations in response to the tested carbamate insecticides among the eight individual species of cyanobacteria and green algae.

Summary and Conclusion

It was also seen that qualitative and quantitative occurrence of heterocystous and non- heterocystous cyanobacteria was decreased considerably with higher doses of pesticide application in the crop fields i.e. more than 100 ppm of Sevin and even at 100 ppm of Endotaf. Furthermore, sheathless heterocystous and unicellular forms of tested cyanobacteria are more sensitive to the Sevin and Endotaf pesticides application than the heterocystous ensheathed and non-heterocystous ensheathed forms. The ensheathed cyanobacterial forms were resistant to the studied pesticides which is probably due to the presence of sheath outside the cell wall of these algae, which protects them from the adverse effect of the toxic agrochemicals. This suggest that, indiscriminate use of these pesticides may cause adversative effects on the occurrence and survival of nitrogen fixing cyanobacteria of various crop fields, which has a direct influence on total productivity.

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