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TOLERANCE OF ALGAL POPULATION IN RICE SOIL TO CARBOFURAN APPLICATION

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FURADAN 3G (3% carbofuran; 2,3-Dihydro-2, 2-dimethyl-7-benzofuranyl *N*-methylcarbamate), the most effective systemic insecticide against rice brown planthopper (*Nilaparvata lugens* Stal.), has been extensively used in rice cultivation. Recent reviews indicate that carbofuran affects soil microbial populations and various elemental transformations, mediated by micro-organisms, of importance to the soil fertility[1,2]. Several workers[3,4] reported its toxicity to pure cultures of algae. However, the influence of carbofuran on native populations of soil algae, a major ecologically beneficial group of soil microflora, has not been studied. An attempt was therefore made to determine the effects of carbofuran on quantitative and qualitative occurrence of algal population in flooded and non-flooded conditions of soil, as existing in rice culture.

Twenty-gram portions of air-dried and sieved black soil (pH 7.4, organic matter 1.82%, total nitrogen 0.044%), collected from a fallow rice field, were taken in test tubes (25 × 150 mm) and treated with aqueous solution of the commercial formulation of carbofuran (50 WP from Rallis India Ltd., Bangalore). Final concentrations were 0, 0.5, 1, 2 and 5 kg a.i. ha^{-1} on a dry weight basis[5]. For each treatment, one set of tubes was maintained at 50% water-holding capacity to provide non-flooded conditions while another set was flooded with distilled water to provide 1:1.25 soil to water ratio[6]. The tubes were incubated at room temperature (27 ± 4°C). After 10 and 20 days of the insecticide treatment, the soil samples, maintained under the two water regimes, were withdrawn for estimation of algae by enrichment culture technique followed by the most-probable number (MPN) method[6]. Fiducial limits for the MPN values of viable cells at the 95% confidence level were also calculated as outlined earlier[5]. Most of the algal forms were identified at least to genus and the frequency of their occurrence was also recorded. No attempt was made to determine the actual number of filaments/cells of each alga which appeared in the MPN tubes.

Application of carbofuran, at 0.5 and 1 kg ha^{-1} levels, to soil under non-flooded conditions either did not affect or slightly enhanced the algal population (table 1). There was also a slight increase in the population size at 2 kg ha^{-1} level in 10 days after treatment but this concentration exhibited toxic effect towards the end of 20 days. Highest level (5 kg ha^{-1}) was significantly toxic to the algae at the start. Thus, only 8 out of 30 MPN tubes, maintained for this concentration, were scored positive for algae after 10 days. Carbofuran application up to 2 kg ha^{-1} level under flooded conditions of the soil significantly increased the algal populations and this trend continued for 20 days. On the contrary, application of carbaryl, a closely related methylcarbamate, to soil under flooded conditions was found to have an inhibitory effect on the population size of algae while a significant enhancement in algal population was observed with its addition up to 25 ppm to non-flooded soil[6]. Although carbofuran is known to yield significant amounts of carbofuran phenol upon its hydrolysis in predominantly anaerobic (flooded) conditions of soil[7], it would seem that the hydrolytic product of carbofuran had no toxic effect on soil algae. However, slight algal toxicity was observed with 5 kg ha^{-1} level. It is evident from these results that application of carbofuran, close to field doses, in rice cultivation would result in the enhancement of algal population in soil.

The soil algal populations belonged to four species of Chlorophyta and 9 of Cyanophyta (table

Table 1 *Algal population (per gram) in untreated and carbofuran-treated soil samples maintained under non-flooded and flooded conditions*

Carbofuran application (kg ha^{-1})	Sampling time, days after application: 10 — MPN ($\times 10^3$)	10 — 95% fiducial limits: Upper	10 — 95% fiducial limits: Lower	20 — MPN ($\times 10^3$)	20 — 95% fiducial limits: Upper	20 — 95% fiducial limits: Lower
			Non-flooded soil			
0	15.9	29.2	8.7	13.6	25.1	7.4
0.5	18.5	33.9	10.1	15.9	29.2	8.7
1.0	15.9	29.2	8.7	13.6	25.1	7.4
2.0	18.5	33.9	10.1	11.7	21.6	6.4
5.0	4.6	8.4	<4.1	11.7	21.6	6.4
			Flooded soil			
0	11.7	21.6	6.4	8.7	16.0	4.7
0.5	18.5	33.9	10.1	10.2	18.6	5.5
1.0	18.5	33.9	10.1	10.2	18.6	5.5
2.0	15.9	29.2	8.7	10.2	18.6	5.5
5.0	10.2	18.6	5.5	6.4	11.7	<4.1

2). In general, the filamentous blue-greens, viz. *Anabaena variabilis, Lyngbya gracilis, Nostoc punctiforme* and *Phormidium tenue,* were consistently occurring in all carbofuran-treated (at all concentrations) soil samples, maintained under two water regimes, suggesting that the filamentous cyanobacteria are generally favoured upon carbofuran application to soil. This finding supports the reported enhancement in survival, growth and nitrogen fixation by a blue-green alga, *Nostoc muscorum*, when carbofuran was added at 25 ppm in culture medium[3]. However, unicellular forms such as *Chlorococcum humicola*, *Gloeocystis gigas*, *Scenedesmus bijugatus* and *Synechococcus elongatus*

Table 2 *Qualitative occurrence of soil algae at the end of 20 days after carbofuran treatment under two water regimes*

Organism	Non-flooded: 0 *	0.5	1	2	5	Flooded: 0	0.5	1	2	5
Chlorella vulgaris	+	−	+	−	−	−	−	−	−	−
C. humicola	−	+	−	−	−	−	+	−	−	−
G. gigas	−	+	−	−	−	−	+	−	−	−
S. bijugatus	+	+	−	−	−	−	+	−	−	−
A. variabilis	++	++	++	++	−	++	+	++	++	++
Lyngbya gracilis	++	+++	+++	+++	+++	++	+++	+++	++	++
Nostoc linckia	+	+	−	−	−	++	+	−	−	−
N. punctiforme	++	++	+	+	++	+	−	++	+	+
N. muscorum	+	−	+	−	−	−	−	−	+	−
Oscillatoria sp.	+	−	+	+	+	+	−	+	−	−
Phormidium faevolarum	+	+	+	+	−	−	+	+	−	+
P. tenue	++	++	++	++	+++	++	++	+++	+++	+++
S. elongatus	−	−	−	−	−	+	+	−	−	−

* Rate of carbofuran application, kg ha^{-1}; − = absent; + = common; ++ = more abundant; +++ most abundant.

appeared especially in flooded soil samples when carbofuran was treated only at 0.5 kg ha^{-1} level, the reason for which is not clearly understood. This observation is in contrast to the reported total restriction of unicellular algae with the addition of carbaryl to the soil[6]. Thus, the present investigation reveals that carbofuran application, at close to field rates, is likely to increase the populations of cyanobacteria which have been particularly implicated in the nitrogen economy of agricultural soils. However, the impact of inert material, present in the commercial formulation, on the soil algal flora must be clearly understood although it is likely that the carrier, in general, causes no biological effect.

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INDUCTION OF SPORULATION IN *ALTERNARIA PORRI IN VITRO*

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ALTERNARIA PORRI (Ellis) Cif. is world wide in distribution causing purple blotch disease, mainly in onion and garlic. The difficulty in sporulation of *A. porri* on culture media as well as on host under normal conditions has been reported[1–4]. In the present investigation, out of the 5 isolates of *A. porri* isolated from onion (*Allium cepa* L.)[5], none could sporulate on the conventional media.

In the present studies some specific methods suggested by Sproston and Setlow[6] and Srinivasan *et al*[7] for inducing sporulation in various fungi were also employed. None, except Srinivasan *et al*'s[7] method in modified form could induce sporulation in isolate Ap-5 of *A. porri*. In the modified method, leaves of 30-day-old seedlings of *Pennisetum americanum* (L.) Leeke were cut into small bits of one cm, suspended in distilled water and autoclaved at 1.045 kg/cm^2 pressure for 15 min. After absorbing the excess moisture on sterile blotters, 3 leaf pieces were transferred to the surface of sterilized calcium carbonate agar medium (Calcium carbonate, 3 g; agar, 15 g; distilled water 1 l) contained in a petri dish. A 2 mm mycelial disc of each isolate cut out from active growing mycelium of 7-day-old culture of *A. porri* grown at 25 ± 1°C on Czapek's agar were placed on the surface of each piece of the leaf and the plates were incubated at 25 ± 1°C. After 48 h plates were examined daily under the steriobinocular. Sporulation occurred in Ap-5 isolate of *A. porri* after 4 days of incubation on pearl millet leaf bits. The spores obtained by the above method were found pathogenic when spray inoculated on leaf/seed-stalk of onion cv 'Nasik Red'.

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