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# Effects of Ekalux Ec-25 on Akinete Germination and Sporulation of *Pithophora kewensis*

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**ABSTRACT.** The effect of the organophosphorus insecticide Ekalux Ec-25 (quinalphos) was studied on akinete germination and sporulation of the green alga *Pithophora kewensis* (*Cladophorales*). Initiation of akinete germination was delayed by 1–3 d with 0.025–0.1 % concentrations of the pesticide used. The percentage of akinete germination was found to be markedly affected by the treatment with insecticide, except with the concentration of 0.25 % Ekalux Ec-25, where percent germination was observed to be higher than in the control. Generally, the time taken for the initiation of akinete formation increased and percent sporulation decreased with the increase of concentrations from 0.025 to 0.1 % of Ekalux Ec-25.

*Pithophora*, a filamentous green alga (*Cladophorales*) occurs abundantly in eutrophic water bodies including the rice fields of the Varanasi district and forms thick, free-floating mats, causing a great nuisance to the habitat. It is extremely difficult to control the growth of the alga because of its resistance to presently applied herbicides as it forms akinetes that are probably resistant to the herbicide.

Agrawal (1983) and Chaudhary and Singh (1986) investigated the effects of copper sulfate and mercuric chloride, respectively, on the growth of *Pithophora* spp. Ware *et al.* (1968) suggested that the filamentous alga *Cladophora* may be used as an indicator of DDT contamination in water as it accumulated higher residues of DDT than higher plants did. Christie (1969) showed that 100 ppm malathion (an organophosphorus compound) had an insignificant effect on *Chlorella pyrenoidosa*. Similar results were also obtained by Moore (1970) in the flagellate *Euglena gracilis*. An increase in algal populations of the standing water of rice fiels was observed by the use of diazion for insect control (Sethunathan and McRae 1969). The use of pesticides and herbicides pose the threat of their side effects on non-target organisms and such effects are not completely understood. Shirashu and co-workers (1976) suggested that many of these chemicals may be carcinogenic and mutagenic.

While studying the effects of Ekalux Ec-25 (quinalphos as active ingredient) on the rice crops, the senior author observed that the recommended doses of this insecticide, besides controlling pests, also checks partially the nuisance growth of *Pithophora*. Since very little information is available concerning the effects of insecticides on akinete germination and sporulation in green algae in the present investigation an attempt was made to assess the effects of the rice-field pesticide Ekalux Ec-25, a commercial organophosphorus insecticide with quinalphos (O,O'-diethyl-O''-quinoxalin-2-yl thionophosphate) as the active ingredient on the germination and formation of akinetes in *Pithophora kewensis*. It is a contact insecticide of proven efficacy against a wide range of insect pests.

## MATERIALS AND METHODS

The alga *Pithophora kewensis* WITTR. was collected during the rainy season from the rice fields of the *Banaras Hindu University Campus*. The mature akinetes were thick-walled and dark-green to black in colour. One-year-old mature akinetes were harvested from the main filaments by homogenization at low speed and then by centrifugation. The isolated akinetes were separately washed with sterilized distilled water before treating them with the desired concentrations of pesticide solutions prepared in Chu-10 medium (Chu 1942). Equal amounts of akinetes were exposed to different concentrations, *viz.* 0.025, 0.05, 0.075 and 0.1 % of Ekalux Ec-25 solution for 2 h. The treated akinetes were washed 5–6 times with double distilled water through repeated centrifugation and were subsequently inoculated on 1 % agarized Petri plates prepared with Chu-10 nutrient medium. The plates were incubated in the culture chamber with c. 2 klx light intensity and 16:8 light-to-dark cycle. For each treatment a control was also maintained.

To find the time required for the initiation of germination and sporulation, % akinete germination and % akinete formation, regular observations were made with the help of a binocular microscope.

## RESULTS

Akinete germination began after 6 d with an increase of its size, emergence of protuberance(s) on the wall and later on growing into germination tube(s). The number of germination tubes per akinete ranged from one to four. A cross wall was laid down at the base of protuberance and in due course the latter elongated and developed into a branched filament by vegetative growth. Rhizoids were also observed in some growing germlings.

The initiation of fresh akinetes on the newly developed branched filaments took place after 17 d of inoculation of the akinetes. Akinete formation started with the migration of protoplasmic contents into the upper portion of the filaments and formation of cross walls, separating the dark-green akinetes from the rest of the cell. The akinetes were generally formed in a basipetal succession, *i.e.* from the lower to the upper (younger) portion of the filament.

TABLE I. Effect of Ekalux Ec-25 on the germination of akinetes of *P. kewensis*

| Concentration | Akinete germination on different days, % | | | | | | |
|---|---|---|---|---|---|---|---|
| % | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 0 | 12.1 | 44.5 | 52.8 | 58.6 | 58.6 | — | — |
| 0.025 | 15.4 | 48.8 | 56.5 | 63.2 | 68.3 | 68.7 | — |
| 0.05 | — | 17.5 | 36.8 | 40.6 | 42.5 | 43.8 | — |
| 0.075 | — | 13.5 | 26.8 | 29.5 | 34.4 | 35.5 | 36.2 |
| 0.1 | — | — | — | 10.2 | 16.4 | 20.8 | 21.3 |

The delay in germination due to pesticide treatment varied from 1 to 3 d. Maximum delay of 3 d in germination was observed with 0.1 % Ekalux (Table I). The control akinetes, as well as those treated with 0.025 % insecticide solution showed akinete germination after 6 d, while with 0.1 % Ekalux solution, the first germination appeared after 9 d. The maximum percentage of germination (68 %) after 10 d was recorded in akinetes treated with a 0.025 % insecticide solution, while in the control the percent germination was only 59 % after 10 d. With higher concentrations (0.05–0.1 %) the percentage of germination was dose-dependent, showing gradual reduction in the percentage of akinete germination with increase in concentration. The minimum percentage of germination was 21 % after 12 d of inoculation of akinetes treated with 0.1 % Ekalux Ec-25 (Table I).

TABLE II. Effect of Ekalux Ec-25 on sporulation of *P. kewensis* after 32 d of inoculation of akinetes

| Concentration % | Initiation of sporulation d | Akinete formation % |
|---|---|---|
| 0 | 17 | 48.6 |
| 0.025 | 17 | 45.7 |
| 0.05 | 18 | 42.3 |
| 0.075 | 22 | 31.5 |
| 0.1 | 28 | 18.6 |

In the control, akinetes began to form on the newly developed filaments after 17 d and reached the maximum after 32 d (Table II). The delay in initiation time increased and the percentage of sporulation decreased with the increase of concentration of the pesticide. At 0.1 % Ekalux Ec-25, the time required for the initiation of fresh akinete was noted to be 27 d, with a concomitantly reduced level of sporulation (19 %).

**DISCUSSION**

Ekalux Ec-25 was found to be toxic for the akinetes of *P. kewensis* at the concentration range of 0.05 to 0.1 % when it affected the time of initiation and the percentage of akinete germination as well as the initiation and extent of sporulation. Exceptionally, at 0.025 %, the germination percentage increased consistently on all days of observation. Thus stimulation of germination may be attributed to an alteration in the permeability of akinete wall and/or some biochemical changes inside the akinete, favouring the process of germination of spores. In the case of blue-green algae, stimulation of growth at a low concentration of machete was associated with an increase in the levels of RNA and photosynthetic pigments (Shuter 1979). Diazion, an organophosphorus compound used in the rice fields was also shown to promote the proliferation of algal populations (Sethunathan and McRae 1969). Pandey and Kashyap (1986) also reported that at low concentrations (0.1 to 1.0 mg/L) machete stimulated growth in three cyanobacteria.

Agrawal (1983) and Chaudhary and Singh (1986) made more or less similar observations with $CuSO_4$ and $HgCl_2$ treatments, respectively, and reported a delay in germination, reduction in germination, delay in sporulation and reduction in the extent of sporulation in *Pithophora* spp. Sarma and Agrawal (1980) and Sarma *et al.* (1983) showed the effects of UV irradiation on the initiation and germination of spores/akinetes of *Stigeoclonium pascheri* and *Pithophora kewensis*, respectively. Changes in the karyology of green algae were recorded by Srivastava and Sarma (1978), in *Oedogonium gunnii* by using the pesticides Dimecron and Nuvan and by Sarma and Abhayavardhani (1980), in *Spirogyra paradoxa*, by administering two organophosphorus insecticides Ekatin and Anthio.

Genetic variation, enzymic inactivation and metabolic inhibition are reported to cause lethal effects in organisms. Ekalux Ec-25 was reported to cause an injurious effect on chlorophyll, carotenoid, protein and ascorbic acid contents of *Oryza sativa* (Agrawal *et al.* 1986*a,b*). Ascorbic acid is known to regulate various physiological and biochemical processes in plants and help them to resist stress conditions (Freebairn and Taylor 1960). Accumulation of phenol during periods of stress is a common observation. Increased phenol levels have also been reported to inhibit $CO_2$ fixation, oxidative phosphorylation and active respiration, besides causing chloroplast disintegration (Howell 1974). The degradation of phycobilins and chlorophyll *a* occurred at a machete concentration of 5.0 mg/L in *N. muscorum*, 2.5 mg/L in *A. nidulans* and 10 mg/L in *A. doliolum* (Pandey and Kashyap 1986). Furthermore, the decrease in protein content was found to reduce the photosynthetic potential of plants due to lack of repair processes.

While experiments have been conducted and plausible explanations afforded for the toxicity of plants in the vegetative phase, the cause(s) of spore toxicity is not yet known. Since the chances of physiological and biochemical changes similar to those in vegetative cells are quite meagre in dormant spores it seems that the pesticide imbibed by spores in dormant condition affected biosynthetic processes and/or activities during germination of akinetes. This seems reasonable as akinetes exhibit greater resistance than vegetative cells do, apparently owing to their thick chitinous wall (cf. Agrawal 1983). Genetic variation caused by Ekalux Ec-25 could be another reason for spore toxicity in *Pithophora*.

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