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Observations on the Effect of the Herbicide Isoproturon on Aquatic Targets and Associated Organisms

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Aquatic weeds, particularly *Eichhornia crassipes*, have become a major nuisance in the management of freshwater bodies in many parts of India (Varshney and Singh 1976). Of the various methods that have been tried to control this noxious weed, chemical control is the most effective (Gopal and Sharma 1981; Julian 1984). Since the actions of most chemicals are not confined to the target organisms alone, it is desirable to know their effects on aquatic ecosystem as a whole (Way and Chancellor 1976). In an attempt to evaluate the effect of various herbicides on water-hyacinth, the present communication reports the effects of the urea herbicide isoproturon. The large scale use of this herbicide for the control of broad leaf weeds in wheat, barley, and other agricultural crops in many countries including India (Smith and Libingstone 1981; Bhatia *et al.* 1981) may lead to an increase in its concentration in aquatic ecosystem through agricultural runoff. The effect of this herbicide has not been evaluated for the control of water-hyacinth. Preliminary results of its effect on this noxious weed and associated organisms are reported here.

MATERIALS AND METHODS

A small freshwater pool of seasonally dried up river with no throughflow of about 9 x 4 m with an average depth of 0.3 m was selected for the present study. It contained a natural growth of aquatic macrophytes like *Eichhornia crassipes* (Mart) Solms and *Ipomoea aquatica* Forsk, besides other natural flora and fauna. The herbicide Isoproturon (50% active ingredient a.i. wettable powder) N-(4-isopropyl phenyl)-N,N-dimethyl urea, was obtained from Paushak Ltd., Baroda, India. The pool and its biotic components were studied regularly for a week before herbicide application. In

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an attempt to select a suitable concentration of the herbicide for field application, in a preliminary experiment different concentrations (0-5 mg/L) were tried in glass troughs containing 20 L of water with water-hyacinth plants under laboratory conditons. The herbicide was added by dissolving the required amount in the pool water at 2.5 mg/L a.i. concentration after calculating the total volume of pool water (Rao 1983). Water samples were collected in 1-L glass bottles from three different sites before application of herbicide. A composite sample was prepared by mixing the three samples and analysed for water qualities and initial plankton community. For estimation of plankton community a known quantity of water sample was centrifuged and concentrated sample was fixed in 4% formalin. Further, plankters were counted in haemocytometer chamber (average of ten counts) and identified. Water and plant samples were collected from the same site on every third day from the treated pool for further analysis. The important water parameters analysed were (average): temperature (31°C), pH (8.4), dissolved oxygen (5.6 mg/L), chloride (112.4 mg/L), phosphate (0.8 mg/L), nitrate (0.27 mg/L) and hardness (187 mg/L). Plant density was measured by laying 50 X 50-cm quadrat at the study sites. An average leaf area of green and nongreen parts were determined by plotting the leaf on a graph paper (reading of ten leaves). Chlorophyll contents were analysed by extracting the acetone soluble pigment from known quantity of leaf (Duxbury and Yentsch 1956). Standard deviation was calculated from three replicates of each parameter. Fish experiments were conducted in laboratory glass aquaria using *Rosbora deniconius* and *Poecilia reticulata* as test fish, obtained from Malaria Research Centre, Nadiad. Ten fish fingerlings of almost equal size and age after initial acclimatization were transferred in duplicate sets to laboratory aquaria. Known amount of herbicide was dissolved in the aquaria containing 20 L of tap water to get final concentration of 0.0 (control), 2.5 and 5.0 mg/L a.i. of the herbicide. A constant aeration for 1 hr was given every day. To keep the herbicide concentration constant the marked level of aquarium was maintained once in a day by adding tap water (hardness 195 mg/L, pH 7.5). Length, weight and survival of test fish were recorded initially and after 10 and 20 d.

RESULTS AND DISCUSSION

Effects of the herbicide on the growth pattern of two aquatic weeds are shown in Table 1. It was observed that *E. crassipes* density decreased by 34% by the third day and on sixth and ninth days it decreased by 46 and 78%, respectively. The density of *I. aquatica*, however,

Table 1: Effect of exposure to 2.5 mg/L concentration of isoproturon on *E.crassipes* and *I. aquatica*

Days	Density/m² (No.)			Leaf Area (cm)			Green area (cm)			Chlorophyll-a ($mg\ g^{-1}$)		
	X	SD	%I/D	X	SD	%I/D	X	SD	%I/D	X	SD	%I/D
E. crassipes												
0	27.0	± 4.2	--	47.6	± 3.7	--	47.6	± 3.7	--	1.21	± 0.03	--
3	17.8	± 4.0	-34	28.0	± 1.5	-41.1	9.7	± 1.7	-79.5	0.89	± 0.12	-26.7
6	14.6	± 2.6	-46	21.4	± 2.8	-55.0	4.0	± 1.2	-91.5	0.4	± 0.14	-67.0
9	5.25	± 2.3	-78.7	10.2	± 1.7	-78.5	1.5	± 0.5	-96.8	0.18	± 0.06	-84.8
I. aquatica												
0	3.0	± 0.8	--	11.5	± 1.5	--	11.5	± 1.5	--	1.66	± 0.09	--
3	2.2	± 0.4	-25	17.0	± 1.4	+47.8	17.0	± 1.4	+47.8	1.65	± 0.10	-2.3
6	3.0	± 1.2	--	25.0	± 1.6	+117.3	25.0	± 1.63	+117.3	1.78	± 0.08	+5.74
9	4.8	± 2.16	+46	34.5	± 0.8	+200	34.5	± 0.8	+200	1.93	± 0.12	+14.5

SD = Standard Deviation, I = Increase (+), D = Decrease (-)

remained unaffected until it increased to 46% on ninth day. The visible effect of herbicide on leaf was leaf-tip die-back followed by chlorosis. The average leaf area of *E. crassipes* decreased initially from 47.6 cm to 10.2 cm, a total decrease of 78% on ninth day. In contrast *I. aquatica* leaf area increased up to 200%. When the green area of the treated plant leaves was measured, it reduced drastically compared to the control. In *E. crassipes* the green part decreased from 47.6 to 1.5 cm, a decrease of 96% by ninth day. Chlorophyll-a content decreased from 1.21 to 0.18 mg g^{-1} fresh weight of leaf tissue a decrease of about 85% on ninth day. *I. aquatica* showed little effect, after a slight decrease on third day, showed 5.0 and 14.0% increase subsequently. Further on twelfth day *E. crassipes* plants become whitish and no parameters could be measured. A regular observation for more than a month revealed no sprouting or regeneration of water hyacinth plants in the treated pool. The occurrence of the more common phyto- and zooplankters collected before and after application of herbicide to the pool are shown in Table 2. The phytoplankton are represented by the members of Cyanophyceae, Chlorophyceae, Euglenophyceae and diatoms. The total counts of different groups shown in Table 3 revealed that the pool before treatment was dominated by euglenoids over other groups (E>Ch>D>Cy). However, herbicide treatment changed the algal composition. Euglenoid dominance was overtaken by diatoms; other algal species were also affected

Table 2. Composition of phytoplankton and zooplankton of the pool

Plankton	Pretreatment	Days		
		3	6	9
Phytoplankton :				
Chroococcus minor	+	+	–	+
Merismopedia glauca	+	+	+	+
Microcystis aeruginosa	+	–	–	+
Oscillatoria tenuis	+	+	+	+
Cosmarium occidentale	+	+	+	+
Schroederia setigera	+	+	+	+
Pediastrum simplex	+	–	–	–
Tetraedon trigonum	+	–	–	–
Scenedesmus abundans	+	+	–	+
S. quadricauda	+	+	+	+
Closterium monoliferum	+	+	–	+
Euglena acus	+	+	–	–
E. minima	+	–	–	+
Phacus armatus	+	–	–	+
P. circumplexus	+	–	–	+
P. caudatus	+	+	–	+
Navicula sp.	+	+	+	+
Nitzschia sp.	+	+	+	+
Zooplankton:				
Paramecium sp.	+	+	+	+
Brachionus calvciflorus	+	+	+	+
Trichocerca longiseta	+	+	+	+
Nauplius (larvae)	+	+	+	+
Cyclops sp.	+	+	+	+

+ = Presence, – = Absence.

(D>E=Ch>Cy). The phytoplankton population decreased from 29 X 10^4 to 12 X 10^4 cells mL^{-1} on ninth day a decrease of about 59%. On the contrary zooplankton density remained unaltered on final count. The total count showed a slight increase by third and sixth days ($31 \times 10^4 mL^{-1}$) but remained almost the same on ninth day ($28 \times 10^{-4} mL^{-1}$). The results of herbicide treatment on two test fish in aquaria are shown in Table 4. The length, weight and survival of the test fish were recorded on first, tenth and twentieth days. All the fish survived in all the concentrations tested. Weight and length of the test fish were not affected greatly up to 20 d, however chronic effects were not measured.

Isoproturon like other urea herbicides is translocated through the root system and inhibits photosystem II

Table 3 Plankton composition and pattern of algal groups of the isoproturon treated pool.

Days	Phyto-plankton $No. X10^4 mL^{-1}$	Zooplankton $No. X10^4 mL^{-1}$	Phyto-plankton $No. X10^4\ mL^{-1}$	Phyto-plankton Pattern
0	29	29	E = 10 Ch = 9 D = 8 Cy = 2	E>Ch>D>Cy
3	22	30	E = 6 Ch = 6 D = 8 Cy = 2	D>E=Ch>Cy
6	19	31	E = 5 Ch = 5 D = 8 Cy = 1	D>E=Ch>Cy
9	12	28	E = 3 Ch = 3 D = 5 Cy = 1	D>Ch=E>Cy

E=Euglenoid; Ch=Chlorophyceae; D=Diatom; Cy=Cyanophyceae

Table 4 Effect of isoproturon on growth parameters of 20 fish/treatment

Concentration	Weight(g/fish) Days			Length (cm/fish) Days		
	0	10	20	0	10	20
R. daniconius						
Control	11.6	12.9	13.9	4.1-6.0	4.3-6.2	4.4-6.4
2.5 mg/L	10.7	12.0	13.0	4.1-5.7	4.3-5.8	4.5-6.0
5.0 mg/L	11.0	12.2	13.1	4.2-6.3	4.4-6.5	4.4-6.8
P. reticulata						
Control	2.6	3.4	4.6	2.2-3.6	2.3-3.8	2.6-4.1
2.5 mg/L	2.5	3.5	4.6	1.7-3.5	1.9-3.8	2.1-4.3
5.0 mg/L	2.4	3.4	4.6	2.4-3.6	2.5-4.0	2.8-4.2

(Ashton and Crafts 1981). The herbicide at 2.5 mg/L, within a short span of 9 d, showed about 80% mortality on whole plant basis and 85% on chlorophyll basis, indicating its inhibition potent. Complete mortality followed and no regeneration was observed until the next month; however, a more detailed study of such a hardy weed may be useful. This herbicide is known to persist for different time periods in soil (Mudd *et al.* 1983) and complete degradation was observed within a crop season of wheat (Randhava and Sandhu 1989). However, its persistance in water and sediment has not been studied. In *I. aquatica* lack of absorption through root system, which remain anchored in the bottom or marshy land, might be the reason that it remained unaffected throughout the present study. The decrease of euglenoids and other phytoplankton due to photosynthetic inhibition by herbicide treatment has also been observed previously (Sikka and Pramer 1968; Wright 1978). This indicates the difference in tolerance by various groups of algae and could be used for selective control of a particular alga or group of algae. The non-toxic nature of the herbicide to other biotic components like zooplankton and fish is also evident from these preliminary results.

The zooplankton remained unaffected throughout the experimental period. Similarly, test fish when exposed to two times higher concentration of herbicide in aquarium they did not show mortality and survived for all the 20 d. Although indirect effect of urea compounds on fish in aquatic system is known, the chronic effect of this herbicide needs to be studied. The measurements of length and weight showed little difference from the control set of the test fish. Our results indicate that isoproturon may prove to be a selective and potent inhibitor for *E. crassipes* and safe at the concentration used for other macrophytes, phytoplankton, zooplankton and fish.

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