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EVALUATION OF BUTACHLOR FOR CONTROL OF SUBMERGED MACROPHYTES ALONG WITH ITS IMPACT ON BIOTIC COMPONENTS OF FRESHWATER SYSTEM

***[1]A. Chattopadhyay, [1]S. Adhikari, [2]S. P. Adhikary and [3]S. Ayyappan**

[1]Central Institute of Freshwater Aquaculture, BBSR-751002, Orissa, India

[2]P.G. Dept. of Botany, Utkal University, BBSR-751004, Orissa, India

[3]D.D.G. Fisheries, Krishi Anusandhan Bhavan, New Delhi, India

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ABSTRACT

In this investigation, the efficacy of the herbicide butachlor, (N-butoxymethyl-2 chloro-2^1, 6^1 diethyl acetanilide) was tested against few common submerged macrophytes namely Hydrilla (*Hydrilla verticillata* (L.) Royale), Najas (*Najas minor* All.), Nechamandra (*Nechamandra alternifolia* (Roxb.) Thwaites) and Ottelia (*Ottelia alismoides* (L.) Pers.) of freshwater fish ponds. Almost complete decay of Hydrilla, Nechamandra and Ottelia was achieved at 7.5 L of active ingredient/ha/m butachlor within 15 days while the herbicide showed no negative effect on Najas. However at the same concentration of butachlor, total mortality of zooplankton and water fern Azolla (*Azolla caroliniana* Lamarck) occurred within seven days. In case of few freshwater fish species like Rohu (*Labeo rohita),* Channa *(Channa punctatus),* Anabas *(Anabas testitudineus)* and Heteropneustes *(Heteropneustes fossilis)*, total mortality occurred upto 90 days after application of the same dose of butachlor but fish survived beyond 120 days of herbicide application indicating degradation of the herbicides.

Key words: *Hydrilla verticillata, Najas minor, Nechamandra alternifolia, Ottelia alismoides, Azolla caroliniana*, butachlor

INTRODUCTION

The use of pesticides whether herbicides or insecticides is indispensable in modern agriculture technology to control pests or weeds for the production of more food and management of public health, both in developed and developing countries. Today about 4500 pesticides are in general use all over the world, out of which 25 have high toxicity potential to a wide range of flora and fauna of economic importance (Adhikary and Sahu, 2001). Most of these are not readily degradable but persists for a considerable period, thereby affecting aquatic biota, specially fish, which are very important due to their nutritive food value (Annon, 1962). Herbicides are extensively used to control aquatic macrophytes in different parts of the world. The present investigation was taken up to test the efficacy of butachlor against some dominant submerged freshwater angiospermic macrophytes and also to study the impact of this chemical on some important biotic components like fish, zooplankton and aquatic fern.

*Corresponding author-Email: *amitachattopadhyay@yahoo.com*
Tel: +91 67 4246 5446, Fax: +91 67 4246 5407

MATERIALS AND METHODS

Determination of herbicide effect on submerged macrophytes

Herbicide selection

Butachlor (50% EC) bearing trade name Hunter TM, manufactured by Hindustan Pulverising Mills, India was used for the experiments. The selected doses were 0.5 mL/10L water, 1.0 mL/10L water and 1.5 mL/10L water which are equivalent to 2.5 L of active ingredient(a.i.)/ha/m (Litres of active ingredient per hectare per meter), 5.0 L of active ingredient/ha/m and 7.5 L of active ingredient/ha/m concentrations, respectively.

Experimental set-up

The experiments were carried out in 20L glass jars using 10L of dechlorinated tap water under

constant light and temperature conditions. The experiments were maintained in triplicates for each concentrations and also for control. Four dominant freshwater submerged weeds namely *Hydrilla, Najas, Nechamandra* and *Ottelia* collected from fish ponds were selected for the study. 30 g of wet biomass for each of the first three species and fresh individual plants weighing 50 ± 5 g for the fourth species were incubated in each jar at different concentrations of the herbicide. A soil base of 1kg soil/10L water was maintained at base of each experimental jar. The soil was also collected from the weed infested ponds and spread uniformly on the bottom of the glass jars. After addition of herbicide observations were recorded in terms of biomass, upto 15 days or until the plant material decayed, which ever was earlier.

Determination of herbicide effect on Azolla caroliniana

The study was conducted in 25 L glass jars using 20 L of dechlorinated tap water under constant light and temperature conditions. Dose for butachlor was 7.5 L a.i./ha/m. As this particular concentration was significantly effective in controlling macrophytes, its impact on other biotic factors of freshwater system was studied. Treatments and control were maintained in triplicates. In each jar 200 g of wet biomass of Azolla, an aquatic fern often cultured for biofertilization of fish ponds, were incubated for a period of 7 days after which data was recoreded in terms of biomass of Azolla.

Determination of herbicide effect on zooplanktons

Zooplanktons collected from fish ponds were cultured in laboratory conditions in glass jars using N, P and K in the ratio 2: 1: 10 and cowdung using pond water following standard methods. Dominant species of the plankton population were *Daphnia* sp, *Cyclops* sp. *a*nd *Moina* sp. The experiments were conducted in 10L glass jars using dechlorinated tap water under constant light and temperature regimes. Dose for butachlor was 7.5 L of active ingredient/ha/m. Triplicates were maintained for treatment and control. Zooplanktons were counted collectively on the first, 3rd, 5th, and 7th day of the experiment using Nauber haemocytometer chamber under a microscope.

Determination of butachlor effect on survivability of some freshwater fish species

The following common freshwater fish were selected for the experimental study:

1. *Rohu* of average weight 25 and average length 15 cm.
2. *Channa* of average weight 48.5 and average length 9.5cm.
3. *Anabas* of average weight 24 and average length 9.5cm.
4. *Heteropneustes* of average weight 15.5 and average length 18.2cm.

Experimental Set-up

Experiments were carried out in semi – pond conditions created using 2kg soil base per 20 L. dechlorinated tap water in 25L glass aquaria. Dose for the herbicide was 7.5 L a.i./ha/m. Three sets of replications were maintained for the treatment as well as for control. Number of fish kept in each jar were 10 for *Rohu*, 6 for *Channa*, 6 for *Anabas* and 4 for Heteropneustes, respectively. Fish were added to the system on the first, 7th, 15th, 30th, 45th, 60th, 75th, 90th, and 120th days. Each time, the observation of fish behaviour and survivability were made.

Water analysis

Some important physico-chemical parameters of water like pH, temperature, electrical conductivity, total alkalinity, total hardness, ammonia and phosphate were determined before and after additions of herbicide to experimental jars using standard laboratory methods (APHA, 1998).

The physico-chemical condition of water in these experiments ranged between pH (7.4 to 8.3), temperature (26 to 32°C), electrical conductivity (0.120 to 0.463 mmhos/cm), total alkalinity (80 to 150 mg/L as $CaCO_3$), total hardness (50 to 130 mg/L as $CaCO_3$), total ammonia (0.01 to 0.03 mg/L) and soluble orthophosphate (0.03 to 0.10 mg/L) for untreated and treated water, respectively.

Statistical analysis

The data were statistically analyzed using analysis of variance (ANOVA) to determine significant differences in change of biomass of various weeds between control and different doses of butachlor at $P<0.05$ level (Zar, 1996).

RESULTS

The effect of different concentrations of butachlor on the changes in the biomass of the submerged macrophytes namely *Hydrilla*, *Najas*, *Nechamandra* and *Ottelia* after 15 days of incubation are presented in Table 1. In case of *Hydrilla*, ninety percent biomass inhibition was observed with 7.5 L a.i./ha/m of butachlor within 15 days followed by forty percent and thirtynine percent biomass inhibition with 5.0 L a.i./ha/m and 2.5 L a.i./ha/m butachlor respectively during the same period of time. The maximum biomass inhibition at 7.5 L a.i./ha/m of the herbicide was statistically significant in comparison to the other concentrations (C.D = 4.05, P = 0.05). There was no negative effect on biomass inhibition of Najas with any of the doses of butachlor upto 15 days. Butachlor at concentrations of 2.5, 5.0 and 7.5 L a.i./ha/m inhibited biomass upto 46, 57 and 74 percent respectively within 15 days of incubation for *Nechamandra*. The biomass inhibition of this macrophyte at the highest concentration of butachlor was statistically significant (C.D = 2.49, P = 0.05). The effect of butachlor on biomass of *Ottelia* was most noticible as compared to the other macrophytes. All the three doses of the herbicide could effectively inhibit growth of this brood-leaved macrophyte. The plant was decayed within 8 days at 2.5 L a.i./ha/m butachlor. For 5.0 L of active ingredient/ha/m and 7.5 L of a.i./ha/m of butachlor the time period of decay of *Ottelia* was 6 days and 4 days respectively. During the course of decay of the macrophytes due to application of herbicides, certain morphological changes were observed. The leaves lost their green colouration and turned yellowish. Apical growth was arrested. The stems were also discoloured. In *Hydrilla*, the stems became narrow and slender and the internodal space between the leaf whorls increased. After decay the entire plant biomass sank under water. The impact of butachlor on azolla and zooplankton are as indicated in Table 2. The entire biomass of Azolla was completely decomposed and sank at the bottom of the experimental jars within seven days of treatment with the herbicide indicating its highly toxic nature. In case of the control, there was insignificant reduction in biomass after seven days of incubation. The herbicidal effect on zooplankton survivability was significant in the

Table 1: Effect of butachlor on the biomass of *Hydrilla*, *Najas*, *Nechamandra* and *Ottelia* after 15 days of incubation under laboratory conditions

Macrophytes	Butachlor concentration (L of active ingredient/ha/m)	Initial biomass of the plant (g)	Final biomass of the plant (g) mean±S.D, n=3	Percent increase (+)/ decrease (-) of biomass	Critical difference C. D. (P=0.05)	Observations
Hydrilla	Control	30	32.0±1.0	+6.6	4.05	Plant healthy and intact.
	2.5	30	18.3± 1.7	-39.0		Plant material decomposes and sinks to bottom of the jar.
	5.0	30	18.0±1.0	-40.0		
	7.5	30	3.0±1.0	-90.0		
Najas	Control	30	30±0.2	Negligible	-	No growth
	2.5	30	29±2	-	-	No significant reduction in biomass
	5.0	30	31±1	-	-	Slight increase in biomass
	7.5	30	31±1	-	-	Slight increase in biomass
Nechamandra	Control	30	31.6±0.4	+5.3	2.49	Plant healthy intact
	2.5	30	16.3±0.7	-45.9		Plant material decomposes and sinks to bottom of the jar.
	5.0	30	13.0±1.0	-56.6		
	7.5	30	7.3±0.6	-75.6		
Ottelia	Control	50 ± 5	76±6	+52	-	Plant healthy throughout the experiment
	2.5	50 ± 5	Plant decayed	-		Plants became yellow within two days of application of the herbicides.
	5.0	50 ± 5	Plant decayed	-		Complete decay of plants occurred on further incubation upto 8, 6 and 4 days at 2.5, 5.0 and 7.5 L of active ingredient/ha/m concentrations respectively.
	7.5	50 ± 5	Plant decayed	-		

Table 2: Effect of butachlor on Azolla and zooplanktons upto 7 days of incubation in the laboratory.

Sample	Butachlor concentration (L of active ingredient/ha/m)	Biomass/ Number	Observation			
			1st day	3rd day	5th day	7th day
Azolla	Control (without butachlor)	Biomass (g)	200±5	-	-	170 ± 5
	7.5	Biomass (g)	200±5	-	-	Plant decayed
Zooplankton	Control (without butachlor)	Count/L	600±30	680±20	720± 20	785 ± 20
	7.5	Count/L	600±20	330±20	0	0

sense that the count/L reduced from 600 ± 20/L on the first day to 0/L on the 5th day in the butachlor treated jar. The count was 330 ± 20/L on the 3rd after treatment. In case of control initial count of 600 ± 30/L on the first gradually increased to 680 ± 20/L on t he 3rd day, 720 ± 20/L on the 5th day and 785 ± 20/L on the 7th day of the experiment. The effect of butachlor on survivability of different fish species was as indicated in Table 3. All the four different species of fish exposed to the herbicide treated water showed similar survivability pattern irrespective of their morphological difference. In all cases there was total mortality upto 90th day of observation. However, on the 120th day, there was 100 % fish survivability.

Certain erratic behavioural patterns of fish was noticed during the exposure period to herbicides. The fish exhibited unrest and a peculiar tumbling motion before they died. Even though mortality occurred on the different days of observation, there was gradual increase in the time period during which the fish survived in the herbicide treated water with advancement in the day of observation. On the first day mortality occurred within one hour in all cases, where as on the 30th day fish died after 5-6 h, on the 90th day few fish in some experimental jars even survived for 24 h. Beyond 120th day no mortality occurred upto period of 4 days after which the experiment was terminated. In the control jars, there was almost no mortality during the experimental period.

DISCUSSION

From the result it is evident that nearly 100 % biomass of *Hydrilla* and *Nechamandra* decayed with the highest concentration i.e. 7.5 L a.i./ha/m of butachlor within 15 days. The lower doses were not so effective as far as these macrophytes were concerned. Similar observation was also made by Mansor *et al*., (1988) while they tested the effectiveness of Aquathol –K on the water weed *Hydrilla*. They reported that 100% injury was achieved within 10 days with highest concentration 2.0 mg/L of Aquathol-K while with lower concentrations of 0.5 mg/L and 0.1 mg/L, 100% injury was never achieved even within 25 days. Butachlor had no injurious effect on *Najas* with any of the concentration upto 15 days. While Ottelia was completely decomposed even at the lowest concentration 2.5 L a.i./ha/m of butachlor within 8 days and 100% injury was obtained with 4 days at the highest concentration 7.5 L a.i./ha/m. This may be due to very broad leaves of *Ottelia* where butachlor possibly got greater surface area and acted immediately. This could be further supported by the fact that butachlor was ineffective in controlling growth of *Najas* which have very narrow spine like leaves. Butachlor is an organochlorine compound. Among pesticides organochlorines have the greatest inhibitory effect on photosynthesis and respiration of macrophytes (Ramachandran *et. al.*, 1984). Several studies have examined the effect of atrazine of various macrophytes (Jones and Winchel, 1984; Jones *et al*., 1986; Delistraty and Hershner, 1984). This substance caused photosynthetic inhibition of 1% (at 20µg/L) and 50% (at 95 µg/L) in *Potamogeton perfoliatus, Ruppia maritima L., yriophyllum spicatum* and *Zannichellia palustris* L. (Jones and Winchel, 1984). Butachlor is a systemic herbicide. Theoritically, systemic herbicides are translocated absorption sites to critical points in the plant. Because death occurs more slowly, oxygen demand does not occur as quickly and nutrients are released over a longer time period. This may cause fewer environmental problems because the ecosystem has more time to assimilate the oxygen demand and nutrient release.

A more thorough kill is expected if the herbicide is

Table 3: Effect of butachlor on survivability of different types of freshwater fish species

Conc. of Butachlor (L of active ingredient/ha/m)	Fish types and number added on each day	Observations								
		0	7th	15th	30th	45th	60th	75th	90th	120th
Control (without butachlor)	*Labeo rohita* (10 fish added on each day) *Channa punctatus* (6 fish added on each day) *Anabas testitudeneus* (6 fish added on each day) *Heteropneustes fossilis* (4 fish added on each day)	All fish survived	All fish survived	All fish survived	All fish survived	All fish survived	All fish survived	All fish survived	All fish survived	All fish survived
7.5	*Labeo rohita* (10 fish added on each day) *Channa punctatus* (6 fish added on each day) *Anabas testitudeneus* (6 fish added on each day) *Heteropneustes fossilis* (4 fish added on each day)	All fish died immediately	All fish died within an hour	All fish died within an hour	All fish died within 5 to 6 hours	All fish died within few hours	All fish died within few hours	All fish died after 10 to 12 hours	Most fish died, within 10 to 12 hours, few survived for 24 hrs.	All fish survived upto 4 days after which experiment is terminated

translocated to critical growth points in the plant (Nichols, 1991). Unfortunately, systemic herbicide movement in submerged plant is not well defined. In addition, if the application rates are too high, systemic herbicides act like contact herbicides (Nichols, 1991). They stress the plant so much that the herbicides are not translocated to critical plant growth areas. Limited movement (i.e. contact) herbicides kill exposed plant tissue. Triazine herbicides, for instance, inhibit photosynthesis almost immediately (Robson, 1983). Oxygen slumps caused by bacterial breakdown of plant matter are compounded by plant respiration. Oxygen is not replenished with photysynthesis and nutrient release begins quickly with contact herbicide use (Daniel, 1972). Limited movement or contact herbicides are not translocated to underground tissues, which can have great reproductive potential. Only the portion of plant above the sediment is damaged. However, systemic herbicides could potentially be more environmentally desirable in an aquatic plant management programme because they may provide longer lasting results and cause fewer environmental problems when used (Nichols, 1991). A fair amount of information of aquatic herbicides is available because of their use as weed control. Most herbicides are taken up within 1 h by roots and shoots (Forney and Davis, 1981; Jones *et al*., 1986). They are probably translocated in the phloem stream with a generally greater basipetal transport than acropetal (Welsh, 1977). Simazine aherbicide, however, is known to be a 'xylem-mobile' herbicide and results reported by Thomas and Seamon (1968) suggest basipetal translocation of this compound. On the other hand, Diquat and Paraquat are not translocated in either direction, perhaps because of strong adsorption and abnormal physiological responses (Thomas and Seamon, 1968). These authors also indicate movements to the growing tips of *Potamogeton nodosus* (via the phloem transport) of the 'phloem-mobile' herbicide endothal. There is no evidence of acropetal movement by the xylem-mobile herbicides Atrazine and Diuron. The reduced movement within the plants of these substances is probably explained by the absence of a transpiration stream in submerged species unlike emergent and floating-leaved ones.

Results showed that the highest concentration i.e. 7.5 L a.i./ha/m of butachlor when applied to Azolla the entire biomass of this floating aquatic fern degraded and the herbicide may have acted in one of the above mentioned mechanism on this pteridophytic macrophyte. Effect on zooplankton which is the main fish food organism, is also indicative of its highly toxic nature. There are reports that population of *Daphnia magna* (Straus) were reduced due to technical grade Bromoxynil Octanoate herbicide (Buhl *et al*., 1993). Effect of butachlor on survivability of different species of fish indicate that this organochlorine herbicide is extremely toxic in nature. Fish mortality on the 90th day is suggestive

of the fact that the toxicity persists in the aquatic system even for such long period of time. However, after 120th day fish in the experimental jars survive indicating that toxic effect have been removed may be due to herbicidal degradation (Chatterjee *et al.*, 2004). The herbicide has most probably broken down into non-toxic components. There are several reports of aquatic toxicity in India and other countries. Researchers have tried to asses toxicity of different chemicals including insecticides and herbicides on aquatic environment from time to time. Acute toxicity of pesticides like Endosulfon, Malathion and Copper sulphate at different concentrations to fresh water prawns *Macrobrachium rosenbergii* were reported (Natarajan *et al.*, 1992). There are also reports of the effects of the herbicide 2, 4-D on the bottom fauna of fish ponds (Sarkar, 1991). Exposure of common carp *Cyprinus carpio* to sublethal concentrations of Endosulfon showed that fish responded with decrease in levels of haemoglobin and haemotocrit, significant elevation in blood glucose and little variation in the serum protein (Chandrasekar and Jayabalan, 1993). The result also indicate that the herbicide butachlor persists in the aquatic system for along period of time. Persistence of Butachlor were studied at two levels of application in three different soils at three different sites under three moisture regimes air dry, field capacity, and submergence (Prakash and Suseela Devi, 2000). Based on this investigation it may be concluded that butachlor is an efficient aquatic herbicide and could help to remove submerged aquatic macrophytes especially the broad leaved ones from the freshwater bodies in very less time. However, if this herbicide is recommended in controlling weeds from fish ponds, then stocking of fish should be done at least after four months (i..e. 120 days) of its application to the ponds.

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REFERENCES

Adhikary, S. P., Sahu, J. K., (2001). Internation of cyanobacteria of rice fields to agrochemicals. Algal Biotechnology. Ed. P. C. Trivedi, Pointer Publishers, Jaipur, India, 98-115.

APHA, (1998). Standard methods for the examination of water and waste water. 20th Ed. American Public Health Association, Washington, D.C.

Arnon, (1962). The wealth of India: Raw material of volume IV, supplement Fish and Fisheries, CSIR, New Delhi. 132.

Buhl, K. J., S. J. Hamilton, Schmulbach, J. C., (1993). Acute toxicity of the herbicide bromoxymil to Daphria magna. Environ. Toxicol. Chem., **12**: 1455-1468.

Chandrasekhar, S., N. Jayabalan, (1993). Haematological responses of the common carp, *Cyprinus carpio* L. exposed to the pesticide endosulfan. Asian Fish Sci. **6** (3): 331-340.

Chatterjee, Amita, S. Adhikari, Adhikary S. P. and Ayyappan, S. (2004). Fish as bioindicators for waiting periods of pesticides. Indian Journal of Fisheries.

Daniel, T. C., (1972). Evaluation of diquat and endothall for the control of watermilfoil (*Myriophyllum exalbescens*) and the effect of weed kill on the nitrogen and phosphorus status of a waterbody. Ph.D. Thesis. University of Wisconsin, Madison, 131.

Delistraty, D. A., Hershner, C., (1984). Effect of the herbicide atrazine on adenine nucleotide levels in *Zostera marina L.* (eelgrass). Aquat. Bot., **18**: 353-369.

Forney, D. R., Davis, D. E., (1981). Effect of low concentrations of herbicides on submerged aquatic plants. Weed Sci., **29**: 667-685.

Jones, T. W., Winchel, L., (1984). Uptake and photosynthetic inhibition by atrazine and its degradation products on four species of submerged vascular plants. J. Environ. Qual., **13**: 243-247.

Jones, T. W., W. M. Kemp, P. S. Estes and Stevenson, J. C., (1986). Atrazine uptake, photosynthetic inhibition and short-term recovery for the submerged vascular plants. *Potamogeton perfoliatus*, L. Arch. Environ. Contam. Toxicol. **15**: 277-283.

Mansor, M., J. Joyee and Thyer, D. (1988). Effect of Aquathol K on *Hydrilla verticillata* (L. F.) Royle. Pertanika., **11**(2): 191-194.

Natarajan, E., R. S. Biradar and J. P. George, (1992). Acute toxicity of pesticides to giant freshwater prawn *Macrobrachium rosenbergii* (De Man). J. Aquacult. Tropics., **7** (2):183-188.

Nichols, S. A., (1991). The interaction between biology and the management of aquatic macrophytes. Aquat. Bot., **41**:225-252.

Prakash, N. B., Suseela Devi, L., (2000). Persistence of Butachlor in soils under different moisture regimes. Journal of the Indian Society Soil Sci., **48** (2): 249-22.

Robson, T., (1983). Localized control of submerged aquatic weeds with herbicides. In: W.W. Jletcher (Editor), Recent Advances in Weed Research Common Wealth Agriculture Bureaux, London, 171-180.

Sarkar, S. K., (1991). Effect of the herbicide 2, 4-D on the bottom fauna of fish ponds. Prog. Fish. Cult. **53** (3): 161-165.

Thomas, T.M., D.E. Seamon, (1968). Translocation studies with endothal 14C in *Potamogeton nodosus* Poir. Weed Res., **8**: 321-326.

Welsh, R. P. H., (1977). Studies on the uptake, translocation and accumulation of trace metals and phosphorus in aquatic plants. Ph.D. Thesis, Westfield college, University of London, 331.

Zar, J. H., (1996). Biostatistical Analysis, 3rd Ed. Prience Hall Int. Inc., London.