



## Herbicides effect on fish mortality and water quality in relation to chemical control of alligator weed

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### ABSTRACT

Effect of three herbicides namely 2,4-D, metsulfuron-methyl and glyphosate was evaluated on fish mortality and water quality in relation to control of aquatic form of alligator weed (*Alternanthera philoxeroides*; Family, Amaranthaceae). All herbicides caused fish mortality and affected water quality after application, but it was highest in 2,4-D treated tanks followed by glyphosate and metsulfuron-methyl. Herbicide did not cause fish mortality at 1 DAA (days after application) but it caused at 7 DAA and increased corresponding to increase in concentration and days. Fish mortality was recorded lowest in herbicides treated tanks that were having only water but no weeds. Significantly higher fish mortality occurred in 2,4-D treated tanks having weeds. This reflected that fish mortality was more due to decaying of weeds, which decreased dissolved oxygen drastically in the water tanks. Herbicides did not affect fish development because growth and weight of fish was highest in water tanks treated with herbicides having no weeds. All the herbicides significantly decreased pH in treated tanks than control at 0 and 1 DAA, however, it was resumed towards normalisation in due course. The decrease in pH was least in the tanks having weeds and treated with metsulfuron-methyl followed by glyphosate and 2,4-D. Further, decrease in pH was less in water tanks having no weeds than having weeds. All the herbicides significantly decreased the dissolved oxygen (DO) at 0 day in water tanks with and without weeds except metsulfuron-methyl in the tanks having no weeds. Decrease in DO was more prominent in 2,4-D treated tanks followed by glyphosate and metsulfuron-methyl. Dissolved oxygen was least affected in tanks having no weeds.

**Key words:** Alligator weed, Chemical control, Fish mortality, Water quality

Alligator weed, *Alternanthera philoxeroides* (Family: Amaranthaceae) is a native of the Panama river system in North-Eastern Argentina and is believed to have been introduced in India from Indonesia and Myanmar. It is a noxious weed in Brazil (Abud 1985), Australia (Krake *et al.* 1999, Milvain *et al.* 1995), New Zealand, UK (Arthington *et al.* 1986) and USA (Rhodes 1983) and is capable of infesting terrestrial wet lands and aquatic habitats. From India, it was first reported in 1965 from Bihar (Maheswari 1965) and since then has spread to all states of India and in some states, it has assumed the alarming situation. It has been found to affect drainage systems severely In Jabalpur in India (Sushilkumar and Bhan 1996), Guwahati and Jorhat (Assam) and in Shilong (Meghalaya), this weed was found abundantly on the roadside as terrestrial weed in high moisture regime area, thus threatening local biodiversity (Sushilkumar *et al.* 2009). In many countries, this weed has been proclaimed as a state-prohibited, which reflects its seriousness in terms of its threat to public interest. In USA, it has become a menace in navigation and United State army had to

spend huge money to clear the path for navigation from the infestation of this weed. Alligator weed has been reported to cause major blockages of water flow in water ways such as irrigation canals, rivers and drainage system. It is capable to readily establishes and covers extensive surface area of lakes and ponds.

Few herbicides have been tried to control alligator weed in other countries among which 2,4-D, glyphosate and metsulfuron-methyl have been used widely (Melvain *et al.* 1995, Langeland 1986, Sandberg and Burkhalter 1983, Sutton 1974). The use of herbicide in aquatic system is increasing worldwide, but so far Govt. of India has not recommended any herbicides for weed control in aquatic situations owing to fear of their non-target effect and very less information about the impact of herbicides on the aquatic ecosystem. Metsulfuron-methyl is a sulfonylurea group herbicide and used as alone or in a mixture to control broad-leaf weeds in cereal crops while glyphosate is a commercial formulation of N-{phosphonomethyl} glycine, which contains thenisopropylamine salt that makes it more soluble in water and its use more convenient in aquatic system, whereas 2,4-D is a broad-spectrum

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non-selective herbicide that effectively controls most of the annual and perennial herbaceous plant and deep root perennial species.

A common concern about the use of herbicides is about their effect on water quality and aquatic fauna especially fish besides non-target effects on other microorganism. To address this concern, in the present study, effects of herbicides, viz. 2,4-D, glyphosate and metsulfuron-methyl have been reported and discussed on fish mortality and growth and on water quality while doing chemical control of the alligator weed. The effect of herbicides on alligator weed control and on its regrowth has been dealt by Sushilkumar *et al.* (2008b) in other studies.

### MATERIALS AND METHODS

The experiment was conducted during 2004-2005 at Directorate of Weed Research, Jabalpur in water tanks having surface area of 2.54 m<sup>2</sup> with one meter depth. Shoots of alligator weed with 40 cm length were cut from naturally infested ponds and brought to the institute for establishment in water tanks. A 10 cm layer of soil was placed on the floor of each tank along with 4 kg FYM before filling the water. In each water tank, 2500 cut shoots were put, based on the survey of per meter square population in the severely infested pond. The cut shoots were allowed to establish for one month. This formed a shoot and leave mat above the water surface and interwoven root mat beneath the water as occurred in natural conditions. Twenty number of fish fingerlings of species *Rohito labio*, having average total length (7.20 cm) and weight (9.91 g) were released in each tank before 7 days of spray of herbicides to acclimatize them. Fresh fish was added in case of any mortality during period of the acclimatization.

On such established alligator weed mat, herbicides were sprayed in different concentrations by knapsack sprayer with flat fan nozzle at the rate of 500 litre per hectare. The herbicide treatments included 2,4-D ethyl ester at 1.5, 2.0 and 2.5 kg/ha, glyphosate at 1.5, 2.5 and 3.0 kg/ha and metsulfuron-methyl at 0.016, 0.020 and 0.024 kg/ha. In control, tanks were sprayed with water only. To see the effect of herbicides on water only, a treatment was kept without weed and herbicides were sprayed on water surface only. The fish mortality was counted daily by observing any dead fish over the water surface and amidst the mat of alligator weed. Total number of dead fish was computed at an interval of 0, 1, 7, 14, 21, 30 and 60 days after application (DAA) of herbicides. All the live fishes were removed after 60 days from the tanks and their total length, standard length and

weight were measured to see the overall growth in tanks treated with herbicides having weeds, no weeds and control without herbicides. The experiment was replicated thrice in a completely randomized block design. The water samples were taken from treated and untreated water tanks at 0, 1, 7, 14, 21, 30 and 60 DAS for water quality, which was determined by following AOAC methods (1970).

## RESULTS AND DISCUSSION

### Effect of herbicides on fish mortality

Herbicide did not cause fish mortality at 0 and 1 days after application (DAA), but it started to cause mortality after 7 days, which increased corresponding to increase in herbicide concentration and days. Metsulfuron-methyl in all concentrations proved to be the best herbicide followed by glyphosate and 2,4-D. It was found that mortality of fishes was significantly less in those herbicides treated tanks having no weeds. However, significantly higher fish mortality occurred corresponding to increase in dose of 2,4-D. Fish mortality was recorded 20, 26 and 46% at 1.5, 2.0 and 2.5 kg/ha doses of 2,4-D at 60 days, respectively, while it was about half in glyphosate and even less in metsulfuron-methyl treated tanks (Table 1). Only 10% fish mortality was observed in 60 days in the tanks having no weeds, but was sprayed with the herbicides. No fish mortality was recorded in control at 30 DAA but about 6.60% mortality was observed at 60 DAA.

The length and weight of fishes were found significantly high in all the herbicide treated tanks than the control. This clearly reflected that growth of fish is drastically affected in the water bodies having cover of weeds over the water surface. Length and

**Table 1. Effect of herbicides on fish mortality at different days after application (DAA)**

Herbicide	Dose (kg/ha)	Progressive fish mortality (%) at different DAA						
		0	1	7	14	21	30	60
2,4-D	1.5	0.0	0.0	6.6	13.2	13.2	13.2	20.0
	2.0	0.0	0.0	6.6	26.5	26.5	26.5	26.6
	2.5	0.0	0.0	9.9	36.5	39.8	39.8	46.6
	2.5*	0.0	0.0	10.0	10.0	10.0	10.0	10.0
Glyphosate	2.0	0.0	0.0	6.6	6.6	6.6	6.6	13.3
	2.5	0.0	0.0	6.6	6.6	6.6	6.6	16.6
	3.0	0.0	0.0	13.2	13.2	13.2	13.2	23.3
	3.0*	0.0	0.0	10.0	10.0	10.0	10.0	10.0
Metsulfuron-methyl	0.02	0.0	0.0	5.0	5.0	5.0	5.0	6.6
	0.02	0.0	0.0	5.0	5.0	5.0	5.0	10.0
	0.02	0.0	0.0	8.3	8.3	8.3	8.3	10.0
	0.02*	0.0	0.0	10.0	10.0	10.0	10.0	10.0
Control		0.0	0.0	0.0	0.0	0.0	0.0	6.6
LSD (p= 0.05)		0.0	0.0	8.9	19.7	22.5	22.5	19.7

\*Spray of herbicides on water surface in tank having no weeds

weight of fishes were significantly higher in the water tanks having no weeds, but treated with highest dose of herbicides than the water tanks having weeds and treated with herbicides (**Table 2**). Significantly higher fish mortality was recorded between 14 to 21 days coincide with the decomposition of weeds due to action of herbicides. This implies that alone herbicide is not responsible for fish mortality because it did not cause significant mortality than the control. More fish mortality was caused due to lowering of water quality by the action of herbicides due to decomposition of weeds. Deivasigamani (2015) found that glyphosate showed significantly lesser fish mortality than 2,4-D Na salt with mortality percentage of 23.0, 16.0 and 20.0 and 46.0, 33.33 and 20.00 for common carp, mrigal and rohu, respectively at 96 h. Satya *et al.* (2016) did not observe mortality of fingerlings with

**Table 2. Weight, standard length and total length of fish in different treatment after 60 days after application**

Herbicides	Dose (kg/ha)	Standard length (cm)	Total length (cm)	Total weight (g)
2,4-D	1.5	8.67	10.9	13.7
	2.0	9.43	11.2	13.9
	2.5	9.45	11.8	16.5
	2.5*	10.02	13.9	23.8
Glyphosate	2.0	9.05	11.4	13.3
	2.5	9.70	12.1	17.1
	3.0	8.95	11.3	18.7
	3.0*	11.64	14.44	29.0
Metsulfuron- methyl	0.016	9.56	12.1	14.5
	0.020	9.16	11.6	15.8
	0.024	9.25	11.8	16.0
	0.024*	11.95	14.8	29.4
Control		7.92	10.0	9.40
LSD (p=0.05)		0.26	0.23	0.20

\*Spray of herbicides on water surface in tank having no weed. Before spray of herbicides, average standard length, total length and weight of fish was 7.20 and 9.9 cm/fish and 19.4 g/fish

**Table 3. Effect of herbicides on pH at different days after application (DAA)**

Herbicides	Dose (kg/ha)	pH at different DAA							
		0	1	7	14	21	30	45	60
2,4-D	1.5	6.2	6.5	6.8	6.9	6.9	7.0	7.4	7.6
	2.0	6.2	6.3	6.5	6.6	6.8	6.9	7.0	7.4
	2.5	6.0	6.2	6.4	6.5	6.7	6.8	6.9	7.1
	2.5*	6.9	6.9	7.0	7.0	7.1	7.4	7.7	7.8
Glyphosate	2.0	6.5	6.7	6.9	7.0	7.0	7.2	7.4	7.6
	2.5	6.4	6.6	6.7	6.9	6.9	7.1	7.2	7.5
	3.0	6.3	6.4	6.5	6.8	6.8	7.0	7.1	7.4
	3.0*	7.0	7.0	7.1	7.1	7.2	7.6	7.8	7.9
Metsulfuron- methyl	0.016	6.7	6.9	7.1	7.2	7.2	7.4	7.6	7.8
	0.020	6.6	6.8	6.9	7.1	7.1	7.3	7.5	7.5
	0.024	6.5	6.6	6.7	7.0	7.0	7.2	7.3	7.6
	0.024*	7.0	7.0	7.1	7.2	7.2	7.6	7.9	7.9
Control	-	7.2	7.2	7.4	7.5	7.5	7.7	7.9	8.1
LSD p=0.05)		0.16	0.17	0.16	0.18	0.18	0.16	0.16	0.16

\*Spray of herbicides on water surface in tank having no weed

all the tested herbicides with different concentrations except spraying of glyphosate at 10 ml/L + 2,4-D Na salt at 2.5 g/lit. In our experiment, reason of more mortality of fish in high dose of 2,4-D (2.5 kg/ha) may attributes of its ester property, which caused fast deterioration of weed by 14 days leading to decrease in dissolve oxygen. However, the same dose of 2,4-D when applied only on water without weed did not cause significant mortality of fish. This further suggests that in large water body, fish mortality may not be due to direct effect of herbicides, instead mortality of fish may be due to decrease of water quality parameters due to decomposition of weeds by action of herbicides.

### Effect of herbicides on water quality

Herbicides influenced water quality in terms of pH, dissolve oxygen (DO), and biological oxygen demand (BOD) corresponding to increase in concentrations and time after spray.

**Effect on pH:** It was observed that pH was decreased in tanks having weeds than the control corresponding to increase in concentrations of herbicides, but pH decrease was less in tanks having only water. All the herbicides significantly decreased pH than control at 0 and 1 DAA. The decrease in pH was minimum in metsulfuron-methyl treated tanks followed by glyphosate and 2,4-D. However, pH tend to be increased corresponding to increase in time but it varied herbicide to herbicide. In tanks treated with higher dose of 2,4-D (2.5 kg/ha) having weeds, pH increased from 6.0 at 0 DAS to 6.9 at 45 DAA, while in tanks having no weeds, the pH increased from 6.9 at 0 DAA to 7.0 at 7 DAA. In control, pH increased from 7.2 to 8.1 by 60 days (**Table 3**). This suggests that pH decrease was high in the presence of weed, while in the absence of weeds, decrease was low. This might be due the release of chemical contents of plants in the water due to deterioration of weeds by action of herbicides.

**Effect on dissolve oxygen:** Decrease in dissolve oxygen (DO) level may be fatal for the aquatic life. Fishes are most susceptible with decrease in DO level. All the herbicide treatments significantly decreased the DO at '0' day in the tanks having or not having weeds except metsulfuron-methyl in tanks having no weeds. The fast decaying of weed biomass after herbicides application started at 5 to 10 days, which was responsible for more lowering the DO and mortality of fish. Decrease in DO was more pronounced in 2,4-D treated tanks followed by glyphosate and metsulfuron-methyl. A decrease trend of DO was observed in all the treatments including

control corresponding to increase in time from the date of application. DO was least affected in tanks having no weeds (**Table 4**). This further confirms that decrease in DO was mainly due to the decaying of weed owing to the action of herbicides. DO was brought down below 4 ppm in 2,4-D 2.5 kg/ha treated tanks at 14 DAS, which might have resulted high increase in fish mortality from 10 to 37% due to oxygen depletion (**Table 1**). Decaying of alligator weed was observed slow in metsulfuron-methyl treated tanks than the 2,4-D and glyphosate, which might have resulted less decrease in DO hence, less mortality of fish.

**Effect on BOD:** Biological oxygen demand (BOD) is also an important water quality parameter for existence of aquatic life particularly microbes, which are essential for aquatic ecology. Less BOD has been considered better for aquatic ecosystem. BOD increased significantly at '0' day in all the treatments than control including water tanks having no weeds. This increase of BOD even in the tanks having no weed reflects that BOD is directly affected by herbicides. BOD increase was highest in 2,4-D followed by glyphosate and metsulfuron-methyl and was at par with control in metsulfuron-methyl treated tanks at 1 DAA, but it increased with the increase in time (**Table 5**).

In general, there was deterioration of water quality parameters with increase of time in herbicide treated and untreated tanks with weed or without weed. This might be due to water stagnation in the tank up to longer duration. It was presumed that water quality deterioration might be less in large aquatic body treated with herbicides in patches. Sushilkumar *et al.* (2005) did not observed mortality of fishes when glyphosate was sprayed in half part of full lotus infested pond, leaving other part unsprayed in a fish culture pond. Reduction in water quality due to lower dissolved oxygen might have been the primary cause for fish mortality in all the herbicides treated tanks having weeds. Olaleye and Akinyemiju, (1996) have also reported the effect of glyphosate on fish composition and abundance during control of water hyacinth in delta of Nigeria. Kannan and Kathiresan (2002) observed minimum fish mortality in the glyphosate treated water tanks followed by 2,4-D in relation to water hyacinth control. They observed lowest dissolve oxygen (2.5 ppm) in tubs sprayed with 2,4-D 1.0 kg/ha at 15 DAA. They also observed reduction in pH and DO by paraquat (0.90 kg/ha), 2,4-D (1.00 kg/ha) and glyphosate (2.20 kg/ha) compared to the untreated control.

**Table 4. Effect of herbicides on dissolve oxygen (DO) at different days after application**

Herbicides	Dose Kg/ha	DO (mg/l) at different DAA							
		0	1	7	14	21	30	45	60
2,4-D	1.5	6.4	6.0	5.6	5.2	4.8	3.6	2.4	2.3
	2.0	5.6	5.6	4.8	4.4	3.2	2.4	2.0	1.9
	2.5	4.8	4.8	4.0	3.6	2.4	1.6	1.2	1.1
	2.5*	7.0	7.0	6.9	6.5	6.5	5.8	5.6	4.5
Glyphosate	2.0	7.2	7.2	6.4	5.6	5.6	4.8	4.0	3.2
	2.5	6.4	6.4	5.6	5.6	4.8	4.0	3.2	2.4
	3.0	6.4	6.4	4.8	4.8	4.0	3.2	2.4	1.6
	3.0*	7.0	7.0	6.9	6.6	6.6	5.6	5.9	4.8
Metsulfuron-methyl	0.016	8.0	8.0	7.2	6.4	6.4	5.6	4.8	4.0
	0.020	7.2	7.2	6.4	6.4	5.6	4.8	4.0	3.2
	0.024	7.2	7.2	5.6	5.6	4.8	4.0	3.2	2.4
	0.024*	8.1	8.1	8.0	7.2	7.0	6.8	6.3	5.3
Control		8.2	8.2	8.1	8.0	7.5	7.2	6.5	5.8
LSD (p=0.05)		0.16	0.21	0.17	0.19	0.20	0.16	0.18	0.17

\*Spray of herbicides on water surface in tank having no weeds

**Table 5. Effect of herbicides on BOD of water at different days after application (DAA)**

Herbicides	Dose Kg/ha	BOD (mg/l) at different DAS							
		0	1	7	14	21	30	45	60
2,4-D	1.5	100	110	130	150	170	190	200	210
	2.0	100	110	140	165	180	190	210	220
	2.5	120	130	150	180	190	200	215	240
	2.5*	60	90	100	120	130	150	160	170
Glyphosate	2.0	100	104	126	148	168	180	190	200
	2.5	100	106	128	150	170	184	198	204
	3.0	100	108	130	156	174	190	200	210
	3.0*	90	100	110	124	136	148	158	162
Metsulfuron-methyl	0.016	90	100	116	130	146	158	160	170
	0.020	90	100	118	134	150	160	164	172
	0.024	90	100	120	138	156	168	172	178
	0.024*	90	100	110	124	136	148	158	162
Control		60	100	110	120	130	140	150	160
LSD (P=0.05)		6.48	3.25	2.71	2.83	2.46	2.43	4.42	2.83

\*Spray of herbicides on water surface in tank having no weeds

In our experiment, all treatments influenced the pH of treated water and also affected dissolved oxygen content. The slight reduction in water quality in the herbicides treated tanks can be attributed to the metabolic processes and to the decaying of organic matter after its death. Sushilkumar, *et al.*, (2005) reported that 2,4-D, glyphosate (2.0 kg/ha) and metsulfuron-methyl (0.012 kg/ha) influenced the water quality in a fish culture pond at Jabalpur, India. The pH was significantly reduced in all the treatments over control but it was least affected by metsulfuron-methyl and maximum by glyphosate and 2,4-D. Detailed study done after the spray of glyphosate in large area in fish culture pond, they found that pH and DO did not change significantly up to 7 days but increased after 15 days corresponding to the decaying of treated weed biomass. Maximum and

longer control in context of regrowth of alligator weed was also found by metsulfuron-methyl as has been reported by Sushilkumar *et al.* (2004, 2008). Metsulfuron-methyl at 16 and 24 g/ha was found effective to control aquatic and terrestrial form of alligator weed in tropical situations as it persisted for about 2 months in the soil sediments (Sushilkumar *et al.* 2008b).

Results unequivocally revealed metsulfuron-methyl as most promising herbicide to control alligator weed in context to less deterioration of water quality parameters as well as less fish mortality. Therefore, metsulfuron-methyl may be recommended to control alligator weed.

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