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EFFECTIVENESS OF ALMIX IN CONTROLLING AQUATIC WEEDS AND FISH GROWTH AND ITS CONSEQUENT INFLUENCE ON WATER AND SEDIMENT QUALITY OF A POND

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Almix

Aquatic weeds

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Anabas sp.

Limnologiocal and Soil parameters

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ABSTRACT

Application of Almix™ 20 WP at a dose of 8 g/ha (0.4 g in 6.0 litre water) was investigated in a pond to control few aquatic weeds like Nymphoides indica, Enhydra fluctuens and Alternanthera phyloxeroides. Effectiveness of almix application in controlling the Alternanthera phyloxeroides was 71.97%, for Enhydra fluctuens and Nymphoides indica were only 61.85% and 53.19% respectively. The two air breathing teleosts, Channa punctatus (Bloch) and Anabus testudineous (Cuvier) gained a total body weight over 48.09% and 41.08% respectively when maintained in the same pond.The activities of digestive enzymes, like amylase, lipase and protease were not changed significantly.The limnological studies depict that the phosphate concentration was increased in sediment (from 43.66 to 173.51 kg/ha) whereas decreased in water (from 0.1 to 0.02 mg/l), for nitrate-nitrogen it was increased both in water (from 0.07 to 0.09 mg/L) and sediment (from 25.09 to 37.63 kg/ha) and in case of potassium it was also increased both in water (from 10.50 to 16.10 mg/L) and sediment (from 307.54 to 367.65 kg/ha) and the organic carbon content also increased both in water and sediment.

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INTRODUCTION

Aquatic weeds create serious problems in aquacultural production activities. Herbicides are now widely used in weed controlling in agricultural fields and in aquatic bodies. Constant flow of agricultural runoff containing herbicides into aquatic system can also lead to contamination of aquatic system. The indiscriminate use of herbicides, careless handling, accidental spillages or discharge of untreated effluents into natural water ways may cause harmful effects on the fish population and other aquatic lives and may contribute long term effects on environment. The direct application of herbicides in aquatic system leads to loss of macrophytes and non-target organisms also such as fish through loss of habitat and food supply (Ervnest 2004). Almix™ is a recently used herbicide of the sulfonylurea group for controlling the sedges and broad leaf weeds that stifle the growth of crops. Almix™ 20 WP (Weight Percentatage) herbicide is a combine product of Metsulfuron Methyl 10% and Chlorimuron Ethyl 10%. Almix™ shows systematic action and works through both contact and residual soil activity, hence provides weeds control for a longer period. It enters the plant body *via* contact through its leaves and from the soil through its roots (Anonymous, 1996). Sulfonylurea herbicides are very effective inhibitors of plant cell division. They inhibit acetolactate synthase (ALS) a key enzyme in the pathway of branched chain amino acids (leucine, isoleucine and valine) in plants (Ray 1984). Three aquatic macrophytes namely, *Nymphoides indica, Enhydra fluctuens, Alternanthera philoxeroides* were taken for this study and two air-breathing carnivorous fishes namely, *Channa puntatus* and *Anabas testudineous,* were selected to study the fish health. The objective of this work is to study the effectiveness of almix herbicide in controlling aquatic weeds and its influence on fish growth and soil and water quality in an aquatic body.

MATERIALS AND METHODS

The present experiment was conducted in a 500 m^2 pond adjacent to agricultural farm of the University of Burdwan, Burdwan. Fish species, *Channa puntatus* and *Anabas testudineous* were maintained in a cage in the pond. Almix™ 20WP herbicide was applied for a period of 30 days with a sprayer in every third day at a dose of 8 g/ha. Sampling was done at an interval of 10 days. Regular monitoring and analysis of limnological parameters of water was performed as per APHA 1998. Enzymological analysis of fish tissues for protease activity (Snell and Snell 1971), amylase activity (Bernfeld, 1955), lipase activity (Cherry and Crandall, 1932) and total protein content by Lowry *et al.*, (1951) were done to study the fish health and growth. Some plant biochemical parameters *e.g.,* protein (Lowry *et al.*, 1951), sugar by Anthrone method, proline (Bates *et al.,* 1973) phenol (Malick and Singh 1980) and total chlorophyll (Arnon 1949) were also measured before and after the application of herbicide in the pond to study the effectiveness of almix in controlling aquatic weeds. Effectiveness of almix was measured by calculating the reduction percentage of total chlorophyll content of the selected plant after treatment.

RESULTS

Analysis of plant biochemical parameters

In the present study results of plant biochemical analysis revealed that the protein, sugar and chlorophyll content of leaves of all three selected plant species, gradually decreased after application of almix (Table-1) but phenol and proline content gradually increased in all three plant species after treatment. In *N. indica* sugar content before treatment was 556.63 mg/g but after 30 days of treatment it was reduced to 29.9 mg/g. In *E. fluctuens* sugar content was 90.77 mg/g before treatment but after 30 days of treatment it was about 24.19 mg/g. In *A. philoxeroides* sugar content was 89.09 mg/g before treatment and after 30 days of treatment it was reduced to 16.62 mg/g. In case of *N. indica* protein content was reduced from 15.13 mg/g to 7.35 mg/g after treatment but for *E. fluctuens,* there was a sharp decline in protein content from 44.62 to 18.49 mg/g whereas for *A. philoxeroides* it was reduced from 14.79 to 5.76 mg/g after final treatment. In *N. indica* proline content before treatment *i.e.,* in control condition was 0.11 mg/g but it was 0.13 mg/g in the 10^{th} day,

Table 1: Results of plant biochemical parameter analysis

Parameters	Plants species	Before treatment (Mean±SD)	Exposure period		
			10th Day (Mean ±SD)	20th Day (Mean±SD)	30th Day (Mean±SD)
Protein (mg/g)	*N. indica*	44.62±0.731	28.56±0.771	21.06±0.670	18.49±1.145
	E.fluctuens	15.13±0.840	12.08±0.880	11.11±2.074	7.35±0.789
	A.philoxeroides	14.79±1.216	10.09±1.138	8.81±1.501	5.76±0.650
Sugar (mg/g)	*N. indica*	556.63±4.581	148.21±1.610	51.01±1.873	29.90±3.441
	E.fluctuens	90.77±1.486	62.03±1.794	45.52±1.467	24.19±2.324
	A.philoxeroides	89.09±1.562	36.00±1.963	29.79±2.017	16.62±1.730
Phenol (mg/g)	*N. indica*	1.25±0.358	1.11±0.184	2.18±0.122	3.82±0.304
	E.fluctuens	0.39±0.078	1.66±0.142	2.12±0.122	2.92±0.174
	A.philoxeroides	0.24±0.058	1.17±0.068	2.68±0.115	4.9±0.373
Proline (mg/g)	*N. indica*	0.11±0.017	0.13±0.017	0.23±0.036	0.31±0.053
	E.fluctuens	0.05±0.027	0.07±0.020	0.17±0.020	0.20±0.020
	A.philoxeroides	0.07±0.010	0.14±0.027	0.14±0.010	0.17±0.026
Chlorophyll –A (mg/g)	*N. indica*	2.06±0.076	1.38±0.070	1.2±0.087	1.04±0.053
	E.fluctuens	5.22±0.035	3.34±0.036	3.23±0.020	1.88±0.063
	A.philoxeroides	1.85±0.027	0.80±0.036	0.53±0.053	0.37±0.044
Chlorophyll –B (mg/g)	*N. indica*	1.31±0.027	1.26±0.026	0.80±0.026	0.59±0.036
	E.fluctuens	3.24±0.036	2.01±0.070	1.27±0.036	1.11±0.027
	A.philoxeroides	1.16±0.083	0.96±0.026	0.89±0.026	0.47±0.027
Total Chlorophyll (mg/g)	*N. indica*	3.76±0.026	2.79±0.036	2.18±0.036	1.76±0.035
	E.fluctuens	8.86±0.026	5.59±0.052	4.73±0.026	3.38±0.056
	A.philoxeroides	3.46±0.026	2.14±0.030	1.6±0.436	0.97±0.026

0.23 mg/g in the 20th day, 0.31 mg/g in final day of treatment and for *E. fluctuens*, it was 0.05 mg/g in control condition but after treatment it was increased up to 0.31 mg/g whereas for *A. philoxeroides* it was increased from 0.07 to 0.17 mg/g. In the present study, phenol content in selected weed species was increased gradually after treatment. In *N. indica* phenol content before treatment was 1.25 mg/g and was gradually increased up to 3.82 mg/g. In *E. fluctuens* phenol content before treatment was 0.39 mg/g but after 30 days of treatment it was increased gradually up to 2.92 mg/g.In *A. philoxeroides* phenol concentration was increased from 0.24 mg/g to 4.9 mg/g after treatment. In *N. indica* total chlorophyll content before application of herbicide was 3.76 mg/g but after final application it was reduced to 1.76 mg/g. In *E. fluctuens* total chlorophyll was reduced to 1.11 mg/g after application which was 8.86 mg/g before application of herbicide. In *A. philoxeroides* before application of herbicide total chlorophyll content was 3.46 mg/g but after application of herbicide it was gradually reduced to 0.97 mg/g.

Study of fish growth

Application of almix did not cause any harm to fish health and growth. In the present study average weight of experimental fishes *C. punctatus* in cage was 24.62 g and average length was 10.3 cm before application of herbicide. But after herbicide application average weight was 37.46 g and length was 14.8 cm. In case of *A. testudineus* average weight and length were 21.42 g and 7.9 cm respectively; on final day, weight and length gained was found as 30.22 g and 10.3 cm respectively (Table- 2).

Enzymological study reported that the digestive enzymes *viz.,* amylase, protease, lipase activities was not altered significantly in the selected tissues of alimentary canal and liver of both the fishes before and after application of almix. In case of *C. punctatus* maximum protease activity was observed in stomach (0.057 ¼g/min/mg protein) in first day of observation and after 30 days there was no significant change ($p < 0.05$) in protease activity. In the other regions of digestive system variation in protease activity was negligible. In *A. testudineus* maximum protease activity was observed in liver (0.022 ¼g/min/mg protein) in control condition. After 30 days of treatment no significant ($p < 0.05$) alteration was observed in control as well as treated condition. Maximum amylase activity had been shown in the oesophagus (0.029 ¼g/min/mg protein) in control condition in *C. punctatus*. After treatment with almix its activity altered to 0.037 ¼g/min/mg protein. In *A. testudineus* amylase activity was maximum in oesophagus (0.034 ¼g/min/mg protein) in control and treated condition it was changed to 0.036 ¼g/min/mg protein. In this experiment, lipase activity was maximum in stomach in both the treatment and control condition in both the fishes. In case of *C. punctatus* in control condition, the maximum lipase activity was 0.038 ¼g/min/mg protein, which was slightly increased (0.039 ¼g/min/mg protein) after the treatment with almix in 30 days. In *A. testudineus* maximum lipase activity was 0.050 ¼g/min/mg protein in control fish and after treatment it was 0.051 ¼g/min/mg protein in stomach. The change of enzyme activity before treatment and after 30 days of treatment was not significant at $p < 0.05$.

Study of sediment quality

The different parameters of pond sediment are represented in Table 4(a). In the present study, the organic carbon contentment of the pond sediment was gradually increased after application of herbicide. Organic carbon content was high both in before treatment (1.81%) and after 30 days of treatment with almix, it was 2.66%. The available nitrogen of pond sediment was gradually increased after application of herbicide from 25.09 kg/ha in control condition to 37.63 kg/ha in 30^{th} day of treatment. Available phosphorous content in the pond sediment was drastically increased after treatment with almix herbicide due to deposition as well as exchange capacity from 43.66 kg/ha in control to 173.51 kg/ha after final treatment. The available potassium of this pond was increased after application of almix. The potassium content in the pond sediment before treatment was about 307.54 kg/ha and in 10^{th} day it was 330.67 kg/ha whereas in 20^{th} day it was increased up to 348.99 kg/ha and after final treatment increased up to the 367.65 kg/ha. In this study, the value of pH and temperature of sediment remained more or less same after treatment with almix herbicides. The Pearson's correlation matrix was carried out to find out relationship between two or more sediment parameters using Minitab-15 software. The correlation matrix is presented in Table 4(b).

Limnological study

The limnological parameters of pond water are shown in Table 5(a). In the present study, the conductivity of water was gradually increased after treatment and ranged between 360 μS cm^{-1} before treatment to 420 μS cm^{-1} on 30^{th}day. The chloride concentration was increased from 34.04 mg/L to 55.06 mg/L. The concentration of dissolved oxygen was more or less same through the entire period of experiment which was very essential for the fish growth. From this study, it was revealed that the BOD value was gradually increased during the time period from 3.3 mg/L in control to 5.2 mg/L at the final. The Nitrate–nitrogen concentration did not show any remarkable change during the entire study and was more or less same. The phosphate concentration in water was gradually decreased after treatment with almix during the study period. The potassium concentration of water was gradually increased during the entire study after application of almix herbicides, ranged from 10.50 mg/L in control to 16.10 mg/L at the final. The Pearson's correlation matrix was carried out to find out relationship between two or more limnological parameters using Minitab-15 software. The correlation matrix is presented in Table 5(b).

DISCUSSION

Weeds are undesirable plants to human being at a particular time and place cause serious problems to agricultural, aquatic systems. In agriculture, they are causing huge reductions in crop yields as well as increase cost of cultivation, reduce input efficiency, interfere with agricultural operations, impair quality, act as alternate hosts for several insect pests, and nematodes. Weeds also affect human and cattle health, aquatic ecosystem, grasslands, paddy-cum-fish culture *etc*. In this work effectiveness and potentiality of one

Table 2: Results of fish growth

Species of fish	Avg.weight (g)		Avg.length (cm)	
	Control (Before treatment)	After treatment (after 30 days)	Control (Before treatment)	After treatment (after 30 days)
C. punctatus	24.62±1	36.46±1	10.3±0.5	14.8±0.5
A.tesdudineus	21.42±1	30.22±1	7.9±0.5	10.3±0.5

Table 3: Results of enzymological study of fish tissues

Parameters	Types of fish	Type of tissue	Control (Mean±SD)	Treatment (Mean±SD)
Protein (mg/g)	*C. punctatus*	Liver	40.34±1.659	42.54±1.893
		Intestine	22.32±1.722	23.24±1.278
		Stomach	22.36±0.785	24.52±0.527
		Oesophagus	20.56±0.376	22.46±0.679
	A. testudineus	Liver	56.44±1.202	57.31±0.370
		Intestine	26.32±1.180	26.84±0.593
		Stomach	20.46±0.745	22.52±0.420
		Oesophagus	20.12±0.912	21.23±0.574
Amylase (μg maltose liberated/mg protein/min)	*C. punctatus*	Liver	0.024±0.003	0.020±0.002
		Intestine	0.0176±0.0001	0.0199±0.001
		Stomach	0.0198±0.003	0.0202±0.001
		Oesophagus	0.0294±0.009	0.0375±0.013
	A. testudineus	Liver	0.0246±0.005	0.0251±0.021
		Intestine	0.0342±0.007	0.0348±0.004
		Stomach	0.0052±0.001	0.0053±0.0002
		Oesophagus	0.0348±0.004	0.0358±0.005
Protease (μg tyrosine liberated/mg protein/min)	*C. punctatus*	Liver	0.0176±0.001	0.0185±0.001
		Intestine	0.0122±0.0004	0.0127±0.005
		Stomach	0.0574±0.0004	0.0581±0.0003
		Oesophagus	0.0112±0.001	ND
	A. testudineus	Liver	0.0224±0.0004	0.0223±0.0004
		Intestine	0.0172±0.0003	0.0178±0.001
		Stomach	0.0156±0.001	0.0161±0.0002
		Oesophagus	0.0074±0.0003	0.0081±0.0002
Lipase (μg fatty acid liberated/mg protein/min)	*C. punctatus*	Liver	0.0228±0.0002	0.0232±0.0004
		Intestine	0.0217±0.0002	0.0219±0.0001
		Stomach	0.0384±0.0004	0.0387±0.0002
		Oesophagus	0.0262±0.0002	0.0268±0.0002
	A. testudineus	Liver	0.030±0.002	0.0304±0.001
		Intestine	0.0338±0.0004	0.0344±0.003
		Stomach	0.0498±0.0004	0.0505±0.004
		Oesophagus	0.0136±0.0002	0.0142±0.0003

sulfonylurea group herbicide namely almix on aquatic weed control and its impact on fish health,water quality and sediment quality of aquatic body were studied. Sulfonylureas represent one of the largest classes of herbicides with 27 different active ingredients currently registered around the world. Sulfonylurea herbicides are very effective inhibitors of plant cell division; they inhibit acetolactate synthase (ALS) a key enzyme in the pathway of branched chain amino acids (leucine, isoleucine and valine) in plants (Ray, 1984). Although each sulfonylurea structure has its own physical, chemical and environmental properties, there are many positive characteristics that these molecules have in common which have contributed to their commercial success. As a class, sulfonylurea herbicides control a wide range of annual and perennial grasses and broad

Table 4(a): Results of sediment parameters

Parameters	Before Treatment	10th Day of Treatment	20th Day of Treatment	30th Day of Treatment
pH	7.18	7.13	7.15	7.23
Temperature (^{0}C)	31.8	31.5	31.7	31.6
Available Potassium as K_2O (kg/ha)	307.54	330.67	348.99	367.65
Available Nitrogen (Kg/ha)	25.09	27.55	28.23	37.63
Organic Carbon (%)	1.81	2.05	2.37	2.66
Organic Matter (%)	3.11	3.53	4.08	4.59
Available Phosphorous as P_2O_5 (Kg/ha)	43.66	55.69	65.22	173.51

Table 4(b): The Pearson's correlation matrix of pond sediment parameters

	pH	Temperature	Available Potassium	Available Nitrogen	Organic Carbon	Organic Matter	Available Phosphorous
pH	1.000						
Temperature,	0.208	1.000					
Available Potassium	0.460	-0.443	1.000				
Available Nitrogen	0.760	-0.394	0.885	1.000			
Organic Carbon	0.539	-0.351	0.994	0.902	1.000		
Organic Matter	0.540	-0.354	0.994	0.903	1.000	1.000	
Available Phosphorous	0.827	-0.311	0.840	0.994	0.867	0.869	1.000

Table 5(a): Results of limnological parameters

Parameters	Before Treatment	10th Day Of Treatment	20th Day Of Treatment	30th Day Of Treatment
pH	7.53	7.67	7.3	7.18
Temperature (^{0}C)	22.3	22.0	22.2	23.1
Conductivity (μS cm^{-1})	360	380	410	420
TDS (mg/L)	216	228	246	252
Total Hardness as $CaCO_3$ (mg/L)	200	166	150	160
Total Alkalinity as $CaCO_3$ (mg/L)	164	100	112	112
Chlorides as Cl^- (mg/L)	34.04	60.07	35.04	55.06
Dissolved Oxygen (mg/L)	5.6	5.2	5.6	5.8
BOD (mg/L)	3.3	3.1	4.8	5.2
COD (mg/L)	20	24	30	38
Ortho Phosphate as $PO_4^{=}$ (mg/L)	0.1	0.05	0.04	0.02
Nitrate-Nitrogen as NO_3^-N (mg/L)	0.07	0.07	0.09	0.09
Potassium as K (mg/L)	10.5	11.4	14.0	16.1

leaf weeds (Russell *et al.,* 2002). Sulfonylureas are noted for their high specific activity, which is reflected in the very low application rates required to obtain economic levels of weed control. Soil and foliar performance of sulfonyl urea herbicides are influenced by many environmental factors (Green and Strek, 2001). These factors include soil type, rainfall, humidity, temperature, light, soil moisture, and wind. Weather conditions can influence sulfonylurea herbicides before, during, and after application. In the present, study different biochemical parameters like protein, sugar, chlorophyll, phenol, proline content of three different aquatic weed species *e.g., N. indica, E. fluctuens, A. philoxeroides* were studied to analyze the performance of almix herbicide. The results of plant biochemistry depict the sharp decline in protein, sugar and chlorophyll content of the weeds after treatment. Proline and phenol contents increased after treatment due to certain stress of almix application, as because phenol and proline are released more by plants in stress condition.In this study phenol and proline concentration were increased gradually due to herbicidal stress which are in consonance with earlier works. After the study of plant biochemical parameters it can

Table 5(b): The Pearson's correlation matrix of limnological parameter of pond water

	pH	Temp	Cond	TDS	T H	TA	Cl	DO	BOD	COD	$PO_4^{=}$	$NO_3^{-}N$	K
pH	1.000												
Temp	-0.796	1.000											
Cond	-0.843	0.601	1.000										
TDS	-0.843	0.601	1.000	1.000									
T H	0.493	-0.140	-0.865	-0.865	1.000								
T A	0.144	-0.010	-0.653	-0.653	0.886	1.000							
Cl	0.150	0.210	0.243	0.243	-0.327	-0.688	1.000						
DO	-0.887	0.823	0.505	0.505	-0.037	0.315	-0.375	1.000					
BOD	-0.980	0.726	0.929	0.929	-0.655	-0.327	-0.052	0.778	1.000				
COD	-0.870	0.793	0.958	0.958	-0.714	-0.548	0.342	0.609	0.920	1.000			
$PO_4^{=}$	0.616	-0.487	-0.934	-0.934	0.907	0.857	-0.554	-0.214	-0.743	-0.900	1.000		
$NO_3^{-}N$	-0.940	0.598	0.943	0.943	-0.745	-0.404	-0.086	0.688	0.985	0.885	-0.763	1.000	
K	-0.912	0.776	0.971	0.971	-0.722	-0.508	0.239	0.656	0.957	0.994	-0.878	0.930	1.000

be concluded that the performance of almix herbicide in aquatic weed control is praiseworthy to mention. Effectiveness in controlling the *A. phyloxeroides* is 71.97% but for *E. fluctuens* and *N. indica* are only 61.85% and 53.19% respectively according to the study which indicates the specific underlying mechanism also cited with it. Herbicide toxicosis on the aquatic organism is a big threat and is sometimes dreadful to the industrialized and highly urbanized modern society and the importance is growing rapidly with the increasing number of cumulative effect and biomagnification quality of these xenobiotics in which man is confronted with. The interaction of herbicide with biological system includes the concept of dose threshold, time of exposure and 'one molecule one hit' type of theory of toxicity. Study of the toxic effects of pollutant(s) on the selected specimen(s) is of another prime importance in detection and monitoring the aquatic pollution. Different authors like Llyod, 1960; McCarty *et al.*, 1978; Datta and Sinha, 1989; Ghosh and Chakraborty, 1990, in different occasion studied the effects of different xenobiotics on fish alimentary canal. Sulfonylurea herbicides inhibit specific plant enzyme like, acetolactase synthase which is not found in animals and have very low toxicity to animals (Brown, 1990; Meister, 1997). Present study shows that almix herbicide has no adverse effect on digestive enzyme activity of *C. punctatus* and *A. testudineus* when it was applied in natural condition at a field application dose (8 g/ha). It was also observed that weights of fishes of both the species were increased normally after 30 days of treatment with almix in natural condition. So it can be concluded that almix herbicide has no adverse effect on fish growth. In this work, sediment and water quality of aquatic body was also studied to analyse the effects of almix on sediment and water.The present study shows that organic carbon percentage was gradually increased after treatment in sediment. COD and BOD were also gradually increased after treatment. This was due to the degradation of weeds by the action of herbicide. Potassium and phosphate in sediment also gradually increased after treatment.This is probably due to mixing of degraded plant parts in bottom sediment of the pond. According to the present study, dissolved oxygen in water was more or less same and was within its desirable condition for the better growth of fish. Potassium content was gradually increased with treatment due to decomposition of the plant parts. In the present study, nitrogen content was more or less same. Chlorides are very important for maintaining the good water quality as well as fish productivity. Present study showed that the chloride concentration of the water was increased after treatment but it was within the desirable limit. The present work indicated that total alkalinity of water was gradually decreased after treatment in water whose importance is very significant, which might have caused due to carbonates and bicarbonate salts. Likewise, total hardness showed decline in concentration and is suitable for better fish growth. So, it was established that sulfonylurea herbicides are very effective in weed controlling. Metsulfuron methyl and chlorimuron ethyl which are the constituent of almix are safe and have no residue as detected after harvesting the crop (Sanyal *et al.,* 2006). So, it can be used in controlling aquatic weed safely, since it is mainly used in terrestrial weed control. Almix, also, has no significant adverse impact on fish like *C. punctatus* and *A. testudineus* growth. Water quality of the pond water did not show any adverse change in the physico-chemical parameters with the application of herbicide, almix. Finally, we suggest that it can be applied in aquacultural field like paddy-cum-fish-culture system for controlling weeds. This study will be able to open up new *vista* for those farmers in the countries like India, Bangladesh and some South-Eastern Asian countries where paddy-cum-fish-culture system gained a potential alternative approach to support the socio-economic development of those people.

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