HERBICIDE RESIDUES IN WATER AND THEIR IMPACT ON FISH

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Received: 05.12.14
Accepted: 25.03.15

Herbicides *viz.* paraquat, 2, 4-D and glyphosate at the recommended rates of application were applied in glass aquariums/ and concrete tanks containing water, fish, sediment and weeds *Salvinia/ Eichhornia/ Alternanthera* according to the treatments. Persistence of herbicides in the different components of the aquatic system were estimated and histological alterations in fish organs *viz.* gills, hepatopancreas and muscles were recorded. Under open condition, persistence of residues was considerably lower than that of the samples taken from aquarium. Paraquat and 2, 4-D residues in water samples taken from the open concrete tanks were below the detectable level by 15 to 60 days after spraying, depending upon the climatic conditions. Residues of 2, 4-D and paraquat estimated in the fish samples were below the acceptable daily intake level of 0.01 and 0.002 mg kg^{-1} bodyweight respectively. Histological studies indicated that application of 2,4-D caused greater impact on fish than that of paraquat and glyphosate.

Key words: *Salvinia, sediment, fish, herbicide residues, histology*

Three herbicides *viz.* paraquat (1,1'-dimethyl-4,4'- bipyridilium dichloride), 2,4-D (2,4-dichlorophenoxy acetic acid) and glyphosate (N-phosphonomethyl glycine) are usually used for the control of major aquatic weeds like *Salvina and Eichhornia* . As per the classification of World Health Organisation, paraquat is a highly toxic chemical with LD_{50} of 157 mg kg^{-1}. The corresponding figures for 2,4-D and glyphosate are 375 and 4320-7600 mg kg^{-1} respectively (RSC,1987). Studies conducted at Thrissur center of All India Coordinated Research Programme on Weed control indicated that spraying these herbicides @ 0.75 – 1.0 kg ha^{-1} gave good control of *Salvinia, Eichhornia* as well as the perennial weeds like Panicum, Sacceolepis etc. and resulted in a saving of Rs. 4000-6000/ha in paddy cultivation over hand weeding (KAU, 2002). However, several reports from various parts of the world showed that there are chances for accumulation of these herbicides in the different components of aquatic system *viz.* water, sediment, fish and other aquatic organisms. In view of the need to elucidate the dissipation pattern of these herbicides in the aquatic environment and to determine the safety of herbicide application for aquatic weed control, the present study was taken up under All India Coordinated Research Programme on Weed Control, Kerala Agricultural University, Thrissur, Kerala during the period from 2003- 2011.

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Materials and Methods

A total of four experiments were conducted to find out the persistence of herbicides *viz.* paraquat, 2, 4-D and glyphosate in aquatic system and its impact on fish. Studies on persistence of herbicides consisted of three separate experiments (i) paraquat in concrete tanks (ii) 2, 4-D in glass aquariums and concrete tanks (iii) glyphosate in plastic basins. The first two experiments were conducted in the Herbicide Residue Laboratory of All India Coordinated Research Programme on Weed Control, College of Horticulture, Kerala Agricultural University, Thrissur. Due to the difficulty in analyzing glyphosate residues using gas chromatograph, the experiment was conducted at Gharda Chemicals Limited, Mumbai after proper standardization of the analytical technique using HPLC.

In order to study the histological alterations in fish organs, the fish were grown in glass aquariums containing water and fish only so as to observe the changes in fish at maximum possible concentration of herbicides in water (0.4, 0.5 and 0.6 mg/litre for paraquat. 2,4-D and glyphosate respectively)

Details of the study

1. Paraquat residues

Concrete tanks each of 1m diameter and 1m height were constructed to grow *Salvinia* and fish together. The tanks were coated inside with epoxy adhesive so as to make favorable environment for the growth of fish. Fine soil @ 5 kg/tank was put at the bottom of the tank and water level was maintained to a height of 75cm. Fresh *Salvinia/ Eichhornia/ Alternanthera* collected from the local ponds were grown in these tanks. After attaining its full coverage over the water surface**,** a fish population (genera *Tilapia*) of 12 nos./tank were introduced. After one week, paraquat was sprayed over the aquatic weed mat @ 1.0kg/ha. There were four treatments (as given in Table 1) and four replications. Estimation of paraquat residues in water (30 mL sample), sediment (5g) and fish samples(one whole fish of approximately 10 g was collected , homogenized and 1g was taken for analysis) of the aquatic systems was done at different intervals as mentioned in Table 1 after standardisation of colorimetric technique *viz.* sodium dithionite method (KAU, 2004). *Salvinia, Eichhornia, Alternanthera* (5g homogenized sample of each material), fish (1g homogenized sample) and sediment (5g air dried) samples used for the study were subjected to residue analysis for paraquat, before introducing the materials into the tank. Three samples were analysed for each material , immediately after collection so as to ensure the absence of paraquat residues in these materials. The same study was conducted in four years from 2008 to 2011 and the average values were taken for comparison of data.

2. 2,4-D residues

Radiotracer technique was employed to study the dissipation pattern of 2,4-D residues in water, fish, *Salvinia* and sediment. A fresh water ecosystem was developed using glass aquarium tanks (60cm x 30cm x 30cm). The tanks received 45L fresh water after keeping a fine soil layer of 2cm at the bottom and a mat of 100g *Salvinia* collected from local paddy field areas. Small fishes (*Tilapia sp.)* were collected from local fisherman and were released in the aquarium tanks (12 nos./tank). Labelled 2,4-D (2,4-D 2 C-14 specific activity 0.5 mCi/m.mole obtained from Board of Radiation and Isotope Technology, Mumbai) mixed with technical 2,4-D dissolved in acetone was added to the tanks to give ^{14}C activity of approximately 2000 dpm/ mL of water so as to get a detectable count for the treatment viz.1.0 kg $ha^{-1.}$ Three replications were kept for each treatment and the water level was maintained at fixed level. *Salvinia*, sediment and fish samples used for the study were subjected to residue analysis for 2,4-D residues , before introducing the materials into the tank. Three samples were analysed for each material , immediately after collection so as to ensure the absence of 2,4-D residues in these materials. Samples of water (5mL), fish (one whole fish of approximately 10g was collected , macerated and apportioned into parts of 1g each) and *Salvinia* (approximately 5g was collected , homogenized and apportioned into parts of 1 g each), sediment (10 g was collected and apportioned into 5 parts of 1 g each after air drying) collected from each of the treated tanks at different days after spraying (DAS) as mentioned in the Table 2 were subjected to ^{14}C analysis by suspension counting with

Cocktail W (10 mL/sample) using Wallac 1409 liquid scintillation counter. Percentage of the applied radioactivity remaining in the samples taken at different periods were calculated and the residue content was estimated from these data.

The study was repeated in the concrete tanks kept under open condition as done for the estimation of paraquat residues except that *Salvinia* was only selected for the study.

3. Glyphosate residues

In order to simulate field condition of a water body infested with aquatic weeds, plastic basins of 45 cm diameter were purchased from local markets of Dombivili. *Pistia* plants (common aquatic weed) and mud were also procured from local markets. The basins were filled to 80% of the capacity with tap water after keeping a mud layer of 2cm at the bottom. *Pistia* plants (30 Nos.) were introduced in to the basin to form a trim mat on the water surface. Water level was maintained upto one week and glyphosate was applied @ 1.0 kg ha^{-1}. Three replications were included in the study. Five hundred milliliter muddy water samples from each basin was collected for estimation of glyphosate residues. Prior to estimation of glyphosate residues, standardisation of the method suggested by Bardalaye *et al.* (1985) was done using a Partisil column in a high performance liquid chromatography (HPLC) equipped with a UV detector at different fortified levels.

4.Histopathological Studies

In order to study the histological alterations in fish organs, the fish were grown in glass aquariums containing water (40 L) and fish (7 nos. *Tilapia* sp.) so as to observe the changes in fish at maximum possible concentration of herbicides in water (0.40, 0.50 and 0.60 ppm respectively for paraquat, 2,4-D and glyphosate respectively) . The herbicides at the above concentrations were applied to water and kept as such for one week without changing water. One sample fish was taken form each tank and kept outside in a tray till it died. Tissues such as gills, liver and pancreas (hepatopancreas) and muscles were removed immediately and fixed in formalin. Tissue sections were prepared by routine histopathological technique to study the microscopic changes in the different regions. This work was done at the Departments of Veterinary Anatomy and Histology and Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy.

Results and Discussion

Persistence of herbicides

Paraquat residues

The residue data pertaining to years 2008 to 2011 were averaged and presented in Table 1. Water samples in all the treatments attained the residue below the detectable levels by 60 DAS. At 30 DAS, the residues were above the permissible limits in all the treatments. Pooled analysis of the data was not done because the residue levels at 30 and 60 DAS were homogeneous with respect to all the treatments During the initial period of sampling, water samples recorded variations in the residue. Paraquat concentration in the water samples at one day after spraying ranged from 0.160 to 0.482 ppm depending on the target weeds on which the herbicide was applied. About 65.00 to 70.00 per cent of the herbicide applied for aquatic weed control dissipated from water during the initial 20 days period. However, the rate of dissipation at later period was low, but the concentration attained below detectable level (BDL) by 60 DAS. As per the European Economic Community Directive (1988), the permissible limit of any pesticide in drinking water is 0.1µg L^{-1}(0.0001 ppm). Since the content of paraquat residue in the water samples at 30 DAS was very much higher than the permissible limits it is necessary to provide at least 2 months waiting period. Sediment (0.1ppm) and fish samples (0.4 ppm) recorded residues which indicated that there is a chance of accumulation of residues in the bottom sediment. Since paraquat is a cationic herbicide, it can bind to negatively charged sediment particles.

2, 4-D residues

Results of the studies (Table 2) indicated that application of 2, 4-D at recommended rate of application of 1.00 kg ha^{-1} over *Salvinia* did not affect the growth of fishes in the system. The quantity of residues present in the fish

body at 60 DAS was very much lower than the acceptable daily intake of 0.01 mg per kg body weight (RSC, 1987). However, in the water samples, 0.25 % of the applied herbicide remained in water at 360 DAS indicating the slow degradation of the herbicide in water under closed condition. Several reports indicated very high persistence of 2, 4-D in still water (USEPA, 1988). Sediment fraction retained 10.5% of the applied activity at 120 DAS.

In the open concrete tanks, 2, 4-D residues were considerably lower than that of concrete tanks. The quantity detected at 7 days after spraying was 0.005ppm and that of 15 DAS was below the detectable level. Since this experiment was conducted in the monsoon season, rain occurred at 10 DAS which resulted in the dilution of residue. Therefore, under field condition, persistence of residues at the recommended level of 2, 4-D is short, especially during the rainy season.

Glyphosate residues

Both gas chromatography (GC) and HPLC procedures for determination of glyphosate residues are very tedious. Since this chemical compound possesses three polar functional groups (phosphonic acid, carboxylic acid and secondary amine) and it is not soluble in common organic solvents, derivitization is required, which is a complicated one. Among the various GC procedures outlined by Bardalaye *et al*., (1985) formation of 2-chloroethyl –n-heptafluorobutyryl ester of glyphosate is comparatively simple and hence tried in the experiment. However, at the concentration used in the experiment (1ppm) the analysis with this GC method did not give any result. HPLC was tried at fortification levels *viz.* 10, 40 and 200 ppm and found that more than 90 % of the applied glyphosate is adsorbed on to mud portion. The method failed to give result at lower concentrations of glyphosate.

Histological alterations in fish

Histological changes in gills, liver and pancreas (hepatopancreas) and in muscles due to herbicide application (Fig 1A and B) indicated that the herbicide application did not make any

Table 1. Persistence of paraquat residues in the aquatic system

Treatments Paraquat (1.0 kg/ha)	*Residues (µg $mL^{-1)}$							
	1 DAS	5 DAS	10 DAS	20 DAS	30 DAS	60 DAS	Fish (60 DAS)	Sediment (60 DAS)
No weed (free water surface)	0.482	0.446	0.167	0.125	0.054	BDL	NA*	
Salvinia	0.198	0.158	0.062	0.05	0.044	BDL	0.40	0.10
Eichhornia	0.160	0.143	0.098	0.050	0.030	BDL	NA	
Alternanthera)	0.232	0.160	0.089	0.050	0.036	BDL	NA	

Average of 4 years data ; NA: Not analysed

Table 2. Residues of 2, 4-D expressed as percentage of applied radioactivity under closed condition

Components of aquatic system	* Percentage of the applied ^{14}C activity remaining in the samples							
	1 DAS	7DAS	15 DAS	21 DAS	30 DAS	60 DAS	120DAS	360DAS
Water	64.88	62.93	14.16	2.18	0.74	0.47	0.27	0.25
Fish	0.03	0.03	0.41	0.43	0.47	0.46	0.11	NA
Salvinia	0.61	0.19	0.57	0.49	0.46	0.26	NA	
Sediment	NA						10.54	NA

NA: Not analyzed ; *Average of three replications

change in the fish gills. However, in the liver, vacuolation of cytoplasm was seen in hepatic cells with all herbicide treatments. Application of 2, 4-D resulted in necrotic changes also. The muscle fibres showed loss of striations with glyphosate and 2, 4-D application. In the case of 2, 4- D, fragmentation of muscle fibres was also seen. No loss of striations was observed with paraquat. The results indicated that application of 2, 4-D caused greater impact on fish than that of paraquat and glyphosate.

Figure 1: Histological alterations in fish organs due to herbicides in water at the maximum possible concentration

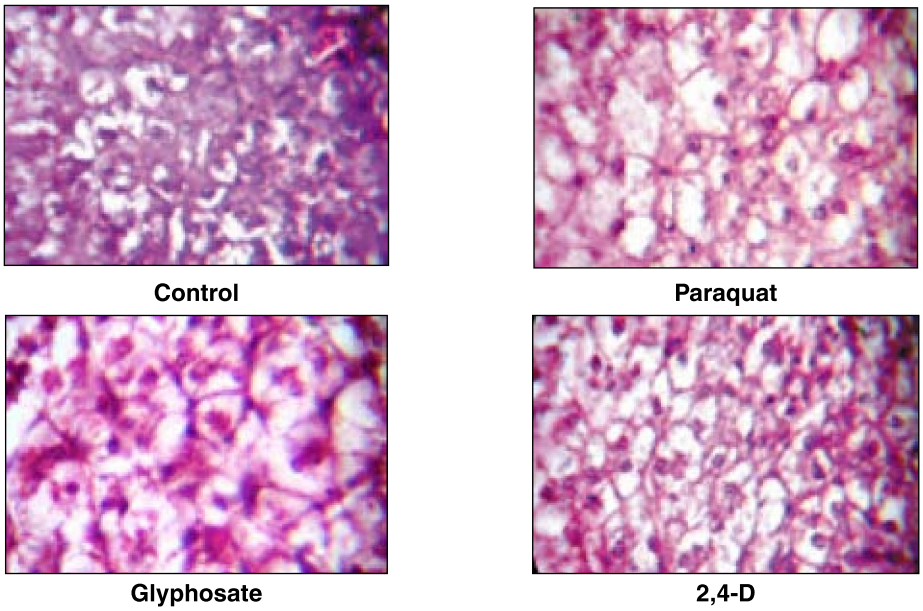

Fig.1. A. Liver (Hepatopancreas). H& E. x 400.
* Fatty changes in liver with herbicide treatments, maximum in paraquat
* 2, 4-D treatment showed focal necrotic changes also

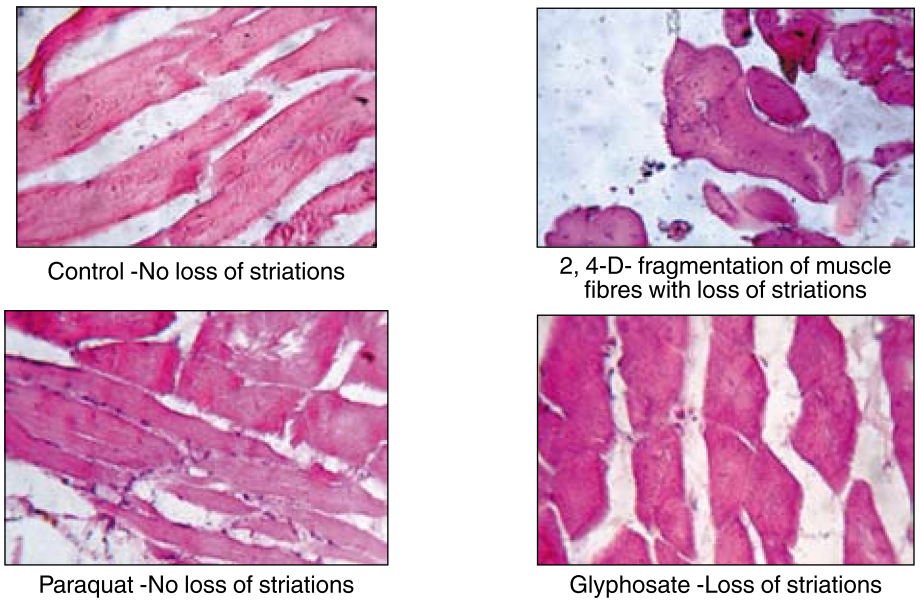

Fig. 1. B. Muscles. H& E. x 400.

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