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Effect of density and dose of herbicide on entry, translocation and root exudates in water hyacinth

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ABSTRACT

Spraying of foliar herbicide to control water hyacinth may lead to mortality of the fish by root exudation of herbicide or accidental drift of herbicide. To overcome herbicide-divalent cations complex formation in hard water at improper pH, increasing herbicide dosage per drop, either by increase of dosage or by reduce the spray volume, was one of the techniques. Using radioactive ^{14}C-glyphosate and ^{14}C-2,4-D, effect of dosage on herbicide retained on the cuticle as dried out residue, distribution with in plant system and moved out as root exudates was assessed. Twice the recommended concentration (2x) of herbicide per drop and drop density per fed spot significantly increased the dried residue on the cuticle 67 to 91 and 68 to 79% over total activity recovered compared to x dose for glyphosate and 2,4-D, respectively. 2,4-D (2x) significantly increased the amount of herbicide cuticle loading and mobility within cuticle but differences in translocation to different parts was not significant except in root (2x had lower activity than x). Similarly, significant difference between 2x and x for glyphosate was observed for cuticle loading and root exudates. At 2x dosage, root exudates of both herbicides was more after 8 days. Half-life in pond water was around 5 days for both herbicides compared to normal water 7 and 11days of glyphosate and 2,4-D respectively. The chances of hherbicide toxicity to fishes were unlikely by foliar applied herbicides.

Key words: Aquatic weed, Chemical control, Dosage, *Eichhornia crassipes,* Herbicide

Eichhornia crassipes is a floating weed with rosettes of leathery, waxy, green leaves attached to thick, spongy (inflated for floatation) petiole. Dark feathery roots that typically hang suspended in the water below the plant and have attractive lavender flowers. Water hyacinth was introduced to remove heavy metal toxicity from pond but owing to its high transpiration rate it has become a threat to irrigation water. Mortality of fish was observed when herbicide was used to kill water hyacinth. High density of fine drops of foliage herbicide known to increase dried residue, entry and translocation in various weed species compared to single coarse drop (Anonymous 2005). Reducing volume of spray 194 to 24 l/ha or enhanced dosage eliminated the antagonistic effect of ionized glyphosate chelating with calcium, magnesium present in hard water spray solution (Buhler and Burnside 1983). Thus with this background, experiments were conducted 1) to assess the effect of dosage and density of labelled herbicide on entry, translocation, root exudates and 2) to assess the half-life of herbicide in different sources of water.

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MATERIALS AND METHODS

Young water hyacinth was allowed to float in plastic pots of size 30 cm diameter with 30 cm depth and one litre mark level indicator for 30 days. Daily water loss due to evapo-transpiration was assessed and Hebbal Lake pond water was added to maintain one liter level at 9:00 h.

Preparation of ^{14}C-glyphosate

^{14}C-methyl labeled glyphosate with specific activity of 0.01 mci/mole was procured from Sigma Aldrich and mixed with 1 µl 2000 ppm non radioactive glyphosate (Roundup 41 SL). From this stock, to 10 µl of ^{14}C-glyphosate, 1 µl of 1.64 kg/ha Roundup (10 µl/l) was mixed. Similarly, 20 µl of ^{14}C-glyphosate stock µlof 3.28 kg/ha of glyphosate (20 ml/l) was mixed.

^{14}C-glyphosate -x (1.64 kg/ha –recommended)
^{14}C-glyphosate -2x (3.28 kg/ha–twice recommended)

Preparation of ^{14}C-2,4-D

^{14}C-carboxyl labeled solid 2,4-D with specific activity 92 mci/mmol was dissolved in 15 µlethanol. Then volume was make up to 1.5 ml with 5000 ppm of 2,4-D (non-radioactive) sodium salt (80%). From this stock, to 10 µl of labeled 2,4-D, 1 µl of 3.2 kg/ha (10 g/l) 2,4-D and to 20 µl of labeled 2,4-D 1 µl of 6.4 kg/ha of 2,4-D (20 g/l) was added to obtain different treatment.

^{14}C-2,4-D -x (3.2 kg/ha)

^{14}C- 2,4-D -2x (6.4 kg/ha)

These four treatments were given to 30 days old water hyacinth with five replications. For x and 2x dosage, 5 and 10 drops of 0.2 µl were placed on fully expanded young leaf. Thus varied drop density and concentration of herbicide per drop affect was assessed on dried residue, entry into cuticle, the plant system and exudates of herbicide from root.

Measurement of radioactivity in root exudates

At regular interval of two days water sample of one ml was drawn near the root zones of water hyacinth to assess the radioactivity that has moved out of plant system.

Measurement of radioactivity

After 10 days, plants were harvested and separated into different plant parts *viz*., 1) washing the fed spot (dried out residue on the cuticle); 2) fed spot of the fed leaf (partitioning coefficient between different cuticle layers and desorption from cuticle); 3) translocation to other portion of the fed leaf (within fed leaf); 4) other leaves and 5) root. Aqueous extract of plant parts using pestle and mortar, volume was made up using water and acetone used as washing material between samples. Radio-activities (counts per minute -CPM) was measured using Liquid Scintillation Counter (LSC) of make Wallac- Model 1409 with scintillation liquid of 3 ml with or without plant (10 µl) or water (50µl) sample and eq (1). Radioactivity was expressed in CPM/100 mg of organ weight, except dried out residue CPM/10 ml washed water.

CPM = (R/aliquot) x volume made up ———(1)

R indicates radioactivity of sample after correction for blank was multiplied by a factor (eq.2) to give correction for LSC dilution factor. The LSC was unable to detect over lapping of radiation within a microsecond which occurs due to high concentration of radioactive compound present.

LSC dilution factor correction = 4E-12x^2 + 4E-06x + 0.054 —(2)

Where x indicated observed activity CPM of the sample.

Measurement of radioactivity in water residue

Pond water might contain microbes compared to normal water to degrade the herbicides. Herbicide (2x) was added to the beaker containing 25 ml of normal or pond water with five replications. The initial CPM/25 ml of ^{14}C-glyphosate and ^{14}C-2,4-D were 1504 and 1214, respectively. At regular interval of two days for ten days, later on at 5 days intervals, samples of one ml were drawn and activity was recorded for 20 days. Whenever samples were drawn, one ml of normal or pond water was added. Per day, 3.2 ml of water was evaporated and thus activity of herbicide can be concentrated. To compensate the radioactivity measured was given a correction factor (CF) using the following equation (3)

CF = (Q/25)/ 0.025 ———(3)

Where, Q indicated quantity of water remaining in the beaker on the day of radioactivity measured, 25 is the quantity of water initially present and 25µl is the aliquot taken for the radioactivity measurement. Treatment was replicated five times and data were statistically analyzed using RBD design.

Half life and persistence of herbicides in water

Regression between periodic herbicide mean radio-activity in water against time was computed. The model performance was judged by coefficient of determination (R^2) and residue sum of square (RSS = S (observed-predicted response) 2) observed that first order exponential equation fitted well (eq 4) with high R^2 and low RSS values (0.1-0.45). However, forcing a=Y showed low R^2, poor fit still, half life was computed using a=Y in (eq 5).

$Y= a\ e^{-bt}$ ——————(4)

Half life (days) = [ln (Y) –ln(Y/2)]/b———————(5)

Where Y/2 is half of initial activity of respective herbicides, “a” indicates the Y axis intercept and “b” denotes slop (rate of degradation) of best fit regression curve. Statistical analysis of all data was carried out using RBD with five replications.

RESULTS AND DISCUSSION

Increase drop density and concentration 2x of both herbicides per drop per fed spot enhanced the dried out residue significantly on the cuticle than recommended (x) (Table 1). The per cent dried out reside increased to 91.2 and 79.5 for glyphosate and 2,4-D than x dose 67.2 and 67.9, respectively. Radioactivity in fed spot showed significant penetration of herbicide into the cuticle in 2,4-D but not for glyphosate, indicating 2,4-D movement across cuticle layers block than glyphosate. Entry of herbicide into cuticle depends on diffusibility of herbicide from drop, and mobility of herbicide across different layers

Table 1. Effect of dosage of radioactive herbicide (CPM/100 mg) on entry and translocation in water hyacinth (10 days after feeding)

Treatment	Dried residue	Fed spot	Rest of fed leaf	Other leaves	Root	Water	Total
^{14}C-glyphosate x	2057 (45.4^{c})	320(17.8^{c})	407(20.18^{a})	87 (9.32)	155 (12.45^{a})	35^{ab}	3061 (55.32^{d})
^{14}C-glyphosate 2x	6166 (78.5^{a})	334(18.3^{c})	345(18.55^{a})	111 (10.54)	62 (7.88^{bc})	44^{ab}	7062 (84.0^{b})
^{14}C- 2,4-D x	3368 (57.9^{b})	1186(34.4^{b})	114(11.03^{b})	173 (12.87)	85 (9.23^{b})	29^{b}	4955 (70.39^{c})
^{14}C-2,4-D 2x	6546 (80.9^{a})	2187(45.9^{a})	98 (9.85^{b})	147 (11.8)	52 (7.20^{c})	55^{a}	9085 (95.3^{a})
LSD (P=0.05)	(6.03)	(10.53)	(3.12)	(NS)	(1.44)	19.3	(7.11)

Figures in the parentheses are square root transformed values

*Superscripts indicate Duncan's range test, treatments with same alphabet are non-significant

of cuticle by partitioning coefficients, for this availability of free herbicide molecule plays an important role. Herbicides forms complex with divalent cation *viz.*, Ca^{++}, Mg^{++}, Fe^{++} present in hard water at improper pH spray solution or 2,4-D bound to epoxides and glyphosate with Ca^{++} present in the cuticle layers, thus free herbicide molecules gets reduced and antagonize the herbicide movement across cuticle. Further, divalent cations deposits as dried out residue from glandular trichome exudates by the process of guttation was reported (Carole and Philip 1982). Alkaline pH (6 or 10) ionizes the herbicides having carboxylic (COOH) in the drop and form herbicide-cation complex thus free herbicide molecules are not available for cuticle penetration (Patrick and Duane 1984).

Higher mobility of glyphosate from fed spot to rest of the fed leaf in both concentrations than 2,4-D was observed suggesting that glyphosate was more translocation type than 2,4-D. Translocation from fed leaf to other leaves was not affected by increased dosage of herbicides. However, in root 2x of herbicides had significantly less herbicide than x dose for both herbicides. Water near to the root zone recorded significantly more 2,4-D but the per cent of total in water due to root exudates ranged 0.4 to 1.1. This suggested that much of the herbicide remained as dried out residue with little or negligible as root exudates.

Root exudates of herbicide to the water near the root zone increased in 2x significantly after 8 days (Table 2) but still the root exudates was 1.1 per cent of total activity recovered. Thus herbicides remain in water hyacinth and do not pollute the water body, therefore herbicides toxicity to fishes were unlikely by foliar applied herbicide.

Residue of ^{14}C-herbicide in normal or pond water indicate drastically reduction of both herbicide in pond water than normal water (Fig. 1). Pond water infested with water hyacinth microbes which degrade the herbicide faster than normal water. Accidental drift of herbicides spray droplet into the water lost its effect within 5 days. Half-life of glyphosate and 2,4-D was around 5.2 days in the pond water whereas it was around 8 and 12 days for glyphosate and 2,4-D in normal water (Table 3). Kirkwood (1979) suggested high adsorption of glyphosate to soil sediment (major sink) which cause slow degradation by microbes (low population in soil than in water). Half life of glyphosate may be >35 days, but it was 1.5 -11.2 days in water (Newton *et al.* 1994). Unlike, 2,4-Dichlorophenol degraded product of 2,4-D which is 15 times more toxic than 2,4-D (www.24d.org), degraded product of glyphosate seemed to be non toxic to fish (U.S. EPA RED 2005). Much of the herbicide gets remained in water hyacinth and accidental drift of the herbicide into the water will be degraded by five days. Thus it was inferred that mortality

Table 2. Effect of dosage of radioactive herbicide (CPM/µl) on periodic herbicide root exudates into water from water hyacinth

Treatment	Days after treatment				
	2	4	6	8	10
Glyphosate x	25.4	27.0	30.4	35.6^{b}	38.7^{b}
Glyphosate 2x	34.9	36.4	36.7	44.0^{ab}	60.6^{a}
2,4-D x	20.6	23.9	29.0	29.3^{b}	35.4^{b}
2,4-D 2x	29.0	31.0	40.7	53.5^{a}	54.6^{ab}
LSD (P=0.05)	NS	NS	NS	17.8	19.3

*Superscripts indicate Duncan's range test, treatments with same alphabet are non-significant

Table 3. Half-life of ^{14}C-herbicide in water (bore well or pond) as estimated by first order exponential equation

Treatment	Type of water used	Exponential equation	R^2	Half-life (in days)
^{14}C-glyphosate	Normal water	$1504e^{-0.09t}$	0.671	7.7
	Pond water	$1504e^{-0.13t}$	0.672	5.3
^{14}C-2,4-D	Normal water	$1214e^{-0.06t}$	0.822	11.5
	Pond water	$1214e^{-0.13t}$	0.130	5.3

Normal water- tube well water of Main Research Station, Hebbal. Pond water- water from *Eichhornia* infested Hebbal Lake

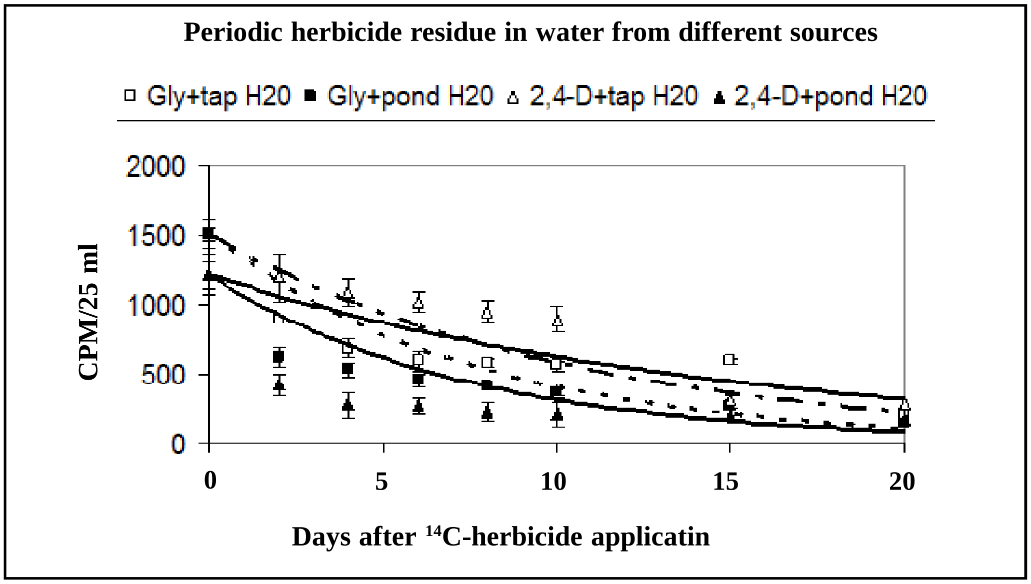

Fig. 1. Radioactive herbicide (2x) decay in different sources of water. Solid line represents 2,4-D and dotted line indicates glyphosate degradation pattern. Error bar indicates replication variations

of the fish in herbicide treated pond is not due to herbicide toxicity but decaying plant material which depletes the oxygen in water, therefore instead of increasing the dose of herbicide the good adjuvants should be used to enhance the penetration of herbicide.

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