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USE OF ISOTOPIC TRACERS IN STUDIES ON ^{14}C-GLYPHOSATE PERFORMANCE ON *CYPERUS ROTUNDUS* IN POT AND FIELD CONDITIONS

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Abstract

The effect of surfactants and oil on bioefficacy of the herbicide, glyphosate in controlling *Cyperus rotundus* L. was evaluated using potted plants. A mixture of the commercial formulation, "Roundup" with 0.2% Triton X-100, 1% diesel oil and 1% of 4% aqueous ammonium sulfate produced the most penetration into the leaf. The results of the field experiments suggested that this mixture applied at a rate of 1.5 kg/ha glyphosate amended "Roundup" can effectively control *C. rotundus* in the field.

1. INTRODUCTION

The methods of formulating an agrochemical, particularly the addition of surfactant adjuvants, can have a profound influence on the efficiency of control of the target plant, by affecting uptake, redistribution and persistence and consequently its ultimate biological performance. Optimising the performance of most commercial pesticide formulations is still done primarily by trial and error but work on the effect of surfactants on the uptake of neutral model organic compounds with disparate physico-chemical properties offers a more rational guideline for adjuvant selection and formulation design using a predictive response surface model approach [1, 2].

Glyphosate, a non-selective, systemic, post-emergence herbicide, controls *Cyperus rotundus,* purple nutsedge, to some extent under many conditions [3–7]. The commercial formulations and application specifications are usually designed to give acceptable performance on a range of species. It is possible to improve performance on particular targets by manipulating formulation and application factors such as the addition of surfactants [8]. Surfactants of different properties may enhance the activity of glyphosate by improving penetration and translocation [9,10] or reduce it by antagonizing uptake [11].

The aim of the studies was to:

(a) standardize the type and concentration of the additives to be used with the "Roundup"commercial formulation of glyphosate for effective penetration in *C. rotundus* under pot conditions.

(b) to assess the time course of penetration over 24 hours.

(c) and finally to apply the results of the pot experiments to studies under field conditions.

2. MATERIALS AND METHODS

2.1.Laboratory experiments

2.1.1. Estimation of the amount of Triton X-100 needed to emulsify 1% oil in Roundup in the presence of ammonium sulfate

The basic mixture used consisted of 54 mL, water. 25 mL "Roundup" (diluted 1:12 to give 30 mg a.e./mL). 1 mL oil (diesel was used through out being the cheapest). 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 mL Triton X-100 was added to 100 mL of the above mixture which was then shaken and allowed to stand over night. The lowest quantity of Triton X-100, that maintained the emulsion was selected. Besides Triton X-100, two other locally available detergents were also tried.

2.1.2. 24 h penetration studies

Cyperus rotundus tubers were collected, selected by weight to ensure uniformity, germinated and plants were grown in the pots as reported earlier. Six uniform plants were selected when they had 6 leaves and the 2nd oldest leaf were used for the operation in all studies and each treatment was repeated on four different days. The herbicide application was done at the same time of the day on each occasion.

The six treatments applied were:

A. "Roundup" alone: 2.5 µL ^{14}C-glyphosate solution + 25 µL "Roundup" diluted 1:12 + 72.5 µL water (i.e. 0.185 KBq/0.005 µCi labelled glyphosate which was one-tenth of the activity used before).

B. "Roundup" + ammonium sulfate: As for A above but replaced 25 µL of the water with 25 µL 4% $(NH_4)_2SO_4$.

C. "Roundup" + Triton X-100: As for A. with addition of the amount of Triton X-100 selected from experiment 1.

D. "Roundup" + Triton X-100 + $(NH_4)_2SO_4$: As for B with the addition of Triton X-100 as in C.

E. "Roundup" + Triton X-100 + oil: As far C but replaced 1 µL of the water with 1 µL oil.

F. Roundup + Triton X-100 + oil + $(NH_4)_2SO_4$: As far E but replaced 25 µL of water with 25 µL 4% $(NH_4)_2SO_4$.

As described previously, 8 × 0.5 µL drops were applied on either side of the midrib in a 2 cm area starting 10 cm from the tip without touching the leaf. Four µL aliquots were added in to 2 scintillation vials to check the application rates. After 24 hours, the treated area was painted with 6% cellulose acetate in 9:1 acetone/water. The dried cellulose acetate was removed with tweezers and dissolved in 2 mL glacial acetic acid and 5–10 mL "Permaflour" was added for counting. Cellulose acetate was also applied to untreated leaves.

2.1.3. Modification of treatment studies

The best treatment from experiment 2 was selected and formulation No. F was modified by replacing 1% diesel oil with 0.5% or 2% diesel oil or with 1% glycerol.

2.1.4. Short term penetration studies
Formulations D and F were selected for the above experiment and penetration was measured over 1 hour and 2 hours following the revised protocol.

2.2. Field experiments

2.2.1. Layout

The best modified formulation found in the pot experiments was tested at rates of 1.5, 0.75 and 0.5 kg/ha a.e. glyphosate and compared with 1.5 kg a.e. glyphosate in unamended "Roundup". The application rate was 200 L/ha using a spray pump made by GLORIA 172-R Germany with a nozzle flow rate 500 ml/min at a pressure of 6 bar. There were two times of application, 8 a.m. and 5 p.m. for both the cropped (with maize) and uncropped plots.

The design was split split in RCBD with 4 replications plot (cropped/uncropped); the size of the cropped plots was 5 × 5 m and that of the uncropped 5 × 3m. There were untreated control + 4 glyphosate treatments × 2 times of application × cropped/uncropped = 5 × 2 × 2 = 20 treatments with 4 replicates, to give 80 plots. In addition, the cropped plots included a hand-weeded control, to make a total of 84 plots. The experimental area was assessed to confirm a natural stand of *Cyperus*. The area was ploughed, the seed bed prepared and irrigated following local agricultural practices. Two days before glyphosate application, the number of *Cyperus* shoots were counted in three 0.25 × 0.25 m quadrats per plot. Seeds of the maize variety (China) were sown three days after glyphosate applications.

2.2.2. Assessment

Weed control was visually estimated on the *Weed Science* scale of 0, 100, where 0 = no weed reduction or injury and 100 complete weed reduction, 4 and 8 weeks after treatment. In uncropped plots *Cyperus* was harvested from half the plot after four weeks and the other half after 8 weeks. Fresh and dry weights were recorded. Yield of the maize crop was measured and all vegetation (crop + weed) was cut to 2 cm above the ground and visual observations were recorded after two weeks and the field was further assessed after eight weeks. The effect of the treatment on tuber formation of *Cyperus* was noted ten weeks after cutting the vegetation.

2.2.3. Statistical analysis

The data collected were subjected to the analysis of variance (ANOVA) and Duncan's New Multiple Range Test [12].

3. RESULTS AND DISCUSSION

During the experimental period between May and September, 1995 daily temperature minima were 22–30°C and maxima 35–48°C. Relative humidities ranged from 62–70% in the morning and 45–56% in the evening. There were 3 showers in September.

The results of the studies made to select the best emulsifier that can hold and maintain the emulsions to be applied weed indicated that 0.02 and 0.05% of Triton X-100, could emulsify 1% diesel oil so 0.02% was chosen. The two locally available surfactants tested were no better than 0.02% Triton-100.

TABLE 1. EFFECT OF FORMULATION ON PENETRATION OF GLYPHOSATE

Treatments	% Recovery of ^{14}C-Glyphosate from cellulose acetate on four different days				
	Day 1	Day 2	Day 3	Day 4	Mean
A	52.6	50.7	54.2	56.3	53.4 b
B	64.1	62.7	56.6	61.7	61.3 a
C	51.1	53.3	47.6	55.8	52.0 b
D	35.7	38.4	33.9	34.2	35.5 d
E	41.7	51.7	48.2	50.6	47.9 c
F	34.0	35.3	30.9	31.8	33.0 e

Means in the same column followed by different letters are significantly different ($p < 0.05$).

The 24 hours penetration studies are presented in Table1. Out of six treatments during four different timings, treatment F produced the best glyphosate penetration followed by D. Treatments A and C were not significantly different and D and E gave more penetration but values were lower than for F. The results of further modification in the selected formulation are presented in Table 2. There was no difference between 2% diesel oil and 1% but 0.5% produced lower penetration. Replacement of diesel oil by glycerol did not improve performance. Therefore 1% diesel oil was chosen.

TABLE 2. MODIFICATION OF TREATMENT MIXTURES

Treatments	Recovery of ^{14}C-glyphosate from cellulose-acetate						
	1	2	3	4	5	6	Average
M-1	48.5	46.2	45.6	48.5	42.9	48.0	46.6 a
M-2	36.9	39.3	38.3	46.3	40.9	41.3	40.5 b
M-3	34.5	35.1	37.6	32.1	32.7	34.5	34.4 c

Means in the same column followed by different letters are significantly different ($p < 0.05$).
Treatments:
M - 1 = Mixture with Glycerol.
M - 2 = Mixture with 0.5% diesel oil.
M - 3 = Mixture with 2% diesel oil.

The shorter term studies over 1 and 2 hours (Table 3) did not show differences between D and F so did not discriminate between formulations as effectively as the 24 h study. However, in tropical conditions, rainfastness is important for glyphosate so the shorter term assessment may have more practical value but this point was not explored further.

TABLE 3. SHORT TERM PENETRATION STUDIES

Treatments	Time (h)	% recovery of ^{14}C-glyphosate from cellulose-acetate					
		1	2	4	5	6	Average
D	1	70.2	73.8	77.9	71.0	76.2	73.4 b
	2	60.6	56.3	61.2	53.5	56.0	57.3 c
F	1	65.9	70.2	67.9	72.3	69.5	69.3 b
	2	55.8	52.7	58.1	56.3	53.1	55.1 c

Means in the same column followed by different letters are significantly different ($p < 0.05$).

TABLE 4. BEFORE SPRAYING GLYPHOSATE, NUMBER OF *CYPERUS* SHOOTS IN THREE 0.25 m × 0.25 m QUADRATS PER PLOT (MORNING)

Treatments	Uncropped plots				cropped plots			
	plot-1	plot-2	plot-3	plot-4	plot-1	plot-2	plot-3	plot-4
Control	9 7 10	6 10 8	7 5 8	8 6 11	8 6 5	10 7 6	8 8 10	7 5 11
Two days before 1st treatment	8 8 10	12 6 7	4 6 7	5 8 12	7 8 12	9 6 4	6 8 7	5 9 9
Two days before 2nd treatment	3 7 10	8 6 7	7 6 11	8 8 10	7 6 6	8 4 3	9 8 4	6 8 5
Two days before 3rd treatment	11 7 5	9 6 5	8 6 5	9 7 8	12 7 9	10 6 9	7 5 12	8 6 9
Two days before 4th treatment	10 8 6	8 7 6	8 6 10	6 8 9	8 9 9	7 5 13	9 8 10	11 7 8

TABLE 5. BEFORE SPRAYING GLYPHOSATE, NUMBER OF *CYPERUS* SHOOTS IN THREE 0.25 m × 0.25 m QUADRATS PER PLOT (EVENING)

Treatment	Uncropped plots				Cropped plots			
	plot-1	plot-2	plot-3	plot-4	plot-1	plot-2	plot-3	plot-4
Control	8 7 10	7 4 9	7 8 10	5 9 8	10 8 10	8 8 8	9 12 7	10 8 7
Two days before 1st treatment	11 6 8	7 9 5	6 8 11	6 12 8	9 11 7	6 9 8	6 9 10	11 10 8
Two days before 2nd treatment	9 6 9	6 8 8	10 5 9	7 8 11	8 12 9	10 8 6	8 10 10	7 11 9
Two days before 3rd treatment	10 8 7	8 5 9	8 7 8	5 9 5	7 12 9	8 9 7	7 9 12	9 9 6
Two days before 4th treatment	5 8 8	7 7 11	8 10 9	10 10 8	8 6 10	8 9 9	8 5 9	4 9 7

The assessment of weed population in the field plots made two days before herbicide application showed that the population was uniform over the whole site (Tables 4 and 5).

The percent weed control ratings recorded in Table 6 show that the maximum efficacy was given by 1.5 kg/ha amended "Roundup", followed by 1.5 kg/ha unamended 'Roundup". The difference between these treatments was highly significant and both were significantly better than the other three. These treatment differences were the same for plants treated at 4 and 8 weeks, for the cropped and uncropped plots and for the morning and evening times of application.

TABLE 6. WEED CONTROL RATING OF GLYPHOSATE FORMULATIONS APPLIED ON *CYPERUS ROTUNDUS*

Treatment	Uncropped plots					Cropped plots				
	Morning		Evening		Average	Morning		Evening		Average
	4 week	8 week	4 week	8 week	Average	4 week	8 week	4 week	8 week	Average
Control	0	0	0	0	0 e	0	0	0	0	0 e
1.5 kg/ha unamended "Roundup"	60	60	60	60	60 b	60	60	60	60	60 b
1.5 kg/ha amended "Roundup"	80	90	80	90	85 a	80	90	80	90	85 a
0.75 kg/ha amended "Roundup"	30	30	30	40	32 c	30	30	30	30	30 c
0.5 kg/ha amended "Roundup"	20	20	20	20	20 d	20	20	20	20	20 d

All values are average of four replicates.
Means in the same column followed by different letters are significantly different ($p < 0.05$).

TABLE 7. FRESH WEIGHT OF *CYPERUS* PLANTS IN PLOTS TREATED WITH DIFFERENT CONCENTRATIONS AND FORMULATIONS OF GLYPHOSATE

	Morning plots		Evening plots		Av. weight
	After 4 week	after 8 week	After 4 week	after 8 week	
Control	2.2	2.4	2.1	2.5	2.3a
1.5 kg/ha unamended "Roundup"	1.8	1.6	1.7	1.6	1.7b
1.5 kg/ha amended "Roundup"	1.1	1.9	1.1	1.0	1.1c
0.75 kg/ha amended "Roundup"	1.8	1.7	1.9	1.6	1.8b
0.5 kg/ha amended "Roundup"	2.5	2.1	2.3	2.2	2.3a

* The values are average of 4 plots.
Means in the same column followed by different letters are significantly different ($p < 0.05$).

TABLE 8. DRY WEIGHT OF *CYPERUS* PLANTS IN PLOTS TREATED WITH DIFFERENT CONCENTRATIONS AND FORMULATIONS OF GLYPHOSATE

Treatment	Morning plots		Evening plots		Average weight	Weight loss
	After 4 week	After 8 week	after 4 week	After 8 week		
Control	0.66	0.68	0.62	0.68	0.66a	71.6 a
1.5 kg/ha unamended "Roundup"	0.62	0.50	0.53	0.51	0.54c	68.6 b
1.5 kg/ha amended "Roundup"	0.33	0.32	0.30	0.29	0.31 d	71.5 a
0.75 kg/ha amended "Roundup"	0.58	0.53	0.57	0.53	0.55c	68.7 b
0.5 kg/ha amended "Roundup"	0.69	0.59	0.65	0.60	0.63b	72.0 a

The values are average of 4 plots.
Means in the same column followed by different letters are significantly different ($p < 0.05$).

TABLE 9. EFFECT OF DIFFERENT CONCENTRATIONS OF GLYPHOSATE ON MAIZE YIELDS

Treatment	Average weight of 10 maize plants (kg)			Average weight of 10 maize cobs (kg)		
	Morning (plots)	Evening (plots)	Average	Morning (plots)	Evening (plots)	Average
Control	5.54	4.610	5.07 e	2.23	2.13	2.18 e
1.5 kg/ha unamended Roundup"	9.70	9.72	9.71 b	3.52	4.20	3.91 b
1.5 kg/ha amended "Roundup"	11.57	12.59	12.08 a	5.05	4.80	4.93 a
0.75 kg/ha amended "Roundup"	9.23	6.41	7.84 c	3.22	2.65	2.94 c
0.5 kg/ha amended "Roundup"	7.5	5.86	6.68 d	2.77	2.52	2.65 d

* These values are average from four plots.
Means in the same column followed by different letters are significantly different ($p < 0.05$).

The fresh weight data of the plants in the uncropped pots (Table7) show that 1.5 kg/ha amended and unamended "Roundup" and 0.75 kg/ha amended "Roundup" significantly reduced fresh weight of *Cyperus* after 4 weeks compared with controls but the 0.5 kg/ha amended treatment did not. There were no differences between plots treated in the morning and evening. Plants in the control plots increased in weight at 8 weeks but the treated plants did not. Corresponding measurements were not made in the cropped plots because visual assessment (Table 6) indicated no differences between cropped and uncropped plots.

Dry weight data (Table 8) follow he same pattern except the 0.5 kg/ha amended treatment also gave a significantly lower value than control.

The results presented in Table 9 show that the yield of maize plants and cobs in all treatments treated was significantly higher than the control with the amended "Roundup" at 1.5 kg/ha the highest followed by the unamended "Roundup" at 1.5 kg/ha. Even the treatments which did not produce a measurable effect on *Cyperus* increased total maize yield with plots treated in the morning plot producing higher weights than those sprayed in the evening. However, there was no significant difference in the yield of cobs.

4. CONCLUSIONS

Amendments could enhance the toxicity of the "Roundup formulation of glyphosate against *C. rotundus* in both pot and field experiments. The improved efficacy of glyphosate against the weed may be attributed to faster penetration and translocation of the compound in the plant caused by the additives as indicated by the tracer studies.

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