

## Effect of Adjuvants and their Concentration on Rainfastness of Glyphosate

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### ABSTRACT

Greenhouse studies were conducted to examine the effect of non-ionic 'Induce' and the organosilicone 'Silwet L-77' adjuvants on the rainfastness of glyphosate applied to a broadleaf weed-wild mustard. Glyphosate was tank mixed with and without adjuvant at three different concentrations. No rainfall and six simulated rainfall treatments at 30 min, 1, 2, 4, 6 and 24 h after glyphosate treatment were applied to the treated plants. With the addition of either adjuvant, absorption of <sup>14</sup>C glyphosate was enhanced, which presumably led to increased rainfastness as indicated by good control of wild mustard plants even when simulated rainfall occurred 30 min after application. Addition of both adjuvants also improved translocation of <sup>14</sup>C glyphosate. Simulated rainfall immediately after glyphosate application washed off chemical from the foliage, leaving not enough chemical to have phytotoxic effects. Simulated rainfall that occurred 30 min after application did not affect the efficacy of glyphosate as a significant amount of <sup>14</sup>C glyphosate had already been absorbed. Use of the organosilicone adjuvant achieved higher per cent control of weeds than the non-ionic adjuvant, presumably through increased rainfastness of glyphosate. Addition of non-ionic adjuvant 'Induce' at 0.25% in and organosilicone 'Silwet L-77' at 0.1% was better in achieving effective control of wild mustard than other rates of adjuvants with glyphosate under different simulated rainfalls. Thus, addition of an adjuvant helped in absorption and translocation of <sup>14</sup>C glyphosate as quickly as 30 min after treatment.

**Key words :** Glyphosate, adjuvant, rainfastness, wild mustard, mortality

### INTRODUCTION

Due to frequent rainfall and warm weather in Florida (USA), weeds grow throughout the year. Citrus is a major crop and in the early years of establishment e. g. 0 to 5 years, weed management is essential to allow better growth and development of citrus trees (Jackson and Davies, 1999). Glyphosate is a non-selective highly effective systemic herbicide used largely for total vegetation control (Jackson and Davies, 1999). Once within the plant system, the herbicide is translocated and lethal to the whole plant resulting in effective weed control. Rainfall soon after glyphosate application may reduce its efficacy significantly. Rainfastness of glyphosate-containing aqueous solutions is of great importance in south-east Asia, Florida, and similar geographic locations where frequent and intense rainfall often occurs.

The presence of an ionic or non-ionic adjuvant may provide more efficient utilization of glyphosate as compared to glyphosate with no adjuvant (Singh 1995; Singh *et al.*, 2004). Addition of adjuvants generally improves the efficacy of glyphosate by increasing the

absorption and translocation of the chemical in plants (Sharma and Singh, 1999; Sharma *et al.*, 2007). Adjuvants increased the activity of glyphosate by 15-29% compared to glyphosate alone by lowering the surface tension and contact angle resulting in increased uptake and translocation of glyphosate in the test plants (Singh and Singh, 2005). Lowest surface tension and contact angle were observed with "L-77" followed by "Induce" (Singh and Singh, 2006). A number of studies have been published outlining the beneficial effects of silicone adjuvants in reducing the critical rain-free period after foliar application of herbicide (Reddy and Singh, 1992b; Roggenbuck *et al.*, 1993). Silicone adjuvants also increase the efficacy of foliar applied herbicides in the absence of rainfall (Reddy and Singh, 1992a; Singh and Mack, 1993). Henry and Shaner (2007) have observed substantial differences between wash off and adjuvant level treatments.

Rapid absorption of active ingredient and, thus, reduction in critical rain-free period, in the presence of organosilicone adjuvants, has been attributed to stomatal infiltration due to reduced surface tension of the solution (Stevens *et al.*, 1991; Buick and Field, 1992). Adjuvants

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can penetrate cuticles rapidly and in substantial quantities, thus, enabling the co-penetration of adjuvant and active ingredient (Holloway and Stock, 1990; Sharma *et al.*, 1996).

Martin and Green (2004) reviewed the required rain-free periods for many herbicides which were listed in specific time on product labels. However, rainfast information on glyphosate labels is vague and varies with the product. Approximately 60% of the labels indicate "rainfall or irrigation occurring within 6 h after application may reduce effectiveness of glyphosate. Heavy rainfall or irrigation within 2 h after application may wash chemical off the foliage and a repeat treatment may be required." The remaining product labels indicated that heavy rainfall or irrigation occurring soon after application may wash chemical off the foliage and a repeat treatment may be required for adequate control.

The "rainfastness" or rain-free period of a pesticide is the point at which rainfall or irrigation no longer reduces the performance of the product. After the sprays have dried, or the active ingredient (a. i.) has absorbed into the plant, chemical cannot be washed off from the plant foliage (DiFonzo, 2004). Information on rainfastness is important since the product must be held on the leaf for a certain amount of time to get adequate uptake of the a. i. into the plant. Glyphosate is the most commonly used post-emergence herbicide in citrus weed management. Due to frequent rainfall conditions in the state of Florida, a practical concern to growers is how quickly the herbicide becomes rainfast following an application. Therefore, a study was conducted to examine the rain-free period after application of glyphosate formulated with two different adjuvant types and concentrations under different levels of simulated rainfall.

## MATERIALS AND METHODS

### Plant Material

The experiment was conducted at the University of Florida, Citrus Research and Education Center, Lake Alfred, Florida, USA under greenhouse conditions maintained at day/night temperature of 25/16°C ( $\pm 0.5^\circ\text{C}$ ), relative humidity of about 70/100% and natural day light conditions. The greenhouse reduced photosynthetically active radiation to 1200  $\mu\text{mol}/\text{m}^2/\text{s}$  at mid day. Wild mustard (*Sinapis arvensis*) was sown in 10 x 15 cm plastic pots in potting medium on March 9, 2007. The study was repeated and wild mustard was planted again

on May 9, 2007. Herbicide treatments were applied at four fully expanded leaf stage of wild mustard seedlings. All plastic pots were fertilized with a 20-20-20 N-P-K fertilizer two weeks after sowing and again before treatment to have optimum growth of the plants.

### Simulated Rainfall Treatment

A rainfall simulator was designed with two Quick Fulljet with a rectangular spray pattern. Nozzles were mounted 91.4 cm apart and 152.4 cm above the ground on a 1.9 cm wide pipe at an angle of 10 degrees from the vertical, with the flow of water directed upwards. The water pressure in the simulator was maintained at 34.5 kPa. The spray droplets from the nozzles after going upwards fall back to the ground in uniform spectrum simulating rainfall at the rate of 7.5 cm/h. This simulated rainfall is similar to the typical heavy intensity of rainfall events in Florida.

### Rainfast/Efficacy

Wild mustard was used to evaluate the effect of simulated rainfall, adjuvants and their concentrations on the rainfastness of glyphosate. Glyphosate was applied as Credit<sup>®</sup> (isopropylamine salt) (4.0 lb a. i./gal) at 0.84 kg a. e./ha. Glyphosate was tank mixed with a conventional non-ionic adjuvant Induce at 0, 0.05, 0.25 and 1.0% v/v and an organosilicone adjuvant 'Silwet L-77' at 0, 0.02, 0.1 and 0.5% v/v, separately. The treatment solutions were prepared immediately before use and applied using a pressurized air Chamber Track Sprayer. The sprayer was fitted with Teejet 8003 flat fan spray nozzle delivering 20 gal/A at 22 psi pressure. After spraying, the plastic pots containing plants were returned to the greenhouse as stated earlier. Glyphosate ( $\pm$ adjuvant) applications were followed by a simulated rainfall treatment immediately after herbicide application, at 30 min, 1, 2, 4 and 24 h after the herbicide application. A treatment having no simulated rainfall was also included. Simulated rainfall was created with 7.5 cm of water delivered over a period of one hour on top of the plants after herbicide treatment application. The plastic pots were watered daily to avoid water stress. Visual observations for the phytotoxic effect of glyphosate on the treated plants were recorded as per cent control weekly for two weeks after simulated rainfall treatment. Zero per cent indicated no weed damage and 100% indicated complete death.

## <sup>14</sup>C Glyphosate Absorption Study

Absorption studies were also conducted to confirm the rainfast efficiency of the adjuvants. Glyphosate was used at 0.84 kg a. e./ha. <sup>14</sup>C glyphosate with a specific activity of 23.87 mCi/mmol was utilized. Plants were transferred to a growth chamber a week prior to receiving the treatments. In the growth chamber, the average photosynthetic photon flux density (PPFD) of 200 mE/m<sup>2</sup>/S at the plant level; day/night temperatures of 25/16°C (±0.5°C) and the relative humidity of 55/70% (±5%), respectively, were maintained. Each plastic pot contained a single seedling representing one replication and there were four replications for each treatment. The experiment was repeated once. The third fully expanded leaf at the 4-leaf stage of wild mustard was carefully covered by aluminum foil, then the plants were sprayed with glyphosate at 0.84 kg a. e./ha with or without adjuvant using a chamber track sprayer as mentioned earlier. After removing the aluminum foil, 5 x 2 µl droplets (10 µl in total) of <sup>14</sup>C glyphosate (±adjuvant) were applied to the adaxial leaf surface of the test leaf. The quantity of <sup>14</sup>C glyphosate applied to the leaf was determined by dispensing same number of droplets directly into scintillation vials, with sufficient <sup>14</sup>C activity to be detected after plant combustion. The plants were harvested at 30 min, 1, 2, 4 and 24 h after treatment. Treated plants were dissected into three sections : (i) treated leaf, (ii) untreated plant tissue above ground and (iii) plant roots. In order to recover all un-absorbed <sup>14</sup>C-glyphosate, the treated leaf was washed twice with 4 ml of water : ethanol (1:1 v/v) and then rinsed twice with 3 ml of ethanol to wash any remaining epicuticular waxes on the leaf surface. A 200 µl sub-sample from each washing was dispensed into a vial containing 7 ml of scintillation liquid and then radio-assayed using a liquid scintillation counter (LSC). Plant samples were oven-dried at 50°C for 48 h and were combusted utilizing a Biological Oxidizer (Model#500T). <sup>14</sup>CO<sub>2</sub> in oxidized samples was trapped in Carbon-14 cocktail and quantified by LSC to determine absorption and translocation of <sup>14</sup>C glyphosate. Foliar absorption was defined as <sup>14</sup>C activity present in plant tissues after washing the treated leaf, and translocation as the amounts of total <sup>14</sup>C activity which traveled out of treated leaf into the rest of the plant. Both values were expressed as a percentage of the applied dose. Basipetal translocation was defined as the <sup>14</sup>C activity detected from the roots of wild mustard. The recoveries were calculated by adding radioactivity in all

the assayed fractions which were 92 to 97%, background activity was subtracted. The experiment was conducted as completely randomized design with a factorial arrangement of treatments with two factors; weed and two herbicides (+adjuvant) with four replications. The study was repeated.

In both studies (efficacy and absorption), the percentage data were arcsine-transformed before analysis to stabilize the variance. In each study, the data of two repeated studies were combined after performing a test of homogeneity of variance. As transformation of data did not alter the data interpretation, the untransformed original percentage data were used in the analysis. The data were analyzed using factorial ANOVA and means were separated at 5% level of significance by Duncan's multiple range test.

## RESULTS AND DISCUSSION

### Efficacy/Rainfastness of Glyphosate

Rainfast is a function of how rapidly a lethal dose of the applied herbicide is absorbed before being washed off from the foliage. The per cent control data were recorded at 1 and 2 weeks after treatment (WAT) and maximum effect of the treatments was observed at 2 WAT. A significantly higher per cent control of wild mustard was obtained with organosilicone 'Silwet L-77' than non-ionic 'Induce' when the effects of the treatments were averaged across these adjuvants as seen in the interaction effects (Table 1). When the effect of the treatments was averaged across the concentrations, there was no significant difference among concentrations but the interaction between no adjuvant and different concentrations of adjuvant on the per cent control of wild mustard was significant (Table 1). Further, it was observed that the immediate simulated rainfall treatment after the application of glyphosate ± adjuvant treatments washed off significant amount of the chemical from the foliage (Fig. 1). The amount of chemical remained on the foliage was not enough to cause phytotoxic effect. Per cent control of wild mustard when averaged across the simulated rainfall treatments (30 min, 1, 2, 4, 6 and 24 h) was significantly different at 1 WAT supporting the rainfastness of glyphosate (Table 1). At 30 min simulated rainfall treatment per cent control was less than the other intervals of time. There was no significant difference in per cent control among the simulated rainfall treatments at 2 WAT when averaged across these

Table 1. Effect of different concentrations of adjuvants and simulated rainfall at various times on per cent control of wild mustard

Treatments	Simulated rainfall applied after glyphosate treatment																							
	No rain		Immediate rain		30 min		1 h		2 h		4 h		6 h		24 h		Mean							
	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks								
No adjuvant	61 <sup>kl</sup>	78 <sup>g</sup>	7 <sup>m</sup>	11 <sup>bij</sup>	68 <sup>ijk</sup>	82 <sup>efg</sup>	61 <sup>kl</sup>	80 <sup>fg</sup>	581	82 <sup>efg</sup>	67 <sup>kl</sup>	82 <sup>efg</sup>	66 <sup>kl</sup>	82 <sup>efg</sup>	73 <sup>bij</sup>	80 <sup>fg</sup>	98							
Induce 0.05%	79 <sup>d-h</sup>	93 <sup>a-d</sup>	13 <sup>m</sup>	13 <sup>h</sup>	72 <sup>bij</sup>	87 <sup>def</sup>	82 <sup>b-h</sup>	91 <sup>a-d</sup>	86 <sup>ef</sup>	92 <sup>a-d</sup>	82 <sup>b-h</sup>	93 <sup>a-d</sup>	89 <sup>ef</sup>	91 <sup>a-d</sup>	86 <sup>ef</sup>	94 <sup>a-d</sup>	78							
Induce 0.25%	88 <sup>e-f</sup>	96 <sup>ab</sup>	6 <sup>m</sup>	5 <sup>j</sup>	78 <sup>e-h</sup>	88 <sup>cde</sup>	88 <sup>e-f</sup>	92 <sup>a-d</sup>	89 <sup>ef</sup>	94 <sup>a-d</sup>	88 <sup>e-f</sup>	93 <sup>a-d</sup>	88 <sup>e-f</sup>	94 <sup>a-d</sup>	89 <sup>ef</sup>	93 <sup>a-d</sup>	79							
Induce 1.0%	89 <sup>e-f</sup>	93 <sup>a-d</sup>	7 <sup>m</sup>	6 <sup>ij</sup>	74 <sup>g-j</sup>	89 <sup>bcd</sup>	85 <sup>e-f</sup>	82 <sup>efg</sup>	89 <sup>ef</sup>	90 <sup>a-d</sup>	87 <sup>e-f</sup>	94 <sup>a-d</sup>	89 <sup>ef</sup>	93 <sup>a-d</sup>	89 <sup>ef</sup>	94 <sup>a-d</sup>	78							
L-77 0.02%	91 <sup>a-d</sup>	95 <sup>abc</sup>	5 <sup>m</sup>	5 <sup>j</sup>	84 <sup>uv-g</sup>	93 <sup>a-d</sup>	90 <sup>e</sup>	94 <sup>a-d</sup>	91 <sup>a-d</sup>	94 <sup>a-d</sup>	92 <sup>abc</sup>	94 <sup>a-d</sup>	83 <sup>abc</sup>	94 <sup>a-d</sup>	93 <sup>abc</sup>	94 <sup>a-d</sup>	81							
L-77 0.1%	91 <sup>a-d</sup>	98 <sup>a</sup>	7 <sup>m</sup>	11 <sup>bij</sup>	87 <sup>uvf</sup>	98 <sup>a</sup>	90 <sup>e</sup>	97 <sup>ab</sup>	89 <sup>ef</sup>	98 <sup>a</sup>	89 <sup>ef</sup>	97 <sup>ab</sup>	91 <sup>a-d</sup>	97 <sup>ab</sup>	92 <sup>abc</sup>	98 <sup>a</sup>	83							
L-77 0.5%	92 <sup>abc</sup>	98 <sup>a</sup>	8 <sup>m</sup>	12 <sup>hi</sup>	89 <sup>uvf</sup>	94 <sup>a-d</sup>	90 <sup>e</sup>	93 <sup>a-d</sup>	92 <sup>abc</sup>	94 <sup>a-d</sup>	94 <sup>a</sup>	94 <sup>a-d</sup>	94 <sup>a</sup>	96 <sup>uv-d</sup>	93 <sup>ab</sup>	94 <sup>a-d</sup>	83							
Mean	84	93	8	9	79	90	84	90	16	92	86	92	86	92	88	92								

In treatment column, all the treatments were tank mixed with glyphosate at 0.84 kg a. e./ha. min or h—Means simulated rainfall treatment was applied minutes or hour after treatment application. Data points followed by the same superscript within the columns and rows are not significant.

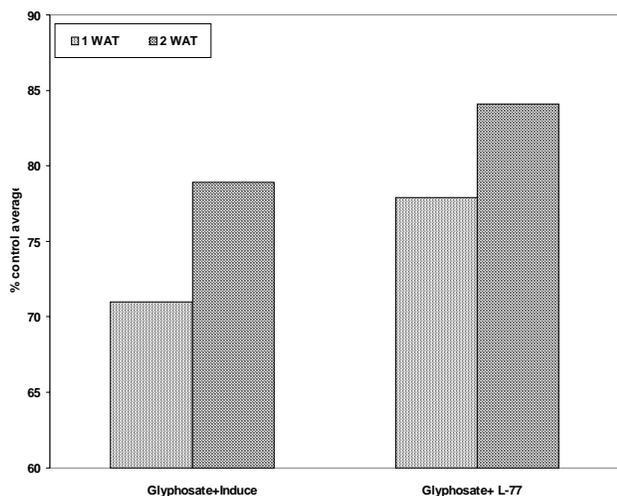


Fig. 1. Effect of glyphosate, adjuvant and simulated rainfall treatments when the effects were averaged across adjuvant only on per cent control of wild mustard [LSD (5%) : 1 WAT=1.5, 2 WAT=0.8] WAT=Week after treatment application.

Note : The data were analyzed by factorial ANOVA with three factors as adjuvant, concentration and simulated rainfall. ANOVA gave averaged data points of all the treatments across a particular factor.

treatments; however, at 1 WAT there was a significant difference between 30 min simulated rainfall treatment and rest of the other timings.

The effect of herbicide treatment was more pronounced at 2 WAT (Table 1). Addition of either adjuvant significantly improved the per cent control of wild mustard over no adjuvant treatment. Belles *et al.* (2004) recorded that 1-aminomethanamide dihydrogen tetraoxosulfate (AMADS) adjuvant increased the glyphosate efficacy on velvetleaf by accelerating the initial rate of herbicide absorption. Feng *et al.* (2000) compared rainfastness of different glyphosate formulations and found that Roundup Ultra was more rainfast than the other formulations of glyphosate, attributing this phenomenon to the more rapid absorption of Roundup Ultra formulation.

The concentration of 0.25% v/v of Induce and 0.1% v/v of L-77 was better than the other concentrations used in improving the rainfastness of glyphosate in wild mustard under all the simulated rainfall treatments except the immediate rainfall treatment (Table 1). Higher concentrations of Induce (0.5%) and of L-77 (0.5%) did not improve the per cent control of wild mustard over their lower concentrations. It confirmed that application

of adjuvant at CMC level had maximum effect on the efficacy of glyphosate. The data on the efficacy indicated that the addition of adjuvant enhanced the rainfast of glyphosate and hence improved the per cent control of wild mustard. Martin and Green (2004) reviewed that heavy rainfall or irrigation within 2 h after application may wash chemical off the foliage and a repeat treatment may be required. Singh (1995) reported that organosilicone adjuvant tank mixed at 0.125% increased per cent control of weeds and improved rainfast.

### <sup>14</sup>C Glyphosate Absorption Study

The addition of either of the adjuvants significantly enhanced absorption of <sup>14</sup>C glyphosate and the addition of organosilicone 'Silwet L-77' to glyphosate achieved significantly higher absorption of <sup>14</sup>C glyphosate over non-ionic adjuvant 'Induce' (Fig. 2). Absorption of <sup>14</sup>C glyphosate increased until 4 h but not significantly thereafter. Total translocation after 30 min was significantly lesser than other harvesting times regardless of treatment. There was no significant increase in translocation until 24 h when glyphosate with no adjuvant was applied. Translocation also increased until 24 h when glyphosate with Induce was applied. Total translocation of glyphosate+Silwet L-77 treatments were significantly higher than the translocation values obtained when <sup>14</sup>C glyphosate or <sup>14</sup>C glyphosate+Induce, were applied. Further, the increase in translocation of <sup>14</sup>C glyphosate with L-77 was significantly higher at 4 and 24 h harvest times than the other harvest times (Fig. 3). Reddy and Singh (1992b) reported the benefit of silicone adjuvants in reducing the critical rain-free period after herbicide application. Basipetal translocation of <sup>14</sup>C glyphosate was 2 to 3% in case of glyphosate only, 5 to 7% in glyphosate+Induce and 6 to 8% in glyphosate+L-77 (Fig. 4) which indicated significantly higher with treatments containing adjuvants. Further basipetal translocation was significantly higher with 'Silwet L-77' than with the addition of Induce.

Feng *et al.* (1998) compared the absorption and translocation of different glyphosate formulations in velvetleaf (*Abutilon theophrasti* Med.) and found that formulations containing a proprietary ethoxylated tallowamine adjuvant were absorbed more rapidly and translocated to a greater extent than a formulation containing an alkyl polyglucoside. There was a positive correlation between leaf damage caused by the ethoxylated tallowamine adjuvant and glyphosate

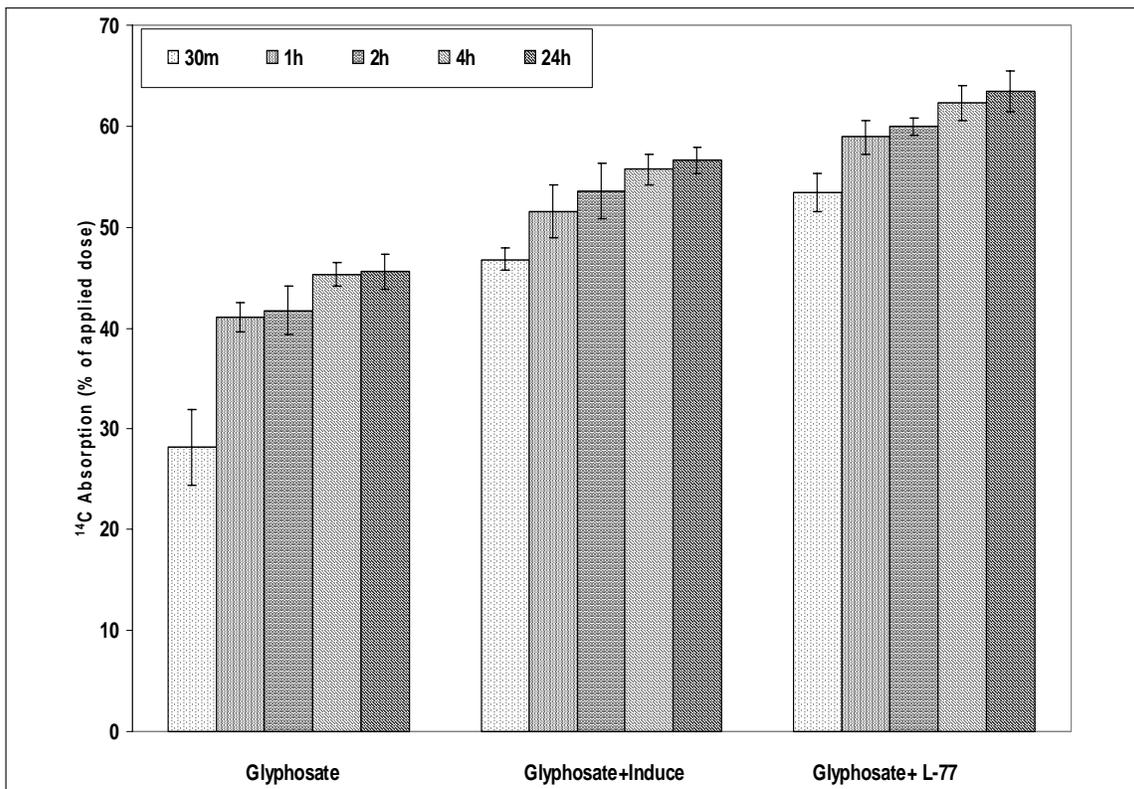


Fig. 2. Effect of adjuvants on the <sup>14</sup>C glyphosate absorption in wild mustard when harvested at different time intervals (min or h-Treated plants were harvested minutes or hour after treatment application).

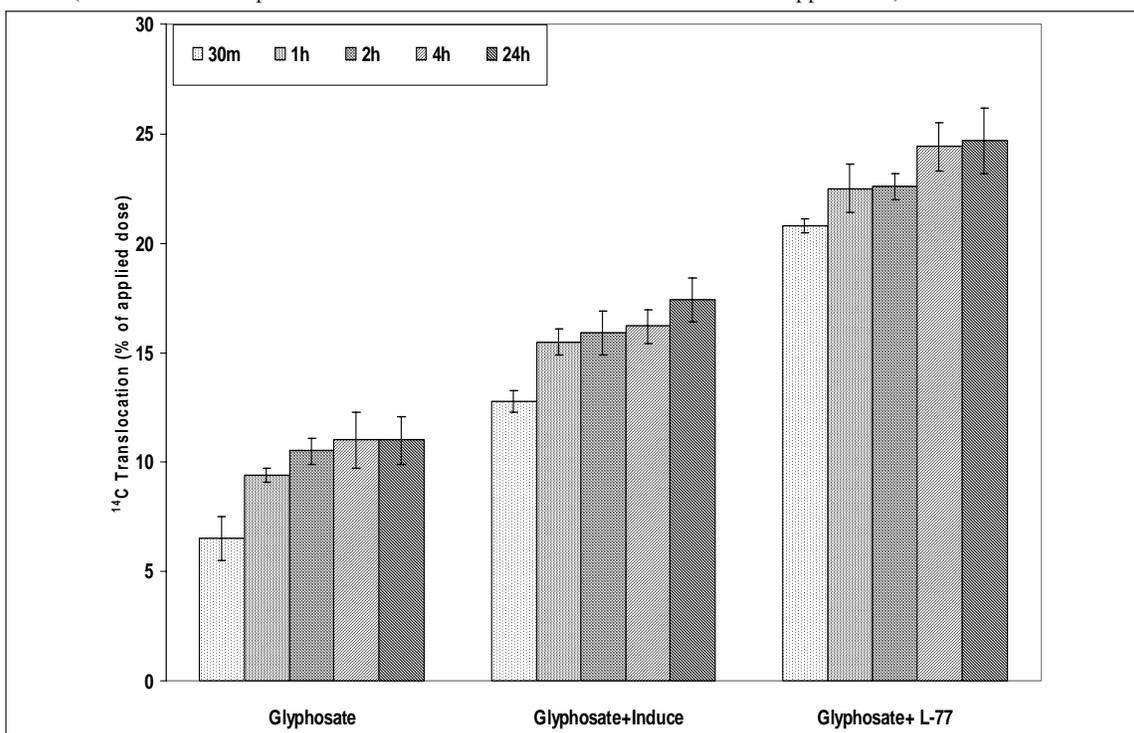


Fig. 3. Effect of adjuvants on the <sup>14</sup>C glyphosate translocation in wild mustard when harvested at different time intervals.

absorption in velvetleaf (Feng *et al.*, 1998; Ryerse *et al.*, 2004), which suggests that the adjuvant disrupts the cuticle and the epidermal cell membranes. This disruption may facilitate the absorption of glyphosate into the leaf tissue. Singh *et al.* (2004) also reported enhanced efficacy of glyphosate with ethoxylated tallowamine against *Parthenium hysterophorus* and other weed species. The enhanced rate of absorption led to increased rainfast and greater glyphosate translocation out of the treated leaf (Feng *et al.*, 2000).

This study confirmed that the addition of adjuvant helped in improving the rainfastness of glyphosate. With the addition of adjuvant, absorption was enhanced which led to increased rainfast or per cent control of wild mustard plants even when simulated rainfall occurred 30 min after application of glyphosate. Addition of adjuvant also improved translocation of  $^{14}\text{C}$  glyphosate from treated leaf to the rest of the plant. It was concluded that rainfall immediately after glyphosate application washed off almost all the herbicide from the foliage leaving not enough chemical to be phytotoxic on the foliage, the plant control was negligible. Rainfall

occurring 30 min after application did not affect the efficacy of glyphosate as significant amount of glyphosate was absorbed. Both non-ionic adjuvant 'Induce' and organosilicone 'Silwet L-77' at a concentration of 0.25 and 0.1%, respectively, were effective in improving rainfast and efficacy of control than the other concentration. These concentrations were critical micelle concentration points for these adjuvants (Sharma *et al.*, 1996). However, Roggenbuck *et al.* (1990) reported that organosilicone adjuvants became progressively less effective at lower rates with rainfast falling off fairly rapidly as the rate was decreased. Consistent infiltration of the solution through stomata occurs at higher concentrations (0.5% v/v) of 'Silwet L-77', even though the adjuvant effectively reduces the surface tension at lower solution concentrations (Field and Bishop, 1988). Organosilicone 'Silwet L-77' adjuvants significantly reduced the surface tension and contact angle of solutions and provided a faster absorption of glyphosate (Singh, 1995; Sharma and Singh, 1999, 2007; Singh and Singh, 2005, 2006). Thus, organosilicone adjuvants improve the rainfastness of

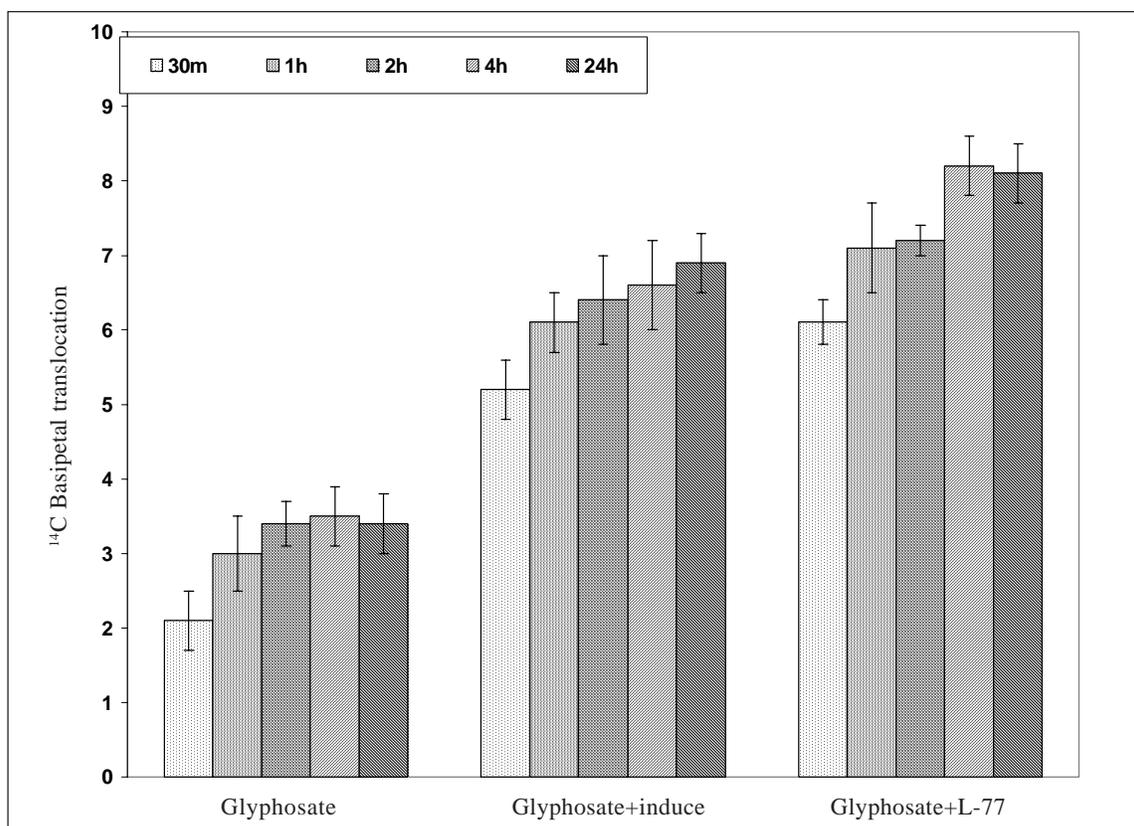


Fig. 4. Effect of adjuvants on the  $^{14}\text{C}$  glyphosate basipetal translocation in wild mustard when harvested at different time intervals.

glyphosate (Singh, 1995). In conclusion, addition of adjuvant to glyphosate significantly improved rainfastness of glyphosate over glyphosate applied with no adjuvant even after 30 min of rainfall. However, in previous studies, it was reported that rain-free period was 6 h. A rain-free period of 2 h after treatment may wash off the chemical from the foliage and a repeat application will be required (Martin and Green, 2004). Among non-ionic 'Induce' and organosilicone 'Silwet L-77', Silwet L-77 plus glyphosate was found better than other treatments to improve rainfastness.

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