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Techniques to reduce pollution by enhancing cuticle loading and entry of herbicide

R. Devendra*, R.C. Asok[1], H.G. Jalendra Kumar[1], S.B. Manjunatha[1] and T.V. Ramachandra Prasad

AICRP on Weed Control, University of Agricultural Sciences, Bangalore, Karnataka 560 065

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ABSTRACT

Technique enhancing herbicide entry into the plants leads to reduction in dosage and therefore pollution. Higher density of fine droplets along with surfactant increased the herbicide entry points, than coarse droplets for same total spray volume. But fine droplets evaporate faster resulting in herbicide getting deposited as dried crystals on leaf surface. To overcome this problem, several adjuvants were tried to increase the time required for evaporation. Jaggery (2%) increased the evaporation period (27-162%) on different weed species. Dried out deposition of radioactive glyphosate from fine drops and along with TritonX 200 was more but showed enhanced glyphosate entry into the plant system. Whereas, aqueous extract (2%) of soap nut fruit (*Sapindus emarginatus*) which acts as bio-surfactant and antioxidant increases the time taken for drying and therefore enhances the translocation of glyphosate to bulb of *O. latifolia,* which was on par with TritonX 100 surfactant (petroleum product). This approach seems to be more beneficial than increasing the dosage of herbicide per droplet.

Key words: Adjuvants, Dried-out deposit, Foliar herbicide

Deposition of droplet on the foliage is the prerequisite for effective uptake of foliar applied herbicide. More density of fine droplets covering the entire leaf surface area and having the same volume of a single coarse droplet, leads to increased entry points and enhanced cuticle loading of the foliar applied herbicide (Devendra *et al.* 2009). However, finer the droplet with surfactant shortens the evaporation period of the droplet, reduced herbicide entry time significantly due to spreading of the droplet (Ashok 2007). Further, Shweta (2009) showed that increasing the dosage of herbicide per drop (x and 2x of radioactive glyphosate, 2,4-D) increased the dried out deposit 67 to 91% and 68 to 77% of glyphosate and 2,4-D on foliage of water hyacinth (*Eichhornia crassipes*). All the radioactive herbicide studies indicated 60-85% of foliar applied herbicide remains as dried out deposit on the epi-cuticular wax. Adjuvants were added by company along with formulations or by farmers as tank mix to aid as wetting, spreading, deposit building, emulsifying, deflocculating, wetting of the leaf cuticle, increase spray retention, protection of the herbicide in the spray solution, promote rainfastness, acts as a co-penetrant *etc.* (Hazen 2000, Penner 2000).

***Corresponding author:** devendra.wc.uasb@gmail.com

[1]*Department of Crop Physiology, University of Agricultural Sciences, Bangalore, Karnataka 560 065*

Adjuvants *viz.*, TritonX 200 (acts as surfactant and humectants), PEG 400 (humectant), Arial (detergent), ethephon or glyphosate (senescence inducing agents), 8 hydroxy quinoline (anti-callose accumulating agent in phloem sieve element); Sure shot **(**rain fastness agent) along with Meera 71.

WS granular formulation of glyphosate, citric acid or ammonium sulphate (anti-chelating agent) prevent chelating of ionized glyphosate molecule with divalent cations Fe^{++}, Ca^{++}, Mg^{++} *etc.* present in the hard water were tried to enhance cuticle loading and translocation of foliar herbicides from fed leaf to the underground plant parts of *Cyperus rotundus* and *Oxalis latifolia*. TritonX 100 or 200 (TX 100 or TX 200) was the best surfactant for glyphosate with ethephon concentration 5000 ppm was not economical, Sure shot did not protect the herbicide droplet against rain wash off. Jalendra Kumar 2004 Further, he showed that *Cyperus rotundus* being easy to wet but difficult to control having predominantly underground plant parts whereas, *Oxalis latifolia*itis difficult to wet, but responds to surfactant. Thus these two weed species were used to test the effectiveness of the adjuvants.

Experiments with surfactant showed that varied ethylene oxide (EO) repeats, less EO for sethoxydim (lipid soluble) and more EO for glyphosate (water soluble) enhanced their potency by enhancing spread, co-penetration, cuticle loading and increased movement across cuticle

(Devendra *et al.* 2001). In-spite of negative relation between surface tension of spray solution and droplet spread (Devendra *et al.* 2000), low surface tension of 67.7 mN/m by Laffmul DA (vegetable oil concentrate) enhanced the efficacy of glyphosate, glufosinate, whereas high surface tension of 76.4 mN/m by ethoxylated castor oil enhanced the efficacy of imazapyr and oxyfluorfen may protect them from photo-oxidization by UV rays of sun and no effect on 2,4-D and imazethapyr as reflected by dry weight reduction bioassay of *C. rotundus* and *O. latifolia* (Devendra *et al.* 2004). Hence, single surfactant for all herbicide can not be recommended. Root absorbs silicon as silicic acid, gets converted it as silicon gel in leaf blade and deposits beneath thin cuticle (0.1mm) as cuticle-Si double layer as thick as 2.5mm (Ma and Yamaji 2006). Silicon surfactant known to enhance entry of herbicide and protect the droplet wash off by rain by forming glass like substance over droplet.

With this in background, experiments were conducted to assess 1) the effect of different anti-drift flood jet nozzles and TritonX 200 on droplet spread on different weed spp, 2) effect of adjuvants on droplet drying period on different weed species foliage and 3) effect of bio-surfactant *Sapinduse marginatus* extract and silicon (rain fastness) on ^{14}C-glyphosate dry deposit, entry and translocation in *Oxalis latifolia*.

MATERIALS AND METHODS

Effect of varied nozzle with or without surfactant on weed foliage droplet deposition

Leaf bits of 2.5 x 1.5 cm (length x width) from weed species *viz., Cyperus rotundus, Oxalis latifolia, Lagas camollis, Digitaria marginata,Dactyloctenium aegyptium, Parthenium hysterophorus* and *Chromolaena odorata* were adhered to glass slides with double sided sticker tape. Care was taken so that upper surface of leaf was exposed to spray. The slides were sprayed *in vitro* using varied orifice nozzles (Table 1) with or without TritonX 200, from a height of 0.5 m with 15 PSI pressure. Spray was replicated three times on different sets of all species leaf bits. Spectrum of droplets deposited on the foliage was viewed from top through binocular microscope. Diameter of droplet and frequency of deposition was recorded using ocular micrometer attached to one eye piece. Drop diameter was measured with fixed magnification (10x optical lens with 1.8 zooming) and expressed in millimeter (mm) (eq 1) by giving calibration conversion factor 0.057 obtained from stage micrometer.

Drop diameter (mm) = No. of division of ocular micrometer x 0.057 ——(1)

Mean of five spray were used to arrive at Number Median Diameter (NMD) using eq 2.

Median = Lower valve of group where median lies + (B/D) x C ——(2)

Where B = 50–A, A indicates cumulative percentage of preceding interval of group where median lies (which was identified by Median = (n+1)/2, n is the total number of observations or total of all frequency), C is the range of median interval and D is the cumulative percentage for the median interval. NMD values were statistically analyzed using factorial CRD.

Effect of adjuvants on weed species foliage droplet drying period

Under laboratory conditions on the foliage of different weeds (Table 2), single coarse drop 5 µl of glyphosate (1.5 kg /ha) with or without different adjuvants *viz.*, TritonX 200 (0.05%), 8-hydroxy-quinoline (1mM), citric acid (0.01%), ammonium sulphate jaggery or sucrose (2% each) were placed on upper surface and droplet drying period (min.) were recorded. Thrice repeated and factorial CRD was used for statistical analysis.

Effect of fine drop

TritonX 200, extract of *Sapinduse marginatus* and soluble silicic acid on ^{14}C-glyphosate dried out deposit, entry and translocation in weed species were studied. Thirty day old seedling were raised in ice-cream cups of size 10 cm diameter at brim and 7.5 cm depth filled with red loamy soil: sand: FYM in 3:1:1 proportion with required NPK , and were irrigated and used for this study.

Preparation of ^{14}C-glyphosate with or without adjuvants

^{14}C-glyphosate with specific activity of 0.01 mci/mole obtained from Sigma-Aldrich was mixed with 1 ml 2000 ppm unlabeled (cold) glyphosate (Roundup 41SL). From this stock, 10 µl of labeled glyphosate was taken and 1 µl of cold glyphosate with or without adjuvants (Table 3 and Fig.1) was added to obtain various treatments. Methane and aqueous extract of *Sapindus emarginatus* were prepared by boiling 20 g dried fruit rind in methanol or water in 100 ml for 20 min and aged for over night and filtered using Whatman filter paper and diluted (2%) in the cold glyphosate.

Technique of feeding

Single drop of 1 µl or 5 drops of 0.2 µl of ^{14}C-glyphosate with or without adjuvants were carefully placed on fully expanded young leaf of weeds, 4th or 5th from top. Placed droplet was marked with water proof maker pen. After 72 h of feeding, plant parts *viz.*, fed leaf, other leaves, stem and root/bulb/tubers were separated. After separating the fed spot from fed leaf, fed spot was washed off by dipping in water and the activity was assessed as dried out deposit. Remaining portion of fed leaf, other leaves, stem, root, bulb/tubers were macerated using passel and mortar in known amount of distilled water and a known aliquot was assessed for radio activity, which was measured using Liquid Scintillation Counter (Wallac Model 1414) and expressed as CPM/organ fresh wt. or CPM/g fresh wt. In different weed species, per cent activity of finer drop with or without TX200 over single coarse drop of glyphosate alone was calculated for dried out deposit and total of different plant parts (Fig.1). Factorial CRD was used for statistical analysis with three replicated data.

RESULTS AND DISCUSSION

Nozzle effect on droplet deposition

Diameter of the droplet deposited on different weed species foliage was significantly more compared to without surfactant (TX200) for both nozzles. Whereas, on *D. aegyptium* and *C.odorata,* droplet spread was not significant with TX200 when sprayed with 0.8 mm nozzle but significant with 2.4 mm nozzle (Table 1). Highest droplet diameter was recorded with wider orifice nozzle sprayed along with surfactant on all weed species foliage. Data suggest that, spraying with narrow orifice nozzle, even with surfactant, deposited droplet will not cause run off from the leaf surface and NMD was close to recommended drop diameter (100 mm NMD) for foliar applied translocative herbicide. Further, volume of solution for 64 finer drop droplets of 125 mm diameter is equal to single coarse drop of 500 mm diameter and finer droplets cover the entire leaf surface, thus more herbicide entry points was available (Manjunatha 2003).

Adjuvant effect on dried out deposit and ^{14}C-glyphosate uptake and translocation

Small droplet with surfactant, significantly enhanced dried out deposit and entry into the plant system of ^{14}C-glyphosate at the fed spot than without surfactant due to spread of the droplet on the foliage, except in *D. marginata* and *D. aegyptium* (Fig.1). Mean time for droplet evaporation over weed species with or without TX200 was 9–60 m and was 36% less in finer drop (2 µl) than coarse drop (5 µl). Further, with TX200, evaporation time was 6, 12.5, 12.8 and 18% less over without from fine drop on *O. latifolia*, *D. marginata*, *C. rotundus* and *L. mollis* foliage, respectively (Ashok 2007). Thus, with finer drop with the surfactant, droplet dries out fast and herbicide molecules may not be able to load into the cuticle fully.

To prolong the period of droplet drying on the foliage, various adjuvants were tried. Amongst adjuvants, jaggery (2%) delayed drying significantly compared to other adjuvants in all weed species except in *C. rotundus* (Table 2). Further, jaggery increased sucrose level in the spray solution which might increase phloem loading in the foliage and enhances translocation to other plant parts. Biomass reduction of *O. latifolia* and *C. rotundus* bioassays showed significant reduction with TX200 + jaggery than glyphosate alone (Ahok 2007).

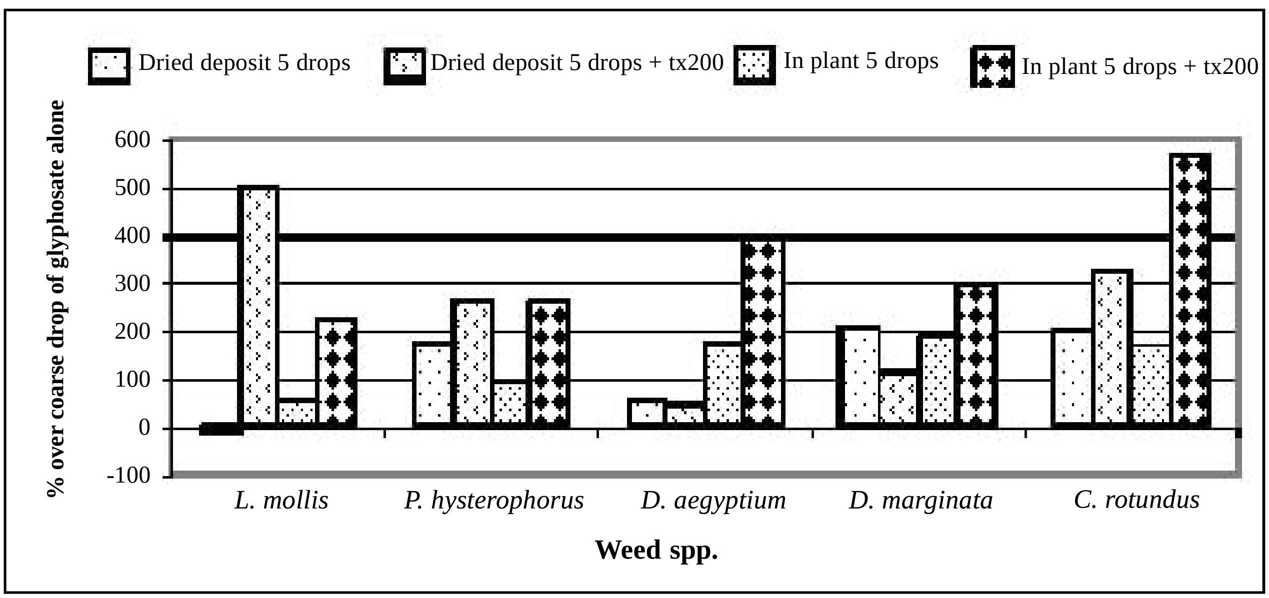

Fig. 1 Effect of finer drop deposition and surfactant on per cent dried out and in the plant system of glyphosate after 72 h feeding

Table 1. Effect of spray fixed with different nozzles, with or without TritonX 100, on Number Median Diameter (NMD) of droplet deposited on weed species foliage

Weed Species	Anti-drift flood jet nozzle having varied orifice size			
	0.8 mm		2.8 mm	
	Water	Water + TritonX 200	Water	Water + TritonX 200
C. rotundus	469^{l}	1025^{de}	$720^{g\text{-}k}$	1593^{a}
O. latifolia	$627^{i\text{-}l}$	844^{fg}	639^{gh}	1375^{b}
L. mollis	563^{kl}	$750^{g\text{-}j}$	$1023^{d\text{-}f}$	1225^{bc}
D. marginata	562^{kl}	$781^{g\text{-}i}$	781^{fh}	1094^{cd}
D. aegyptium	$639^{h\text{-}k}$	$657^{h\text{-}k}$	844^{fg}	1083^{cd}
P. hysterophorus	$594^{j\text{-}l}$	812^{gh}	$614^{i\text{-}l}$	1083^{cd}
C. odorata	$594^{j\text{-}l}$	$666^{g\text{-}l}$	$1000^{d\text{-}f}$	1083^{cd}
		LSD (P=0.05) 180		

In a column, the figures followed by same alphabet do not differ significantly.

Table 2. Effect of adjuvants on droplet (5 µl) drying period (minute) on different weed species

Adjuvants to glyphosate (1.5 kg/ha)	*O. latifolia*	*D. marginata*	*L. mollis*	*C. rotundus*
Glyphosate alone	51^{c}	50^{c}	$17^{e\text{-}g}$	$22^{d\text{-}g}$
TritonX 200 (0.05%)	48^{c}	50^{c}	$17^{e\text{-}g}$	28^{d}
8 Hydroxy-quinone (0.5%)	49^{c}	45^{c}	15^{g}	$22^{d\text{-}g}$
Ammium sulfate (2%)	45^{c}	49^{c}	$24^{d\text{-}f}$	26^{de}
Sucrose (2%)	94^{b}	92^{b}	16^{fg}	$24^{d\text{-}g}$
Jaggery (2%)	132^{a}	131^{a}	42^{c}	28^{d}
Citric acid (0.01%)	131^{a}	127^{a}	$18^{e\text{-}g}$	25^{de}
		LSD (P= 0.05) 8.75		

In a column, the figures followed by same alphabet do not differ significantly.

Table 3. Effect of soapnut (*Sapinduse marginatus*) extract (2%) and silicon (0.4%) on ^{14}C-glyphosate dry deposit on cuticle, entry and translocation in *Oxalis latifolia*

Treatment	DD	Fed spot	x10^{2} CPM/organ weight			x 10^{2} CPM/g organ weight		
			Fed leaf	Other leaves	Bulb	Fed leaf	Other leaves	Bulb
Glyphosate	115	25	25^{lm}	$146^{f\text{-}h}$	517^{c}	$123^{h\text{-}j}$	$173^{a\text{-}e}$	$158^{d\text{-}h}$
Glyphosate + ME (2%)	126	29	52^{klm}	$61^{i\text{-}m}$	909^{b}	$145^{e\text{-}i}$	$125^{h\text{-}j}$	$197^{a\text{-}c}$
Glyphosate + AE (2%)	96	16	173^{fg}	$131^{g\text{-}i}$	1352^{a}	$170^{a\text{-}f}$	$162^{c\text{-}g}$	203^{ab}
Glyphosate + silicon (0.4%)	135	20	$78^{h\text{-}m}$	$53^{j\text{-}m}$	368^{d}	$176^{a\text{-}e}$	$172^{a\text{-}f}$	$188^{a\text{-}d}$
Glyphosate + TritonX100 (0.05%)	99	22	29^{1e}	217^{f}	390^{d}	$167^{b\text{-}f}$	$140^{e\text{-}i}$	204^{a}
LSD (P=0.05)	NS	NS			71.5			36.4

ME and AE indicates methane and aqueous extract of *Sapindus emarginatus*.

In a column, the figures followed by same alphabet do not differ significantly.

Interestingly, *C. rotundus* foliage had maximum spread with TX200 118% when sprayed with 0.8 mm nozzle compared to other species (Table 1). Maximum spread led to more entry points and maximum ^{14}C-glyphosate entry (56.9%) in *C. rotundus* than other species (Fig.1) since droplet evaporation time was same in all species with or without TX200 (Table 2). Whereas, on *L. mollis* and *D. marginata,* spread of droplet with TX200 was same (33 and 38%, respectively), but drop evaporation period was faster on *L. mollis* (66%) than *D. marginata*. Thus per cent dried out deposit with fine drops + TX 200 was 50.2% on *L. mollis* than 11.7 on *D. marginata*. Thus spreading on the *C. rotundus* and prolonged evaporation on *D.marginata* foliage drastically reduced the dried deposition on these weeds foliage than *L. mollis*.

TritonX 100 or 200 is not available in all places therefore, an attempt was made to use the extract of soapnut fruit (*Sapinduse marginatus*) to assess its ability to enhance enrty and translocation of radioactive glyphosate in *O. latifolia*. Relatively low radioactivity was recorded in dried deposit at fed spot of glyphosate with aqueous extract of soap nut fruit and significantly increased glyphosate in fed leaf and bulbs compared to glyphosate alone. This treatment was on par with TritonX 100 (Table 3). Soapnut fruit is easily available to the farmers at all places. Soapnut fruit extract acts as bio-surfactant, antioxidant and better Fe^{2+} chelating agent than tocopherol and ascorbic acid, (Sirkanth and Muralidharan 2010). Thus combination of adjuvants, jaggery and aqueous extract of soapnut fruit seems to be beneficial in aid entry and translocation of foliar herbicides and its efficacy.

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