International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(1): 521-526

Received: 17-11-2017
Accepted: 21-12-2017

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Glyphosate selectivity by non-transgenic methods and efficacy of weed control in cotton (*Gossypium hirsutum* L)

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Abstract
Glyphosate is a non-selective, broad spectrum and systemic weedicide and cannot be sprayed directly on crop without protective mechanism. Application of glyphosate at early stage of weed growth requires minimal dose. Higher dose also could be used by employing protective mechanism to crop plants. Glyphosate phytotoxicity could be reduced by pre-conditioning of plants by chemicals. Keeping the above facts in view, this experiment this experiment was conducted during 2013-14 at Central Institute for Cotton Research, Coimbatore with objective to assess glyphosate based weed control in cotton by achieving crop selectivity for glyphosate. Higher rate of crop phytotoxicity grade of 2.5 and 2.0 were registered by application of 2.5 ml/L of glyphosate along with 0.625 ml/L of Quizalofop ethyl at 20 and 40 DAS without protective mechanism (T6). The same treatment, with pre conditioned application of NiCl @0.01% on crop plants two days before application of glyphosate combination observed less phytotoxicity grade of 1.25 for both 20 and 40 DAS (T8). The significantly least weed DMP was estimated with application of glyphosate @ 5 ml/L at 20 and 40 DAS with protective mechanism(T9). Manual weeding at 20 and 40 DAS (T2) had registered significantly the highest seed cotton yield (2087kg/ha). The results was on par with application of glyphosate @ 5 ml/L at 20 and 40 DAS with protective mechanism (T9) and also comparable with glyphosate @ 2.5 ml/L with 0.625 ml/L of quizalofop ethyl at 20 and 40 DAS with protective mechanism/ pre conditioned by application of NiCl on crop plants (T7 & T8). Application of Glyphosate @5.0 ml/L at 20 & 40 DAS with protective mechanism registered the significantly highest net return (Rs. 61, 875/ ha) and benefit cost ratio (2.56).

Keywords: Cotton, Phytotoxicity, Weed control, Selectivity, non-transgenic

Introduction
Cotton (*Gossypium hirsutum* L.) is one of the most important commercial crop of India, cultivated in 10.5 m ha with a production of 35.1 million bales of lint in 2016-17 with productivity of 568 kg ha^{-1}. Weed infestation in cotton has been reported to offer severe competition and causing yield reduction to the extent of 85 per cent (Sankaranarayanan *et al.,* 2012) [18]. Being a rainfed and long-duration crop, weeds flourish in many flushes and compete with the crop for nutrients, moisture, light, space *etc.* and also harbour insects, pests and diseases (Anderson 1983) [2]. Thus, effective, broad spectrum and cheaper post emergence chemicals for weeding in cotton are need of the hour. Glyphosate is non selective, cheaper, broad spectrum and systemic weedicides (Riaz *et al.,* 2007) [16]. The selectivity of the chemical was attempted by covering crop plants using PVC pipes cut through length wise. At later stages the selectivity was achieved by using protected shield attached to nozzle of delivery system of the sprayer to avoid direct chemical contact to crop plants. The other approach includes reducing the dose and improving the efficiency by adjuvant/ tank mixing with other herbicides were addressed. A novel method of managing phytotoxicity by chemical pre conditioning was explored. Weed control efficiency, effect on growth characters, yield attributes, yield and economics were worked out for different methods.

Materials and Methods
A field experiment was conducted during 2013-14 at Central Institute for Cotton Research, Coimbatore (N 11.01°, E 76.93° with an altitude of 427.6 m above MSL) on a clay loam soil, low in available N, medium in available P and high in available K with a pH 8.4 and EC 0.3 dS/ m. Soil tests on micronutrients showed 0.41, 1.0, 3.14, 2.41 and 0.06 ppm of Diethylene Triamine Penta Acetic acid (DTPA)-extractable zinc, copper, manganese, iron and boron

(Hot water extract), respectively. The experimental site is a double cropped irrigated upland with cotton cultivated during August to March. The *hirsutum* genotypes *viz*. 'Suraj', was planted at high density (45 X 15 cm, 1, 48,000 plant/ha). The recommended level of 30:13.0:24.9 kg/ha of N, P, and K were applied for high density planted genotypes equal quantity of N was top dressed at 45 Days After Sowing (DAS) after ensuring the sufficient soil moisture in the field. The crop was grown under irrigated condition and irrigated through ridge-furrow from August to March as per requirement. The crop was harvested manually to obtain sample plants (s) and per plot yield. Randomly selected plants were used for biometric observations from each replication and also to determine fiber quality parameters.

Selectivity of glyphosate (Roundup ® Glyphosate 41% SL, Monsanto Chemicals of India Limited, India) was attempted by five methods includes two physical and three chemical methods. In physical methods of protective mechanism; Cotton seedling was covered without any physical damage by Polyvinyl chloride (PVC) pipes with 6 inch diameter and 20 feet length which was cut half in length wise which could cover a single row and the chemical was applied. At later stages (40 DAS), the chemical was sprayed with protected shield attached to the nozzle of delivery system to avoid direct chemical contact with crop plants. In chemical methods, pre conditioning of cotton plant by 0.01% *nickel chloride* (NiCl) on two days before glyphosate application was followed. The other approach includes reducing the dose and improving the efficiency by adjuvant (glyphosate (Roundup @ 0.6 and 1 ml/L of spray fluid) was tank mixed with 100mM of ammonium sulphate (AMS))/tank mixing with other herbicides (sub lethal dose Glyphosate (Roundup @ 2.5 ml/L of spray fluid) with quizalofop ethyl (Targa super@0.6 ml/L) (Targa super (quizolofop ethyl 5% EC, Dhanuka Agritech Limited,Gurgaon, Haryana - 122002, India.) were attempted.

The experiment was laid out in randomized complete block design (RBD) with eight treatments including two control (un weeded control and manual weeding) and replicated thrice. The gross plot size adopted was 38.6 Sq. meter. Treatments consisted of glyphosate based weed control methods viz., T3 Glyphosate (Roundup @0.6 ml/L) + 100mM ammonium Sulphate at 20 & 40 DAS, T4. Glyphosate (Roundup @1 ml/L) + 100mM ammonium Sulphate at 20 & 40 DAS, T5. Glyphosate (Roundup @2.5ml L^{-1}) at 20 & 40 DAS, T6. Glyphosate (Roundup @2.5 ml/L) + Quizalofop ethyl (Targa super @ 0.625ml L^{-1}) at 20 & 40 DAS, T7. Glyphosate (Roundup @2.5 ml/L) + Quizalofop ethyl (Targa super @0.625 ml/L) at 20 DAS (covered by PVC pipes) & 40 DAS (protective shield), T8. Glyphosate (Roundup @2. ml/L) + Quizalofop ethyl (Targa super @0.625 ml/L) at 20& 40 DAS +0.01% NiCl two days before Glyphosate spraying, T9. Glyphosate (Roundup @5.0 ml/L) at 20 DAS (covered by PVC pipes) & 40 DAS (protective shield) and compared with two controls. (T1. Un weeded Control & T2.Manual weeding).

Hand operated knapsack sprayer fitted with a flat fan type nozzle (WFN 40) was used for spraying the herbicides. Phytotoxicity rate based on visual estimates of crop response and weed control were recorded 10 days after glyphosate application. Foliar chlorosis, necrosis, and plant stunting were considered when making the visual evaluations (Frans *et al.,* 1986) [6]. Phytotoxicity was estimated using a scale of 0 to 10 with 0 = no specific symptom and 10 = mortality. Weed control was estimated using a scale of 0 to 10 with 0 = no weed control and 10 = complete weed control. The weeds falling within the frames of 0.5 m × 0.5 m the quadrate were collected, shade dried and later dried in hot-air oven at 80°C for 72 hrs. Weed control efficiency (WCE) was calculated as per the procedure given by Mani *et al.,* (1973) [10]. The seed cotton yield obtained from the net plot area at each picking was recorded and expressed in kg ha^{-1}. The data were statistically analyzed following the procedure given by Gomez and Gomez (2010) [7] for randomized block design. The data pertaining to weeds were transformed to square root scale of (X + 2). Whenever significant difference existed, critical difference was constructed at five per cent probability level. Such of those treatments where the difference are not significant are denoted as NS.

Experimental Results

Weed Population

The Weed flora observed in the experimental trial before imposing weed control treatments at 20 DAS consisted of *Trianthema portulacastrum, Amaranthus viridis, Datura metal, Cyprus rotundus, Panicum repens, Parthenium hysterophorus, Cynodon dactylon, Cleome gynandra*and others. The relative density (%) of 52.6, 26.7 and 20.6 per cent of broad leaved, grasses and sedges observed respectively. Amongst different weed species recorded, *Trianthema portulacastrum* (50.2%) was predominant followed by *Panicum repens* (29.1%) and *Cyprus rotundus* (9.5%).

Selectivity by non-transgenic method

Phytotoxicity rate of different treatments were assessed 10 days after application of treatments. The results revealed that among treatments the highest rate of crop phytotoxicity grade of 2.5 and 2.0 were registered by application of 2.5 ml/L of glyphosate along with 0.625 ml/L of quizalofop ethyl at 20 and 40 DAS without protective mechanism. The same treatment, when pre conditioned by application @ 0.01% of NiCl on crop plants two days before application of glyphosate reduced phytotoxicity as recorded as grade of 1.25 for both first and second spray done at 20 and 40 DAS respectively. The cation of Ni may be interacted with glyphosate and formed the chelated compounds and reduces the effectiveness of glyphosate on cotton plants subsequently phytotoxicity. Due to the relatively high *in vitro* affinity of Ni^{2+} to glyphosate (Motekaitis *et al.,* 1985) [11]. and its role as an ethylene biosynthesis inhibitor in plants; Ni may interact with glyphosate in crops directly at a chemical and/or indirectly at a functional level as resulted in reduction of shoot biomass by glyphosate was significantly (by 40%) prevented by foliar NiCl(BaharYildiz Kutman *et al.*,2013) [3] Spraying. Foliar Ni application completely protected the plants from the effects of glyphosate on stem elongation. Foliar Ni applications with sufficiently high concentrations enhanced the resistance of wheat to glyphosate (Bahar Yildiz Kutman *et al.,* 2013) [3]. The presence of several divalent cations including Ca, Mg, Mn, and Zn in spray solutions was shown to lower the herbicidal effect of glyphosate. (Thelen *et al.,* 1995) [21].

Application of higher dose of glyphosate @ 5 ml/L at 20 and 40 DAS not caused phytotoxicity because of cotton plants were covered by PVC pipes. At later stages (40 DAS), glyphosate was sprayed with protected shield attached with nozzle to delivery system in order to avoid direct chemical contact to crop plants. The seedlings of cotton was covered by long necked aluminum tumbler and glyphosate was applied directly on crop field observed effective weed control without phytotoxicity to crop plants (Sankaranarayanan and

Rajendran, 2014) [17]. Tank mixing of glyphosate with 100mM ammonium sulphate/quizalofop ethyl @ 0.625 ml/L at 20 & 40 DAS enhanced the phytotoxicity rate on crop plants as compared to lower dose (T3) or glyphosate (T5) alone treatments.

Weeds dry matter production and weed control efficiency

The results revealed that significantly highest weed dry matter production of 452.4 and 1062 kg /ha respectively at 40 and 60 DAS in control (unweeded plot). The significantly highest weed control efficiency of 65.8 and 66.4 per cent and the least DMP of 154.6 and 356.4 kg/ ha were estimated with manual weeding at 40 and 60 DAS respectively; which was on par with treatments having application of glyphosate @ 5 ml at 20 and 40 DAS respectively. The results of manual weeding also comparable with glyphosate @ 2.5 ml + 0.625 ml/L of quizalofop ethyl at 20 and 40 DAS without and with protective mechanism and pre conditioned by application of NiCl @0.01% on crop plants(T6, T7 & T8). Reed *et al.,* (2015) [15] reported that flumioxazin tank-mixed with glyphosate showed the range of good (80 to 89%) to excellent (> 90%) control of annual bluegrass. These tank-mixtures were also more effective than application of glyphosate/ flumioxazin alone. Similar result by post-emergence herbicide in cotton was documented by Ali *et al.,* (2005) [1]. The treatment of quizalofop ethyl was observed better control of graminae especially with *Cynodon dactylon* (L.). Patil (2007) [14] reported effective control of grassy weeds by the application of quizalofop-ethyl at 1.0 litre/ ha on 35 DAS.

Growth characters

Direct applications of sub lethal dose of glyphosate on actively growing cotton plants observed that main stem height, no. of nodes, no. of squares, no. of sympodia and LAI were significantly affected. However, the control (unweeded) had registered significantly less growth characters because of competition by weeds, which suppressed the growth of plants. Among the treatments, manual weeding (T2) showed significantly highest plant height (92.6 cm), number of nodes (22.6), number of bolls (5.0), number of monopodia (1.8), number of sympodia (17.8) and leaf area Index (7.8). These results were on par with treatments having application of glyphosate @ 5 ml/L at 20 and 40 DAS with protective mechanism (T9). The results of manual weeding also comparable with glyphosate @ 2.5 ml with 0.62 ml/L of quizalofop ethyl at 20 and 40 DAS with protective mechanism and pre conditioned by application of NiCl @0.01% on crop plants (T7 & T8). These findings were in accordance with the results of Panwar *et al.,* (2001) [12]. Better growth characters were observed in the treatments resulted in effective weed control having no or less phytotoxic effect. Which were evidenced by less weed DMP and higher weed control efficiency. The formation of poorly soluble glyphosate − metal complexes by pre conditioning of plants by applying of 0.01% NiCl two days before glyphosate reduced the translocation within plant tissues(Cakmak *et al.,* 2009) [4].

Seed cotton yield

Manual weeding at 20 and 40 DAS had registered the significantly highest seed cotton yield (2087 kg /ha) due to effective weed control along with pulverization of soils by hoeing could helped for better growth of the plants, thus ultimately resulted into higher seed cotton yield. The results was on par with application of glyphosate @ 5 ml/L at 20 and 40 DAS with protective mechanism (T9) and also comparable with glyphosate @ 2.5 ml/L with 0.625 ml/L of quizalofop ethyl at 20 and 40 DAS with protective mechanism/ pre conditioned by application of NiCl on crop plants (T7 & T8). The treatments had good control of weeds, ultimately leads to higher growth characters, yield attributes and seed cotton yield; Cotton yield was largely improved by weed management methods with better weed control efficiency; Grichar *et al.,* (2004) [8] recorded that glyphosate application system resulted 96 per cent weed control. Wilcut *et al.,* (1996) [22] also reported that glyphosate controlled a broad spectrum of annual and perennial grasses, sedge, and broadleaf weeds and might be a viable alternative to other commonly used herbicides. Glyphosate @ 0.6 and 1 ml/L along with 100 mM ammonium sulphate at 20 & 40 DAS produced significantly lesser yield because of poor weed control, ultimately leads to lesser crop growth characters and seed cotton yield. Glyphosate applied at lower doses not effective in controlling sedge weed, *Cyperus rotundus*and some broad leaved weeds like *Parthenium hysterophorus*and *Commelina benghalensis*. Koger and Reddy (2005) [9] reported that minimum dose of glyphosate provided marginal or no control of weeds like Bermuda grass (*Cynodon dactylon* (L.) Pers.). The results of glyphosate @ 2.5 ml with 0.625 ml/L of quizalofop ethyl at 20 and 40 DAS (T6) observed reduced yield as compared to the same treatment had protective mechanism/ pre conditioned by application of NiCl @0.01% on crop plants (T7& T8). Glyphosate applied as post emergence directly to crop plants resulted in 19 % of yield reduction compared with the weed-free non treated cotton (Edenfield *et al*., 2005) [5]. Pre conditioning of cotton by 0.01% of NiCl before glyphosate application@ 2.5 ml with 0.625 ml/L of quizalofop ethyl at 20 and 40 DAS reduced the phytotoxicity effect and numerically improved the seed cotton yield as compared to without pre conditioning. Glyphosate can form complexes of various stabilities with divalent metal cations (Motekaitis *et al.,* 1985) [18]. Due to such interaction, the presence of several divalent cations including Ca, Mg, Mn, and Zn in spray solutions were shown to lower the herbicidal effect of glyphosate (Thelen *et al.,* 1995) [21]. The formation of poorly soluble glyphosate − metal complexes may relevant in plant and resulted in less translocation within plant issues (Cakmak *et al.,* 2009) [4]. Unweeded check recorded the lowest seed cotton yield was due to severe weed competition.

Table 1: Phytotoxicity grading and weed dry matter production as influenced by weed control methods

Treatments	10 Days after first spray		10 Days after second spray		Weeds DMP(kg/ ha)		Weed Control Efficiency (%)	
	Crop	weed	Crop	weed	40 DAS	60 DAS	40 DAS	60 DAS
T1. Un weeded Control	0	0	0	0	452.4	1062	0.0	0.0
T2.Manual weeding	0	10	0	10	154.6	356.4	65.8	66.4
T3 Glyphosate @0.6 ml/L + 100mM ammonium Sulphate at 20 & 40DAS	1.5	5.75	0.75	3	356.8	758.7	21.1	28.6
T4. Glyphosate @1 ml/L + 100mM ammonium Sulphate at 20 & 40DAS	2	7.5	1.75	6.5	256.3	528.3	43.3	50.3
T5. Glyphosate @2.5 ml/L at 20 & 40 DAS	2.25	7.5	1.75	6.5	225.6	506.3	50.1	52.3
T6. Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625ml/L at 20 & 40 DAS	2.5	7.5	2	6.5	206.3	428.3	54.4	59.7
T7. Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625 ml/L at 20 DAS(covered by PVC pipes) & 40 DAS (protective shield)	0.0	7.5	0	7.0	203.3	432.6	55.1	59.3
T8 Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625 ml/L at 20 & 40 DAS +0.01% NiCl two days before glyphosate spraying)	1.25	7.75	1.25	7.5	200.8	436.2	55.6	58.9
T9. Glyphosate @5.0 ml/L at 20 DAS (covered by PVC pipes) & 40 DAS (protective shield)	0	9.75	0	9	186.2	386.2	58.8	63.6
SEd					27.1	45.2	5.3	5.0
CD (5%)					59.2	98.6	11.6	11.0

DMP- Dry Matter Production

Table 2.Growth characters, yield attributes and seed cotton yield as influenced by weed control methods

Treatments	Plant height (cm)	Nodes	No. of bolls	No of monopodia	No. of Sympodia	LAI	Boll weight(g)	Yield (Kg /ha)
T1. Un weeded Control	59.3	14.3	1.3	0.5	10.3	2.4	4.0	459
T2.Manual weeding	92.6	22.6	5.0	1.8	17.8	7.8	4.3	2087
T3 Glyphosate @0.6 ml/L + 100mM ammonium Sulphate at 20 & 40DAS	66.4	19.1	3.8	0.8	13.6	4.6	4.4	1400
T4. Glyphosate @1 ml/L + 100mM ammonium Sulphate at 20 & 40DAS	72.3	20.8	4.0	1.3	14.3	5.6	4.5	1558
T5. Glyphosate @2.5 ml/L at 20&40 DAS	74.6	21.0	4.0	1.0	14.0	5.8	4.6	1600
T6. Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625 ml/L at 20 &40 DAS	74.3	21.3	4.3	1.2	14.4	6.3	4.6	1628
T7. Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625 ml/L at 20 DAS(covered by PVC pipes) & 40 DAS (protective shield)	72.6	20.6	4.6	1.2	16.3	6.3	4.5	1650
T8 Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625 ml/L at 20 & 40 DAS +0.01% NiCl two days before glyphosate spraying)	71.6	20.9	4.6	1.2	17	6.4	4.3	1659
T9. Glyphosate @5.0 ml/L at 20 DAS(covered by PVC pipes) & 40 DAS (protective shield)	82.6	22.6	4.8	1.6	17.5	7.3	4.5	2030
SEd	2.8	1.8	0.3	0.3	1.5	0.6	0.2	200
CD (5%)	6.2	4.0	0.6	0.6	3.2	1.4	NS	440

LAI-Leaf Area Index

Table 3.Economics as influenced by weed control methods

Treatments	Total CC(Rs ha-1)	GR (Rs ha-1)	NR (Rs ha-1)	B/C ratio	Weeding cost (Rs ha-1)
T1. Un weeded Control	19600	23000	3400	1.17	0
T2.Manual weeding	45900	104500	58600	2.28	45900
T3 Glyphosate @0.6 ml/L + 100mM ammonium Sulphate at 20 & 40DAS	31700	70000	38300	2.21	2700
T4. Glyphosate @1 ml/L + 100mM ammonium Sulphate at 20 & 40DAS	33350	78000	44650	2.34	2750
T5. Glyphosate @2.5ml L^{-1} at 20 & 40 DAS	33800	80000	46200	2.37	2800
T6. Glyphosate @2.5ml L^{-1} + quizalofop ethyl @ 0.625 ml/L at 20 & 40 DAS	34275	81500	47225	2.38	2975
T7. Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625 ml/L at 20 DAS(covered by PVC pipes) &40 DAS (protective shield)	35350	82500	47150	2.33	3850
T8 Glyphosate @2.5 ml/L + quizalofop ethyl @ 0.625 ml/L at 20 & 40 DAS +0.01% NiCl two days before glyphosate spraying)	34675	83000	48325	2.39	3075
T9. Glyphosate @5.0 ml/L at 20 DAS(covered by PVC pipes) & 40 DAS (protective shield)	39625	101500	61875	2.56	39625
SEd	2359	10129	3817	0	379
CD (5%)	5224	22428	8452	0.34	840

CC- Cost of Cultivation, GR-Gross Return, NR-Net Return, B?C ratio- Benefit Cost Ratio

Economics

The total weeding cost was highest in manual weeding (Rs.10, 000/ ha), but significantly lesser (Rs.2, 700 to 4,325/ ha) in all other treatments because of minimal quantity of herbicides used in addition to less cost associated with glyphosate. The total cost of cultivation was highest in manual weeding (Rs.45, 900/ ha), which was followed by application of glyphosate @ 5.0 ml/L at 20 and 40 DAS with protective mechanism (Rs.39, 625/ ha). The above said treatments had harvested higher seed cotton yield, thus incurred higher picking cost, and ultimately leads to higher cost of cultivation. The increase in seed cotton yield in the best treatments might be due to less weed competition thus increase growth attributes like plant height, sympodial branch, dry weight of plants, number of bolls $plant^{-1}$ which resulted in higher yield. These results were in conformity with the findings of Patel *et al.,* (2013) [13] and Shelkey *et al.,* (2013) [20], Scroggs *et al.,* (2007) [23] observed maximum seed cotton yield in glyphosate post program which included three applications of glyphosate with protective mechanism. Higher seed cotton yield (2030 kg ha^{-1}) in addition to less weed control cost (Rs 4, 325/ha) resulted in the highest net return (Rs 61, 875/ ha) and benefit cost ratio (2.56) was achieved by application of glyphosate @5.0 ml/L at 20 & 40 DAS with protective mechanism edge over the manual weeding.

Conclusion

Selectivity of glyphosate on crop plants could be achieved by imposing physical protection mechanism to crop plants. By using this protection, application of Glyphosate (Roundup @5.0 ml/L) at 20 DAS (covered by PVC pipes) & 40 DAS (protective shield) recorded better weed control and net return in cotton when compare with all other weed control methods.

Acknowledgements

The authors duly acknowledge the facilities and support provided by The Director, CICR, Nagpur, Maharashtra, India.

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