International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 228-231

Received: 10-09-2017
Accepted: 14-10-2017

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Residual effect of Glyphosate-tolerant transgenic maize herbicides on growth and yield of succeeding crop

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Abstract
Transgenic stacked maize hybrid was developed for preventing yield losses of maize crop to improve productivity. In this view, the field experiments were conducted to study the carry over effect of glyphosate K salt applied in preceding transgenic stacked maize hybrids (Mon 89034X NK 603) in succeeding green gram in the experimental site of Tamil Nadu Agricultural University, Coimbatore during *kharif*, 2009 and *rabi*, 2009-10. Glyphosate was applied as post emergence at 900, 1800 and 3600 g a.e ha^{-1} in Hishell and 900 M Gold transgenic maize hybrids and it was compared with non-transgenic counterparts with pre-emergence application of atrazine at 0.5 kg/ha followed by HW on 40 DAS. Succeeding green gram crop was sown immediately after the harvest of herbicide tolerant transgenic stacked maize hybrids. Observations like germination percentage, visual phytotoxicity, plant height, total weed density, dry matter production and yield were recorded. The final results revealed that, both post emergence application of glyphosate and pre-emergence application of atrazine did not have any residual effect on succeeding crop.

Keywords: herbicide residue, transgenic maize, succeeding crop, yield

Introduction
Glyphosate is a foliar applied, broad spectrum, post emergence herbicide capable of controlling annual and perennial grasses and dicotyledonous weeds (Franz *et al*., 1997) [1]. Glyphosate was classified as a herbicide after it was discovered by J.E. Franz in 1971 at Monsanto and was commercialized under the trade name Roundup. Today, glyphosate is sold as an isopropylamine salt, trimethylsufonium (trimesium) salt, sesquisodiumsalt, potassium salt and ammonium salt under several hundred trade names by Monsanto and other chemical companies (WSSA, 2007) [2].

Herbicide-tolerant plants are produced by the stable insertion of a gene that expresses a modified plant synthase protein in the receptor plant that is tolerant to particular herbicides (LeBrun *et al.,* 1997) [3]. In the early 1980s, the tools for producing transgenic crops were becoming available. Introduction of transgenic crops made resistant to broad-spectrum, non-selective herbicides was rightfully perceived as a better strategy in terms of weed management and market share. The two herbicides that fitted this approach best were glyphosate and glufosinate. Both compounds are amino acid analogues that have molecular targets in amino acid biosynthesis pathways. Herbicide-resistant crops were the first major wave of transgenic crops (Duke, 2005) [4]. The glyphosate-tolerant maize event NK 603 was produced by two copies of the *cp4 epsps* gene was introduced into the maize genome to produce Roundup Ready corn event NK603. The *cp4 epsps* gene derived from the common soil bacterium *Agrobacterium* sp. strain *CP4* encodes for the naturally glyphosate tolerant EPSPS protein.NK603 has high tolerance to recommended field application rates of glyphosate, and the transgenic insertion neither created nor was linked to negative parameters that could affect human and animal health, the environment or yield performance.

In general, glyphosate is moderately persistent in soil. It is relatively immobile in most soil environments as a result of its strong adsorption to soil particles. Ghassemi *et al*. (1982) [5]. Muller *et al*. (1981) found that glyphosate degrades at very low temperatures and does not adversely affect nitrogen fixation, nitrification or denitrification activity. With this view the following experiments was conducted to study the residual effect of glyphosate applied in transgenic maize on succeeding green gram crop.

Materials and Methods

Herbicides used in maize (Succeeding crop)

Pre-emergence herbicide atrazine at 0.5 kg ha^{-1} was sprayed at 3 DAS in the respective plots of conventional maize hybrids, whereas potassium salt of glyphosate at different doses as per treatment schedule were sprayed as post-emergence in the respective plots of transgenic maize hybrids during 2 to 4 leaf stage of weeds (20-25 DAS of maize). The properties of herbicides (*viz.*, atrazine and potassium salt of glyphosate) used in the study

Residue Crop

To study the residual effect of herbicides applied to maize, the succeeding crop of green gram was raised without disturbing the layout of maize experimental field. After removing the maize crop, the follow up crop was sown and all the recommended package of practices were followed to raise the residue crop.

*Germination percentage***:** Germination was recorded at 10 DAS by counting the number of seeds germinated and expressed as percentage to total seeds sown. The values were transformed into arc sine values and analysed statistically.

*Dry matter production***:** Dry matter production (DMP) was estimated at 25 and 50 DAS and at harvest. Five plants were removed from the sample rows, air dried and then oven dried at $80^0 \pm 2^0$C till a constant weight was recorded. Dry matter production was expressed in kg ha^{-1}.

Yield attributes and yield

Number of pods per plant: The number of matured pods per plant was counted from the randomly selected five plants for each treatment and the mean expressed as number of pods $plant^{-1}$.

Number of grains per pod: The number of grains per pod was counted from the randomly selected five pods of plants for each treatment and the mean value expressed as number of grains pod^{-1}.

Test weight: The weight of 100 grains drawn at random from the five randomly selected plants was recorded and expressed in grams.

Grain yield: Grain yield of green gram in each net plot area was weighed and expressed in kg ha^{-1}.

Results and Discussion

Germination: Germination of the residue green gram was not affected due to the application of herbicides to the preceding maize crop. There was no significant variation in germination percent among the weed management treatments on the germination of residual crops in both the seasons of study (Table 1).

Plant height: In the succeeding green gram after *kharif*, 2009, at 25 DAS, taller plants were recorded with application of POE glyphosate at 3600 g a.e ha^{-1} in preceding transgenic 900 M Gold maize hybrid (T_6). This was comparable with all other weed control treatments employed in the transgenic (T_1, T_2, T_3, T_4 and T_5) and non-transgenic maize hybrids (T_7, T_{10}, T_{13} and T_{15}). At 45 DAS and at harvest, there is no significant differences were observed on plant height. However, in both the stages plant height was shorter in unweeded check (T_{14}) when compared to other treatments. During *rabi*, 2009-10, significantly taller plants were observed with application of POE glyphosate at 3600 g a.e ha^{-1} in preceding transgenic Hishell maize hybrid (T_3). This was comparable with all other weed control treatments employed in the transgenic (T_1, T_2, T_3, T_4 and T_5) and non-transgenic maize hybrids (T_7, T_{10}, T_{13} and T_{15}). At 45 DAS as well at harvest stage, shorter greengram plants were observed with preceeding unweeded plot of CoHM 5.

Dry matter production: In the succeeding green gram after *kharif* season maize, higher dry matter production was observed with glyphosate at 3600 g a.e ha^{-1} in transgenic Hishell and was comparable with all other weed control treatmets employed in preceding maize crop. Unweeded control recoded lower dry matter production at 25 DAS. During 45 DAS, glyphosate at 1800 g a.e ha^{-1} in transgenic Hishell (T_3) recorded significantly higher dry matter production of green gram. This was comparable with all other treatments applied in both the transgenic and non-transgenic hybrids. Similar trend was also observed at the time of harvest with increased amount of dry matter production over time. Both the stages unweeded control recorded lower dry matter production of succeeding green gram. During *rabi*, 2009-10, significantly higher dry matter production was recorded with glyphosate at 3600 g a.e ha^{-1} in transgenic Hishell and was comparable with all other weed control treatments employed in preceding maize crop (Table 1). Unweeded control recoded lower dry matter production at 25 DAS and 45 DAS. Whereas, at the time of harvest, application of PE atrazine at 0.5 kg ha^{-1} *fb* HW recorded in CoHM 5 (T_{15}) recorded significantly higher dry matter production of succeeding green gram. Other weed control treatments in the transgenic (T_1, T_2, T_3, T_4, T_5 and T_6) and non-transgenic maize hybrids (T_7, T_{10} and T_{13}) also recorded higher and significantly comparable dry matter production. But, unweeded control like T_9 and T_{14} also recorded higher dry matter production.

Yield Attributes

Number of pods per plant: In succeeding green gram after *kharif*, 2009 maize, all weed control treatments recorded significantly higher and comparable number of pods per plant. Whereas, no significant difference was observed in succeeding green gram after *rabi*, 2009-10 maize (Table 2).

*Number of seeds per pod***:** During both the seasons, application of POE glyphosate at 900 g a.e ha^{-1} in transgenic Hishell recorded significantly higher number of seeds per plant, and was comparable with all other weed control treatments. Unweeded control like T_8, T_{12} and T_{16} during *kharif* and T_8, T_{11} and T_{13} during *rabi* showed higher number of pods per plant, while compared to other unweeded checks.

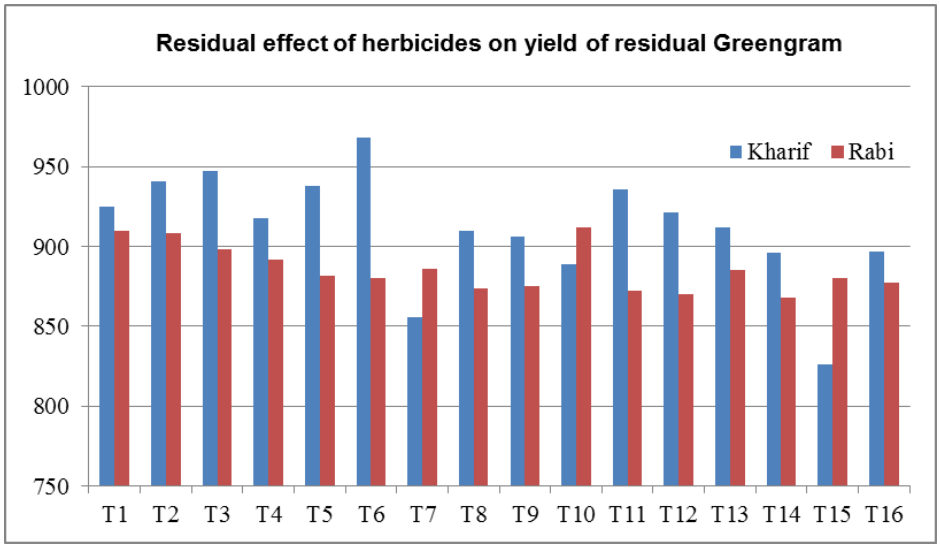
*Test weight***:** Weed management practices did not show any significant difference in test weight of succeeding green gram among different treatments at both the seasons. However, lesser test weight was recorded under unweeded checks (T_9 for *kharif* and T_{12} for *rabi*).

*Grain yield***:** In the succeeding greengram after *kharif* maize 2009, among the weed management practices, POE application of glyphosate at 3600 g a.e ha^{-1} in transgenic 900 M Gold maize hybrid recorded higher grain yield of green gram (T_6) and lesser grain yield was recorded PE application of atrazine at 0.5 kg ha^{-1} *fb* HW in CoHM 5 maize hybrid (T_{15}) (Table 2). Whereas, in the succeeding greengram after *rabi* maize 2009, different weed management practices did not exhibit any significant variation in the grain yield of succeeding green gram.

Discussion

Post emergence application of glyphosate in transgenic maize hybrids did not affect the germination per cent of succeeding green gram. This might be due to inactive of glyphosate in

soil. Franz *et al*. (1997) [6] reported that, crops can be planted or seeded directly into treated areas of glyphosate because it has no pre-emergent activity even when applied at high rates. In non-transgenic maize hybrids with PE application of atrazine also not exhibit any germination variations in succeeding green gram. In contrast with current result, Saghir and Choudhary (1967) [7] who reported that, atrazine residual activity in soil could be harmful to subsequent sensitive crops such as cereals, sunflower, rapeseed and sugar beet. Whereas, the injury to following crops is influenced by herbicide adsorption to soil and the rate of degradation. If an herbicide is strongly adsorbed to soil then it will not be very available for plant uptake.



The different herbicidal treatments did not show variation in plant height, yield attributing parameters like number of pods per plant, pod length, number of grains per pod and test weight and yield. With respect to weed density of succeeding green gram, lower weed density was recorded with glyphosate applied plots in preceding maize crop, which might be due to timely weed control at before seed production of weeds in maize and reduced the weed seed bank. This might have led to lower density of weeds compared to other treatments. Walker and Oliver (2008) [8] who had reported that, minimum number of glyphosate application effectively reduced the population of weed seed production thus reduced the weed intensity.

Table 1: Residual effect of herbicides on germination and plant height (cm) of residual Greengram - *kharif*, 2009

Treatments	*kharif*, 2009				*rabi*, 2009-10			
	Germination (%)*	Plant height (cm)			Germination (%)*	Plant height (cm)		
		25 DAS	45 DAS	Harvest		25 DAS	45 DAS	Harvest
T_1 - T. Hishell POE glyphosate @ 900 g ha^{-1}	89.3	17.0	24.2	41.2	90.67	15.8	23.1	40.3
T_2 - T. Hishell POE glyphosate @ 1800 g ha^{-1}	85.3	16.8	23.9	43.2	89.00	15.8	22.5	41.7
T_3 - T. Hishell POE glyphosate @ 3600 g ha^{-1}	88.3	18.0	23.0	42.8	91.00	17.0	23.4	41.2
T_4 - T. 900 M Gold POE glyphosate @ 900 g ha^{-1}	81.3	16.7	22.8	39.8	89.00	15.5	22.2	39.5
T_5 - T. 900 M Gold POE glyphosate @ 1800 g ha^{-1}	87.3	16.9	24.4	42.1	88.67	16.0	23.2	40.5
T_6 - T. 900 M Gold POE glyphosate @ 3600 g ha^{-1}	88.0	17.3	24.0	40.8	91.33	16.9	23.1	39.7
T_7 - Hishell PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	87.7	16.8	23.3	41.6	89.33	16.0	22.5	39.8
T_8 - Hishell No WC and no IC	87.0	14.8	22.0	39.1	89.67	13.2	20.0	37.6
T_9 - Hishell No WC and only IC	86.0	15.3	21.6	39.6	91.33	13.9	20.1	38.9
T_{10} - 900 M Gold PE atrazine @ 0.5 kg ha^{-1}+ HW+ IC	85.3	17.1	24.0	43.1	90.00	16.4	23.2	42.2
T_{11} - 900 M Gold No WC and no IC	89.0	15.7	21.7	39.8	89.00	14.7	20.0	38.9
T_{12} - 900 M Gold No WC and only IC	87.3	15.9	22.0	38.9	89.00	14.9	21.1	37.8
T_{13} - Proagro PE atrazine 0.5 @ kg ha^{-1} + HW+ IC	83.7	16.7	23.1	40.8	90.67	15.4	22.4	40.3
T_{14} - Proagro 4640 No WC and no IC	87.0	15.1	21.6	38.8	89.33	14.4	20.5	38.4
T_{15} - CoHM 5 PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	88.7	16.8	22.8	41.2	90.67	15.8	22.6	41.2
T_{16} - CoHM 5 No WC and no IC	86.0	14.9	21.8	40.4	91.00	14.2	19.9	38.2
SEd	-	0.7	1.5	2.4	-	1.1	1.9	2.4
CD (P=0.05)	-	1.3	NS	NS	-	2.1	NS	NS

* Data not analysed statistically

Table 2: Residual effect of herbicides on germination and plant height (cm) of residual Greengram - *kharif,* 2009

Treatments	*kharif,* 2009				*rabi,* 2009-10			
	Dry Matter Production at Harvest (kg ha^{-1})	Yield attributes			Dry Matter Production at Harvest (kg ha^{-1})	Yield attributes		
		No. of pods plant^{-1}	No. of seeds pod^{-1}	Test weight (g)		No. of pods plant^{-1}	No. of seeds pod^{-1}	Test weight (g)
T_1 - T. Hishell POE glyphosate @ 900 g ha^{-1}	1645	18	9.3	5.48	1520	17	9.3	4.95
T_2 - T. Hishell POE glyphosate @ 1800 g ha^{-1}	1736	18	8.9	5.23	1498	17	9.2	5.13
T_3 - T. Hishell POE glyphosate @ 3600 g ha^{-1}	1712	17	9.1	4.85	1566	18	8.8	4.98
T_4 - T. 900 M Gold POE glyphosate @ 900 g ha^{-1}	1685	18	9.0	4.98	1568	17	9.0	5.31
T_5 - T. 900 M Gold POE glyphosate @ 1800 g ha^{-1}	1630	18	9.2	5.36	1574	17	9.1	5.16
T_6 - T. 900 M Gold POE glyphosate @ 3600 g ha^{-1}	1648	17	9.2	5.30	1543	16	9.0	4.98
T_7 - Hishell PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	1732	18	9.1	5.10	1566	18	8.8	5.23
T_8 - Hishell No WC and no IC	1212	14	8.8	4.79	1325	16	8.9	4.89
T_9 - Hishell No WC and only IC	1327	14	8.3	4.58	1453	16	8.2	4.52
T_{10} - 900 M Gold PE atrazine @ 0.5 kg ha^{-1}+ HW+ IC	1620	19	9.0	4.95	1557	15	9.2	4.89
T_{11} - 900 M Gold No WC and no IC	1305	15	8.1	4.69	1298	15	8.6	4.60
T_{12} - 900 M Gold No WC and only IC	1398	13	9.0	4.86	1423	16	8.0	4.46
T_{13} - Proagro PE atrazine 0.5 @ kg ha^{-1} + HW+ IC	1700	18	8.9	5.27	1576	17	9.0	5.19
T_{14} - Proagro 4640 No WC and no IC	1367	14	8.3	5.00	1463	15	8.6	4.58
T_{15} - CoHM 5 PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	1689	16	9.0	5.00	1583	16	9.0	5.20
T_{16} - CoHM 5 No WC and no IC	1234	13	8.7	4.82	1323	15	8.3	4.61
SEd	77.0	1.1	0.3	0.49	71.3	1.7	0.2	0.45
CD (P=0.05)	154	2.2	0.58	NS	143	NS	0.49	NS

Acknowledgement
The authors thank Monsanto India Pvt. Ltd., Mumbai for financial assistance rendered for carrying out the research work

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