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D Ravisankar
Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

C Chinnusamy
Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

P Muthukrishnan
Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
D Ravisankar
Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Influence of post emergence application of glyphosate on crop phytotoxicity and soil microbial status in transgenic maize

D Ravisankar, C Chinnusamy and P Muthukrishnan

Abstract
Field experiments were conducted at Tamil Nadu Agricultural University, Coimbatore to study the weed and crop phytotoxicity and soil weed seed bank of transgenic stacked maize hybrids named Hishell and 900 M Gold and the treatments were, glyphosate as early post emergence application (POE) at 900, 1800 and 3600 g a.e ha^{-1} compared with non-transgenic counterpart maize hybrids applied with pre-emergence (PE) atrazine at 0.5 kg ha^{-1} followed by one hand weeding (HW) on 40 DAS and with and without insect management. The results revealed that the rate of phytotoxicity development and time to destruction of weeds was increased over the increase of glyphosate doses from 900 g a.e ha^{-1} to 3600 g a.e ha^{-1} without affecting the crop growth. Post emergence application of glyphosate at 900 and 1800 g a.e ha^{-1} in herbicide tolerant transgenic maize resulted in more number of bacteria, fungi and actinomycetes. Whereas, in higher dose (3600 g a.e ha^{-1}) initial reduction of microbial population was observed and it recovered within 45 days after glyphosate spraying. PE application of atrazine exerted a significant detrimental effect on soil bacteria, fungi and actinomycetes and reduced the microbial count in herbicide treated plots upto 30 DAHS. Thereafter, microbial population started to recover slowly.

Keywords: Phytotoxocity, Soil Microorganism, Glyphosate, Transgenic Maize

1. Introduction

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) [1]. 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. Various post-emergence type herbicides used for weed control in soybean, canola, or corn can cause crop injury and ultimately yield loss. Crop injury is more severe when the crop is under stress or unfavourable environmental conditions occur. In contrast, crop injury is reduced with the use of herbicide tolerant crops.

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) [2]. Muller *et al*. (1981) [3] found that glyphosate degrades at very low temperatures and does not adversely affect nitrogen fixation, nitrification or denitrification activity. Soil studies have determined glyphosate half-lives ranging from 3 to 130 days. A study on the effects of glyphosate on microbial biomass (Stratton and Stewart, 1992) [4] revealed that glyphosate generally had no significant effect on the numbers of bacteria, fungi or actinomycetes in forest soil and overlying forest litter. There was no effect of glyphosate on *in situ* respiration in most of the treated systems while the remainder showed an increase in respiration. Wardle and Parkinson (1992) [5] reported that antagonistic interactions between the fungal species were eliminated by glyphosate suggesting that the herbicide might influence overall soil fungal community structure. Means (2007) [6] detected a significant increase in soil Fusarium within two weeks after glyphosate was applied at recommended rates in the field. Meriles *et al.* (2006) [7] verified that the population growth and sporulation of soil *Fusarium* spp. increased after glyphosate application to soils containing maize or peanut crop residues compared with lower *Fusarium* populations in similar fallowed soils lacking crop residues. In culture based studies, indicates that isolated *Fusarium* spp. From soil were able to metabolize glyphosate and use it as a phosphorus source (Castro *et al.*, 2007) [8].

A follow-up study verified that long-term exposure of soil microorganisms to glyphosate led to a fungal community dominated by *Fusarium* spp. (Krzysko-Lupicka and Sudol, 2008) [9].

Materials and Methods

The research was conducted with glyphosate resistant maize hybrids during kharif 2009 and rabi 2009-2010 seasons at experimental site of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The experiment was laid out in randomized complete block design (RBD) with sixteen treatments and replicated thrice. Treatments consisted of post emergence application of glyphosate at 900, 1800 and 3600 g a.e/haat 2 - 4 leaf stage of weeds (approximately 25 Days After Sowing of transgenic maize hybrids) in transgenic maize hybrids. In non-transgenic maize hybrids viz., Hishell, 900 M Gold, COHM 5 (local test hybrid) and Proagro (national test hybrid) with pre-emergence application of atrazine 0.5 kg/ha at 3 Days After Sowing (DAS) followed by one hand weeding (HW) at 40 DAS along with insect control was done by whorl application of carbofuran at 1.0 kg a.i/ha at 20 DAS.

Observations

Phytotoxicity Assessment

Visual scoring for control of weeds and phytotoxic symptoms (yellowing / chlorosis / stunting/scorching) in maize crop were done on 7, 14 and 21 DAHS.
The scoring scale followed is given in below table.

Phytotoxic symptom scoring and rating on weeds and crop (Rao, 2000) [10]

Crop injury symptom	Rating	Effect
No injury, Normal	0	None
Slight stunting, injury or discoloration	1	Slight
Some stand loss, stunting / discoloration	2	Slight
Injury more pronounced but not persistent	3	Slight
Moderate injury, recovery possible	4	Moderate
Injury more persistent, recovery doubtful	5	Moderate
More severe injury, no recovery possible	6	Moderate
Severe injury, stand loss	7	Severe
Almost destroyed few plants surviving	8	Severe
Very few plants alive	9	Severe
Complete destruction	10	Complete

Soil Microbial analysis

The standard serial dilution plating technique of Pramer and Schemidt (1965) was adopted for the estimation of microbial population and expressed as colony forming units (CFU) g^{-1} of soil. The different types of microorganisms were enumerated using differential media favouring the growth of bacteria, fungi and actimomycetes as shown in below table.

Particulars	Author	Method
Total bacteria (CFU x 10^6)	Collings and Lyne (1968)	Standard plating technique using Nutrient agar medium
Total fungi (CFU x 10^3)	Martin (1950)	Standard plating technique using Martins Rose Bengal agar medium
Total actinomycetes (CFU x 10^4)	Kenknight and Muncie (1939)	Standard plating technique using Kenknights medium

Results and Discussion

Weed flora

Weed flora in the soil weed seed bank was predominantly consisted of four species of broad-leaved weeds, three species of grasses and a sedge weed. The predominant broad leaved weeds were *Trianthema portulacastrum*, *Cleome gynandra*, *Boerhaavia diffusa* and *Digera arvensis*. Among the grassy weeds *Cynodon dactylon, Dactyloctenium aegyptium* and *Cyanotis axillaris* were the dominant ones. *Cyperus rotundus* was the only sedge weed.

Crop Phytotoxicity

The phytotoxic symptom of herbicides on maize was observed during both the seasons of *kharif*, 2009 and *rabi*, 2009 (Table 1). There was no phytotoxicity observed in both transgenic and non-transgenic maize hybrids due to different herbicidal treatments. Regarding phytotoxicity, there was no phytotoxic symptom observed in transgenic hybrids due to application of various doses of glyphosate at 900, 1800 and 3600 g a.e ha^{-1} at throughout the crop growth. The reason was pointed out by Nida *et al.*, (1996)[11] resistant to glyphosate was developed by insertion of a bacterial gene, along with a promoter, which encodes for a version of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) having greatly reduced affinity for glyphosate. Parkar (1999)[12] revealed that no injury was recorded in maize crop due to application of POE glyphosate product at various levels of concentrations. Importantly, growers have perceived the lower potential for crop phytotoxicity to be a significant benefit for transgenic crops. Similarly, in non-transgenic hybrids, PE application of atrazine at 0.5 kg ha^{-1} also did not show any phtotoxic symptoms.

Effect of Herbicide Residues on Soil Microorganisms

Biological properties like bacteria, fungi and actinomycetes of the soil were significantly influenced by weed management practices (Table 2 and 3). In transgenic maize hybrids, POE application of glyphosate at 900 and 1800 g a.e ha^{-1} in both transgenic maize hybrids was recorded more number of bacteria and fungi at 7, 15 DAHS and 60 DAHS compared to 3 DAHS. Whereas, higher dose of glyphosate at 3600 g a.e ha^{-1} recorded reduced population at 7 DAHS and 15 DAHS compared to 3 DAHS but it was recovered at 40 DAHS. Regarding the population of actinomycetes, there was no influence of glyphosate on actinomycetes population at all stages of observation. In non-transgenic maize hybrids, experimental results revealed that the application of PE atrazine at 0.5 kg ha-1 showed a significant detrimental effect on soil bacteria, fungi and actinomycetes and reduced the microbial count in all the samples collected in the herbicide treated plots at 3, 7 and 15 DAHS. Thereafter, at 45 DAHS, microbial density started to recover slowly with atrazine applied plots. In plots that devoid herbicidal treatment, the microbial density was continuously increased.

Application of herbicide in agriculture may have side effects on biological equilibrium following the changes in soil environment. Herbicides applied in crop fields for weed control are likely to have effects of the microorganisms in the rhizosphere of crops, weed and in soil (Lewis *et al*., 1988) [13]. In transgenic maize hybrids, POE application of glyphosate at lower doses like 900 and 1800 g a.e ha^{-1} recorded with more number of bacteria, fungi and actinomycetes. This might be due to glyphosate applied directly on the weeds that added organic material to the soil, during decomposition of organic material microbial population might have been increased.

Haney *et al*. (2000)[14] who had reported that glyphosate was available to soil and rhizosphere microbial communities as a substrate for direct metabolism leading to increased microbial biomass and activity. Higher doses of glyphosate with 3600 led to slight reduction in microbial population as observed at initial stages and recovered within 45 days. The results corroborate with the observations of Weaver *et al*. (2007) [15] who had reported that glyphosate had only small and transient effects on the soil microbial community, even when applied at greater than field rates.

In non-transgenic maize hybrids, microbial population was declined immediately after application of atrazine at 0.5 kg ha^{-1}. The counts of bacteria, fungus and actinomycetes recorded at 3 DAHS revealed that soil microbes are more sensitive to atrazine. The reason for the inhibitory effect of atrazine was due to direct application of to the soil and hence the amount of herbicide residues that comes in contact with soil was more. The reduction in the microbial count was slowly recovered with 45 days after herbicide application.However, due to our limited knowledge of the linkage between microbial community structure and function, more work needs to be done on a case-by-case basis to further evaluate the effects of transgenic plants on soil microorganisms and soil ecosystem functions.

Table 1: Weed control rating in transgenic and non-transgenic maize (mean scores of two seasons)

Transgenic Maize with Treatments	**3 DAHS**			**7 DAHS**			**15 DAHS**		
	BLW	**G**	**S**	**BLW**	**G**	**S**	**BLW**	**G**	**S**
T_1 - T. Hishell POE glyphosate @ 900 g ha^{-1}	5.0	3.0	2.0	6.0	6.0	3.0	7.0	7.0	5.0
T_2 - T. Hishell POE glyphosate @ 1800 g ha^{-1}	5.0	5.0	2.0	7.0	7.0	4.0	8.0	8.0	6.0
T_3 - T. Hishell POE glyphosate @ 3600 g ha^{-1}	5.0	5.0	2.0	7.0	7.0	4.0	8.0	8.0	6.0
T_4 - T. 900 M Gold POE glyphosate @ 900 g ha^{-1}	5.0	3.0	2.0	6.0	6.0	3.0	7.0	7.0	5.0
T_5 - T. 900 M Gold POE glyphosate @ 1800 g ha^{-1}	5.0	5.0	2.0	7.0	7.0	4.0	8.0	8.0	6.0
T_6 - T. 900 M Gold POE glyphosate @ 3600 g ha^{-1}	5.0	5.0	2.0	7.0	7.0	4.0	8.0	8.0	6.0
Non-Transgenic Maize with Treatments	**25 DAS**			**35 DAS**			**45 DAS**		
	BLW	**G**	**S**	**BLW**	**G**	**S**	**BLW**	**G**	**S**
T_7- Hishell PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	9.0	9.0	7.0	7.0	7.0	7.0	9.0	9.0	8.0
T_8 - Hishell No WC and IC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T_9 - Hishell No WC and only IC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T_{10} - 900 M Gold PE atrazine @ 0.5 kg ha^{-1}+ HW+ IC	9.0	9.0	7.0	7.0	7.0	7.0	9.0	9.0	8.0
T_{11} - 900 M Gold No WC and IC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T_{12} - 900 M Gold No WC and only IC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T_{13} - Proagro PE atrazine 0.5 @ kg ha^{-1} + HW+ IC	9.0	9.0	7.0	7.0	7.0	7.0	9.0	9.0	8.0
T_{14} - Proagro 4640 No WC and IC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T_{15} - CoHM 5 PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	9.0	9.0	7.0	7.0	7.0	7.0	9.0	9.0	8.0
T_{16} - CoHM 5 No weeding & IC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Data not statistically analysed, DAHS: Days after Herbicide Spray, G: Grasses S: Sedges BLW: Broad leaved weeds

Table 2: Effect on weed management methods on soil in maize during *Kharif* season

Treatments	**Bacteria ($\times 10^{-6}$ CFU g^{-1})**			**Fungi ($\times 10^{-4}$ CFU g^{-1})**			**Actinomycetes ($\times 10^{-4}$ CFU g^{-1})**		
	7 DAHS	**15 DAHS**	**45 DAHS**	**7 DAHS**	**15 DAHS**	**45 DAHS**	**7 DAHS**	**15 DAHS**	**45 DAHS**
T_1 - T. Hishell POE glyphosate @ 900 g ha^{-1}	36.21	38.23	40.53	24.12	25.21	27.32	11.35	12.05	12.65
T_2 - T. Hishell POE glyphosate @ 1800 g ha^{-1}	36.00	39.50	37.94	25.36	26.00	28.54	11.67	11.98	12.32
T_3 - T. Hishell POE glyphosate @ 3600 g ha^{-1}	29.65	34.12	36.97	22.65	24.12	28.00	11.32	11.67	12.41
T_4 - T. 900 M Gold POE glyphosate @ 900 g ha^{-1}	37.12	37.91	39.65	24.00	25.23	29.64	10.69	11.35	12.62
T_5 - T. 900 M Gold POE glyphosate @ 1800 g ha^{-1}	35.24	38.64	40.12	25.31	26.31	28.61	12.00	12.64	13.00
T_6 - T. 900 M Gold POE glyphosate @ 3600 g ha^{-1}	30.21	35.24	37.49	23.12	25.98	29.07	10.64	11.32	12.00
T_7 - Hishell PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	19.62	15.38	29.62	18.32	20.24	26.34	9.07	9.87	11.23
T_8 - Hishell No WC and IC	38.52	40.65	41.08	26.00	26.98	29.64	12.18	12.93	12.97
T_9 - Hishell No WC and only IC	38.61	40.00	40.84	27.12	27.82	30.12	12.41	13.10	13.34
T_{10} - 900 M Gold PE atrazine @ 0.5 kg ha^{-1}+ HW+ IC	18.35	16.21	30.21	21.00	22.65	26.81	9.62	10.00	11.67
T_{11} - 900 M Gold No WC and IC	37.00	39.64	41.32	27.53	28.30	30.14	11.21	11.98	12.38
T_{12} - 900 M Gold No WC and only IC	37.58	38.25	39.64	26.98	27.51	29.85	12.46	13.00	13.78
T_{13} - Proagro PE atrazine 0.5 @ kg ha^{-1} + HW+ IC	18.24	15.32	27.42	21.31	22.00	26.00	8.65	9.57	11..56
T_{14} - Proagro 4640 No WC and IC	36.85	39.21	38.67	27.12	28.31	30.53	11.32	12.31	12.89
T_{15} - CoHM 5 PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	20.85	18.62	26.36	19.21	20.31	25.61	9.71	10.03	11.82
T_{16} - CoHM 5 No weeding & IC	35.94	39.21	38.24	25.32	26.78	29.67	12.00	12.32	13.21
SEd	1.78	1.97	1.27	1.83	2.25	2.13	0.93	0.90	0.90
CD (P= 0.05)	3.57	3.95	4.37	3.68	4.53	4.29	1.86	1.82	1.82

Table 3: Effect on weed management methods on soil in maize during *Rabi* Season

Treatments	Bacteria (x 10^{-6} CFU g^{-1})			Fungi (x 10^{-4} CFU g^{-1})			Actinomycetes (x 10^{-4} CFU g^{-1})		
	7 DAHS	15 DAHS	45 DAHS	7 DAHS	15 DAHS	45 DAHS	7 DAHS	15 DAHS	45 DAHS
T_1 - T. Hishell POE glyphosate @ 900 g ha^{-1}	36.44	40.54	41.35	23.76	25.00	26.50	11.02	11.35	12.31
T_2 - T. Hishell POE glyphosate @ 1800 g ha^{-1}	35.79	39.86	38.76	25.15	25.10	27.72	11.36	11.98	12.56
T_3 - T. Hishell POE glyphosate @ 3600 g ha^{-1}	30.61	34.78	37.79	21.69	24.10	27.18	11.39	12.00	12.50
T_4 - T. 900 M Gold POE glyphosate @ 900 g ha^{-1}	36.16	38.76	40.47	23.04	25.10	28.82	10.92	11.62	11.98
T_5 - T. 900 M Gold POE glyphosate @ 1800 g ha^{-1}	34.28	39.01	40.94	24.35	26.27	27.79	11.00	11.32	11.92
T_6 - T. 900 M Gold POE glyphosate @ 3600 g ha^{-1}	29.25	35.38	38.31	22.16	25.84	28.25	11.67	11.98	12.67
T_7 - Hishell PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	18.66	16.23	30.44	17.36	19.39	25.21	9.21	9.97	10.23
T_8 - Hishell No WC and IC	37.56	41.50	41.90	25.04	26.13	28.82	11.62	12.03	12.62
T_9 - Hishell No WC and only IC	37.65	42.37	41.66	26.16	25.45	29.30	11.62	12.24	12.38
T_{10} - 900 M Gold PE atrazine @ 0.5 kg ha^{-1}+ HW+ IC	17.39	18.35	31.03	20.04	20.51	25.08	9.08	9.67	10.41
T_{11} - 900 M Gold No WC and IC	36.04	40.67	42.14	26.57	27.27	29.32	11.85	12.52	12.82
T_{12} - 900 M Gold No WC and only IC	36.62	38.37	40.46	26.02	27.39	29.03	12.08	12.87	13.00
T_{13} - Proagro PE atrazine 0.5 @ kg ha^{-1} + HW+ IC	17.28	15.98	28.24	20.35	21.34	25.30	9.08	9.36	10.05
T_{14} - Proagro 4640 No WC and IC	35.89	39.86	39.49	26.16	27.66	29.71	11.26	11.87	12.20
T_{15} - CoHM 5 PE atrazine @ 0.5 kg ha^{-1} + HW+ IC	19.89	19.14	27.18	18.25	19.79	24.83	9.10	10.00	10.57
T_{16} - CoHM 5 No weeding & IC	34.98	39.97	39.06	24.36	26.02	28.85	11.65	12.30	12.92
SEd	2.03	2.06	2.17	1.94	1.97	2.16	0.60	0.79	0.55
CD (P= 0.05)	4.09	4.13	4.36	3.86	3.95	4.32	1.21	1.60	1.10

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