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Degradation of Glyphosate in Soil and its Effect on Fungal Population

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Glyphosate application resulted in a decline in soil pH with consequent increase in soil mycoflora suggesting an indirect relationship. Though the composition of mycoflora unchanged, species of aspergilli, fusaria, penicillia and *Trichoderma* were predominant. HPLC, IR analysis revealed the presence of sarcosine derivative as an intermediary of glyphosate degradation in soil.

Key words: *Population dynamics, biodegradation, mycoflora, HPLC*

Introduction

Soil being a dynamic medium for microbial growth serves as a repository of biological resources that can be exploited in the bioremediation of soil pollutants. Microbial activities immensely influence the degradation of various soil pollutants including pesticides and herbicides. Hydrolytic enzymes produced during microbial activities degrade the pesticides (Levanon, 1993)[1]. Effect of the herbicide glyphosate on soil mycoflora varies with the application of the herbicide as well as the composition of soil microflora (Muller *et.al.,* 1981)[2]. Acclimatization of microflora to the herbicide influences the rate of degradation and extent of mineralization of glyphosate (Robertson and Alexander, 1994)[3]. This paper deals with the impact of glyphosate on population dynamics, rate of degradation and the metabolites formed during the degradation.

Materials and methods

The herbicide, glyphosate (Glycel, 41% pure) was sprayed on the foliage of weeds at 0.5M concentration and samples were collected from day 1 to 30 days for herbicide as well as microbial analysis. Mycoflora was estimated as colony forming units per gram soil (g/cfu)from glyphosate treated and control non-treated lots of the University research fields employing soil dilution plate method. Individual colonies of fungi were isolated and sub-cultured on potato dextrose agar slants for identification using standard manuals.

HPLC analysis of glyphosate and metabolites

Soil samples were extracted in distilled water (1g+10mL water) by thorough mixing, separation and centrifugation (125rpm/10min). The supernatant was filtered through Whatman filter paper No.1 and Millipore (0.5µM)filter. The filtrate was analyzed for glyphosate and its metabolites using HPLC system(Shimadzu make, Model LC 10AT,CR 7 Age plus Chromatopac, C-18 column spd 10AiUV detector and the injector 77261). The method for HPLC-UV analysis of glyphosate and metabolites was optimized by scanning their standards and the wavelength of optimum response was used for the determination of residues in soil. Water and methanol mixture 80:20% was used as mobile phase at the flow rate of 1.5mL/min. 10µl of standards and samples were injected and their chromatograms were studied. All the injections were done in triplicate. The concentration of each compound was evaluated using retention time and absorbance.

IR spectral analysis

The liquid sample collected as drain from HPLC method at 3.0-3.4 minutes was placed in a petri dish with KBr powder and oven dried for 5 minutes at 80^{0}C. The dried sample was ground in pestle and mortar by adding fresh KBr powder to get a homogenous mixture. The samples spectra was obtained from IR(FT-IR Spectrophotometer, ABB Bomen MB semi model: MBB-MB104) and analyzed after recording.

Results and discussion

Herbicide treatment affected the fungal population in soil with an initial increase in fungal numbers. However, the overall composition of mycoflora remained the same with the predominance of aspergilli, fusaria, penicillia, and species of *Trichoderma*. Population of *T. asperellum* and *T. pseudokoningii* increased considerably in the presence of glyphosate. Increasing trend continued up to 3 weeks and decreased thereafter. Levanon (1993)[1] reported the involvement of fungi and bacteria in mineralizing the herbicides atrazine and alachlor in soil. Presence of glyphosate in soil had no adverse effect on soil microflora (Rueppel *et al.,* 1977)[4], in fact it has enhanced the fungal population in the present study. Glyphosate application altered the soil pH towards acidic side **(Fig.1)** with consequent increase in the growth and multiplication of mycoflora in general and *Trichoderma* species

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in particular. The results conform to the earlier finding of Cal-A-de *et al*. (1993)[5], in which increase in *Trichoderma* population after herbicide application was reported. Microbial activity and glyphosate degradation followed an inverse relationship and the herbicide completely disappeared by 20th day **(Fig.2)** with corresponding increase in its derivative sarcosine suggesting the glycine pathway of degradation in soil. Muller *et al*. (1981)[2] correlated the increase in microbial activity with the rate of degradation of glyphosate in Finnish agricultural soils. Grunewald *et al*. (2001)[6] have reported the behavior of glyphosate and AMPA in soil, and water with half life of glyphosate ranging from 11-17 days. In the present study, the aurthors report the breakdown of glyphosate in soil through glycine pathway mediated by fungi.

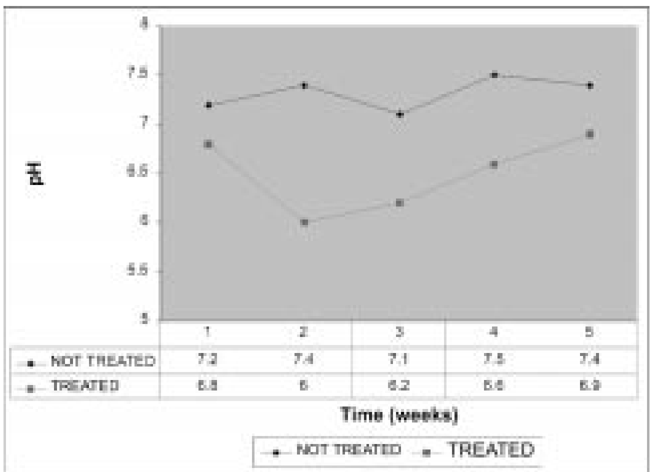

Fig. 1:Variation in pH of soil treated with glyphosate at different intervals

Conclusion

Glyphosate application affected the soil pH and enhanced the fungal population initially. Increased microbial activity rapidly mineralized glyphosate in soil resulting in the release of sarcosine derivative suggesting the glycine pathway of degradation.

Acknowledgement

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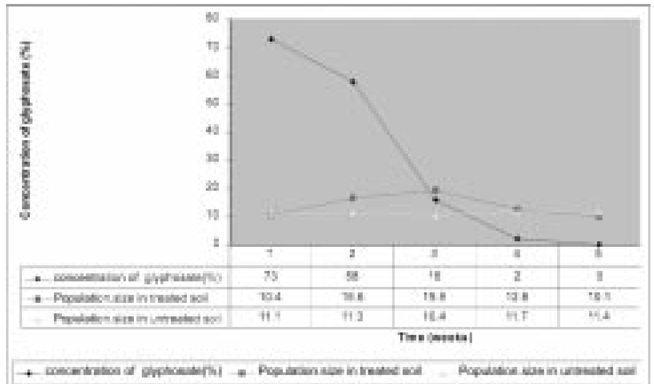

Fig. 2:Variation in glyphosate concentration and population size in soil treated with glyphosate

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