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Research Paper

Effect of herbicide Glyphosate on the microorganisms in tea growing soil

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Abstract

The In vitro and In vitro experiments were conducted at the Plant Pathology laboratory and experimental field of Bangladesh Tea Research Institute (BTRI) during 2008-2012. The study was conducted to find out the effect of glyphosate and its influence on microorganism proportions of soil in four dilutions. In laboratory experiment, recommended dilutions of glyphosate was made by mixing the required quantity formulated product into PDA before solidification. A zero concentration was prepared for control. Soils were collected from 5 different marks of tea field areas. In field experiment, initially soil nutrient status of the experimental area was determined with the consideration of pH, percent Organic carbon and Nitrogen, Phosphorus, Potassium, Calcium and Magnesium in PPM. The herbicide glyphosate was applied @ 3.7 liter ha^{-1}. Soil was collected from the top 0 to 9 cm layers three times at one month interval. Herbicide was applied before flowering and after full ground coverage was obtained. In both cases, for mycofloral analysis serial dilution (10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were same. Colony forming unit g^{-1} of soil was counted after 4-7 days. Results revealed that the pH of soil was moderately acidic with value of 4.8 ± 0.0254, having only 1.03 ± 0.0483% organic carbon, 0.12 ± 0.0042% total nitrogen, 140.95 ± 0.0275 ppm available phosphorus, 45.45 ± 0.0052 ppm potassium, 97.40 ± 0.0134 ppm calcium and 49.40 ± 0.0037 ppm magnesium. Trichoderma sp was dominated for all the time of observations, accounting more than 20% of total colony count. Glyphosate treated sample only Penicillium and Aspergillus sp were recorded contributed only up to 10% in 10^{-3} dilution. In 10^{-4} dilution with control, Phomopsis, Ustulina, Sclerotium, Trichoderma, Rhizoctonia, Penicillium and Aspergillus sp were recorded. Among these seven organisms Trichoderma was dominated for all the time of observations, accounting more than 20% of total colony count. All other organisms were contributing up to 20% identical colony count after two months of herbicide spray. Glyphosate treated sample with same concentration, Sclerotium, Rhizoctonia, Penicillium and Aspergillus sp were recorded contributed only up to 10% while it was up to 20% colony count for the same organisms in 10^{-5} and 10^{-6} dilutions from two months of herbicide spraying. Therefore, it was indicated that the glyphosate has toxic effect on Phomopsis and Ustulina as soil mycoflora. In contrast to the fungal suppression, Sclerotium, Trichoderma, Rhizoctonia Penicillium and Aspergillus sp showed enhancement at higher concentration of herbicide.

Keywords: Glyphosate, tea, soil, Penicillium, Aspergillus, In vitro.

Introduction

Weeds are recurring problem in the nursery, young tea as well as in mature tea plantations. It competes with tea for nutrients, moisture, space and sunlight. Resulting reduced growth and yield of tea. Cramer (1967), Eden (1961), Rahman (1975) and Sana (1989) Estimated crop loss due to weed infestation be 9%, 12%, 6-12% and 9% in Sri Lanka, North-East India, South India and Bangladesh respectively [1,2,3,4]. The weed flora in Bangladesh tea a diversified in nature and composed of a wide spectrum of various monocot and dicot weeds. As many as 36 weed species have so far been recorded [4]. Chemical method of weed control involves use of synthetic herbicides to interfere with the metabolic processes of plants to result in death of weeds [5,6]. Herbicides are substances or cultured biological organism used to kill or suppress the growth of unwanted plants and vegetations [7]. During the past four decades, a large number of herbicides have been introduced as pre or post emergent weed killers in many countries of the world. Glyphosate is generally regarded to be an herbicide with low environmental impact, with low mammalian toxicity. Being water soluble, glyphosate has a low risk of bioaccumulation in food webs. In addition, the phosphate group in glyphosate readily adsorbs to clay and aluminum and iron oxides in soil, which limits losses from the field and entry into aquatic ecosystems or groundwater. Once adsorbed, the compound is rapidly degraded by soil microorganisms [8]. Microbial degradation of herbicides in soils is a function of three key variables. The ability of the microorganisms to degrade the pesticides, the quantity of these microorganisms in the soil, and the activity of the soil microbial enzyme system [9]. Herbicides when applied to the soil for the control of weed in crop fields have enormous effects on microbial activities. Glyphosate [N-(phosphonomethyl)glycine] is the most widely used herbicide in the world. First sold in 1974 under the trade name Roundup by Monsanto, glyphosate works by inhibiting the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase. This enzyme is a critical intermediate, used in the production of three aromatic amino acids vital to plants [8]. Glyphosate is the systemic herbicide that is commonly used to control a broad spectrum of weed in crops and pastures worldwide [10]. Glyphosate is a polar compound known for its strong adsorption to Fe and Al oxides and clay minerals [11, 12].

Due to application of Glyphosate, percent increased of all soil nutrients were improved in an increasing trend with the increasing day intervals [13]. Soil microbes are the driving force behind many soil processes including transformation of organic matter, nutrient release and degradation of xenobiotics [14]. The presence of glyphosate in soil may cause changes to the microbial population and activity of a soil. The presence of glyphosate in a soil was related to a temporary increase in the both number of bacteria in a soil and the overall microbial activity of the soil, although the number of fungi and actinomycetes was not affected [15, 16]. The effect of glyphosate on soil microorganisms has been widely studied, with conflicting results. In part this may be because soil microbial communities are diverse and live in diverse soil ecosystems. A number of studies have found glyphosate has no significant effect on microbial community activity and composition [11, 12, 17, 18]. In recent years, the intensive use of herbicides has increasingly become a matter of environmental concern, partially because of the adverse effects of these chemicals on soil microorganisms. Glyphosate [N-(phosphonomethyl) glycine] is a broad-spectrum, non-selective, postemergence herbicide that is widely used in agriculture. The commercial success of glyphosate as a highly effective herbicide has stimulated several studies on its behavior and persistence in soil [19, 20, 21]. More recently, the effect of glyphosate was evaluated on the microbial community of soils and observed that microbial activity was stimulated in the presence of this herbicide [11, 22].

The intensive use of herbicides is an environmental problem, partially because of the potential hazardous effects of these chemicals on soil biological processes and non-target organisms. These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plants, wildlife and man [23]. The present study was designed to detect changes in soil microbial community structure and activity following glyphosate exposure in the field and the laboratory.

Materials and Methods

In vitro experiment

The *In vitro* experiment was carried out at the Plant Pathology laboratory of Bangladesh Tea Research Institute, Srimangal during 2008-2010. Potato Dextrose Agar (PDA) medium was prepared in the laboratory. Medium and necessary glassware were sterilized in autoclave [24]. Recommended dilutions of glyphosate were made by mixing the required quantity formulated product into PDA before solidification. A zero concentration was prepared for control. The medium is then dispensed uniformly into 90 mm diameter Petri plates. Soils were collected from 15 different marks of tea areas under main farm of Bangladesh Tea Research Institute at 0-9 inches. All collected samples were mixed thoroughly to make a composite sample. For mycofloral analysis, serial dilution agar plating technique was adopted. Soil dilutions taken were 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Colony forming unit g^{-1} of soil was counted after 4-7 days.

Field experiment

The field experiment was carried out at the experimental farm of Bangladesh Tea Research Institute (BTRI) during 2010-2012 to find out the soil mycofloral responses following the exposure to glyphosate in young tea. The soil type of the experimental unit was sandy loam. Initially soil nutrient status of the experimental area was determined with the consideration of pH, percent Organic carbon and Nitrogen, Phosphorus, Potassium, Calcium and Magnesium in PPM. Organic matter was analyzed by dichromate oxidation and titration with ferrous ammonium sulphate [25]. Total nitrogen was determined by [26]. Phosphorus and Potassium were determined by ascorbic acid and flame photometric method respectively. Calcium and Magnesium were analyzed by Atomic absorption spectrophotometric method. Potassium, Calcium and Magnesium, were extracted with 77% Ammonium acetate [13].

The herbicide glyphosate was applied @ 3.7 liter ha^{-1}. Three times soil was collected from the top 0 to 9 cm layers at one month interval from the application of the said herbicide. Herbicide was applied before flowering and after full ground coverage was obtained. For mycofloral analysis, serial dilution agar plating technique was adopted. Soil dilutions taken were 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. In this method Potato Dextrose Agar (PDA) medium was used for the culture of mycofloral proportions. Colony forming unit g^{-1} of soil was counted after 4-7 days.

Results and Discussion

Physico-chemical and mycological parameters of soil

The mean values of physico-chemical parameters in present study are shown in Table 1 and mycological parameters are given in Tables 2, 3 and 4. The micro-organisms are greatly affected by physical and chemical conditions of their environment. Soil is considered to be the most dynamic site of biological interactions. Microbial population and activity in soil can be regulated by soil's physico-chemical characters [27].

Table 1: Values of some selected physico-chemical characteristics of soil (Mean ±S.E. for four observations each)

Parameters	Values
pH	4.8 ± 0.0254
% OC	1.03 ± 0.0483
% N	0.12 ± 0.0042
P (PPM)	140.95 ± 0.0275
K (PPM)	45.45 ± 0.0052
Ca (PPM)	97.40 ± 0.0134
Mg (PPM)	49.40 ± 0.0037

Table 2: Percentage of different fungal species after one month

Name of fungi	Soil dilutions							
	10^{-3}		10^{-4}		10^{-5}		10^{-6}	
	Control	Glyphosate Treated	Control	Glyphosate Treated	Control	Glyphosate Treated	Control	Glyphosate Treated
Phomopsis	+	-	+	-	+ +	-	+ +	-
Ustulina	+	-	+	-	+ +	-	+ +	-
Sclerotium	+	-	+ +	-	+ +	+	+ +	+
Trichoderma	+ +	-	+ +	-	+ + +	+	+ + +	+
Rhizoctonia	+ +	-	+ +	-	+ + +	+	+ + +	+
Penicillium	+	+	+	+	+ +	+	+ +	+
Aspergillus	+	-	+	-	+ +	+	+ +	+

- = No CFU, + = CFU: 1-10%, + + = CFU: 11-20%, + + + = CFU: >20%,

Table 3: Percentage of different fungal species after two month

Name of fungi	Soil dilutions							
	10^{-3}		10^{-4}		10^{-5}		10^{-6}	
	Control	Glyphosate Treated	Control	Glyphosate Treated	Control	Glyphosate Treated	Control	Glyphosate Treated
Phomopsis	+	-	+ +	-	+ +	-	+ +	-
Ustulina	+	-	+ +	-	+ +	-	+ +	-
Sclerotium	+ +	-	+ +	+	+ +	+	+ +	+
Trichoderma	+ + +	-	+ + +	+	+ + +	+	+ + +	+
Rhizoctonia	+	-	+	+	+ +	+	+ +	+
Penicillium	+ +	+	+ +	+	+ +	+ +	+ +	+ +
Aspergillus	+ +	+	+ +	+	+ +	+	+ +	+ +

Table 4: Percentage of different fungal species after three months

Name of fungi	Soil dilutions							
	10^{-3}		10^{-4}		10^{-5}		10^{-6}	
	Control	Glyphosate Treated	Control	Glyphosate Treated	Control	Glyphosate Treated	Control	Glyphosate Treated
Phomopsis	+ +	-	+ +	-	+ + +	-	+ + +	+
Ustulina	+ +	-	+ +	-	+ + +	-	+ + +	-
Sclerotium	+ +	-	+ + +	+	+ + +	+	+ + +	+
Trichoderma	+ + +	-	+ + +	+	+ + +	+	+ + +	+
Rhizoctonia	+ +	-	+ +	+	+ + +	+	+ + +	+
Penicillium	+ +	+	+ +	+	+ + +	+ +	+ + +	+ +
Aspergillus	+ +	+	+ +	+	+ + +	+ +	+ + +	+ +

In the present study, the pH of soil was moderately acidic with value of 4.8 ± 0.0254. In this study, soil procured from tea cultivated land was having only 1.03 ± 0.0483% organic carbon, 0.12 ± 0.0042% total nitrogen, 140.95 ± 0.0275 ppm available phosphorus, 45.45 ± 0.0052 ppm potassium, 97.40 ± 0.0134 ppm calcium and 49.40 ± 0.0037 ppm magnesium. A positive correlation between organic matter percent and fungal populations [28]. High concentration of nutrients (N, P and K) is also due the presence of organic layer rich in mineral elements [29].

In the present study, four dilutions viz., 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were taken of which the 10^{-3} dilution was most concentrated. In this dilution with control, *Trichoderma* sp was dominated for all the time of observations, accounting more than 20% of total colony count. *Sclerotium, Rhizoctonia, Penicillium* and *Aspergillus* sp were also observed, contributing up to 20% after two months of herbicide spray. Glyphosate treated sample with same concentration, only *Penicillium* and *Aspergillus* sp were recorded contributed only up to 10%.

In 10^{-4} dilution with control, *Phomopsis, Ustulina, Sclerotium, Trichoderma, Rhizoctonia, Penicillium* and *Aspergillus* sp were recorded. Among these seven organisms *Trichoderma* was dominated for all the time of observations, accounting more than 20% of total colony count. All other organisms were contributing up to 20% identical colony count after two months of herbicide spray. Glyphosate treated sample with same concentration, *Sclerotium, Rhizoctonia, Penicillium* and *Aspergillus* sp were recorded contributed only up to 10% while it was up to 20% colony count for the same organisms in 10^{-5} and 10^{-6} dilutions from two months of herbicide spraying (Table 3 and 4).

After a study with five commonly used herbicides (2, 4-D, glyphosate, dicamba, atrazine and metsulfuron-methyl) on the growth of rhizobial strains, 2, 4-D and glyphosate in solid medium inhibited and diminished the growth respectively in slow growing rhizobial strains [10]. Glyphosate increased root exudates and stimulated microbial biomass, most notably *Fusarium* spp [30]. Several studies have shown increased microbial biomass in response to glyphosate [22, 31] and it might be expected that a greater microbial biomass would enhance the potential for glyphosate degradation. *Trichoderma, Gliocladium* and culturable total fungi populations were not affected by glyphosate applications [32]. Glyphosate has been found to be toxic to culturable fungi and bacteria in laboratory studies when

added to artificial media [11]. Contrary to these laboratory results, field studies have revealed very few examples of effects on soil micro-organisms.
Information on the actual time and duration of population responses of various important soil-borne fungi after glyphosate treatment is currently limited since it is dependent on numerous parameters such as soil condition, type of hosts involved and soil microbial interactions.

The results of this study showed that the glyphosate has toxic effect on *Phomopsis* and *Ustulina* as soil mycoflora. In contrast to the fungal suppression, *Sclerotium, Trichoderma, Rhizoctonia Penicillium* and *Aspergillus* sp showed enhancement at higher concentration of herbicide. The impact of glyphosate on soil microbes and microbial processes in this study was small and short lived. This microbial resilience, coupled with the lack of soil persistence, indicates that soil quality will not be reduced by glyphosate application in similar agroecosystems.

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