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Influence of Glufosinate Ammonium on Some Soil Properties and Rhizospheric Micro-organisms of Tea Crop (*Camellia sinensis* L.) in Eastern Himalayan Region of India

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effect of Glufosinate Ammonium 13.5% SL on soil physico-chemical properties and microflora population on tea soil of Darjeeling, West Bengal, India was investigated over two years (2014 and 2015). Effect of herbicides on bulk density, water holding capacity, moisture content, soil pH, organic matter content, electrical conductivity, as well as total nitrogen, available phosphorus and available potassium contents were analyzed along with microflora population of rhizosphere soil (total bacteria, actinomycetes and fungi). The experiment was laid out in randomized complete block design with three replications. The treatments comprised of POE application of Glufosinate Ammonium 13.5% SL at 300, 375, 500, 750 g ha^{-1} along with standard check Basta (Glufosinate Ammonium 13.5% SL) at 375, 500 g ha^{-1}, hand weeding (at 25 and 45 DAA) beside untreated control plot. Result revealed that, no significant changes in soil physico-chemical properties were

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observed due to application of tested herbicides. Herbicidal treatments recorded a decreasing trend on soil microbial counts immediately after application up to 20 DAA. Then the microflora population increased gradually after completion persistency period of Glufosinate Ammonium. Overall there was no long term adverse effect of Glufosinate Ammonium 13.5% SL on the microbial population in *Rhizosphere* soil of Tea crop.

Keywords: Herbicide; glufosinate ammonium; physico-chemical property; microflora population; tea soil.

1. INTRODUCTION

Tea is one of the main export commodities of India and during 2013-14; 211.86 million kg of tea worth Rs. 4211.49 million was exported to different countries [1]. The reduction in yield of tea leaves due to weeds can be as high as 12 to 21% [2] depending upon the management practices followed. Besides competing for nutrient, water, light and space, weeds harbour crop pests and pose many operational hazards in tea crop. Further tea crop requires adequate organic matter rich soil to boost up its vegetative growth. Application of huge organic manure invades high weed infection also. Thus, weeding is an important practice for efficient management and sustenance of tea production. Physical way is not at all a better option because of expense involved. Ecosafe organic chemical control scores over other methods [2,3] due to their bio-efficiency, cost effectiveness and ease of operation. But increased use of toxic pesticides in agricultural soils causes contamination of the soil with toxic chemicals. When toxic pesticides are applied, these chemicals may exert certain effects on non-target organisms, including soil microorganisms [4]. The microbial biomass plays a vital role in the soil ecosystem where they fulfill a crucial role in nutrient cycling and decomposition [5]. However, the large-scale use of synthetic herbicides has led to the development of a number of environmental problems, including risks to human health and the induction of weed resistance [6]. During the past four decades, a large number of synthetic herbicides have been introduced for tea crop in many countries of the world but their ecosafe low cost efficiency needs to be investigated. According to Patra et al. [7] Glufosinate Ammonium 13.5% SL can be used safely with no phytotoxic effect at the recommended rate in tea for effective weed management. Glufosinate Ammonium is a non-selective herbicide that controls a broad spectrum of annual, perennial grass and broadleaf weeds including ferns [8] by inhibiting the activity of glutamine synthetase, which converts glutamate and ammonium into glutamine [9] and inorganic nitrogen into organic compounds. Considering these facts, the present experiment was designed to furnish the information regarding the effects of glufosinate ammonium 13.5% SL on physico-chemical properties and microflora density in tea soil.

2. EXPERIMENTAL DETAILS

2.1 Study Area

The experiment was conducted at Kamalpur Tea Estate, Darjeeling district of West-Bengal (88°53' E longitude and 27°2' N latitude), India during 2014 and 2015. Soil pH of the experimental block was 6.60 showing acidic, blackish gray in colour mostly due to high organic matter and poor bases, rich in available major three nutrients.

2.2 Climatic Condition of the Experimental Plot

The rainfall starts during May and remains very erratic thereafter up to October i.e. throughout the period of the monsoon. The mean monthly rainfall is highest in July and lowest in January. The average rainfall is 3000 mm per annum of which around 75% rainfall occurs during June to September. Temperature begins to rise from April and reach maximum in June. It starts dropping from middle of October and gradually attains the minimum in January. The average temperature during summer varies between 11°C to 19°C. The lowest relative humidity is observed in the month of December while the maximum in July.

2.3 Experimental Design and Treatments

The experiment was conducted in randomized complete block design with eight treatments replicated thrice. Each plot size was of 10 m × 10 m. The eight treatments were post-emergence application (POE) of Glufosinate Ammonium 13.5% SL at 300, 375, 500, 750 g ha^{-1} along with standard check Basta (Glufosinate Ammonium 13.5% SL) at 375, 500 g ha^{-1}, hand

weeding (at 25 and 45 DAA) beside untreated control plot.

2.4 Spraying of Herbicides

The tea variety "TV-36" about 10 years old planted with a spacing of 100 cm × 100 cm was selected for this experiment. Application of the herbicides was done on 08.04.2014 and 12.04.2015 in inter- and intra-rows of the experimented matured tea garden with hooded Knapsack sprayer fitted in a flood jet nozzle with a spray volume of 500 L ha^{-1}.

2.5 Data Collection and Analysis

2.5.1 Soil physicochemical properties

Soil samples from the experimental plots were collected from 0-15 cm soil depth at 60 days after application of herbicides (DAA). The soil samples from the different places per replicate were pulled together and then requisite composite samples of each treatment were taken for analysis. The different methods used for analyzing the physical and chemical properties of the experimental soil are presented below (Table 1).

2.5.2 Analysis of microbes

Soil samples from the experimental plots were collected at a soil depth of 0–15 cm on different dates *viz.* initial, 20 DAA, 40 DAA and 60 DAA. The soil samples from different places per replication were pulled together and then requisite composite samples of each treatment were taken for microbial analysis by dilution plating following standard methods. Soil dilutions were prepared in sterile distilled water by constant shaking and plating was done separately in replicates in specific media: Total bacteria (Thornton's agar medium at 10^{-6} dilutions), fungi (Martin's rose bengal streptomycin agar medium at 10^{-4} dilutions), actinomycetes (Jensen's agar medium at 10^{-5} dilutions). The enumeration of the microbial population was done on agar plants containing appropriate media following serial dilution technique and pour plate method [13], plates were incubated at 30°C. The counts were taken on the 3rd day of incubation.

2.6 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) [14] to evaluate the differences among treatments while the means were separated using the least significant difference (LSD) test at the 5% level of significance.

3. RESULTS

3.1 Physicochemical Properties of Soil

The particle size distribution of soil (sand, silt and clay contents); physical properties (bulk density, water holding capacity and moisture content) and chemical properties (pH, electrical conductivity, organic carbon, total nitrogen content, available phosphorus (P_2O_5) and potash (K_2O) contents) at 60 days after application of testing herbicides in experimental field are presented in Tables 2 and 3. Results clearly showed that bulk density (BD), water holding capacity (WHC) and moisture content (MC) of soil did not vary significantly at 60 DAA due to application of testing herbicide Glufosinate Ammonium 13.5% SL. No variations were found among the different textural classes of soil, sand, silt and clay. The soil pH, electrical conductivity (EC), organic carbon, total nitrogen, available P_2O_5 and available K_2O at 60 DAA were not significantly different from each other in both the years (2014 and 2015) against the testing herbicide Glufosinate Ammonium 13.5% SL applied as post emergence. These findings were in line with the findings of Rajkhowa et al. [15] and Ghosh et al. [16] where they documented that different herbicidal applications *Viz.* Carfentrazone-Ethyl 40 DF and Glyphosate as tank Mixture and Glyphosate + 2,4-D has no direct influence on soil physicochemical properties.

3.2 Microbial Properties

The impact of the testing herbicide Glufosinate Ammonium 13.5% SL on soil microflora *viz*. total bacteria, fungi and actinomycetes (Table 4) as recorded at different time of observations (initial, 20, 40 and 60 DAA) are described below.

3.2.1 Total bacteria (CFU x 10^6 g^{-1})

Both the testing Glufosinate Ammonium 13.5% SL and standard check Glufosinate Ammonium 13.5% SL (Basta) did not show any significant influence on the population of total bacteria in rhizosphere soil at initial stage. Significant low population was recorded up to 20 DAA and thereafter, the population started to increase (Table 4). At 20 DAA, herbicide treated plots

Table 1. Methods employed for analyzing physicochemical properties of the experimental soil

Particulars	Analytical method employed
A) Physical properties of soil	
Sand (%), Silt (%) and Clay (%)	International Pipette Method [10]
Bulk density (g cc^{-1})	Field method [11]
Water holding capacity (WHC) (%)	Keen box method [10]
Moisture content (%)	Field method [10]
B) Chemical properties of soil	
pH	pH Meter [12]
Electrical conductivity (EC)	Conductivity meter [12]
Organic carbon (%)	Volumetric Redox Titration Method [12]
Total Nitrogen (%)	Modified Macro-kjeldahl Distillation [12]
Available Phosphorus (P_2O_5) (kg ha^{-1})	Olsen's Method [12]
Available Potash (K_2O) (kg ha^{-1})	Flame Photometer Method [12]

Table 2. Physical properties of experimental soil at 60 days after application of testing herbicides (DAA) in tea soil

Treatments	Bulk density (g/cm^3)		Moisture content (%)		Water holding capacity (%)		Sand content (%)		Silt content (%)		Clay content (%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
T_1	1.427	1.428	14.91	14.85	43.19	43.31	62.50	62.50	24.20	24.20	13.30	13.30
T_2	1.419	1.420	14.70	14.61	43.13	43.28	62.30	62.30	24.60	24.60	13.40	13.40
T_3	1.447	1.450	14.98	14.95	43.42	43.61	62.70	62.70	24.30	24.30	13.00	13.00
T_4	1.439	1.440	14.88	14.83	43.70	44.01	62.30	62.30	24.60	24.60	13.20	13.20
T_5	1.405	1.410	15.04	15.01	43.53	43.76	62.30	62.30	24.20	24.20	13.50	13.50
T_6	1.437	1.440	15.11	15.09	43.41	43.71	62.30	62.30	24.50	24.50	13.50	13.50
T_7	1.426	1.430	14.76	14.79	43.36	43.53	62.50	62.50	24.50	24.50	13.40	13.40
T_8	1.428	1.433	14.90	14.98	43.52	43.69	62.60	62.60	24.50	24.50	13.30	13.30
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS= Non Significant

Table 3. Chemical composition of the experimental soil at 60 days after application of testing herbicides (DAA) in tea soil

Treatments	pH		EC (dSm^{-1})		Organic carbon (%)		Total N (%)		Available P_2O_5 ($kg\ ha^{-1}$)		Available K_2O ($kg\ ha^{-1}$)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
T_1	6.69	6.60	0.146	0.144	0.591	0.592	0.0591	0.0553	26.83	28.56	232.70	233.58
T_2	6.71	6.60	0.144	0.143	0.583	0.593	0.0584	0.0550	25.90	28.31	233.04	233.94
T_3	6.67	6.60	0.142	0.143	0.584	0.603	0.0589	0.0548	27.58	28.94	231.96	233.47
T_4	6.70	6.60	0.148	0.145	0.589	0.578	0.0586	0.0556	26.40	28.45	232.19	233.49
T_5	6.66	6.60	0.143	0.144	0.579	0.596	0.0579	0.0554	26.69	28.71	232.41	233.57
T_6	6.68	6.63	0.145	0.144	0.570	0.584	0.0589	0.0556	26.15	28.33	231.97	233.84
T_7	6.67	6.63	0.148	0.153	0.592	0.614	0.0583	0.0552	27.08	28.86	231.84	233.29
T_8	6.65	6.61	0.146	0.143	0.585	0.593	0.0574	0.0551	27.30	28.43	232.23	233.81
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS= Non Significant, [T_1- Glufosinate Ammonium (GA) 13.5% SL at 300 g ha^{-1}, T_2- GA 13.5% SL at 375 g ha^{-1}, T_3- GA 13.5% SL at 500 g ha^{-1}, T_4- GA 13.5% SL at 750 g ha^{-1}, T_5- Standard check- Basta 13.5% SL at 375 g ha^{-1}, T_6- Standard check- Basta 13.5% SL at 500 g ha^{-1}, T_7- Hand weeding twice at 25 and 45 DAA, T_8- Untreated control]

Table 4. Effect of treatments on soil microflora population at initial, 20, 40 and 60 DAA (pooled over two years)

Treatments	Total bacteria (CFU x 10^6 g^{-1} of soil)				Fungi (CFU x 10^4 g^{-1} of soil)				Actinomycetes (CFU x 10^5 g^{-1})			
	Initial	Herbicide application			Initial	Herbicide application			Initial	Herbicide application		
		20 DAA	40 DAA	60 DAA		20 DAA	40 DAA	60 DAA		20 DAA	40 DAA	60 DAA
T_1	821.67	685.33	821.67	835.67	53.67	39.00	55.33	60.67	1038.00	858.33	1045.33	1062.00
T_2	815.67	672.67	818.33	833.67	53.33	36.67	54.67	60.33	1028.33	855.67	1038.33	1059.67
T_3	823.33	661.33	814.67	833.00	54.00	32.00	54.33	58.33	1030.67	848.33	1037.00	1058.33
T_4	820.67	645.67	809.33	831.67	54.33	29.67	53.33	58.00	1034.00	933.00	1036.00	1049.67
T_5	819.33	671.33	810.33	833.33	53.00	37.00	52.00	60.67	1021.67	853.67	1039.33	1059.00
T_6	814.33	660.00	812.67	832.67	55.00	31.33	54.67	59.33	1029.33	848.67	1038.67	1057.67
T_7	816.67	821.33	822.67	831.00	54.67	55.67	55.33	60.33	1033.67	1040.00	1043.33	1052.33
T_8	813.67	818.67	819.33	826.67	53.67	54.67	55.00	58.67	1031.33	1038.33	1042.00	1049.67
LSD (P=0.05)	NS	20.15	NS	NS	NS	1.75	NS	NS	NS	7.01	NS	NS

NS= Non Significant, [T_1- Glufosinate Ammonium (GA) 13.5% SL at 300 g ha^{-1}, T_2- GA 13.5% SL at 375 g ha^{-1}, T_3- GA 13.5% SL at 500 g ha^{-1}, T_4- GA 13.5% SL at 750 g ha^{-1}, T_5- Standard check- Basta 13.5% SL at 375 g ha^{-1}, T_6- Standard check- Basta 13.5% SL at 500 g ha^{-1}, T_7- Hand weeding twice at 25 and 45 DAA, T_8- Untreated control]

recorded 16.28 to 21.13% lower total bacteria population than control plot. Thus, the adverse influence of the treatments on beneficial bacteria was for a short period of time. Similar result was also found by Bera and Ghosh [17] on the soil microflora (bacteria, fungi and actinomycetes) population as influenced by Bispyribac Sodium 10% SC.

3.2.2 Fungi (CFU x 10^4 g^{-1})

The herbicide Glufosinate Ammonium 13.5% SL, tested in this experiment, showed that there was significant adverse effect on the population of fungi up to 20 DAA in rhizosphere region; which at the later stage started to increase and showed the higher population in all the treatments than the initial stage (Table 4). Ghosh et al. [16] also reported the similar trend of variation in the population of total fungi while working with Glyphosate + 2,4-D for weed control in tea.

3.2.3 Actinomycetes (CFU x 10^5 g^{-1})

Like the bacteria and fungi, all the herbicidal treatments recorded significant detrimental effect on actinomycetes (Table 4) immediately after application upto 20 DAA but at their post persistence period, populations were recovered. At 60 DAA, the herbicidal treatments recorded 1.51 to 3.65% higher population of actinomycetes than initial count. Victor et al. [18] reported that, application of Glufosinate Ammonium on tea significantly reduces the beneficial soil microflora population upto 2-3 weeks of application.

4. DISCUSSION

Based on the results, soil physico-chemical properties did not show any perceptible variation under different herbicidal treatments. The rate of decrease in the population of total bacteria up to 20 DAA was mainly due to competitive influence and the toxic effect as well as different persistence periods of tested organic chemical herbicides in different soil ecosystems [17]. Similarly regarding actinomycetes and fungi the results might be due to the competitive influence of various microorganisms on the population of actinomycetes in the rhizosphere of tea as well as toxic effect of the chemicals applied. The detrimental effect of the organic chemical herbicides on beneficial soil microorganisms was for a short period of time as the microorganisms are able to degrade herbicides and utilize them as a source of biogenic elements for their own physiological processes. The toxic effects of herbicides are normally most severe immediately after application, when their concentrations in soil are the highest. Later on, microorganisms take part in the degradation process; hence the herbicide concentration and its toxic effect gradually decline up to half-life [17]. Then the degraded organic herbicide provides the substrate with carbon, which leads to an increase of the soil microflora density in rhizosphere soil.

5. CONCLUSION

Considering soil microflora population (total bacteria, actinomycetes and fungi) and physicochemical properties, Glufosinate Ammonium 13.5% SL applied at different doses did not show any long run adverse effect on rhizosphere region of Tea crop. Therefore, Glufosinate Ammonium 13.5% SL can be safely recommended for weed control in tea crop.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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