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Soil microbial dynamics as impacted by Diuron, Pyrithiobac sodium and Quizalofop ethyl applied to cotton cultivated in red and black soils

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Abstract
Simultaneous two field experiments were conducted during *kharif* 2018 to study the effect of diuron on soil fungal, bacterial and actinomycetes population dynamics. These experiments were laid out in a Randomized Block Design (RBD) comprising of seven treatments which were replicated thrice. Preemergence herbicides, diuron 80% WP 0.5 kg ha^{-1}, 0.75 kg ha^{-1}and 1.0 kg ha^{-1}; and pendimethalin 38.7% CS 677 g ha^{-1} were sprayed two days after sowing. Tank mix application of pyrithiobac sodium + quizalofop ethyl was done at 2-3 leaf stage of the weeds. The non-chemical weed management treatments included polyfilm, mechanical weeding and unweeded control. Soil microbial populations were found to be adversely affected with the application of varied doses of diuron. The higher dose of diuron recorded greater decline in microbial populations. The impact of diuron was more severe in black soils than in red soils on soil bacterial populations. The average percent reduction in fungal population was more in red soil than in black soil. The impact of diuron on actinomycetes population was more severe in black soil compared to red soils at lower rates of application whereas at higher dose, impact was more severe in red soil than in black soil.

Keywords: diuron, pendimethalin, bacteria, fungi, actinomycetes

1. Introduction

Over the years herbicides have emerged as an indispensable tool in management of weeds. Herbicides use is increasing throughout the globe due to increasing costs of hiring manual labour to do manual weeding, availability of wider-choice of herbicides for specific crops and cropping systems, rapid weed control in crop and in non-crop areas, penetration of herbicides even into developing and under-developed economies etc. Due to intensive research in herbicide discovery and mode of action of herbicides, many new molecules are available to cater the farmers' specific needs. Globally, herbicides constitute 44% of total plant protection chemical consumption followed by the insecticides (22%), fungicides (27%) and other chemicals (rodenticides, acaricides etc) (7%). In India, herbicide contribution has increased to 30% during the last 10 years in managing weeds in the country (Sondhia, 2013) [1].

Because herbicides are usually applied when crops are absent or at early growth stages, most of the spray solution contacts soil. These chemicals may affect non-target soil organisms, including microorganisms. Herbicide-induced changes in abundance, diversity and activity of soil microbial communities may, in turn, influence microorganism-mediated processes that are important to sustainable agriculture, e.g., recycling of plant nutrients and maintenance of soil structure (Lupwayi *et al* 2004) [2]. The generalized use of pesticides in agriculture leads to the contamination of soil and other connected environmental resources. The persistence of pesticide residues in soil is identified as a major threat for in-soil living organisms that are supporting an important number of ecosystem services. Although authorities released pesticides on the market only after their careful and thorough evaluation, the risk assessment for in-soil living organisms is unsatisfactory, particularly for microorganisms (Mauprivez *et al* 2019) [3]. Herbicide spray may cause injury to the crop canopy leading to reduced crop growth and development. Similarly, herbicide spray on the soil surface damages the soil microbial population and thus decreases the soil microbial activity in soil (Hiremath *et al*., 2014) [4].

The influence of diuron preemergence application and tank mix application of pyrithiobac sodium and quizalofop ethyl on soil microbial dynamics is not studied in Indian context. Hence the two field experiments were conducted to assess the impact of these herbicides on microbial activity of soils in cotton cultivated in red and black soils.

2. Material and Methods

2.1 Experimental site and meteorological information

Two field experiments were carried out at College Farm, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana state. The farm is geographically located at 17°19' N latitude and 78°23' E longitude at an altitude of 542.6 m above mean sea level (MSL). The climate of the region is semi-arid. More than 80 % of rainfall is received from South-West monsoon (June-October). Both the field trial sites are located in same experimental farm with in few hundred-meter distance. Red soil experiment site was sandy clay in texture with neutral pH, non-saline and classified as Typic Haplustalf. Black soils site was classified as Vertic

Haplustept with clay loam texture, slightly alkaline pH and non-saline.

Field experiments were laid out in a Randomized Block Design comprising of seven treatments which were replicated thrice. “First Class BG II” cotton hybrid seeds of Bayer company were sown at a seed rate of 2.5 kg ha^{-1}. Seeds were sown at a spacing of 90 x 60 cm. Thinning was done within two weeks of sowing to maintain optimum plant population. Preemergence (PE) herbicides, diuron 80% WP (“Karmex” of Adama India Pvt. Ltd) at 0.5 kg ha^{-1}, 0.75 kg ha^{-1}and 1.0 kg ha^{-1}; and pendimethalin 38.7% CS (“Stomp extra” BASF India Pvt. Ltd (677 g ha^{-1}) were sprayed with knapsack sprayer fitted with flat fan nozzle at two days after sowing. Spray volume applied was 500 lit ha^{-1}. Pyrithiobac sodium 10% EC (“Hit Weed” of Godrej Agrovet India Pvt. Ltd) 62.5 g ha^{-1} + quizalofop ethyl 5 % EC (“Targasuper” of Dhanuka Inida Pvt. Ltd.) 50 gm ha^{-1} were sprayed at 2-3 leaf stage of the weeds. Polyfilm was spread one day before sowing and seeds were sown by making holes on film at designated spacing. Mechanical weeding was done at 20, 40, 60 DAS with power weeder (Honda F300) and an unweeded control was maintained without any weeding from sowing to harvest.

2.2 Enumeration of microbial populations

Bacteria**,** fungal and actinomycetes population was counted by using serial dilution technique (Subba Rao, 1988) [5]. One gram of soil sample was suspended in 9 ml of sterile water in a dilution tube (Tuladhar, 1983) [6] and shaken for 15 min. This constituted 10^{-1} concentration. One millilitre of this suspension was taken by using a fresh sterile pipette and 9 ml sterile water was then added to get 10^{-2} dilution. The sequence was continued till a dilution of 10^{-8} was reached.

Different media was prepared for isolation of micro-organisms. Nutrient agar media (Allen, 1953) [7] for bacteria, rose-bengal agar media (Martin, 1950) [8] for fungi and actinomycetes isolation agar for actinomycetes were used. Freshly mixed suspension (1 ml) was transferred into the sterile petri dish using sterile tip of micro-pipette. For fungi, 10^{-3} to 10^{-6} dilutions, 10^{-5} to 10^{-8} dilutions for bacteria and 10^{-4} to 10^{-7} dilutions for actinomycetes were used. Subsequently, about 15ml of partially cooled appropriate medium was poured into each plate and carefully swirled to thoroughly mix the contents. After the media got solidified plates were inverted and kept in an incubator at respective incubation temperature for different micro-organisms (25 °C for fungi and 37 °C for bacteria, 30 °C for actinomycetes). After specified period of growth (72 hrs for fungi, 24 hrs for bacteria and 48 hrs for actinomycetes), colonies were counted with colony counter and population was enumerated and expressed as colony forming units per gram soil (CFU g^{-1} soil).

3. Results and Discussion

3.1 Effect of diuron on total heterotrophic bacteria:

Population of bacteria was found to be significantly influenced by the varied doses of diuron at days after application of herbicides in both red and black soils (Table 1).

Table 1: Soil bacterial populations [CFU g^{-1} soil (x10^6)] as influenced by varied doses of diuron at different intervals after application in red and black soils

Treatments	Red soil								Black soil							
	Days after herbicide application															
	1	3	5	10	15	30	60	90	1	3	5	10	15	30	60	90
Diuron 80% WP 0.5 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	5.60	5.00	2.30	2.35	5.55	3.95	2.90	3.05	2.85	2.42	1.95	2.50	3.55	2.65	3.10	3.50
Diuron 80% WP 0.75 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	4.20	3.90	2.40	2.70	2.90	2.20	2.90	2.90	2.55	2.41	1.45	2.22	3.45	2.22	3.32	3.65
Diuron 80% WP 1.0 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	2.30	2.20	2.15	2.35	2.60	2.15	3.30	4.25	2.35	1.70	1.20	1.65	3.75	2.00	3.75	3.40
Unweeded Control	4.90	4.85	4.45	4.45	4.75	4.55	4.40	4.60	4.45	4.15	4.20	3.90	4.15	4.65	5.15	4.10

3.1.1 Red soil

Total bacterial colony counts decreased from 1 day after herbicide application (DAHS) to 5 DAHS and thereafter increased upto 15 DAHS in all the diuron treatments. At 1 DAHS, 53.1 % decrease in bacterial population in 1.0 kg ha^{-1} dose and 14.3 % reduction in 0.75 kg ha^{-1} dose were noticed in comparison with control treatment. However, in 0.5 kg ha^{-1} treatment 14.3% increase in bacterial count compared to control was recorded. Even at 15 DAHS, the bacterial counts recorded were lower in 0.75 kg ha^{-1} and 1.0 kg ha^{-1} treatments compared to control treatment.. The bacterial population count recorded on every sampling day was always highest in diuron 0.5 kg ha^{-1} and lowest in 1.0 kg ha^{-1} (from 1 DAHS to 15 DAHS). Significant reduction in population counts and failure to recover to the original population status in 1.0 kg ha^{-1} diuron application treatment clearly indicated its toxicity to bacterial population at higher doses. From 30 DAHS onwards, the bacterial population increased upto 90 DAHS in 0.75 kg ha^{-1} and 1.0 kg ha^{-1} diuron treatments. Complete recovery of soil bacterial population was not observed in any of diuron treatments even at 90 DAHS. Among the different doses of diuron, 1.0 kg ha^{-1} recorded significantly lower total bacterial population at 5, 10, 15, 30 and 60 DAHS.

3.1.2 Black soil

Impact of different diuron treatments on bacterial counts in back soil was more pronounced all diuron treatments at 1, 2, 5 DAHS. Compared to no-chemical (unweeded control), reduction in bacterial population counts recorded in 1.0 kg ha^{-1} was 47.2%, 59.0%, 71.4%, 57.7%, 9.6 % at 1, 3, 5, 10 and 15 DAHS respectively. Whereas in 0.75 kg ha^{-1} dose the percent reduction recorded was 42.7, 41.9, 65.5, 43.1, 16.9 at 1, 3, 5, 10 and 15 DAHS respectively. This shows, most significant reduction in bacterial count occurred at 5 DAHS. Unlike in red soils, even at 0.5 kg ha^{-1} dose also the bacterial counts were reduced significantly due to impact of diuron. The highest total bacterial population was reported in unweeded control at all the intervals of time.

Eventhough by 15 DAHS, the bacterial population recovered significantly, application of post-emergence herbicide has again resulted in significant reduction in bacterial counts. At 30 DAHS, reductions in bacterial counts were 57 %, 52.3 % and 43.0 % in 1.0, 0.75 and 0.5 kg ha^{-1} rates of application respectively. The counts gradually increased up to 90 DAHS. In total, impact of diuron was more severe in black soil compared to red soils. Averaged bacterial count from 1 to 15 DAHS show that, the reduction was 12.3% in red soils and 36.3 % in black soils at 0.5 kg ha^{-1} rate of application. At 0.75 kg ha^{-1}, the average reduction was 42 % in black soils and 31.6 % in red soils. In the highest dose of diuron, the percent reductions are 50.4 and 49.0 % in red and black soils respectively.

3.2 Effect of diuron on total heterotrophic fungi

The fungal population was found to be significantly influenced by the varied doses of diuron and at different days after application of herbicides (Table 2).

Table 2: Soil fungal populations [CFU g^{-1} soil ($x10^3$)] as influenced by varied doses of diuron at different intervals after application in red and black soils

Treatments	Red soil								Black soil							
	Days after herbicide application															
	1	3	5	10	15	30	60	90	1	3	5	10	15	30	60	90
Diuron 80% WP 0.5 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	35.5	29.5	14.5	19.3	33.5	38.0	34.5	25.5	27.5	17.5	7.5	13.5	18.1	18.1	29.5	24.8
Diuron 80% WP 0.75 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	24.1	20.8	14.1	17.1	22.2	35.6	30.8	25.0	26.1	17.8	7.3	12.8	17.8	19.5	29.5	25.2
Diuron 80% WP 1.0 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	21.3	17.5	12.2	15.2	15.5	22.5	28.0	23.5	24.5	16.2	5.5	14.2	21.2	23.5	30.5	23.5
Unweeded Control	36.0	34.4	36.4	33.8	34.5	39.5	43.0	25.5	28.1	27.5	25.5	26.2	27.2	29.5	30.5	26.2

3.2.1 Red soil

The highest total fungal colony forming units were recorded in unweeded control at all the intervals of sampling in both red and black soils. Among the varied doses of diuron, in comparison with the control treatment, 0.5 kg ha^{-1} recorded significantly higher and diuron at 1.0 kg ha^{-1} recorded significantly lower total fungal populations from 1 to 15 DAHS. Lower fungal population counts were recorded at 5 DAHS in all diuron treatments which later recovered up to 15 DAHS and beyond. The percent reduction in fungal count at 5 DAHS was 60.2, 61.3 and 66.5 at 0.5, 0.75 and 1.0 kg ha^{-1} diuron doses respectively. The negative impact was evident from 1 DAHS, where in 1.4 to 40.8 % reduction in fungal population was noticed in various diuron treatments. The population recovery was more rapid in 0.5 kg ha^{-1} and 0.75 kg ha^{-1} compared to 1.0 kg ha^{-1} rate of application. Impact of post-emergence herbicides on fungal population was not evident as indicated by an increase in the population counts after postemergence herbicide application. The fungal population gradually increased up to 60 DAHS and later decreased at 90 DAHS.

3.2.2 Black soil

Among diuron applied treatments, rapid decrease in total fungal Population was observed from 1 DAHS to 5 DAHS, thereafter it increased from 5 DAHS to 60 DAHS and then reduced to considerable limit between 60 DAHS to 90 DAHS. The negative impact of diuron on fungal population was severe at 1 DAHS compared to red soils. However, significant reduction in fungal colonies was observed at 3 and 5 DAHS. At 5 DAHS 70.6 %, 71.4 % and 78.4 % reduction in fungal colonies compared to unweeded control was recorded in 0.5, 0.75 and 1.0 kg ha^{-1} doses respectively. In various diuron treatments, in black soils also, highest total fungal population was recorded in unweeded control at all the intervals of time.

Population reduction as influenced by diuron doses was more in red soils at higher doses (0.75 and 1.0 kg ha^{-1}) than at 0.5 kg ha^{-1} dose. The averaged percent reduction (from 1 to 15 DAHS) was 24.3 %, 43.8 % and 53.3% in red soils and 38.2 %, 39.9 % and 40.0 % % in black soils at 0.5, 0.75 and 1.0 kg ha^{-1} doses respectively.

3.3 Effect of diuron on actinomycetes

The actinomycetes population was found to be significantly influenced by the varied doses of diuron and days after application of herbicides (Table 3).

Table 3: Soil actinomycetes populations [CFU g^{-1} soil ($x10^5$)] as influenced by varied doses of diuron at different intervals after application in red and black soil

Treatments	Red soil								Black soil							
	Days after herbicide application															
	1	3	5	10	15	30	60	90	1	3	5	10	15	30	60	90
Diuron 80% WP 0.5 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	16.9	14.1	11.6	13.3	15.8	17.8	18.0	18.7	10.5	7.9	7.6	6.0	14.1	14.2	13.0	12.1
Diuron 80% WP 0.75 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	14.3	12.8	10.6	13.2	14.7	16.8	16.5	18.7	9.2	8.7	7.4	8.2	12.8	13.5	13.5	12.8
Diuron 80% WP 1.0 kg ha^{-1} as PE *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1} as PoE.	13.1	8.15	7.2	10.1	11.3	13.8	16.2	16.6	11.9	8.9	7.4	9.3	11.0	10.7	10.3	11.7
Unweeded Control	14.5	15.8	15.9	15.8	16.0	18.0	18.5	20.7	12.5	12.8	13.2	15.5	17.3	16.5	16.6	16.7

3.3.1 Red soil
The actinomycetes population reduction as influenced by diuron application was noticed upto 5 DAHS, which later increased upto 15 DAHS. Highest reduction in population of actinomycetes was recorded in the treatment where diuron was applied at 1.0 kg ha^{-1}. The percent population reduction of actinomycetes compared to unweeded control treatments was 9.7, 48.4, 54.7, 36.1 and 29.4 at 1, 3, 5, 10, 15 DAHS respectively in diuron highest dose treatment. The highest total actinomycetes population was reported in unweeded control treatment at all sampling times. At 1, 5, 15 and 30 DAHS, in diuron at 0.5 kg ha^{-1} PE, at 3 and 10 DAHS diuron at 1.0 kg ha^{-1} PE and at 60 and 90 DAHS diuron at 0.75 kg ha^{-1} recorded significantly higher total actinomycetes population among the herbicide treatments.

3.3.2 Black soil
Unlike in bacterial and fungal populations, among different days of sampling the lowest population of actinomycetes as influenced by diuron was noticed at 10 DAHS irrespective of dose of application.

Among various diuron treatments, gradual decline in total actinomycetes population was observed from 1 DAHS to 10 DAHS, thereafter the count increased from 10 DAHS to 30 DAHS and then reduced gradually till 90 DAHS. The highest total actinomycetes population was reported in unweeded control at all the intervals of time. Reduction in actinomycetes population at 10 DAHS was 61.3 %, 47.1 % and 40 % in 0.5, 0.75 and 1.0 kg ha^{-1} treatments respectively.

Overall impact of diuron on actinomycetes population was more severe in black soil compared to red soils at lower rates of application. Averaged actinomycetes count from 1 to 15 DAHS shows that, the average reduction was 7.7 % in red soils and 35.3 % in black soils at 0.5 kg ha^{-1} rate of application. At 0.75 kg ha^{-1} the average reduction is 35.1 % in black soils and 15.7 % in red soils. In the highest dose treatments, the percent reductions are 35.6 and 31.1 % in red and black soils respectively.

The higher concentration of diuron present in the root zone might have hindered the microbial growth in black soils more severely than in red soil.

Prado and Airoldi (2001) [9] showed that, addition of diuron caused a delay in metabolism, and the inhibition reached almost 100 % with the doses above 66.67 micrograms per gram soil. This shows that frequent application of pesticide to soil even in amounts as small as 1.67 µg g^{-1} could almost totally inhibit the microbial activity of soil. Moreover, heavy applications of diuron to soil induce conditions adverse to restoring soil fertility and damage balance in the ecosystem with serious ecological disturbances. The soil fungi were less resistant towards the phenyl urea herbicides applied at field rate which caused a significant depression in number of colony forming units (CFU) for first seven days. The results indicated that these herbicides cause a decline in the population of actinomycetes and fungi (Lone *et al*, 2014) [10]. Fantroussi *et al.* (1999) [11] observed that, bacterial diversity seemed to decrease in soils treated with urea herbicides (diuron), and sequence determination of several DGGE fragments showed that the most affected species in the soils treated with diuron and linuron belonged to an uncultivated bacterial group.

The negative effect of phenyl ureas on actinomycetes and fungi might be due to certain undesirable metabolic products released during the degradation of herbicides. Sorensen *et al*. (2003) [12] showed that during the degradation of phenyl ureas, certain undesirable products accumulate in soil which could be more hazardous to non-target organisms than the herbicide itself. Such a response could also be attributed to the competition between higher bacterial population and relatively smaller fungi and actinomycetes population for available carbon and energy sources.

4. Conclusion
Soil microbial populations were found to be affected with the application of varied doses of diuron. The higher dose of diuron recorded greater decline in microbial populations. The impact of diuron was more severe in black soils than in red soils on soil bacterial populations. The average percent reduction in fungal population was more in red soil than in black soil. The impact of diuron on actinomycetes population was more severe in black soil compared to red soils at lower rates of application whereas at higher dose, impact was more severe in red soil than in black soil.

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