

Current Journal of Applied Science and Technology

39(16): 103-111, 2020; Article no.CJAST.57784 ISSN: 2457-1024 (Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Variations in Soil Urease and Dehydrogenase Activities as Determined by Diuron, Pyrithiobac Sodium and Quizalofop Ethyl Applied to Cotton Cultivated in Red and Black Soils

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Authors' contributions

This work was carried out in collaboration among all authors. Author MMF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TR and TA managed the analyses of the study. Author MM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i1630742 <u>Editor(s):</u> (1) Dr. Nhamo Nhamo, Zimbabwe Open University, Zimbabwe. <u>Reviewers:</u> (1) Paula Ioana Moraru, Romania. (2) Barkissa Fofana, Université Joseph KI-Zerbo, Burkina Faso. Complete Peer review History: <u>http://www.sdiarticle4.com/review-history/57784</u>

Original Research Article

Received 10 April 2020 Accepted 18 June 2020 Published 26 June 2020

ABSTRACT

Activities of soil enzymes *viz.*, urease and dehydrogenase were studied in two field experiments conducted in red and black soils at Professor Jayashankar Telangana State Agricultural University, Rajendranagar during *kharif*, 2018. Different doses of diuron in combination with postemergence tank-mix application of pyrithiobac sodium + quizalofop ethyl were tested. Soil urease activity showed an increasing trend and it increased from the day of PE herbicide application to flowering. Highest urease activity was noticed at flowering stage and then after the activity decreased upto harvest. In diuron treatments, the urease activity decreased substantially upto 5 DAHS, which later increased at flowering. Significant differences were observed in urease activity among all the

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treatments from the day of PE application to the day of postemergence spray in both red and black soils. No significant difference was observed in urease activity due to application of postemergence herbicide in both red and black soils. No significant difference was observed due to the application of pre and postemergence herbicides on the activity of dehydrogenase in both red and black soils but the activity of dehydrogenase increased from the day of PE herbicide application to flowering, exhibited highest activity at flowering stage and there after the activity decreased at harvest in both red and black soils.

Keywords: Urease; dehydrogenase; diuron; pendimethalin; polyfilm; mechanical weeding.

ABBREVIATIONS

PE (Preemergence); DAHS (Days after Herbicide Spray).

1. INTRODUCTION

Natural and anthropogenic factors may affect the soil enzyme activities directly or indirectly. Herbicides are one of the major groups of plant protection chemicals, used to kill or suppress the growth of unwanted plants to reduce the cost of cultivation and also to sustain high yield. Number of herbicides leaves unwanted residues in soil, which are ecologically harmful [1,2]. Preferred herbicides should not only have good efficacy, but also poses minimum adverse effects to crop. ecology and environment [3,4]. Herbicides are extraneous to soil component pools, and are expected to affect the catalytic efficiency and behaviour of soil enzymes which contribute the total biological activity of the soil-plant environment under different states. Various studies have revealed that the herbicides can cause qualitative and quantitative change in enzyme activity [5,6,7]. The enzyme activities are considered to be sensitive to chemical pollutants/agrochemicals and have been proposed as potential indicators for measuring the degree of pollution of contaminated soil [8.9] and referred to as markers of soil environmental purity [10].

Soil urease is one of most active hydrolytic enzymes in the soil, the hydrolysis urea which is applied into the soil to release ammonium that can be used by crops. Soil urease is paid more and more attention because it can be used as important indexes to evaluate soil organic matter and nitrogen application [11] and more quickly response to environment change and agricultural management [12].

Among all enzymes in soil environment, the dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils.

Dehydrogenase is an enzyme that oxidizes soil organic matter by transferring protons and electrons from substrates to acceptors. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellular in the soil and it occurs only within soil bacteria (e.g. genus *Pseudomonas*, with *Pseudomonas entomophilies* as most abundant). Measuring dehydrogenase activity aids in better understanding of the effect of agricultural practices, such as pesticide use, or other management practices on the health of soil, as well as a direct measure of soil microbial activity.

At present, pendimethalin and alachlor are the two pre-emergence (PE) herbicides registered for use in cotton besides diuron [13]. As alachlor is being phased out of use by 2020, pendimethalin will be the sole available preemergence herbicide registered for use in cotton. It is a well-established fact that, using a single herbicide over long period can have adverse effects such as development of resistance in weeds and poor bio-efficacy over long-term usage. Further, continuous use of same herbicide may also negatively influence different properties of soil like soil enzyme activity and soil microbial population. Even though diuron is registered for use in cotton decades back, its usage is very low due to long residual life and phytotoxicity in the cotton as well as succeeding sensitive crops. Diuron belongs to substituted urea chemical family which is used as PE herbicide in cotton. Even though diuron is registered for use in cotton, its usage is very low due to long residual life (80-230 days) which may negatively influence different properties of soil, like soil enzyme activity. Impact of tank-mix postemergence application of pyrithiobac sodium and guizalofop ethyl on soil enzyme activity is also not available in Indian situations. Hence the

present investigation was carried out to determine the effect on soil urease and dehydrogenase due to application of varied doses of diuron in combination with post emergence application of pyrithiobac sodium + quizalofop ethyl at different intervals in cotton cultivated in red and black soils.

2. MATERIALS AND METHODS

2.1 Experimental Site and Meteorological Information

Two field experiments were carried out simultaneously in red and black soils with the same set of treatments 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 within 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.

The experiment was laid out in a Randomized Block Design comprising of 7 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⁻¹and 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 Urease Activity

Urease activity in soil was assayed by quantifying the ratio of release of NH_4^+ from the hydrolysis of urea [14]. Soil sample (5 g) was taken in a 50 ml volumetric flask, after adding 0.2 ml of toluene and 9 ml THAM buffer, the flask was swirled for a few seconds to mix the contents and 1 ml of 0.2M urea solution was added and swirled the flask again for a few seconds. Then the flask was stoppered and placed in an incubator at 37°C. After 2 hours, the stopper was removed, and approximately 35 ml of KCI-Ag₂SO₄ solution was added, swirled the flask for a few seconds, and allowed the flask to stand until the contents have cooled to room temperature (about 5 min). The contents were made to 50 ml by addition of KCI-Ag₂SO₄ solution, the flask was stoppered and inverted several times to mix the contents. NH4+-N was determined in the resulting soil suspension, by pipetting out 20 ml aliquot of the suspension distilling with 0.2 g of MgO for 4 min. The ammonium boric acid complex was titrated against standard 0.05 N H₂SO₄. Controls were performed by following the procedure described for assay of urease activity, but for the addition of 1 ml of 0.2 M urea solution after the addition of KCl-Ag_2SO_4 solution. The urease activity was expressed as μg NH^{+4} -N released g^{-1} 2h^{-1}. The activity of soil urease was determined on the day of PE spray, 5 days after PE spray, 10 days after PE spray, 15 days after PE spray, on the day of postemergence spray, 30 days after PE spray, at flowering stage and at harvest stage.

2.3 Dehydrogenase Activity

Dehydrogenase activity in the soil was determined by the standard procedure [14]. The method involved spectrophotometric determination of the tri Phenyl formazan (TPF) produced when soil is treated with triphenyl tetrazolium chloride (TTC). One gram of soil sample was taken in screw capped glass tubes. To this 50 mg of CaCO₃ was added followed by addition of 2.5 ml of distilled water and 1 ml of 3% TTC. The contents were swirled on vortex mixer for few minutes and incubated at room temperature for 24 h. After 24 h, stopper was removed, 10 ml of methanol was added, and stoppered the tube. Tube was shaken for 30 min and filtered the suspension through a glass funnel into a 25 ml volumetric flask. The filtrate was made to 25 ml with methanol and red colour UV-Visible measured on intensity was 485 spectrophotometer at nm. The

dehydrogenase activity was expressed as μ g of TPF g⁻¹ soil day⁻¹. The activity of soil dehydrogenase was determined on the day of PE spray, 5 days after PE spray, 10 days after PE spray, 15 days after PE spray, on the day of postemergence spray, 30 days after PE spray, at flowering stage and at harvest stage.

3. RESULTS AND DISCUSSION

3.1 Urease Activity (µg NH⁺⁴ -N Released g⁻¹ 2h⁻¹)

General trend in urease activity (UA) showed that it increased with increase in age of the crop. UA increased from the day of (PE) herbicide application to flowering stage where it was highest and there after the activity decreased at harvest (lowest activity). However in diuron treatments, the urease activity decreased substantially at 5 DAHS, which later increased at flowering. Significant differences were observed in urease activity among all the treatments from the day of PE application to the day of postemergence spray in both red and black soils.

3.2 Red Soil

At different days of herbicide application, lowest UA (Table 1) was recorded on 5 days after PE herbicide spray in all the herbicide applied plots. On the day of PE spray and 5 days after PE application, all the non-herbicide treatments were on par and recorded significantly higher urease activity. Highest dose of diuron at 1.0 kg ha⁻¹ recorded lowest urease activity on the day of PE spray, 5 days after PE spray, 10 days after PE spray, 15 days after PE spray and on the day of postemergence spray. Application of diuron at 0.75 kg ha⁻¹ also recorded significantly lower urease activity, compared to lowest dose of diuron and pendimethalin, but significantly higher activity than the highest diuron dosage. The lower dosage of diuron at 0.5 kg ha⁻¹ and pendimethalin treatments also registered significantly lower activity of urease on the day of PE spray and 5 days after PE spray when compared to the non-herbicidal treatments. At 30 days after PE application, at flowering and at harvest, no significant differences were observed among the different treatments.

3.3 Black Soil

Significantly higher urease activity (Table 2) was recorded in all the non-herbicide treatments on

the day of PE spray, 5 days after PE and 10 days after PE application which were on par among those treatments. Diuron at 1.0 kg ha⁻¹ and 0.75 kg ha⁻¹ recorded significantly lower urease activity on the day of spray, 5 days and on 10 days after PE spray. Urease activities recorded from 10 days after PE application to the day of postemergence application in lowest dose of diuron at 0.5 kg ha⁻¹ and pendimethalin were statistically on par with that of non herbicide treatments. At 30 days after PE application, at flowering and at harvest, no significant differences were observed among the different treatments.

It has been reported that the UA showed an increasing trend with the age of the crop [15]. It increased from the day of PE herbicide application to flowering, exhibited highest activity at flowering stage and there after the activity decreased at harvest. Several studies reported decreased UA due to herbicide application [16,17,18].

3.4 Dehydrogenase Activity (μg of TPF g⁻¹ Soil Day⁻¹)

There was no significant difference among the different weed control measures on the activity of dehydrogenase (Figs. 1 and 2) in both red and black soils. But the activity of dehydrogenase increased from the day of PE herbicide application to flowering, exhibited highest activity at flowering stage and there after the activity decreased at harvest in both red and black soils. Dehydrogenase activity was found to be inhibited by herbicide in sandy loam soil [19] while no effect was observed by other workers [20].

The low activity of soil urease and dehydrogenase at early stages of crop may be due the application of herbicides when there is no crop as in PE and early stages of crop as in postemergence, the higher proportion of herbicide may have accumulated in the top layer of soil which is microbiologically active and causes inhibition of microbial populations like bacteria, fungi, actinomycetes and on soil enzymes. The inhibition effect of herbicides towards microbial populations and enzymes reduces with time because of recovery of microbial populations and enzyme activity after initial inhibition due to the adaptation of microbes to herbicides. The partial degradation of the herbicide with time in soil may also be another factor for decrease in inhibition of enzyme activity.

Treatments	On the day of PE application	5 days after PE application	10 days after PE application	15 days after PE application	On the day of postemergence spray	30 days after PE application	Flowering	Harvest
T ₁	18.8	13.2	29.0	40.9	46.0	55.2	66.7	39.3
T ₂	14.3	9.7	28.0	31.6	42.0	54.5	65.1	38.5
T_3	9.2	5.3	16.9	28.9	34.1	52.1	64	36.5
T₄	19.6	12.2	29.0	39.7	46.6	55.3	65.5	37.0
T ₅	17.0	23.2	31.2	42.1	48.0	56.4	73	41.9
T ₆	17.5	25.0	32.6	43.0	48.3	56.6	69.3	36.7
T ₇	18.8	24.5	30.6	42.6	50.1	57	71	42.4
SE(m)±	0.84	1.15	1.05	1.39	1.27	1.65	3.3	2.99
C.D (p=0.05)	2.1	1.9	3.8	3.5	4.45	NS	NS	NS

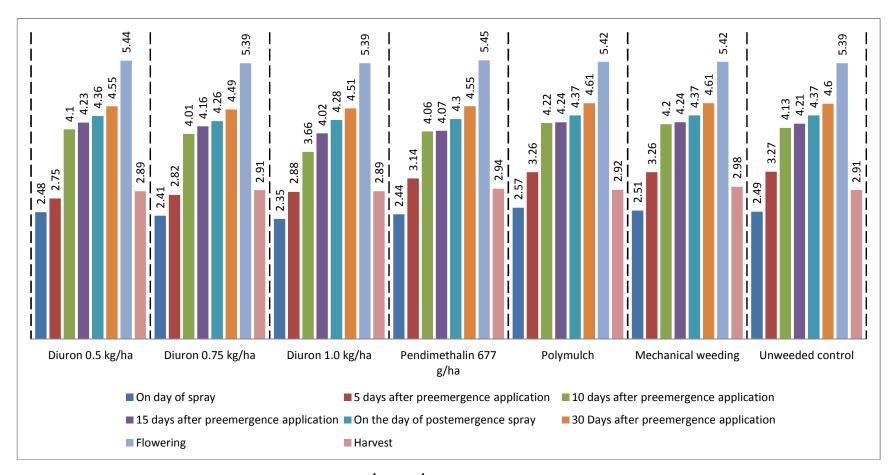
Table 1. Soil urease activity (µg NH4⁺ -N released g⁻¹ 2h⁻¹) as influenced by weed control measures in cotton in red soil

 T_1 -Diuron PE followed by (fb) pyrithiobac sodium + quizalofop ethyl PoE; T_2 -Diuron PE fb pyrithiobac sodium + quizalofop ethyl PoE; T_3 -Diuron PE fb pyrithiobac sodium + quizalofop ethyl PoE; T_4 -Pendimethalin PE fb pyrithiobac sodium + quizalofop ethyl PoE; T_5 -Polyfilm (250 µm thickness); T_6 -Mechanical weeding with power weeder at 20, 40, 60 DAS; T_7 -Unweeded control

Table 2. Soil urease activity (μ g NH₄⁺ -N released g⁻¹ 2h⁻¹) as influenced by weed control measures in cotton in black soil

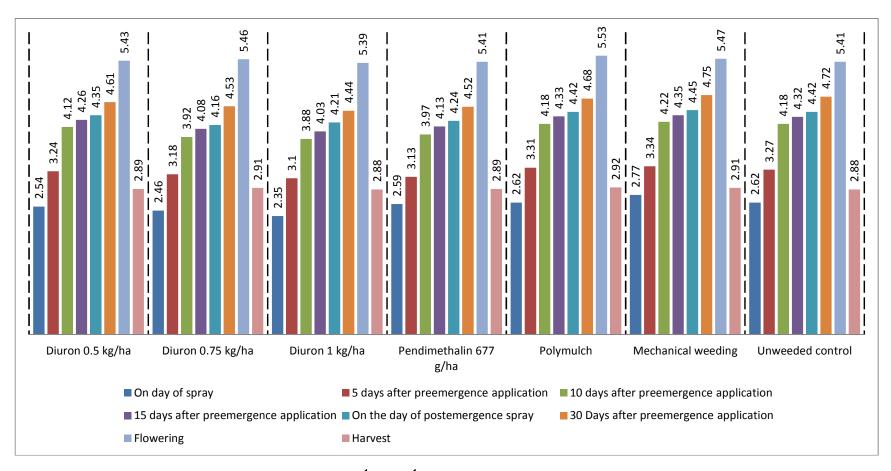
Treatments	On the day of PE spray	5 days after PE application	10 days after PE application	15 days after PE application	On the day of postemergence	30 days after PE application	Flowering	Harvest
					spray			
T ₁	18.0	15.4	29.0	38.0	58.0	66.6	67.7	34.6
T ₂	14.4	10.4	26.4	36.1	55.4	65.5	66.6	33.9
T ₃	12.8	9.0	18.4	32.5	44.2	64.1	65.0	32.9
T ₄	18.5	16.4	28.6	37.6	57.0	69.5	70.0	34.8
T ₅	20.0	22.8	34.1	38.6	61.9	68.0	71.4	34.5
T ₆	19.6	22.0	32.1	38.6	61.2	68.9	71.9	35.0
T ₇	19.1	21.7	32.2	39.3	60.2	67.3	71.8	34.2
SE(m)±	0.81	1.10	1.33	0.85	1.93	2.49	4.32	1.99
C.D (p=0.05)	1.3	1.4	4.11	2.63	5.95	NS	NS	NS

 T_1 -Diuron PE followed by (fb) pyrithiobac sodium + quizalofop ethyl PoE; T_2 -Diuron PE fb pyrithiobac sodium + quizalofop ethyl PoE; T_3 -Diuron PE fb pyrithiobac sodium + quizalofop ethyl PoE; T_4 -Pendimethalin PE fb pyrithiobac sodium + quizalofop ethyl PoE; T_5 -Polyfilm (250 µm thickness); T_6 -Mechanical weeding with power weeder at 20, 40, 60 DAS; T_7 -Unweeded control



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Fig. 1. Soil dehydrogenase activity (µg TPF g⁻¹ soil hr⁻¹) as influenced by weed control measures in cotton in red soil



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Fig. 2. Soil dehydrogenase activity (µg TPF g⁻¹ soil hr⁻¹) as influenced by weed control measures in cotton in black soil

4. CONCLUSION

From the present study it can be emphasized that the application of diuron at varied doses does not shown any significant inhibition on dehydrogenase activity whereas the significant reduction in activity of soil urease was seen on the day of application and it shown an increasing trend with the age of the crop. It increased from the day of PE herbicide application to flowering, exhibited highest activity at flowering stage and there after the activity decreased at harvest. The increase in dose of diuron increased the reduction of urease and dehydrogenase activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/57784