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## THE RESPONSE OF SOIL MICROBIAL ACTIVITIES ON THE APPLICATION OF ATRAZIN AND PARAQUATE IN A GINGER BASED CROPPING SYSTEM

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### ABSTRACT

Herbicides are chemical substances used to control unwanted plants. Its application may lead to significant changes in microbial populations and activities influencing microbial ecological balance affecting soil fertility. The increasing use of herbicides for sustainable agriculture has led to concern about their ecotoxicological effects influencing microbial population and enzyme activities which is an indicator to soil quality. The effect of herbicides (Atrazine and Paraquat) on soil microbial population, metabolic quotient and enzyme activities (amylase, invertase, protease, urease and dehydrogenase) were assessed for four weeks and were analysed for microbial population, metabolic quotient and enzyme activities. The result showed that some viable soil bacteria and fungi were present and the isolates include *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Flavobacterium aquatile*, *Proteus mirabilis*, *Bacillus licheni formis*, *Bacillus cereus*, *Actinomyces bovis*, *Rhizocotinia solani*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium oryzae*, *Rhizopus stolonifer*, *penicillium notatum*, *Rhizopus oryzae*, *Aspergillus oryzae*. The total bacterial count (TBC) in Atrazine and Paraquat treated soil showed a reduction trend from 0 day to 28<sup>th</sup> day after treatment, which ranged from ( $15.8 \times 10^4$  to  $2.7 \times 10^5$ ) and ( $26.4 \times 10^4$  to  $1.9 \times 10^6$ ) cfu/g soil respectively. While that of fungi varies from ( $2.7 \times 10^5$  to  $11.0 \times 10^8$ ) cfu/g soil, ( $3.4 \times 10^5$  to  $14.9 \times 10^8$ ) cfu/g soil respectively. The microbial and fungi populations decreased upon treatment with herbicides when compared with the control. The bacteria recorded the lowest percentage reduction in atrazine and paraquat with 6.7% and 5.2% respectively when compared with the control while the fungi recorded lowest percentage in paraquat (6.3%) and atrazine (13.4%). The gradual increase in microbial counts could be due to their ability to temporarily mineralize and use the herbicides as energy source, which showed initial increase up to 28 days for bacteria and 21 days for fungi. The soil metabolic quotient (qCO<sub>2</sub>) increased across the treatment periods. Herbicides treatment revealed variation in enzyme activities, while the control soil samples gave the highest activity. The result revealed that the herbicides caused transient impact on microbial population and enzyme activities with the application rate used.

**Keywords:** Atrazine, Paraquat, Soil microbial population, Microbial quotient, Enzymatic activities, Ginger crop.

### Introduction

Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms. In particular, they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like carbon and nitrogen, which are required for plant growth. For example, soil microorganisms are responsible for the decomposition of the organic matter entering the soil (e.g. plant litter) and therefore in the recycling of nutrients in soil (Tugel and Lewanski, 2001). Hence it is vital in biological cycles in terrestrial habitat. The soil microbial biomass is considered as active nutrient pool to plants and plays an important role in nutrient cycling and decomposition in ecosystem (De Lorenzo *et al*, 2001). Healthy population of soil microorganisms can stabilize the ecological system in soil (Chauhan *et al*, 2006), due to their ability to regenerate nutrients to support plant growth. Any change in their population and activity may affect

nutrient cycling as well as availability of nutrients, which indirectly affect productivity and other soil functions (Wang *et al*, 2008).

Herbicides are one of the major groups of pesticides, which include substances or cultured biological organisms used to kill or suppress the growth of unwanted plants and vegetation according to Cork and Kruger (1992) in order to minimize the cultivation cost as well as to sustain high yield. Atrazine {2-chloro-4-(ethylamino)-6-isopropylamino-1,3,5-triazine} is a widely used 5-triazine herbicide. It is used as pre-emergence herbicide in control of broad leaf and grassy weeds in a variety of commercial crop as well as road side and fallow fields (Munier-Lamy *et al*, 2002). Early studies on the environmental fate of atrazine have shown that it is transformed slowly by fungi in the soil (Kaufman and Kearny, 1970).

A number of herbicides have not only been introduced as pre- or post-emergence weed killer (Ayasina and Oso, 2006) but also leave unwanted residues in soil, which are ecologically harmful (Riaz *et al*, 2007).

Herbicides not only affect the target organisms, but also microbial communities in soil. These non-target effects may reduce the performance of important soil functions (Hutsch, 2013) and poses a risk to the entire ecological system (Kalia and Gupta, 2004) and by (a) changing their biosynthetic mechanism, (b) affecting protein synthesis, (c) affecting cellular membrane, and (d) affecting plant growth regulators. Herbicides are extraneous to soil component pools, and are expected to affect the catalytic efficiency, behaviour of soil enzymes (Sannino and Gianfreda, 2001) which contribute to the total biological activity of the soil-plant environment under different states (Dick, 1997). The interaction between herbicides and soil microorganisms may be of practical significance because of possible inhibition in microbial activities contributing to soil fertility.

Paraquat is intrinsically biodegradable as demonstrated in laboratory incubation studies with microorganisms in aqueous solution (Ricketts, 1999). Various bacteria and fungi found in soil (e.g. *Corynebacterium fascians*, *Lipomyces starkeyi*, *Aspergillus niger*, *Penicillium frequentans*, *Fusarium* spp. and *Pseudomonas* spp.) have been found to readily metabolise paraquat. According to Summers (1980), in two experiments using cultures of microorganisms extracted from two UK agricultural soils, it was demonstrated unequivocally that radio labelled paraquat was completely degraded in less than three weeks, mainly to carbon dioxide (detected as  $^{14}\text{CO}_2$ ). Other degradation products included ammonia and simple naturally-occurring organic acids. However, in the field, very little applied paraquat is biologically available. When paraquat spray penetrates vegetation to reach the soil it undergoes binding (adsorption) to soil constituents, which is very strong and occurs immediately. This means that only very low concentrations of paraquat are available to any soil organisms (Summers, 1980). Paraquat is toxic to soil fungi and bacteria causing a reduction in some populations (Sahid *et al*, 1992). It has also been found, in combination with diquat, to increase populations of some pathogens, such as *Gaeumannomyces graminis* var. *tritici*, the causal agent of take-all disease of wheat (Sims, 1990). It was found to be toxic to 29 out of 35 strains of the nitrogen fixing bacteria *Rhizobium*, which play an important role in maintaining soil fertility (Martani *et al*, 2001) and (Marino *et al*, 2008). Paraquat is also toxic to the beneficial nitrogen fixing blue-green alga *Cylindrospermum* sp found in rice paddy (Kaur *et al*, 2002). These organisms, chiefly bacteria, fungi and *Streptomyces*, occupy a unique position in biological cycles in terrestrial habitats and are essential for plant growth. They decompose root and crop residues, mobilize the reserves of organic nitrogen and phosphorus, carry out transformations involving a change of oxidation state and bring about many other reactions necessary for crop production. Any

deleterious influence, even of a temporary nature, could have a serious effect upon crop yields. A new class of herbicides, the bipyridyl compounds, was first studied by (Brian *et al*, 1958). Kaneshiro and Zweig (1965) found that diquat (1,1'-ethylene-2,2'-bipyridylum dibromide) at  $2 \times 10^6$  moles inhibited both aerobic and an-aerobic growth of *Rhodospirillum rubrum*. With photosynthetic cultures, diquat affected the synthesis of bacterio chlorophyll more than it did cell mass. Ricketts (1999) Found that several moulds, bacteria and actinomycetes tolerated relatively high concentrations of paraquat (1,1'-dimethyl-4,4'-bipyridylumdichloride), and that some activity degraded the herbicide. The studies reported here were initiated to determine changes in the microbial activity of soil populations following treatment with different rates of paraquat. The aim of this study is to know the effect of paraquat and atrazine on soil microbial population and their responses to microbial quotient and enzymatic activities in the agricultural soil.

## MATERIALS AND METHODS

### Study Site.

The study was carried out in the Agricultural field of National Root Crops Research Institute (NRCRI), Umudike with Latitude of  $05^{\circ} 29' \text{N}$  and Longitudinal rainfall of  $07^{\circ} 33' \text{E}$

### Experimental Set up and Herbicide treatments (Atrazin and Paraquat)

This study was carried out at National Root Crops Research Institute (NRCRI) Umudike. The experiment was laid out in a randomized complete block (RCBD) design, and each plot measured (2mx3m). The herbicides used for the study were paraquat and Atrazin which were applied on the soil at a specified rate. Each plot was replicated 4 times making it a total of 16 plots. The herbicides used for this experiment were the commonly used in the agricultural fields especially in NRCRI Umudike to control weeds and were obtained from a local agricultural store. Paraquat solution were sprayed on the soil surface of the plots using a green nozzle sprayer delivering  $2.1 \text{ lbar}^{-1}$ . The plots were sprayed with atrazine and paraquat herbicides individually, (except control) at company recommended rate and as directed by a weed scientist. Seven days after planting ginger, atrazin was sprayed at the rate of 350ml atrazin + 15 litres of water delivering 87.5ml of atrazin + 3.75 litres of water through a  $2.1 \text{ liter bar}^{-1}$  green nozzle sprayer on a 2mx3m per plot. While paraquat (gramoxone) was sprayed at the rate of 150ml + 7 liters delivering 37.5ml+1.75 liters of water through the same  $2.1 \text{ liter bar}$  green nozzle sprayer on (2mx3m) per plot.

### Soil Sampling Bioassay

Sampling was done in accordance with the general methods for soil microbiological study (Alexander,

1977). Top soil sample was collected from a depth of (0-30) cm from each plot according to Saeki and Toyota (2004). Soil samples from each replication were collected, sieved (2 mm) to remove stone and plant debris and were analysed. The effect of Paraquat and Atrazin herbicide in soil was analysed in response to microbial enumeration and characterization, metabolic quotient ( $qCO_2$ ), and enzymatic activity with respect to the control soil (without treatment) at regular intervals of 0, 7th, 14th, 21st and 28th day after the application of the herbicide for a period of 4 weeks.

### Preparation of media for isolation and enumeration bacteria and fungi

The media used for this study was Nutrient agar and Potato Dextrose agar (PDA). For Nutrient agar, 7g was added to 250 ml of distilled water. It was dissolved completely by heating to boil and sterilized by autoclaving at 121°C for 15 minutes and thereafter allowed to cool to 45°C before dispensing into sterile Petri dishes. Potato dextrose agar was used for the isolation of fungi. It was prepared by 9.5g of PDA into 250ml of distilled water. It was dissolved completely by heating to boil and sterilized by autoclaving at 121°C for 15 minutes and thereafter allowed to cool to 45°C before dispensing into sterile Petri dishes.

### Isolation and enumeration of bacteria and fungi population

The method described by Adarsh *et al* (2007) was used in the isolation of soil microorganisms. 1g of soil sample was dissolved in 10ml of distilled water to make soil suspension. Serial dilutions were made from the filtrates of soil samples, from the diluted samples, 0.1ml of the appropriate dilution of  $10^4$  for 0 day,  $10^5$  for 7<sup>th</sup> day,  $10^6$  for 14<sup>th</sup> day,  $10^7$  for 21<sup>st</sup> day and  $10^8$  were poured into the sterile petri dishes which were in triplicate. Nutrient agar was used for the enumeration of total bacteria by the pour plate method. The agar on the plates were allowed to solidify. Incubation was at 37°C for 24 – 48 h. Bacterial colonies were counted using a colony counter. Plates having 30 – 100 cfu/g (colony forming unit/g) were the only plates considered.

$$\text{Microbial load} = \frac{\text{CFU}}{\text{Volume used} \times \text{Dilution factor}}$$

Where CFU = Colony forming unit (average count)

Volume used = 0.1ml.

The dilution factors were  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$  and  $10^8$ . Inoculates were also collected from each distinct colonies and were inoculated into sterile agar plates following strict asepsis to obtain pure cultures, the resulting sub-culture plates were examined for uniformity as a mark of purity. The pure cultures were then used for microbial characterization and subsequent identification (ATCC, 1992). The morphology of the organisms was recorded as seen on the plates together with the gram stain reaction, spore stain, motility and biochemical tests. (Catalase test,

oxidase test, methyl red, indole test, nitrate reduction test, citrate utilization test, voguesproskauer test, hydrogen sulphide test, spore formation, gelatine hydrolysis and sugar fermentation test) and then characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was thereafter made with reference to the Bergy's manual (1984).

### Fungi Enumeration and Identification

Potato dextrose agar (PDA-oxid) was used for enumeration and isolation of fungi. Incubation was at 25°C for 48 hours. Using taxonomic schemes as described by Cowan (1985). Morphological characteristics were picked for sub culturing. The isolates were characterized and identified. This involves the examination of colonial morphology (Macroscopically) of the isolates and subjecting the colonies to microscopic examination. Fungi isolates observed on PDA plates were characterized and identified by unstained wet mount (Fawole and Oso, 1998). A drop of normal saline was placed on a clean slide. A small amount of the isolate was collected and added to the normal saline on the slide. This was teased out carefully and covered with cover slip. It was then observed microscopically under x40 objective and compared with (Barnett and Hunter, 1992).

### DETERMINATION OF METABOLIC QUOTIENT ( $QCO_2$ )

Metabolic quotient ( $qCO_2$ ) was determined by ratio of soil respiration ( $CO_2$ ) to microbial biomass of carbon (MBC).  $CO_2:MBC = qCO_2, 44:MBC = qCO_2, qCO_2 = 44/MBC$  (mg) as described by Anderson and Domsch (1978).

### Soil Enzyme activity

Amylase activity of Paraquat and atrazin herbicides treated as well as control soil was determined as described by Monty and Amiya (2014) by taking starch as substrate. Invertase activity was determined by spectrophotometric method (Ross and Tate, 1993) by using sucrose as substrate. Protease activity was also determined by spectrophotometer (700nm) with sodium caseinate as a substrate (Monty and Amiya, 2014). Urease activity of the herbicide treated as well as control soil was determined by titration method using 0.005 N  $H_2SO_4$  with boric acid indicator. Dehydrogenase activity was measured following reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to red-coloured triphenylformazon (TPF), which were determined spectrophotometrically.

### DATA ANALYSIS

Data generated from the study were subjected to analysis of variance (ANOVA) and Duncan's multiple test range using SAS software, version 8.0 [Statistical Analysis System (SAS) 1999]

## RESULT AND DISCUSSION.

### Microbial population

The population and distribution of microorganisms in the soil were counted and expressed in colony forming

units per gram soil (cfu/g). There were differences between the different herbicide treated soil based on microbial enumeration. Tables 1 and 2 show the effect of different herbicides on soil microbial population. The total bacterial count (TBC) in Atrazin and Paraquat treated soil showed a reduction trend from 0 day to 28<sup>th</sup> day after treatment, which ranged from (15.8 x 10<sup>4</sup> to 2.7 x 10<sup>5</sup>) and (26.4 x 10<sup>4</sup> to 1.9 x 10<sup>6</sup>) cfu/g soil respectively. Atrazin treated soil had the lowest count on the 7<sup>th</sup> day, while paraquat gave the lowest count on the 14<sup>th</sup> day. However, the control soil had the highest count when compared with the herbicide treated soil. The microbial count in different herbicides and days after treatment showed significant difference (p<0.05). Atrazin and paraquat treated soil had highest counts on the 0 day.

The fungal count in atrazin and paraquat treated soil showed a decreasing trend from 0 day to 7<sup>th</sup> day after treatment and increased up to 28<sup>th</sup> day which varies from (2.7 x 10<sup>5</sup> to 11.0 x 10<sup>8</sup>) cfu/g soil, (3.4 x 10<sup>5</sup> to 14.9 x 10<sup>8</sup>) cfu/g soil respectively. The data showed that the fungal counts were inhibited by both treatment on the 7<sup>th</sup> day and gradually increased with the highest counts on the 28<sup>th</sup> day. Nevertheless, the use of paraquat resulted in higher fungal count as compared to atrazine application while the control showed the highest count without treatment. However, the difference in fungal count, with different treatments days showed a significant difference (p<0.05) reduction when compared with the control soil. The microbial count result showed that the atrazine had the highest count for bacteria while paraquat gave the highest count for fungal. However, the treatment effect on microbial population growth over the five sampling periods exhibited rapid decreasing trends after 0 day, and the reducing effect were observed for full recovery of the microbial population from the initial herbicidal effect on the 21<sup>st</sup> day.

#### **Morphological and cultural characterization of isolates**

A diverse group of soil microorganisms (bacteria and fungi) were isolated during the periods of soil analysis as shown in (Table3). The bacterial and fungal isolates obtained from the treated soil with atrazine, paraquat and control soil samples. The isolates includes *Bacillus subtilis*, *Pseudomonas aerogensa*, *Pseudomonas putida*, *Flavobacterium aquantile*, *Proteus mirabilis*, *Bacillus licheni formis*, *Bacillus cereus*, *Actinomyces bovis*, *Rhizocotinia solani*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium oryzae*, *Rhizopus stolonifer*, *penicillum notatum*, *Rhizopus oryzae*, *Aspergillus oryzae*. In this study, *Bacillus* spp were isolated in high number through soils treated with atrazine and paraquat while *Aspergillus* and *Rhizopus* spp were found to be distributed through the soil treated with atrazine and paraquat. Atrazin treated soil showed the highest bacterial count which indicate that atrazine persistence in the soil is characterized by a moderate,

short, half life of 35 to 50 days (Topp, 2001) although faster atrazine mineralisation has been observed in the soils which had been exposed to this herbicide as normal agricultural practice (Houot,2000). However, some microorganisms have the ability to degrade herbicide, while some others were adversely affected depending upon the application rate/ dose and type of herbicides used (Sebiomo *et al*, 2011). The herbicides effect on soil microbial population cloud be either stimulating or depressive depending on the agrochemicals, mode of application and type, group of microorganisms and environmental conditions. Herbicides decomposition is frequently faster in soil that contain high organic matter may be because of more vigorous microbial activity. Different studies have shown that herbicides can cause qualitative and quantitative change in soil microbial population (Shukla and Mishra, 1997). The fungi population took more time to recover from the detrimental effect caused by herbicides. Herbicides can influence fungal count directly or indirectly by the interaction of fungi with other microorganisms (Araujo *et al*, 2003). Paraquat is also known to be bounded strongly and coherently to soil components such as clay minerals and organic matter and limits the access of microorganism to paraquat in soil (Isenring, 2006). Paraquat adsorption rapidly decreases the bioavailability of herbicide in the soil and the capability of adsorption to deactivate paraquat application (Robert *et al*, 2002). Some microbial species are capable of metabolizing paraquat as a source of carbon (Imai and Kuwatsuka, 1989).

Table 4 shows the effect of herbicide on soil microbial quotient (QCO<sub>2</sub>) in mg/g. There was no significant difference between the treatments across the periods, except on the 21<sup>th</sup> day where control gave the highest value of 28.93 mg/kg which showed significant increment when compared with atrazine and paraquat. However, paraquat value (23.32 mg/kg) compared favourably with the control. Metabolic quotient (QCO<sub>2</sub>) indicates the microbial physiological stress. The low values obtained in the study indicates high microbial physiological stress. The application of paraquate (17.28 mg/kg) was seen to be inhibit the growth of soil microorganisms on day 7 which indicates a high metabolic quotient (QCO<sub>2</sub>). This could be attributed to slow mineralization of the herbicide (Wolf and Martin, 1978). The result is similar to that of Anderson and Domsch (1989), where a low value of the metabolic quotient is an indication of microbial physiological stress. Other values were not significantly affected which can also confirm what Panda and Sahu (2004) stated, that the herbicides generally had no major effect on soil organisms and metabolic quotient, with the exception of butachlor, which was shown to be very toxic to earthworms at agricultural rates.

Table 5, shows the effect of herbicides on enzyme activity on different days after treatment. The

variation in the soil enzyme activities involving (amylase, invertase, protease, urease and dehydrogenase) in different herbicides treated soil are shown in Table 5. Amylase activity in Atrazine treated soil showed an increase trend from 7<sup>th</sup> day (10.80 µg glucose/g soil/hr) to 21<sup>st</sup> day (42.25 µg glucose/g soil/hr). However, the amylase activity in paraquat treated soil was found highest on the 21<sup>st</sup> day (59.00 µg glucose/g soil/hr) and declined gradually on the 28<sup>st</sup> day (11.24 µg glucose/g soil/hr). The difference in soil amylase activity with the herbicides and different days after treatment showed significant difference ( $p < 0.005$ ). The differences in amylase activity in various herbicide treated soil could be due to herbicide induced changes in starch degrading enzyme (Achuba, 2006) and may also be due to unavailability of nutrients inducing stress (Anigboro and Tonukari, 2008). Some microorganisms produce enzymes which can decompose herbicides, which subsequently use the metabolite as a source of biogenous element (Milosevic *et al*, 2001). Nevertheless, certain groups of microorganisms start to decompose herbicides a few days after the application. The invertase activity in atrazine treated soil showed consistent increase from 7<sup>th</sup> day (9.63 µg sucrose/g soil/hr) to 14<sup>th</sup> day (60.32 µg sucrose/g soil/hr) and gradually declined on the 21<sup>st</sup> day (10.24 µg sucrose /g soil /hr) after treatment. Similar trend was exhibited in paraquat treated soil where the invertase activity varies from 7<sup>th</sup> day (5.20 µg sucrose /g soil /hr) to 28<sup>th</sup> day (30.00 µg sucrose /g soil /hr) after treatment. The invertase activity in the soil varied with respect to different herbicides and different days after treatment and showed significant difference ( $p < 0.005$ ) among the samples. The decrease in the soil invertase activity may be attributed to the cellular destruction caused by the toxic substances in herbicides followed by the reduction in nutrient mobilization and glucose level (Perucci and Scarponi, 1994). Invertase activity is of extracellular origin and the death of some microorganisms may cause a decline in the production and excretion of enzyme which leads to the reduction in the soil activity (Perucci *et al*, 1994). Enzyme activity recovery after initial inhibition could be due to growth of microbial population after adaptation or most probably due to increased availability of nutrients due to the degradation of herbicides (Ismail *et al*, 1998). The activity of the protease enzyme in

atrazine treated soil showed an increasing trend from the 7<sup>th</sup> day (28.81 µg tyrosine /g /soil /hr) to 28<sup>th</sup> day (36.24 µg tyrosine /g soil /hr) after treatment. Similar trend was exhibited in paraquat which ranged from (33.54 µg tyrosine /g / soil /hr) to (35.54 µg tyrosine/ g / soil /hr). The variation in the protease activity may depend on the distribution of proteolytic bacteria in the soil (Subrahanyam *et al*, 2011) and the amount of proteinaceous substrate availability and ammonium-nitrogen (NH<sub>4</sub>-N) (Sardans and Penuelas, 2005) in soil organic matter. The urease activity in atrazine treated soil showed an increasing trend from 7<sup>th</sup> day (3.50 µg NH<sub>4</sub><sup>+</sup>/ g soil /hr) to 28<sup>th</sup> day (6.14 µg NH<sub>4</sub><sup>+</sup> g / soil/hr) of incubation (Table 5). Similar trend was shown in paraquat treated soil which varies from (4.10 µg NH<sub>4</sub><sup>+</sup> /g soil/hr) to (6.10 µg NH<sub>4</sub><sup>+</sup> /g soil/hr). The urease activity was lower when compared with other enzymes activities. This led to the credence that native soil urease are mainly extracellular and are persistent because of their association with inorganic and organic colloids (Gianfreda *et al*, 1995). The soil dehydrogenase activity showed an increasing trend from 7<sup>th</sup> day to 28<sup>th</sup> day of incubation for both herbicides treated soil with (1.14 µg TPF/g soil/hr) to (12.44 µg TPF /g soil /hr) (Table 5). The effect of atrazine on soil dehydrogenase activity showed an increasing trend from 7<sup>th</sup> day (2.10 µg TPF/g soil/hr) to 28<sup>th</sup> day (10.26 µg TPF/g /soil/hr) after treatment. The paraquat soil dehydrogenase activity showed similar trend which varied from (1.14 µg TPF/g soil/hr) to (12.44 µg TPF /g soil /hr). The variation in soil dehydrogenase activity with respect to different herbicides and different days after treatment showed significant difference ( $p < 0.05$ ). The increase in soil dehydrogenase activity in herbicides treated soil from 7<sup>th</sup> day to 28<sup>th</sup> day of incubation could be attributed to the increase in microbial community composition with the ability of utilizing the herbicides as source of carbon (Vandans *et al*, 2012).

### Conclusion

The result from the study reveals that the application of two herbicides (Atrazine and Paraquat) altered the microbial populations with respect to different days after treatment and thereby affects the different soil enzyme activities. The toxic effect of the herbicides was evident at the 7<sup>th</sup> day for Atrazine and 14<sup>th</sup> day for Paraquat when compared with the control which was manually weeded.

**Table 1: Effect of different herbicides on total bacterial count (TBC)**

Days	Control	Atrazin	Paraquat
0	35.7x10 <sup>4a</sup>	15.8x10 <sup>4a</sup>	2.6x10 <sup>4a</sup>
7	11.9x10 <sup>5e</sup>	2.7x10 <sup>5e</sup>	3.7x10 <sup>5d</sup>
14	14.9x10 <sup>6d</sup>	5.2x10 <sup>6d</sup>	1.9x10 <sup>6e</sup>
21	21.9x10 <sup>7c</sup>	7.5x10 <sup>7c</sup>	13.5x10 <sup>7c</sup>
28	25.6x10 <sup>8b</sup>	8.7x10 <sup>8b</sup>	14.3x10 <sup>8b</sup>

Values are means of triplicate determination

Values with same superscript letters are not significantly different (p>0.05)

**Table 2: Effect of different herbicides on total fungal count (TFC)**

Days	Control	Atrazin	Paraquat
0	27.3x10 <sup>4a</sup>	9.8x10 <sup>4c</sup>	14.9x10 <sup>4a</sup>
7	9.6x10 <sup>5d</sup>	2.7x10 <sup>5e</sup>	3.4x10 <sup>5d</sup>
14	13.6x10 <sup>6c</sup>	7.2x10 <sup>6d</sup>	8.2x10 <sup>6e</sup>
21	18.6x10 <sup>7b</sup>	10.9x10 <sup>7a</sup>	12.6x10 <sup>7b</sup>
28	2.29x10 <sup>8e</sup>	11.0x10 <sup>8b</sup>	14.9x10 <sup>8a</sup>

Values are means of triplicate determination

Values with same superscript letters are not significantly different (p>0.05)

**Table 3: Bacteria and fungi isolated from herbicides treated and control soils**

BACTERIA	FUNGI
<i>Bacillus subtilis</i>	<i>Rhizocotinia solani</i>
<i>Pseudomonas aerogenosa</i>	<i>Aspergillus niger</i>
<i>Pseudomonas putida</i>	<i>Aspergillus flavus</i>
<i>Flavobacterium aquantile</i>	<i>Penicillium oryzae</i>
<i>Bacillus cereus</i>	<i>Rhizopus stolonifer</i>
<i>Bacillus licheniformis</i>	<i>Penicillium notatum</i>
	<i>Fusarium oxysporum</i>
	<i>Fusarium solani</i>
	<i>Rhizopus oryzae</i>

**Table 4: Effect of Herbicides on soil microbial Metabolic Quotient (QCO<sub>2</sub>) (mg/g)**

Periods	Control	Atrazin	Paraquat	LSD <sub>(0.05)</sub>
0 Day	35.98 <sup>a</sup>	26.63 <sup>a</sup>	28.08 <sup>a</sup>	23.79
7 <sup>th</sup> day	28.07 <sup>b</sup>	21.14 <sup>b</sup>	17.28 <sup>d</sup>	11.96
14 <sup>th</sup> day	24.93 <sup>d</sup>	18.35 <sup>d</sup>	23.23 <sup>b</sup>	12.38
21 <sup>st</sup> day	28.93 <sup>b</sup>	18.78 <sup>d</sup>	23.32 <sup>b</sup>	8.79
28 <sup>th</sup> day	25.93 <sup>c</sup>	20.94 <sup>c</sup>	22.42 <sup>c</sup>	9.62

Values are means of triplicate determination

Values with same superscript letters are not significantly different (p>0.05)



**Table 5: Effect of Herbicides on enzyme activity on different days after treatment.**

Enzyme	Days	Control	Atrazin	Paraquat
AMYLASE	7	32.52 <sup>d</sup>	10.8000 <sup>g</sup>	7.2750 <sup>j</sup>
	14	39.12 <sup>d</sup>	30.4100 <sup>c</sup>	22.1550 <sup>f</sup>
	21	39.54 <sup>d</sup>	9.2550 <sup>i</sup>	59.0050 <sup>a</sup>
	28	42.12 <sup>d</sup>	5.4500 <sup>k</sup>	11.240 <sup>o</sup>
INVERTASE	7	200.24 <sup>d</sup>	9.6300 <sup>i</sup>	5.2000 <sup>m</sup>
	14	424.11 <sup>b</sup>	60.3200 <sup>a</sup>	20.3000 <sup>g</sup>
	21	455.22 <sup>b</sup>	10.2450 <sup>h</sup>	5.7100 <sup>lm</sup>
	28	612.55 <sup>a</sup>	15.5550 <sup>f</sup>	30.0050 <sup>e</sup>
PROTEASE	7	70.72 <sup>d</sup>	28.8130 <sup>e</sup>	33.5400 <sup>d</sup>
	14	73.44 <sup>d</sup>	29.8000 <sup>d</sup>	34.4115 <sup>c</sup>
	21	73.90 <sup>d</sup>	30.2210 <sup>c</sup>	34.6000 <sup>c</sup>
	28	74.82 <sup>d</sup>	36.2400 <sup>b</sup>	35.5405 <sup>b</sup>
UREASE	7	12.12 <sup>d</sup>	3.5000 <sup>m</sup>	4.1000 <sup>n</sup>
	14	12.15 <sup>d</sup>	4.2250 <sup>l</sup>	5.2000 <sup>m</sup>
	21	12.43 <sup>d</sup>	5.3000 <sup>k</sup>	6.1000 <sup>lk</sup>
	28	13.22 <sup>d</sup>	6.1450 <sup>j</sup>	6.4250 <sup>k</sup>
DEHYDROGENASE	7	3.46 <sup>d</sup>	2.1050 <sup>n</sup>	1.1450 <sup>o</sup>
	14	3.72 <sup>d</sup>	5.3000 <sup>k</sup>	3.5000 <sup>n</sup>
	21	4.49 <sup>d</sup>	6.4005 <sup>j</sup>	6.1400 <sup>lk</sup>
	28	4.72 <sup>d</sup>	10.2600 <sup>h</sup>	12.4400 <sup>h</sup>

Values are means of triplicate determination

Values with same superscript letters are not significantly different ( $p > 0.05$ )

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