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**Research Article**

### BACTERIA SOIL PROFILE AND BEHAVIOURAL GROWTH RESPONSE OF RICE PADDY TREATED WITH AGROHERBICIDES IN ABAKALIKI, EBONYI STATE, NIGERIA.

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**Abstract**

*Study of soil bacteria and growth behavioral response of rice paddy treated with Agro herbicides in two seasons in Abakaliki L.G.A. was investigated using standard Microbiological procedures. The results obtained from Bacteria enumeration showed that soil samples from treated rice paddy analyzed during dry season had aerobic plate count ranging from  $3.2 \times 10^9$  cfu/g to  $9.5 \times 10^9$  cfu/g for treated soil samples and  $9.2 \times 10^9$  to  $1.04 \times 10^{10}$  cfu/g for the controls. The result of aerobic plate count from soil samples simulated with Butachlor and Bispyribac-sodium at varying concentrations during rainy season showed lower colony counts on 7 days of application and higher colony counts on 55 days of application. Moreover, result of aerobic plate count obtained from soil samples simulated with mixture of Butachlor and Bispyribac-sodium at varying concentrations had lowest colony count of  $5 \times 10^8$  cfu/g at 3.13ml/333.3ml concentration on 7days and highest colony count of  $4.1 \times 10^9$  cfu/g at same concentration on 55days after applications. The control samples showed colony counts within the ranges of  $8.3 \times 10^9$  cfu/g to  $8.9 \times 10^9$  cfu/g. There is no significant difference between bacteria load of treated and untreated soil samples during dry and rainy seasons at  $p > 0.05$ . The results of isolation and identification revealed that six and five bacteria were isolated from treated soil samples during dry and rainy seasons respectively. Nine bacteria were also isolated from control soil samples studied. The bacterial isolates are of the genera: Bacillus, Pseudomonus, Escherichia, Rhizobium, Azotobacter, Enterobacter, Proteus, Micrococcus, and Staphylococcus. Finally, the result of growth behavioral response of treated rice paddy showed delayed growth 15 days after application in most cases except PRE 3 that extended to 30 days while there were gradual increase in the rice growth with highest growth rates observed 55 days after application. This growth increase could be as a result of degradation of hydrocarbon component of agroherbicides used and soil enrichment by beneficial bacterial isolates initially affected by the agroherbicides.*

**Keywords:** Soil Bacteria, Growth Behavior, Rice Paddy, Agro Herbicides and Abakaliki.

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**INTRODUCTION:**

Herbicides, which are substances used in the control of weeds, have been used for a long time now to eliminate unwanted plants. With the growing world population and consequent increase in the nutritional requirement, the use of herbicides to eliminate weeds that attack important crops like Rice has become more popular. Herbicide usage which were earlier mainly in plantation crops, have now expanded to crops like wheat and rice which account for about 42 and 30% of the total consumption of herbicides, respectively [1].

In Ebonyi State Nigeria, a marked increase in the use of herbicides in rice farming has been observed. Infact, it has become quite rare to see relatively large Rice farmlands where herbicides were not used.

Typically, this comes from a need by farmers for a more convenient and relatively cheaper means to eliminate weeds which pose great challenges to rice farmers.

Herbicides are classified in various ways: according to the activity (selective and non-selective); time of application (pre-plant, pre emergence and post emergence); mechanism of action; and method of application (soil applied and foliar applied) [2].

A lot of these different types of herbicides are commonly available for rural and urban rice farmers in Nigeria to control the scourge of weeds in rice farms.

Although herbicides have been generally successful in the control of weeds in rice farms, many have been reported to have various effects on the soil and its flora.

Most of these herbicides applied accumulate in the top soil layer (0-15cm) where most of the microbiological activities occur [3]. The persistent herbicides like Triazine have been shown to adversely affect some soil microorganisms in terms of population and biomass in long-term exposures [4].

Herbicide application may also kill species of bacteria that combat disease causing microorganisms, thereby upsetting the balance of pathogens and beneficial organisms and allowing the opportunistic disease causing organisms to become a problem [5].

Various studies have also implicated herbicides in causing quantitative and qualitative decline of important soil bacteria responsible for nutrient cycling and fixing, decomposition and other beneficial activities in the soil [6].

In addition, change in the soil microflora has been listed as one of the possible causes of productivity decline in rice cropping systems [7].

In Ebonyi State where Rice cultivation is very important as a means to meet the economic and nutritional requirements of the indigenes and others,

the need for improved soil productivity of which soil bacteria play a huge role, cannot be over emphasized. In the light of all these, it becomes pertinent to investigate the effect of herbicides on soil bacteria of rice farmlands. This will give a clue to the long-term soil productivity for rice cultivation.

**Aim and Objectives****Aim of the Study**

The aim of this study is to investigate soil bacteria and growth behavioral response of rice paddy treated with agro herbicides in two seasons in Abakaliki local government area

**Objectives**

- To determine the bacterial load of the soil samples
- To isolate and identify soil bacteria associated with Rice plant growth.
- To determine the growth rate of Rice plants at different concentrations of herbicides
- To determine the statistical difference of the bacterial load of the treated soil samples and the untreated soil samples.

**MATERIALS AND METHODS:****Study area:**

This study was carried out within Abakaliki, the capital of Ebonyi state in Nigeria. Abakaliki lies within the Latitude of 6°20'N and Longitude of 8°06'E.

The city lies at about 400m above sea level, with two main seasons of dry season, which spans from November to March and Rainy season, which begins in April and ends in October with a short period of reduced rain falls in August. Temperature of Abakaliki in the dry season ranges from 20°C to 38°C. The monthly rainfall ranges from 31mm in January to 270mm in July. Average annual Rainfall varies from 1500mm to 1650mm. The soil type of Abakaliki is mainly clay loam which is usually swampy. These conditions make the area suitable for rice farming.

**Materials:**

The materials that were used in this study include media such as yeast extract mannitol Agar (YEMA); Reagents for biochemical tests; peptone water, distilled water, Petridish, Bursen burner, Microscope, Autoclave, Hand trowel etc. Agroherbicides:

- Jackpot Herbicide (Butachlor 500g/L)

**Manufactured by:**Anhui Zhongbang Biology Engineering Co. Ltd: 6, Zhongbang Shushan District Hefei, China.

- Agriforce (100g/L Bispyribac – Sodium Sc)

**Manufactured by:**Anhui Zhonghon chemical industries Co. Ltd chemical industrial park. Xiangyu, Dougzi Chizou, Anhui, China.

### Collection of Samples

This Research which was conducted in two main phases of dry season and rainy season necessitated the collection of samples as follow:

#### Phase one of Research (Dry season):

Soil samples were collected from three different Rice farmlands at four different points for each of the farmland where agroherbicides were previously applied:

T1- Rice farmland in Nkaliki

T2 – Rice farmland in College of Agricultural Science (C.A.S)

T3 – Rice farmland around College of Health Science (CHS)

And from Two different Rice farmlands at four different points for each farm lands where agroherbicides were not applied which served as the controls:

C1 – Rice farmland in Nkaliki

C2 – Rice farmland around College of Health Science Campus

#### Phase Two of Research (Rainy season):

Soil samples were collected from a Rice farmland around College of Health Science with no prior exposure to agroherbicide.

These soil samples were randomly collected from depths of 0-3cm (Saeki and Toyota, 2004), [8], with sterile Hand trowels.

The soil samples were transported in sterile polyethylene bags to the laboratory.

### Sample Processing

For the both phases of the research, the soil samples were homogenized and sorted out to remove stones and other debris using sterile 2.5mm sieve before analysis.

Ten fold serial dilutions of the samples T1, T2, T3 (soils exposed to agroherbicides) and C1, C2, (soils with no exposure to agroherbicides) were performed using one gram of each different soil samples.

Approximately 1ml aliquot from dilutions of  $10^{-8}$  and  $10^{-9}$  were added on petri dishes and the media – yeast extract mannitol agar was added and incubated at 28°C for 2-5 days.

The observed bacterial colonies were then counted and expressed as colony forming units per gram of soil. The growths were sub-cultured severally to obtain pure isolates and subsequently stored in the refrigerator at 4°C.

### For the phase two of the study:

The soil sample with no prior exposure to agroherbicides were processed and taken to the green house of Biotechnology research unit of EBSU where they were placed in bowls to be used for seedling the rice.

The materials used for the phase two:

Agroherbicides:

Butachlor 500g/L (Pre-emergence, selective)

100g/L Bispyribac-sodium sc (post emergence, selective).

Distilled water

Rice seeds (R8)

These agroherbicides Butachlor (pre-emergence and selective) and Bispyribac-sodium sc (post emergence and selective) were used in this research because they are commonly used by farmers within Abakaliki LGA for treatment of their rice farmlands.

Also, some rice farmers tend to apply both agroherbicides on their farmlands, however, at different stages of rice planting for each agroherbicide.

The Butachlor herbicide (pre-emergence) was applied to the following soil samples in concentrations of:

PRE 1 3.13ml (herbicide)/500ml (water)

PRE 2 3.13ml (herbicide)/333.3ml (water)

PRE 3 3.13ml (herbicide) 166.6ml (water)

Bispyribac-sodium sc herbicide (post emergence) was applied as follows:

POST 1 3.13ml (Herbicide)/500ml (water)

POST 2 3.13ml (Herbicide)/333.3ml (water)

POST 3 3.13ml (Herbicide)/166.6ml (water)

Also, the butachlor was applied on the samples as a pre-emergence, and later, Bispyribac-sodium sc was applied as a post emergence in concentrations as seen below:

MIXED 1 3.13ml

(Herbicide)/500ml (water)

MIXED 2 3.13ml

(Herbicide)/333.3ml (water)

MIXED 3 3.13ml

(Herbicide)/166.6ml (water)

The rate and dosage of application of these agroherbicides were determined taking into consideration the manufacturer's instructions and the size of the bowl used for the rice planting.

Soil samples were taken from all the treatments at intervals of 0, 3, 7, 15, 30, 45 and 35 days after application of the herbicides [9].

Ten fold serial dilutions were done for the soil samples taken at each particular day after application of herbicide.

Approximately 1ml aliquot from dilutions  $10^{-8}$  and  $10^{-9}$  were added on petri dishes, and yeast extract mannitol agar added. The petri dishes were incubated at  $28^{\circ}\text{C}$  for 2-5 days and observed colonies were counted and expressed as colony forming units per gram of soil (cfu/g). The growth were sub-cultured severally and pure isolates stored in the refrigerator at  $4^{\circ}\text{C}$

#### Identification of Isolates

Identification of the test isolates was done based on morphological and biochemical tests; sugar fermentation test (glucose, fructose and lactose), voges proskauer test, catalase test, coagulase test, oxidase test and indole test; including Gram staining reaction and motility tests were done for proper characterization and identification of the bacterial isolates according to [10].

The colonies of the purified bacterial culture were observed for their morphology (colour, shape, elevation).

#### Gram Staining Technique

A smear of each isolates was made on a clean grease-free slide. The smear was air-dried and heat-fixed by waving over a Bunsen flame. The smear was flooded with crystal violet for 1 minute and was then rinsed in a slow running tap water and flooded with Lugo's iodine for 1 minute. The smear was rinsed again in a slow running tap water and then decolourized with absolute alcohol. The smear was covered with safranin for 30 seconds and then washed off under running tap. The smear was allowed to air dry and examined microscopically using oil immersion objective lens [11].

#### Motility Test

The hanging drop technique was used for this test. A drop of each bacterium isolate from 24 hours old culture growth was made on clean glass slide held between two fingers. It was inverted onto a cavity slide and observed microscopically using X40 objective lens for viewing motile bacteria isolates. The directional movement of bacterial cells indicated motility while no movement indicated non-motility [12].

#### Biochemical Tests

##### Catalase Test

This test was used to determine if the organism produced the enzyme catalase or not.

A 24-hour old culture was used to carry out the test.

A sterile wire loop was used to make a homogenous suspension on the slide. A drop of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was added to the suspension on the slide. The occurrence of effervescence indicated a positive

reaction while its absence indicated a negative reaction [13].

##### Urease Test

This test was used to determine the presence of urease in the bacterial isolates. Urease is an enzyme that breaks down urea to ammonia and carbon (iv) oxide, represented by the equation:  $-\text{H}_3\text{NCONH}_2$



Bijou bottles containing 3ml of sterile modified Christensen's urea broth was prepared by slanting. The slants were inoculated with isolates and incubated at  $37^{\circ}\text{C}$  for 24 hours. The development of pink colour indicated a positive result [14].

##### Oxidase Test

A piece of filter paper was placed in a clean petri dish and 2 drops of freshly prepared oxidase reagent (p-aminodimethylaniline) were added. Discrete colonies of each isolate were picked with glass rod and emulsified on the filter paper in the petri dish. The development of blue-black colour/purple within seconds on the filter paper indicated a positive result [15].

##### Coagulase Test

Here, a grease pencil was used to mark a slide into two sections. A loopful of normal saline was placed on each of the marked sections. Smear of the colony of the test organisms were made on the slide and mixed until homogenous suspensions were obtained. A drop of human plasma was added to one of the suspensions and observed for clumping after few seconds [11].

##### Indole Test

A peptone broth was prepared according to the manufacturer's prescription by adding 15g of peptone powder to 1 litre of distilled water. 5ml of the broth was dispensed into McCartney bottles and autoclaved at  $121^{\circ}\text{C}$  for 15 minutes.

The medium was seeded with the isolates and incubated at  $35^{\circ}\text{C}$  for 48 hours. After incubation, there was appreciable growth of the isolates. 1ml of chloroform was added to the broth culture and shaken gently. Also 2ml of Kovac's reagent (p-dimethylaminobenzaldehyde) was added and shaken gently. The bottles were allowed to stand on the bench for 20 minutes to permit the reagent to rise to the top. A red coloration at the top layer indicates indole production while a yellow coloration indicates a negative result [13].

### Citrate Test

Slants of Simmon's citrate agar were prepared in Bijou bottles as prescribe by the manufacturer. Using a sterile straight wire loop, a saline suspension of the test organism was first streaked on the slant and then stabbed (to create anaerobic conditon). The bottles were incubated at 37°C for 72hours. Alkaline pH shown by intense blue colour indicates citrate utilization [15].

### Sugar fermentation Test

The ability of the bacterial isolates to ferment lactose, sucrose, maltose and glucose was tested. 0.5% of each sugar was mixed with 5ml of peptone water in different test tubes and 4 drops of 0.01% phenol red indicator was added and inverted Durham tubes were dropped into the test tubes to identify gas production. The test tubes contents were autoclaved at 121°C for 15 minutes, after which they were seeded with pure isolates and incubated at 37°C for 5 days. Accumulation of gas at the top of the inverted Durham tube and colour change from red to yellow from the bottom to the top indicated positive result [13].

### Hydrogen sulfide (H<sub>2</sub>S) production Test

The isolates were inoculated in triple sugar iron agar (TSIA) slant medium and were incubated at 38°C for 24 hours. Black colour in the agar indicates production of hydrogen sulfide [10].

### Nitrate Reduction Test

An inoculums from a pure culture was transferred aseptically to a sterile tube of nitrate broth. The inoculated tube was incubated at 35°C – 37°C for about 48 hours. 5 drops of sulfanilic acid and 5 drops of N, N-dimethyl-1-naphthylamine were added. The change of colour of broth to deep red within 5 minutes meant that the bacteria had produced nitrate reductase. If colour did not change, the result in inconclusive. Then small amount of zinc would be added to the broth. If the solution remains colourless, then both nitrate reductase and nitrite reductase are present. If the solution turns red, nitrate reductase is not present [10].

## RESULTS:

### Bacterial Enumeration

The population of bacterial colonies were counted and expressed as colony forming units per gram of soil for all the soil samples studied during dry and rainy seasons.

For the phase one study which was carried out during the dry season, the highest population of bacteria observed was in C2 (the second control sample), which had a total of 1.04x10<sup>10</sup> CFU/g, and the least was in T1 (soil sample where agroherbicide was used), which had 3.2x 10<sup>9</sup> CFU/g.

The T2 (Where agroherbicide was used) had the second highest population of bacteria at 9.5 x 10<sup>9</sup> and followed by the C1 (control sample 1) at 9.2 x 10<sup>9</sup>

**Table 1: Population of bacteria from soil samples where agroherbicides were used and where herbicides were not used expressed as CFU/g dry soil**

Samples	CFU/g	Log <sub>10</sub>
T1	3.2x10 <sup>9</sup>	9.51
T2	9.5x10 <sup>9</sup>	9.98
T3	3.5x10 <sup>9</sup>	9.54
C1	9.2x10 <sup>9</sup>	9.96
C2	1.04x10 <sup>10</sup>	10.02

**Key:** C1 = Control 1, C2 = Control 2 (where agroherbicides were not used)

T1, T2, T3, = Soil samples where agroherbicide were used

**For the Phase two of the Study (carried out during the rainy season):**

The data for the total population of bacteria for the PRE1, PRE2, PRE3 and control for the various days samples were obtained (0, 3,7,15,30, 45 and 55); and for post 1, post 2 and post 3 for 0,3,7,15,30, 45 and 55 days after application; and also for mixed 1, mixed 2 and mixed 3 for 0,3,7,15,30, 45 and 55 days after application at different concentrations of the agroherbicides are presented in Table 2.

The results of total aerobic bacteria counts obtained from rice paddy pretreated with Butachlor and post treated with Bispyribac-sodium sc at varying concentrations showed that total aerobic counts were lower after 7 days of application and higher after 55 days of application.

It also revealed that total aerobic bacteria counts obtained from rice paddy treated with mixture of Butachlor and Bispyribac-sodium at varying concentrations had lowest colony counts of 5 x 10<sup>8</sup> cfu/g at 3.13ml/333.3ml after 7 days of application and highest colony counts of 4.1 x 10<sup>9</sup> cfu/g at same concentration after 55 days of application. Mean while total aerobic counts within the range of 8.3 x 10<sup>9</sup> cfu/g to 8.9 x 10<sup>9</sup> cfu/g were obtained from the control sample.

**Table 2: The population of bacteria in (PRE1, PRE2, PRE3, POST1, POST2, POST, Mixed1, Mixed2, Mixed3 and control) paddy rice treatment expressed in colony forming units per gram dry soil (cfu/g) and transformed to Log<sub>10</sub>.**

Treatment	Dose in ml(Herb/ water)	0 DAA	3 DAA	7 DAA	15 DAA	30 DAA	45 DAA	55 DAA
PRE1 Butachlor	3.13ml/ 500ml		3.5x10 <sup>9</sup> (9.54)	8x10 <sup>8</sup> (8.90)	1.3x10 <sup>9</sup> (9.11)	2.9x10 <sup>9</sup> (9.46)	3.7x10 <sup>9</sup> (9.57)	5.4x10 <sup>9</sup> (9.73)
PRE2 Butachlor	3.13mL/ 333.3mL		1.6x10 <sup>9</sup> (9.20)	3x10 <sup>8</sup> (8.48)	1.7x10 <sup>9</sup> (9.23)	2.3x10 <sup>9</sup> (9.36)	3.1x10 <sup>9</sup> (9.49)	4.2x10 <sup>9</sup> (9.62)
PRE3 Butachlor	3.13ml/ 166.6ml		1.2x10 <sup>9</sup> (9.08)	1x10 <sup>8</sup> (8)	1.1x10 <sup>9</sup> (9.04)	1.8x10 <sup>9</sup> (9.26)	2.5x10 <sup>9</sup> (9.39)	2.7x10 <sup>9</sup> (9.43)
POST1 Bispyribac-sodium sc	3.13ml/ 500ml		4.5x10 <sup>9</sup> (9.65)	2.2x10 <sup>9</sup> (9.34)	3.4x10 <sup>9</sup> (9.53)	4.9x10 <sup>9</sup> (9.69)	5.1x10 <sup>9</sup> (9.71)	5.6x10 <sup>9</sup> (9.75)
POST2 Bispyribac-sodium sc	3.13ml/ 333.3ml		4.3x10 <sup>9</sup> (9.63)	3.5x10 <sup>9</sup> (9.54)	3.2x10 <sup>9</sup> (9.51)	3.6x10 <sup>9</sup> (9.56)	4.8x10 <sup>9</sup> (9.68)	5.2x10 <sup>9</sup> (9.72)
POST3 Bispyribac-sodium sc	3.13ml/ 166.6ml		3.9x10 <sup>9</sup> (9.59)	2.4x10 <sup>9</sup> (9.54)	3.2x10 <sup>9</sup> (9.51)	3.6x10 <sup>9</sup> (9.56)	4.8x10 <sup>9</sup> (9.68)	5.2x10 <sup>9</sup> (9.48)
Mixed1 Butachlor+ Bispyribac	3.13ml/ 500ml		2.6x10 <sup>9</sup> (9.41)	1.6x10 <sup>9</sup> (9.20)	9x10 <sup>8</sup> (8.95)	1.0x10 <sup>9</sup> (9)	2.6x10 <sup>9</sup> (9.41)	3.8x10 <sup>9</sup> (9.58)
Mixed 2 Butachlor+Bispyribac	3.13ml/ 333.3ml		1.8x10 <sup>9</sup> (9.26)	5x10 <sup>8</sup> (8.69)	1.5x10 <sup>9</sup> (9.18)	2.3x10 <sup>9</sup> (9.36)	3.2x10 <sup>9</sup> (9.51)	4.1x10 <sup>9</sup> (9.62)
Mixed 3 Butachlor+Bispyribac	3.13ml/ 166.6ml		1.4x10 <sup>9</sup> (9.15)	1.1x10 <sup>9</sup> (9.04)	1.1x10 <sup>9</sup> (9.04)	1.7x10 <sup>9</sup> (9.23)	2.2x10 <sup>9</sup> (9.34)	3.9x10 <sup>9</sup> (9.59)
Control	N/A		8.7x10 <sup>9</sup> (9.94)	8.9 x 10 <sup>9</sup> (9.93)	8.6 x 10 <sup>9</sup> (9.93)	8.8 x 10 <sup>9</sup> (9.94)	8.3 x 10 <sup>9</sup> (9.92)	8.7 x 10 <sup>9</sup> (9.94)

**Key:** DAA = Days After Application

The result of morphological and biochemical characteristics of bacteria isolated from soil samples where agroherbicides were used during the dry season of the study is presented in table 3. A total of six different bacteria were identified. The probable organisms are: *Bacillus sp.*, *Escherichia sp.*, *Rhizobium sp.*, *Azotobacter sp.*, *Micrococcus sp.* and *Staphylococcus sp.*.

For that of the control soil samples (where agroherbicides were not used for both the dry season

and rainy season of the study), Nine different bacteria were identified. The probable bacteria are: *Bacillus sp.*, *Pseudomonas sp.*, *Escherichia sp.*, *Rhizobium sp.*, *Azotobacter sp.*, *Enterobacter sp.*, *Proteus sp.*, *Micrococcus sp.*, and *Staphylococcus sp.* This is as shown in Table 4.

In table 5, the probable bacteria from the soil samples where herbicides were used are: *Bacillus sp.*, *Rhizobium sp.*, *Azotobacter sp.*, *Micrococcus sp.*, and *Staphylococcus sp.*.

**Table 3: The result of morphological and biochemical characteristics of bacteria isolated from soil samples where agroherbicides were used during dry season.**- **Key:** + = Positive; = Negative

Cell/colony morphology	Gram reaction	Motility Test	Oxidase Test	Citrate Test	Catalase Test	Indole Test	Urease Test	H <sub>2</sub> S Production Test	Coagulase Test	Glucose	Sucrose	Lactose	Maltose	Nitrate Reduction Test	Probable Organism
Rod/Large whitish Round colonies	+	+	-	+	+	-	-	-	-	+	+	+	+	+	<i>Bacillus sp.</i>
Opaque, sticky dull white	-	+	-	-	+	+	-	-	-	+	-	+	+	+	<i>Escherichia sp.</i>
Large mucoid milkish elevated colonies/Rod	-	+	+	-	+	+	+	-	-	+	+	+	+	+	<i>Rhizobium sp.</i>
Flat, slimy, paste-like, white/Rod	-	+	-	+	+	+	+	+	-	+	+	+	+	+	<i>Azotobacter sp.</i>
Yellow small smooth round colonies cocci	+	-	+	-	+	-	+	-	-	+	+	+	+	-	<i>Micrococcus sp</i>
Creamy spherical cocci	+	-	-	+	+	-	+	-	+	+	+	+	+	+	<i>Staphylococcus sp</i>

**Table 4: The result of morphological and biochemical characteristics of Bacteria isolates from the controls where agroherbicides were not used.**

Cell/colony morphology	Gram reaction	Motility Test	Oxidase Test	Citrate Test	Catalase Test	Indole Test	Urease Test	H <sub>2</sub> S Production Test	Coagulase Test	Glucose	Sucrose	Lactose	Maltose	Nitrate Reduction Test	Probable Organism
Rod/Large whitish Round colonies	+	+	-	+	+	-	-	-	-	+	+	+	+	+	<i>Bacillus sp.</i>
Large flat yellowish	-	+	+	+	+	-	+	-	-	+	-	-	-	+	<i>Pseudomonas sp.</i>
Opaque, sticky dull white	-	+	-	-	+	+	-	-	-	+	-	+	+	+	<i>Escherichia sp.</i>
Large mucoid milkish elevated colonies/Rod	-	+	+	-	+	+	+	-	-	+	+	+	+	+	<i>Rhizobium sp.</i>
Flat, slimy, paste-like, white/Rod	-	+	-	+	+	+	+	+	-	+	+	+	+	+	<i>Azotobacter sp.</i>
Pink raised/smooth edge	-	+	-	+	-	-	-	-	-	+	-	+	+	+	<i>Enterobacter sp</i>
Small round colonies rod	-	+	-	+	+	-	+	+	-	+	+	-	+	+	<i>Proteus sp</i>
Yellow small smooth round colonies cocci	+	-	+	-	+	-	+	-	-	+	+	+	+	-	<i>Micrococcus sp</i>
Creamy spherical cocci	+	-	-	+	+	-	+	-	+	+	+	+	+	+	<i>Staphylococcus sp</i>

**Table 5: The result of morphological and biochemical characteristics of bacteria isolated from soil samples where agroherbicides were used during rainy season.**

Cell/colony morphology	Gram reaction	Motility Test	Oxidase Test	Citrate Test	Catalase Test	Indole Test	Urease Test	H <sub>2</sub> S Production Test	Coagulase Test	Glucose	Sucrose	Lactose	Maltose	Nitrate Reduction Test	Probable Organism
Rod/Large whitish Round colonies	+	+	-	+	+	-	-	-	-	+	+	+	+	+	<i>Bacillus sp.</i>
Large mucoid milkish elevated colonies/Rod	-	+	+	-	+	+	+	-	-	+	+	+	+	+	<i>Rhizobium sp.</i>
Flat, slimy, paste like, white/Rod	-	+	-	+	+	+	+	+	-	+	+	+	+	+	<i>Azotobacter sp.</i>
Yellow smooth small round colonies cocci	+	-	+	-	+	-	+	-	-	+	+	+	+	-	<i>Micrococcus sp</i>
Creamy spherical cocci	+	-	-	+	+	-	+	-	+	+	+	+	+	+	<i>Staphylococcus sp</i>

**Key:** + Positive

-Negative

**The growth rate observed from the research conducted during the rainy season is presented in table 6.**

The results of growth rate of the rice plants observed after treating the rice paddy with pre, post and mixed agroherbicides at varying concentrations from 0-55 days showed that there was no growth of rice plant on samples PRE 1 and PRE 2 from 3-15 days of application while growth rate on same samples from 30-55 days ranged from 5.2cm to 11.6cm. Samples PRE 3 showed no growth of rice from 3-30 days of application while growth was recorded from 45-55

days of application within the range of 3.5cm to 6.9cm.

Also, post samples 1,2 and 3 recorded growth within 7 days after application while mixed samples 1, 2 and 3 recorded growth from 30-55 days after application. The higher growth rate of 18.5cm was observed after treating rice paddy with POST 1 Bispyribac-sodium sc at 55 days of application while the lowest growth rate of 2.1cm was observed after treatment with mixed 3 agroherbicides at 30 days after application. Meanwhile, control showed highest growth rate of 25.5cm at 55days after application.

**Table 6: Growth rate of the rice plants observed after 3,7,15,30,45 and 55 days of application at various concentrations of agroherbicides.**

Samples	3 Days	7 Days	15 Days	30 Days	45 Days	55 Days
PRE1	N/A	N/A	N/A	5.2	8.7	11.6
PRE2	N/A	N/A	N/A	4.0	6.1	9.3
PRE3	N/A	N/A	N/A	N/A	3.5	6.9
POST 1	N/A	N/A	7.1	11.14	15.3	18.5
POST2	N/A	N/A	6.2	9.3	12.6	15.7
POST 3	N/A	N/A	6.8	9.0	12.5	13.3
MIXED1	N/A	N/A	N/A	5.0	6.2	8.8
Mixed 2	N/A	N/A	N/A	4.2	6.0	6.7
MIXED3	N/A	N/A	N/A	2.1	3.5	5.9
Control	N/A	N/A	6.5	14.8	20.5	25.5

**Key:** N/A = Not Available

The result of the statistical analysis using ANOVA to determine the statistical difference between the bacterial load of the treated soil samples and the untreated soil samples showed no significant difference at ( $p > 0.05$ ).

### DISCUSSION:

The results from this study showed that the agroherbicides: Butachlor and Bispyribac – sodium Sc affected the bacterial population of soil samples of rice farm lands.

From the research conducted during the dry season where soil samples were collected from rice farm lands where agroherbicides were previously applied and from rice farm lands where agroherbicides were not used, the result revealed that the highest population of bacteria was seen at the control sample (which had no agroherbicide) C2 at  $1.04 \times 10^{10}$  cfu/g, followed by the sample from rice farm land where herbicides were applied T2, at  $9.5 \times 10^9$  cfu/g. The 3<sup>rd</sup> highest population of bacteria was the 1<sup>st</sup> control sample C1 at  $9.2 \times 10^9$ , followed by the 3<sup>rd</sup> test sample T3 at  $3.5 \times 10^9$  and the least been the 1<sup>st</sup> test sample T1 at  $3.2 \times 10^9$  cfu/g. This result could mean that the test samples T1 and T3 still had residual effects of the agroherbicides applied on their bacterial population compared to that of the test sample T2. This is in line with works of [9 and 3], who reported the residual effects of some herbicides on bacterial population after days of application.

Apart from the T2 which had the second highest bacterial population, it could be said that the control samples (C1 and C2) had more population of bacteria compared to T1 and T3 soil samples.

For the research conducted during rainy season, the population of bacteria was sampled at 0,3, 7, 15, 30, 45, and 55 days after application of the Butachlor and Bispyribac - sodium sc herbicides at different concentrations.

Also the control sample (which had no agroherbicide) was analysed for its bacterial population, and it showed  $8.7 \times 10^9$  cfu/g at  $\log_{10} 9.94$ , which was the highest population of bacteria observed in the study. This is in agreement with the works of other researchers like [7 and 16], where the controls had significant more populations of bacteria than the samples where agroherbicides were applied.

The least population of bacteria was observed in the sample PRE3 that had Butachlor at the concentration of 3.13ml (herbicide) / 166.6ml (water) on the 15<sup>th</sup> day of application at  $1 \times 10^8$  cfu/g.

The treatments that had mixtures of Butachlor and Bispyribac – sodium sc also recorded varying low bacterial populations compared to the treatments that had only Bispyribac – sodium sc herbicides at the different concentrations and different days samples

were analysed. This could be explained as a result of the synergistic effect of the two agroherbicides (Butachlor and Bispyribac – sodium sc) used on the bacteria resident in the soil as also reported by [9 and 17].

Comparing the individual effect of the herbicides on the bacterial population, it was observed that the Butachlor herbicide used had much more effect on the bacterial population than the Bispyribac – sodium sc herbicides. Several other people (Latha *et al.*, 2010), (Simerjeet *et al.*, 2014) and (Durga *et al.*, 2007), [7, 18 and 9], have also reported this effect and attributed it to butachlor being more difficult to degrade by the microorganisms than the other herbicides.

The effect of the different agroherbicides applied generally increased with increased concentration of the herbicide. Hence, the population of the bacteria decreased with increased concentration of herbicide. For example, from table 2, at 3.13ml (herbicides)/500 ml (water), on the 3<sup>rd</sup> day after application, a  $3.5 \times 10^9$  cfu/g population of bacteria was observed for the PRE1 Butachlor treatment; while at 3.13ml (herbicide)/166.6ml (water) on the 3<sup>rd</sup> day after application of the herbicide,  $1.2 \times 10^9$  cfu/g was recorded for PRE3 Butachlor treatment.

This inverse relationship between the concentration of herbicides and population of bacteria has been reported by other researchers like [19, 3 and 20].

However, it was observed that the population of bacteria generally picked up as the days progressed, [9, 18 and 7].

The Bacteria identified in this study *Bacillus* sp, *Escherichia* sp, *Rhizobium* sp, *Azotobacter* sp, *Pseudomonas* sp, *Micrococcus* sp, *Enterobacter* sp, *Proteus* sp and *Staphylococcus* sp - are bacteria generally observed in soil samples (Vrieze, 2015), [21], and linked with agroherbicide degradation [22 and 23]. *Rhizobium* and *Azotobacter* have been associated with rice plant growth and are capable of fixing Nitrogen to Ammonia for plants development [24].

The growth rate of the plants was observed and seen to have the highest at 25.5cm on the 55<sup>th</sup> day of planting the control sample. It showed an initial no-growth period of about 3- 7 days after seeding and attained penultimate highest growth at 20.5cm.

The least rate of growth was observed in the “mixed 3” sample which had Butachlor and Bispyribal – sodium sc at 2.1cm on the 30<sup>th</sup> day and reached maximum of 5.9cm on the 55<sup>th</sup> day.

Additionally, there were periods of growth latency which was highest at the PRE3 sample on the 30<sup>th</sup> day after seeding. Some others were at PRE1, PRE2 (containing Butachlor herbicides). This scenario has been reported severally by researchers like (Tatiane

et al., 2007), (Fenner et al., 2013), [25 and 26], etc about the ability of some bacteria to degrade herbicides.

The bacteria in the soil were able to degrade the chemical compounds of the herbicides which brought about the germination of the seeds. The growth latency periods observed which matched days samples had low population of bacteria (from Table 2), could be explained as the points the herbicides suppressed the activities of the resident bacteria and the periods the bacteria began to degrade the herbicides [27].

From the result of the statistical analysis carried out using Anova, it showed that there was no statistical difference between the bacterial population of the treated soil samples and that of the untreated soil samples at ( $P > 0.05$ )

### CONCLUSION:

This study revealed the effect of herbicides Butachlor and Bysribac - sodium Sc used as preemergence and post emergence respectively, and as mixture, at different concentrations on the soil bacteria and on the growth rate of the rice plants.

It indicated that with increased concentration of the herbicides, the bacterial population generally decreased and plant growth delayed. This is until the resident bacteria are able to degrade the herbicides and normal growth of the plant would be seen to commence and the Bacterial Load would increase.

### RECOMMENDATION

The following measures are recommended:

- Proper care should be taken by farmers when using herbicides to follow the instructions on their usage as relates to dosage, mode of application etc.
- Herbicides should be used only when absolutely necessary; otherwise, other actions for eliminating weeds should be employed.
- There should be proper and effective government regulation on the types of herbicides available to farmers within the country to ensure their safety to the environment

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