USE OF BACTERIA IN RECLAIMING SOIL CONTAMINATED WITH HERBICIDES 2, 4 DINITROPHENYLACETATE AND 4 – CHLOROPHENYL ETHANAMIDE MIXTURE IN SOIL

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Abstract

An investigation was carried out in search for reclamations of contamination soils using herbicide degrading bacteria. Soil samples were taken from rice farm contaminated with the herbicides solution using mineral salt agar (MSA) without herbicides solution as control. Screening involved purification by cloning three successive times in herbicide supplemented MSA and isolation of pure and subsequent subculture in herbicide supplemented mineral salt broth (MSB). Bacteria growth was determined by colony formation on MSA as well as by optical density (OD) of MSB cultured at 600mm using spectronic – 20. Out of the bacteria strain isolated from these herbicide contaminated soils only one organism showed positive herbicide utilization ability as sole carbon and energy source. Pure cultures of this isolate was characterized using standard technological techniques and identified as Pseudomonas sp. It is therefore recommended that herbicides that are biodegradable, such as synthesized herbicides should be used for weeds control in the farm.

Key words: Herbicide, bacteria, culture, isolation, contamination

Introduction

Weeds are the most underestimated pests in tropical agriculture. They have great influences on human social actions more than other crop pests. The presence of weeds levies a heavy tax on farm operations, food, fiber cost and ultimately the cost of living of the general public. Weeds cause a lot of havoc to agricultural crops, animals and humans (Lavabre, 1991). They compete with crops for growth resources and are persistent and resistant to control measures applied in the farm. They contaminate agricultural produce, reducing the quality of crop market values and extreme cases causes' food poisoning. There are also indications that some weeds exude toxins, which adversely affect crop growth. This is known as Allelopathy (Akobundu, 1987).

Weed control refers to those actions that seek to restrict the spread of weeds and destroy or reduce their population in a giving location (Aldrich, 1984) Food production is a premier human enterprise and weed control is an integral part of this business. Weeds and their control are human activities and human are always face with problems of weeds whenever, the natural vegetation is disturbed. It is particularly difficult to separate works from human affairs and problem of weeds will not disappear if ignore by man.

Weeds control is as old as agriculture. The history of weeds control practices is an account of man's technological advancement and equally how the energy resource for removing weeds have shifted from man to his animals, machines, fossil fuels and finally to chemical energy (Whitten, 2006).

The oldest of weeds control is the pulling of weeds by hands. Generally, Jethro Tull in the 18th Century in England had the idea of sowing crops on rows so that weeds could be destroyed using machinery drawn by horses and he was the first man to use the word 'WEED' as it is used today (Floyd & Craft. (2008). This was the beginning of mechanical weed control and it has been developed over the years to take the form of the tractor powered mechanical weeding.

Cultivation requires tremendous amount of energy in terms of equipment, food and manpower. For this reason, there arose a need to devise a means of achieving optimal food production with minimum amount of energy (Chandler, 2004). Traditional mechanical means of weeds control reached its peak of efficiency by the end of 19th Century when plenty of cheap labour was still available. With the rapid growth of industrialization, the resultant drifts of labour

from the countryside to factories meant a shortage of manpower on the farms and consequently agricultural wages increased. The situation provided the stimulus for the development of more efficient means of weed control and the introduction of chemical weed killers (herbicides) (Audu, 2008)

Herbicides are substances used for killing or adversely affecting plant growth. The practice by which undesirable vegetation (weeds) are killed with herbicide is called chemical weed control (George, 2012). The use of herbicides is one of the solution to problem weeds constitute in agriculture. The total dependence of herbicides by farmers for controlling of weeds shows either improper understanding of the role of herbicides in weed control or outright abuse of the technology. The indiscriminate use and total dependence of herbicides by farmers have led to some adverse effects on human's health as well as contaminating our environment (Smith, 2003). These herbicides are destructive to the environment and could cause cancer to the human body if used more frequently at rates of application well above the recommended rate for weed control. This accumulation may pose serious health hazard to human, animal and plants, because of the toxic effects of these chemical pollutants. Environmental pollution from these developed herbicides is now of serious concern. There is urgent need to clean up polluted environment through biological technique which offers an attractive option that is both cost effective and environmental compactable or friendly. The objective of the investigation is to develop an environmentally compatible process for biological detoxification of these herbicides.

Materials and Methods

The research investigation was carried out in laboratory of Department of Pure and Industrial Chemistry, Faculty of Physical Science, University of Nigeria Nsukka. Two herbicides were formulated 2, 4, Ditrophenyl acetate and 4, Chlorophenyl ethanamide. The mixture 2, 4, Ditrophenyl acetate and 4, Chlorophenyl ethanamide were used to isolate herbicide degrading microorganisms by enrichment culture technique. Soil samples were inoculated aerobically in mineral medium (25 ml) culture flasks at 30 ^{0}C in a Gallenkamp orbital incubator with the mixture of 2, 4, Ditrophenyl acetate and 4, Chlorophenyl ethanamide being used as sole source of carbon.

2, 4 – Dinitrophenylacetate

2, 4 – dinitrophenol (12.0g, 0.05 mole) was dissolved in 10% sodium hydroxide solution in 250 ml conical flask. It was shaken until the nitrophenol dissolved. Redistilled acetyl chloride (10 ml) was carefully added. This was shaken vigorously for about 20 minutes. Great heat was evolved, showing that the reaction is exothermic. This was allowed to cool, then filtered by suction and washed two times with 50 ml of water, dried in air. The cycle product was recrystallized from rectified spirit to give a bright yellow crystals of 2, 4 – denitrophenyacetate with a melting point 250 – 251^{0}C.

4 – Chlorophenyl ethanamide

In a 100 ml round bottom flask equipped with a reflux condenser, chloroanaline (5.1g, 0.05 mole) was placed. A mixture of glacial acetic acid (5ml) and acetic anhydride (5ml) was added. Small amount of of Zinc dust (0.05g) was greatly introduced into the mixture. The mixture was gently boiled for 30 – 45 minutes and the hot liquid was poured in a thin stream into 250 ml beaker containing 125ml of cold water while stream vigorously at room temperature. The crude product was filtered at the pump and watered with little water, drained well and dried upon filter paper in air. The product was recrystallized from water (125ml) tp which methylated spirit (3ml) was added

to give a dirty white crystal of a Chlorophenyl ethanamide with point of 170 – 172^{0}C (LR 172 - 173^{0}C)

Melting points of the compounds synthesized were determined by the use of electrothermal melting point apparatus in open capillaries and uncorrected. Ultraviolet and visible spectra were recorded on a pye – unicam SP8 – 100m spectrophotometer using matched 1 cm quartz cell. Absorption maxima is given in nanometer (nm) nuclear magnetic. Resonance spectral data were obtained on a EM 360/390 NMR Spectrometer. Degraded rate was assessed based on the rate which the medium changes its colour i. e. becoming turbid.

Application of herbicides

Knapsack sprayer (20 liters) was used in the application of the herbicides. The area of the farm chosen was 400m^2. The farm is a rectangular plot of dimension: length (40m) width (10m). The plot was divided into seven equal parts, each (5.14m^2) and the herbicides and their mixture applied in six of them leaving one as a control experiment. The herbicide was applied in each of them in the same concentration for effective comparison of their biological activities on the growth of weeds and rice plants.

Collection of soil samples

Soil samples used for the isolation of the microorganisms were obtained from the rice farm where the herbicides were applied in Ndeaboh town. Samples of soil were collected aseptically using sterile sample bags.

Isolation of microorganisms

Microorganisms were isolated from soil samples after enrichment. Soil sample (1g) was placed in 150ml capacity Erienmeyer flask bearing 25ml of sterile enrichment medium containing in g per liter; Nh_4NO_3, 2.5; $NaHPO_\$$, 1.0; $MgSO_4$, 0.5; $Fe_2(SO_4)_3$, 0.01; $CoCl_3$, 0.01; 0.005; $CaCl_3$, 0.001; KH_2PO_4, 0.0005; $MnSO_4$, 0.0001; $(NH_4)_7MO_7O_{24}.H_2O$, 0.0001; and mixture of 2, 4 – denitrophenyacetate and 4 –chlorophenyl ethanamide 0.04g. Inoculated soil was inoculated for one week. Sample (1ml) from the above culture was subsequently transferred into fresh enrichment medium and inoculated as earlier described above. A third enrichment stage was carried out before isolation on solid isolation media. Medium for microbial isolation was the same as that for enrichment except that agar (15 g/l) was added as a solidifying agent. The streak plate technique was employed for isolation. Inoculated plates were incubated at room temperature (30^{0}C) until growth occurred. Suspected herbicide degraders were purified by passage on sterile isolation medium..

Screening For The Ability Of Isolate To Use The Herbicides As Sole Carbon Source For Growth

Isolate was inoculated (one loopful) into 25 ml of sterile liquid screening medium (same as isolation medium without agar) at a large test tube containing 0.1 ml of the herbicide mixture as sole carbon on the mixture of 2, 4 – denitrophenyacetate and 4 – chlorophenyl ethanamide were indicated by increase in medium turbidity measured at 600 nm using mitton and Ray spectronic – 20 spectrophotometer.

Identification Of 2, 4 – Denitrophenyacetate And 4 – Chlorophenyl Ethanamide

Test employed in identification of the herbicide degraders include Gram staining, oxidase and catalase test, motility test and test growth on acetamide agar were carried out a cording to standard procedure, and motility test was by hanging drop method. Fresh sample from the pure broth culture was used. Acetamide agar was prepared according to the manufacturer's instructions

and dispensed into the petri plates. Inoculated plates was then inoculated at room temperature for 24 – 48 hours then examined for the development of blue colour which confirms the presence of *Pseudomonas sp.*

The incubated mixtures were subcultured once a week. After 1 – 2 weeks, growth was evident in the flask as judged by an increase in optical density (OD) from 0.04 – 0.36 at 600 nm as well as the disappearance of the herbicides in question from the culture medium. The culture consistently degraded the mixture of the synthesized herbicides in subsequent subculture.

Plates of minimal medium containing the mixture of the herbicides were streaked from a subculture. Colonies that appeared during one week incubation were patched and further streaked to isolate single colony. One of the strains degraded the mixture of 2, 4, Ditrophenyl acetate and 4, Chlorophenyl ethanamide as sole source of carbon and energy. The strain was an oxidase and catalase positive, motile, Gram – negative rod. On the basis of these characteristics, the strain was identified as *Pseudomonas sp.*

Results and Discussion

Most of the herbicides in the medium inoculated with bacteria were degraded in two weeks, whereas no loss of the herbicide was noticed in the medium not inoculated over the same period of time.

Generally, when herbicides are applied in an industrial scale over a period of time, they may become recalcitrant perhaps due to accumulation. These chemicals when accumulated in the site (soil) need to be removed by an introducing bioremediation process which is the use of biological agents and their biodegradative ability to reclaim contaminated sites (Lebron, & Gressel, (2008). The biological agents for bioremediation are most frequently microorganisms, commonly bacteria that degrade pollutants. This can be accomplished by stimulating the ability of indigenous microorganisms responsible for the degradation of the herbicides (Akinbundu, 1980). Alternatively, isolated and cultured (genetically engineered) microorganisms such as *Pseudomonas sp* could be used to inoculate the affected soils to enhance the degradation of the herbicides. The ultimate goal of bioremediation is to convert these contaminants into biomass and harmless by – products of microbial metabolism (Walter, 2005).

Conclusion

The importance of herbicides in controlling weeds cannot be overemphasized. Some herbicides tend to contaminate the environment when applied over a period of time. The result obtained from this work indicated that the synthesized herbicides are biodegradable and as such are environmentally friendly. In this event of bio – accumulation of these herbicides in soils, this problem can be solved by stimulating the activity of the indigenous microorganisms responsible for their degradation.

Recommendation

It is therefore recommended that herbicides that are biodegradable, which are also known as environmental friendly such as synthesized herbicides should be used for weeds control in the farm.

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Appendix

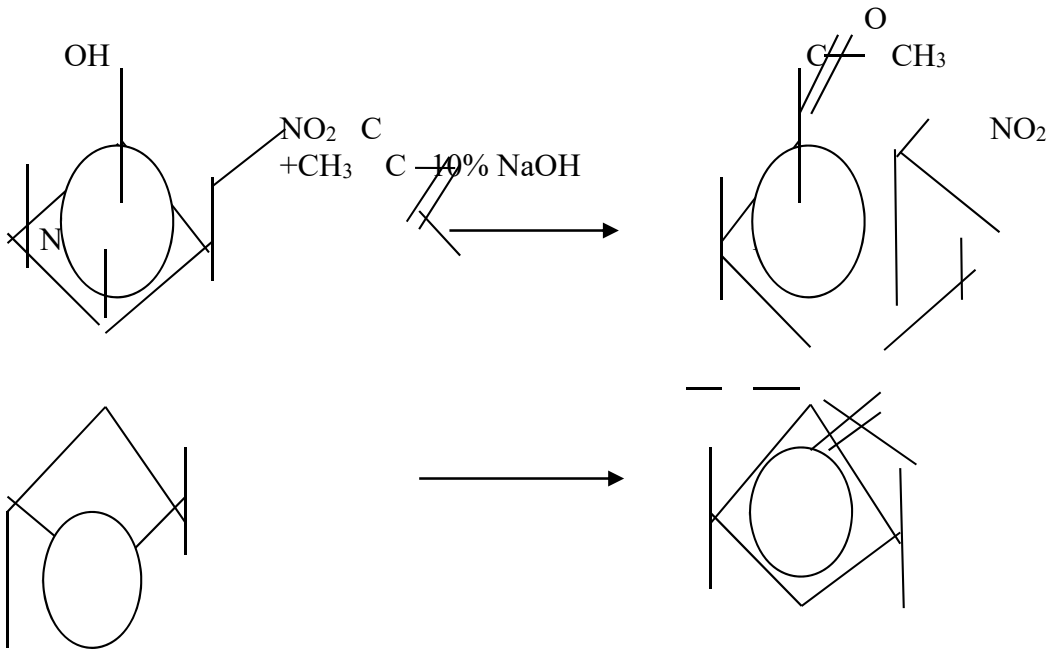

Fig. 12, 4 – Dinitrophenyl Acetate Production

H N C

CH3

+(CH3CO)2^0 CH3COOH

Fig. 2. N – (4 – Chlorophenyl Ethanamide)