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Development of Bio-Herbicides from the Phytotoxic Metabolite of *Pseudomonas Putida*

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***Abstract* - A strain of *Peudomonas putida* capable of producing phytotoxic metabolite was investigated for its biocontrol activity against *Chromolaena odorata, Tridax procumbens* and *Physalis angulata*. The organism was mass produced separately in fermentation media and phytotoxic metabolite was precipitated from the culture. Bioherbicide formulation was produced from metabolite viz the bioherbicide formula (which is made up of ten percent of the metabolite yield dissolved in water to make a total of 100 ml). The formulation was sprayed on the three weeds and their effects on the leaves of the three weeds were expressed as percentage and shown pictorially. The volume of the phytotoxic materials obtained from organism was 20ml. The bioherbicide formular obtained from- P. putida showed complete withering of the *Chromolaena* weed by the 8th day while it took 9 days for *Physalis* and *Tridax* to be completely withered after application. Hence, it has therefore been shown that Phytotaxic metabolic of *P. putida* is efficacious and safe and therefore can be used as an alternative to chemical herbicide and can therefore be optimized for large scale field application.**

Keywords: phytotoxic, metabolite, bioherbicide, biomagnification, withering.

I. INTRODUCTION

The use of plant pathogen for weed control has been reported before the turn of the century. It involves the use of living organisms that has the ability to produce phytotoxins that are capable of inducing disease state in the targeted weed [1]. The termbioherbicide is generally used to refer to herbicide produced from any living organism (e.g. Fungi, Bacteria, Viruses and Protozoans). The phytotoxins produced, can be defined as microbial metabolites that are harmful to plants at low concentration. In many cases, the toxins are low molecular weight compounds belonging to a variety of natural products which are able to diffuse from the site of infection to surrounding tissues or are translocatable within the plant [2].

Since exotic organisms often yield irreproducible result when introduced into different environment hence, the need to isolate local strains of organism for indigenous weeds. This research work provides an alternative way for weed control thereby eliminating poisoning of agricultural products due to improper handling of chemical herbicides. It will create a weed control programme that is environmentally safe. Also from the literature search, work has not been reported on the control of the three weeds using *Pseudomonasputida.*

II. MATERIALS AND METHOD

The weeds used for this experiment were obtained from the back of the cafeteria of Nigeria Stored Products Research Institute (NSPRI) Ilorin. The weeds are: *Tridax procumbens, Chromolaena odorata* and *Physalis angulata.* A fermentation flasks, pipes and buckets forplanting as well as spraying guns were procured for the purpose of this experiment.

Pseudomonas minimal medium which is made up of 20g/l of mixed peptone, 2g/l of potassiumhydrogen tetraoxosulphate, 2g/l of magnesium sulphate heptahydrate and 50ml/l of glycerol wasalso obtained for culturing the organism.

2.1 Soil Collection

Garden soil was collected from the grounds of NSPRI and sterilized in drums by applying heat for six days to kill the seeds of unwanted weeds. The sterilized soil was then divided into eighteen clean pierced buckets. These buckets containing the sterilized soils were then divided into two equal groups of nine buckets per group.

2.2 Pot Preparation

The two groups were labeled as follows: control experiment, and experimental group. The experimental group was also sub-divided into three groups. The three sub groups were labeled *Tridax procumbens, Chromolaena odorata* and *Physalis angulata* respectively.

2.3 Transplanting

The weeds were transplanted in such a way that each sub-group consisted of three pots of each weed. The control experiment pots (group 1) were also transplanted with the three weeds. After transplanting, each pot was sprayed with

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water every day in the morning for two weeks to ensure proper fixing and firmness of the root in the soil and to avoid any doubt of results.

2.4 Bacterial isolate

Slant bottles containing *P. putida* was obtained from the culture bank of Microbiology unit, Ladoke Akintola University of Technology Ogbomosho (LAUTECH). Koch postulate was carried out using the method of Bailey [3] to determine if the organism has pathogenic effect on the three weeds to be evaluated.

2.5 Koch's Assay for *P. putida* activity

Freshly detached leaves from *Tridax*, *Physalis* and *Chromolaena* were surface sterilized using 70% alcohol and then rinsed with distilled water and placed on moist filter paper in glass Petri dishes. Agar plug from slants containing the purified bacteria culture was placed at the center of each leaf. The dishes were sealed to prevent moisture loss, and incubated for three days at room temperature. The development of disease symptoms was observed.

2.6 Inoculum preparation

After Koch's assay was carried out, the strain that induced disease state in all the three weeds was selected. This strain was then mass produced by mass streaking on twelve Petri dishes, containing *Pseudomonas* minimal medium and incubated for three days at room temperature.

2.7 Fermentation

A fermentation flask with four litter capacity was used for this fermentation process.

The flask was sterilized by washing with hundred percent absolute ethanol. Two liters of sterilized *Pseudomonas* minimal medium was poured aseptically into the flask. This flask was then inoculated with the inoculum preparation. Aeration and agitation was supplied to the flask by connecting them to an aerator and the fermentation process was carried out for four days. After four days, the content of the flask was centrifuged to separate the cells from the solution. Phytotoxic metabolite was then precipitated out of these solutions by an equal volume of acetone. The precipitates were dried at room temperature. This method has been used by [4].

2.8 Bioherbicide Formulation

The metabolites produced were used for the bioherbicide formulation. This is made up of ten percent of the metabolite yield dissolved in water to make a total of 100ml. This method is in accordance with the work of Hasan *et al*.(2021)[4] and [2].

2.9 Spraying of the Potted Weeds

Spraying gun was used to spray the bioherbicide formulation on the potted weeds; this process was repeated accordingly in the entire experimental group. The water control was sprayed with water.

2.10 Counting of Leaves

After spraying the number of leaves that turned yellow, brown, rot and wither were observed and recorded for twenty-one days.

III. RESULTS AND DISCUSSION

The yield of the phytotoxic metabolite obtained from the fermentation of *P. putida* was 20 ml. The metabolite obtained from the *P. putida* was a whitish chalk like liquid.

Effectiveness of the bioherbicides formulated from these metabolites was determined by the number of days it took the weeds to wither (Figure 2) and the percentage reduction in leaf number twenty-one days after the application of the bioherbicides (Figure 1). Withering of the leaves was observed in all the experimental groups. The water control experiment which was sprayed with water only was green and flourishing throughout the experimental period. The bioherbicide was highly effective as it resulted in complete withering of the three replicates of each weed between eight and nine days (Plates 1, 2 and3). When applied to *Chromolaena*, complete withering was achieved by the eighth day, while it took nine days for withering to be completed on *Tridax* and *Physalis*.

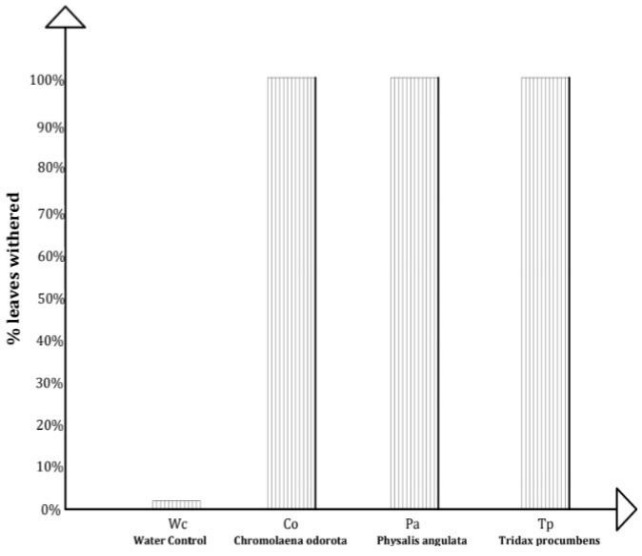

Figure 1: Effect of Innoculants on percentage Leaves Withered

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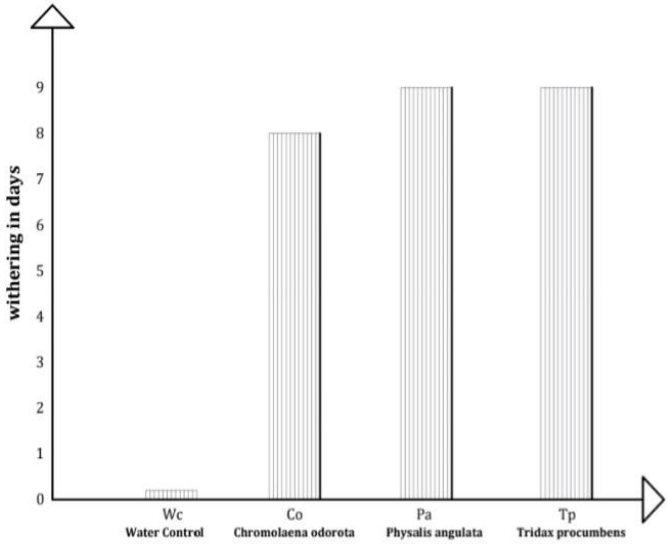

Figure 2: Effect of Innoculants on Withering in Days

The production of phytotoxic metabolite by the *Pseudomonas spp*. has been reported by various researchers. For example, *Pseudomonas hrassicacearum* YC5480 was found to producephytotoxic compounds identified as 2,4 diacetylphloroglucinol (DAPG) and 24-6trihydroxyacctophenol (THA)[5]. The isolates of *Pseudomonas syringae* were also found to produce phytotoxic compounds such as syringomycin and syringopcctin whosemode of action, regulation and biosynthesis by peptide and polyketide synthetases has been elucidated [6].

The weeds in the control pots that were sprayed with water alone were green and flourishing with minimal shedding of leaves as the water sprayed on them did not have any negative effect on the health of the leaves. Water act as a medium for the absorption of dissolved mineral salts and as medium of transport for plant nutrients[7]. The bioherbicide formulation produced by the addition of water to the phytotoxic metabolite of the wild strain of *P. putida* induced chlorosis in the weeds between 8 and 9 days. Hasan *et al.* (2021) described water as the simplest bioherbicide delivery system formulated as sprayable suspension.

Bioherbicide has been shown to have adverse effect on plant growth and this effect may range from morphological aberration, reduction in biomass to stomata abnormalities [8].

IV. CONCLUSION

This study has shown the potential of the bioherbicide produced for the control of the three weeds viz *Tridax procumbens, Chromolaena odorata* and *Physalis angulate* therefore the exploitation of microorganism as agent of weed control is thereby recommended to reduce environmental pollution caused by application of chemical herbicide.

V. RECOMMENDATION

Due to the fact that this research work is based on three weeds, the following recommendations are made:

(i) Further research should be carried out to access the effect of the bioherbicide on more weeds
(ii) Other plant pathogens can be screened for phytotoxic metabolite.
(iii) Bioherbicide with vast host range can also be produced to help in fields infested with different array of weeds.

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