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CONTENTS

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Studies on Herbicidal and Anti-Fungal activity of *Drechslera puttaparthii sp.nov.*

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ABSTRACT: **In this study, a newly discovered species of Drechslera, namely *Drechslera puttaparthii sp.nov* is tested for herbicidal and anti-fungal activity. The fungus is cultured in Bioreactor and Rotary shaker at varying pH for extraction of crude metabolite. The crude metabolite produced by the fungus is then tested for herbicidal and anti-fungal activity. *Parthenium hyterophorus* and *Neurospora species* were chosen to study herbicidal and anti-fungal activity respectively. This present preliminary investigation revealed significant herbicidal activity and anti-fungal activity as observed by wilting of *Parthenium* plant and inhibition of *Neurospora* colonies on agar plate by *Drechslera* metabolites. Best results were obtained by growing *Drechslera puttaparthii sp.nov* at pH 5.4 on Czapex-dox medium. Further work to isolate the compounds responsible for herbicidal and anti-fungal activity is in progress.**

Key words; *Drechslera puttaparthii sp.nov*, Herbicidal activity, Anti-Fungal activity.

Identification of microorganisms that produce bioactive compounds is of great interest in the development of new molecules to fight against many pathogens (Dreyfuss and Chapela, 1994). Fungi produce a wide range of secondary metabolites with high therapeutic value as antibiotics, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor, repellent etc., (Demain, 1999). Secondary metabolites produced from fungi vary in production, function and specifity to a particular fungus (Keller, 2002). These metabolites are being exploited in different fields of medicine and industries (Huisman et al., 2002). Drechslera is a fungal genus well known for the production of phytotoxic secondary metabolites. Some phytotoxins were also isolated from pathogens of grass weeds and proposed as potential natural herbicides. Recently, a novel phytotoxin named drazepinone was also identified as the major phytotoxic compound produced by a strain of *Drechslera siccans*. Chandramohan et al. have shown that *D. giganteana* alone and in combination with two other grass pathogens, *Exserohilum longirostratum and Exserohilum rostratum*, could be used in a bio-herbicide cocktail to control seven different weedy grasses (Charudattan et al., 1991). There are about 30,000 species of plants that are considered to be weeds and are directly or indirectly noxious to humans and their domesticated animals. About 1,600 of these can cause serious crop losses, with many crops having several competing weeds and costing close to $14 billion dollars in losses and control of these pests (Boger and Sandmann, 1989). In order to control weeds and prevent these losses, agriculturalists have turned increasingly to the use of herbicides. As you are well aware through newspaper and TV reports, many of the herbicides have gotten into our drinking water and lakes and have the potential of causing serious health problems. Several cities have recently detected alarming levels of herbicides and other pesticides in their water systems. Bio-herbicides have advantages over chemical herbicides in that they can be more host-specific, preparation costs will be less expensive, and human health hazards can be eliminated (Barrett, 1982; Wapshere et al., 1974). Biological control of weeds is a positive avenue to approach this problem. Our present study is in tune with the ongoing search for isolation of novel bio-herbicidal agents. Since Drechslera species are known to produce herbicidal compounds, we have chosen to study herbicidal potential of *Drechslera puttaparthii sp.nov*. Parthenium hysterophorus, commonly referred to as Congress Grass Weed, is a major problem in the agricultural lands of south India. We selected Parthenium hysterophorus plants growing in adjacent rice fields to test herbicidal activity. In the second part of the study, anti fungal activity of *Drechslera puttaparthii sp.nov* is tested on Neurospora species. Neurospora species are air borne fungi which cause allergy. Some species of Neurospora are laboratory contaminants. For these reasons anti fungal activity of *Drechslera puttaparthii sp.nov* is studied on Neurospora species. Local strains of Neurospora growing as laboratory contaminants in our university were chosen for this purpose of studying anti-fungal activity.

Methods

A. Culturing of drechslera puttaparthii sp.nov in bioreactor: *Drechslera puttaparthii sp.nov* was aseptically inoculated in baby fermentor (1 lit.) which contained Potato Dextrose broth medium amended with tetracycline. It was grown for 5 to 6 days and pH of 5.6 was maintained by adding dilute hydrochloric acid and Sodium hydroxide (NaOH). The crude metabolite was obtained by filtration using Whatman No. 1 filter paper.

B. Culturing of drechslera puttaparthii sp.nov in rotary shaker

a. Potato Dextrose broth medium; In shake culture, *Drechslera puttaparthii sp.nov* was aseptically inoculated in a conical flask which contained Potato Dextrose broth medium amended with tetracycline. It was grown for 5 to 6 days and pH of 5.6 was maintained. The crude metabolite was obtained by filtration.

b. Czapex-dox medium; Using the same shake culture technique, *Drechslera puttaparthii sp.nov* was aseptically inoculated in a conical flask which contained Czapex-dox medium. This Czapex-dox medium is specially used for culturing of fungi, so the addition of antibiotic to the medium can be avoided. Drechslera puttaparthii sp.nov is grown in there different pH of 5.4, 6.3 and 7.2. Fungus is allowed to grow for 5 days after which the crude metabolite was obtained by filtration using Whatman filter paper.

C. Test for herbicidal activity: Herbicidal activity of Drechslera puttaparthii sp.nov is tested on common grass weed *Parthenium histerophorus*. For this purpose healthy Parthenium plants were collected from paddy fields.

Healthy Parthenium plants were placed in beakers containing crude metabolite, extracted from Drechslera puttaparthii sp.nov growing on Czapex-dox medium. Pure water is used as control. The effect of Drechslera extract on Parthenium plant is visually observed after 24 hours of incubation.

D. Test for anti-fungal activity: The agar diffusion test, or the Kirby-Bauer disk-diffusion method, is a means of measuring the effect of an antimicrobial agent against fungi grown in culture. The fungus in question is swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against other fungi at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound. Paper disc diffusion method was used to observe antifungal activity. The paper discs were soaked in the metabolites for an hour or two and air dried discs were aseptically placed in the center of the Petri plate which contained PDA medium seeded with *Neurospora specie,* which is a common laboratory contaminant.

Results

After six days of culturing Drechslera puttaparthii sp.nov in bioreactor (Plate 1) and rotary shaker (Plate 2) in Potato dextrose medium, visible change in the colour (pinkish) of the medium and also the growth of the mycelium was observed. The rotary shaker showed a peculiar globular formation of mycelium (Plate 2). The metabolite from pH of 5.4 (Plate 4) was showing strongest herbicidal activity on *Parthenium* weed. Wilting and yellowing of plant was observed after 12 hours in both the cases (Plate 5). The metabolite from pH 6.3 was showing the same effect after 24 hours (Plate 6). Water as a control did not show any visible effect on *Parthenium* weed. Metabolites produced by Drechslera puttaparthii sp.nov showed significant anti fungal activity against Neurospora species (Plates 7-10). Metabolite started diffusing out of the filter paper disc after 6 hours of incubation (Plate 7). After incubation of filter paper disc impregnated with metabolite for 12 hours, a distinct inhibitory zone is observed around Neurospora colonies growing in Agar medium (Plate 8). Control was maintained using water. It did not show any inhibitory zone. The inhibition zone of metabolite is between 2 - 4 mm diameters.

Figures

A. Culturing of Drechslera puttaparthii in bioreactor and rotary shaker for extraction of metabolites

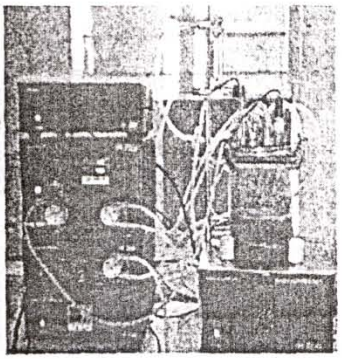


Plate -1

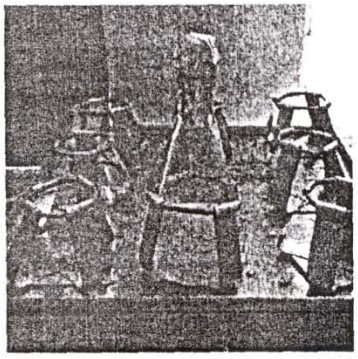


Plate-2

Plate-3

B. Herbicidal activity

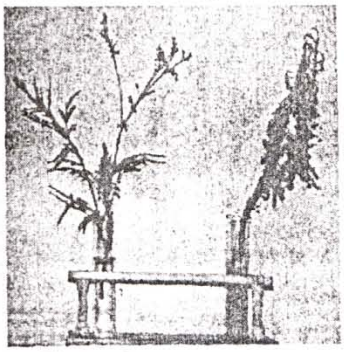


Plate -4
Control (left), pH-5.4 (Right)

Plate-5
pH-5.4 (Left), pH-6.3 (Right)

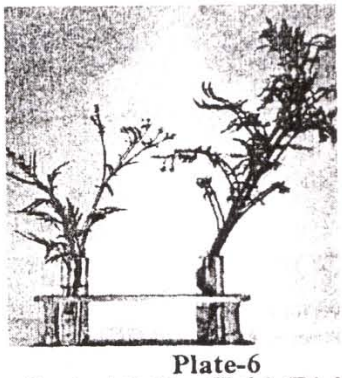
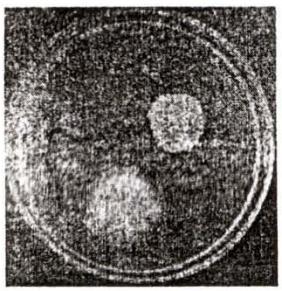


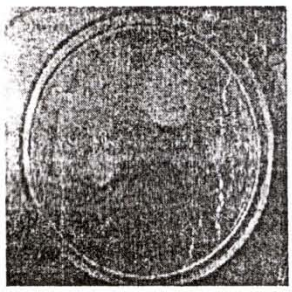
Plate-6
Control (left), pH-6.3 (Right)

C. Anti-fungal activity



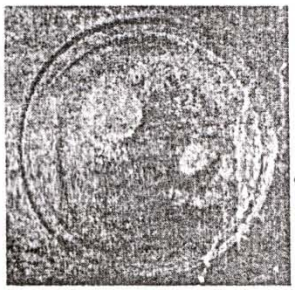
After 6 hrs of Incubation

PLATE 7



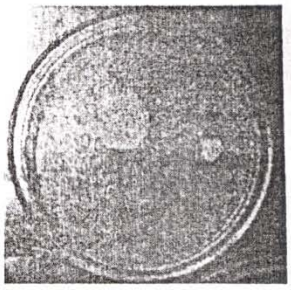
After 12 hrs of Inccubation

PLATE 8



After 18 hrs of Incubation

PLATE 9



After 24 hrs of Incubation

PLATE 10

Discussion

Herbicidal potential of ophiobolins produced by Drechslera giganteana was reported by Heald and Wolf in 2002. In a very active research program on the biocontrol of weeds conducted at the University of Florida under the supervision of Dr. Charudattan, a new fungus *Phomopsis amaranthicola* is able to control pigweed; *Dreschlera/Exerohilum* cocktail is able to control various grasses; *Bipolaris saccharii* is able to control cogon grass. In our present investigation also the crude metabolite obtained from *Drechslera puttaparthii sp.nov* showed that it can serve as a myco-herbicide against *Parthenium* weed *(Parthenium hysterophorus).* It also showed its antifungal activity against *Neurospora species,* which is a common laboratory contaminant. So, it gives an insight that it can be used as a myco-fungicide. This preliminary investigation reveals that *Drechslera puttaparthii sp.nov* can be used as environmentally safe antifungal and herbicidal material, further work needs to be done in this direction.

Aknowledgements

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References

Barrett 1982. Evaluation of *Alternaria cassiae* as a mycoherbicide for sicklepod (*Cassia obtusifolia*) in regional field test. Auburn, AL: Alabama Agric. Exp. Statiion South. Coop.

Boger and Sandmann 1989. Pathogens with potential for weed control. *Inn* R. E.

Charudattan Boyette, C. D. , P. C. Quimby Jr , A. J. Caesar , J. L. Birdsall , W. J. Connick Jr , D. J. Daigle , M. A. Jackson , G. H. Egley ,1991. Adjuvants, formulations, and spraying systems for improvement of mycoherbicides. Weed Technol 10:637–644.

Demain AL.Pharmaceutiically active secondary metabolites of microorgaanisms. Appl Microbiol Biotechnol. 1999 Oct;52 (4):455-63.

Dreyfuss MM, Chapela IH. Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals.B iotechnology. 1994;26:49-80.

Hoagland, ed. Microbes a nd Microbial Products as Herbicides. Am. Che m. Soc. Symp. Ser. No. 439, Washingtonn, DC: ACS Books. pp. 132–154.

Huisman. G.W, C. D. Bo yette, and R. E. Hoagland. 2002. Phytotoxicityy of *Fusarium*, other fungal isolates, and of the phytotoxins fumonisin, fusaric acid and moniliformin to jimsonweed. Phytoprotection 76:17–25.

Keller, C, 2002. Bioherbicidal potential of *Fusarium moniliforme* and its phytotoxin, fumonisin. Weed Sci 39: 673–677. Ser. Bull. 317. 19 p.

Wapshere N., Charudattan, R. , V. J. Prange , and J. T. Devalerio . 1974. Exploration of the use of the "bialaphos genes" for improving bioherbicide efficacy. Weed Technol 10:625–636.