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Evaluation of herbicidal activity of weed pathogenic fungi secondary metabolites as mycoherbicide for Weed *Sida acuta* Burm f

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ABSTRACT
Sida acuta Burm f (common wireweed) is a small, erect, perennial shrub that grows wild in wastelands and along roadsides. It is native to Central America has spread throughout the tropics and sub-tropics in the Pacific, Asia and Africa and has successfully invaded the tropics worldwide, largely as a contaminant in pasture seed. It infests various crops and habitats but has been most problematic in pastures and rangelands. It is declared a Class B (growth and spread to be controlled) and Class C (not to be introduced) weed in the Northern Territory, Australia. We have conducted survey on weed plants for the primary investigation of natural enemies which may became promising mycoherbicidal agents. The fungal isolates such as Alternaria alternata (FGCCW#06), Helminthosporium (FGCCW#53), Bipolaris sp. (FGCCW#11), and Curvularia lunata (FGCCW#21) were screened from diseased leaf spots. Colletotrichum sp. (FGCCW#29) was isolated from diseased foliage, Chaetomium globosum (FGCCW#51) was isolated from diseased stem and Fusarium oxysporum (FGCCW#41) from infected root parts of weed on different fungal growth media. The mycoherbicidal activity of the isolates were examined to select herbicidal activity against host weed. There are various steps viz. selection of suitable microbial strain to final formulation for development of potential mycoherbicide. The main aim of this study to select a potential fungus those producing secondary metabolites with potential herbicidal compound for control of weed. The phytotoxicity of fungal metabolites was assessed via biological tests using the weed Sida acuta and the most promising strain was identified by assay. All isolated fungi and their secondary metabolites were tested on target weed. Fungus FGCCW#53 belonging to the genus Helminthosporium cell free culture broth showed the most pronounced herbicidal activity against Sida actua.
Keywords: Bioprospecting/Sida acuta/Weed pathogenic Fungi/Biological Control/ Mycoherbicides/ Biorational

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I. Introduction

S. acuta , a native to Central America is considered to be one of the most serious weed of pastures and rangelands and has spread throughout the tropics and sub-tropics in the Pacific, Asia and Africa(Parsons and Cuthbertson, 1992). It is a weed of plantation crops, cereals, root crops and vegetables throughout the Pacific and South-East Asia. It is reported as major weed of maize in Mexico, sorghum in Australia and Thailand, tomatoes in the Philippines, onions in Brazil, Pastures in Australia, Fiji, Nigeria and Papua New Guinea, Tea in Taiwan and Sri Lanka, Groundnuts in Ghana, cassava in Ghana and Nigeria, Maize in Ghana, Nigeria and Thailand, Coconuts in Trinidad, Beans in Brazil, Coconuts in Sri Lanka, Pineapples in the Philippines, Sugarcane and groundnuts in Australia, El Salvador and Trinidad, coffee in Colombia, rubber in Malaysia, upland rice in the Philippines and Nigeria, cotton in El Salvador and Thailand, and cowpeas and sweet potatoes in Nigeria (Ham and Eastick, 2004).

The management of this weed control strategies are hand weeding, mechanical weeding and weeding by chemical herbicides. Hand weeding is time consuming and is becoming expensive, while the use of mechanical weeders reduces yield. Although weeds have been eradicated using various cultural practices in current farming methods, the chemical herbicides have been heavily used for weeds control is the most effective and immediate method. Some problems, however, have been emerged in association with heavy use of herbicides such as the appearance of herbicide resistant weeds and the contamination of soil and ground water by chemical residues which lead to the environmental pollution (Boyette 1994).

Biological control of weeds using plant pathogens is a practical and environmentally sound method of weed management. A variety of herbaceous, woody, climbing, aquatic, and parasitic weeds have been shown to be capable of being controlled by plant pathogens (Charudattan 1991).

Biological control with plant pathogens is an effective, safe, selective and practical means of weed management that has gained considerable importance (Charudattan1986; Flint & Thomson 2000; Pemberton & Strong 2000; Bouda et al. 2001). The study on survey, isolation and pathogenicity of fungal pathogens on weeds can explores the biological control methods and the finding of new biocontrol agents for the control of some serious agricultural weeds commonly compete with agricultural crops, pasture and open field weeds. Weed pathogenic fungi that are basically classified as necrotrophs, hemibiotrophs and biotrophs constitute one of the main infectious agents in weeds, causing alterations during developmental stages including post-harvest, gaining nutrients from the plants they invade and, therefore, resulting in huge economic damage. The application of spores as mycoherbicide have several environmental limitations so application of secondary metabolites is good alternative as weed control. Fungi are rich sources of thousands of secondary metabolites (SMs), which consist of low-molecular weight compounds (the number of the described compounds exceeds 100,000) that are usually regarded as not essential for life while their role are quite versatile (Perez-Nadales et al., 2014; Scharf et al., 2014). Fungal metabolites are divided into four main chemical classes viz., polyketides, terpenoids, shikimic acid derived compounds, and non-ribosomal peptides. Fungal metabolites in addition to synthetic chemicals are possible source of new herbicidal substance. Bialaphos was developed as the first practical herbicide of actinomycetes origin. Herbicide that inhibits photosynthesis has a remarkable selective toxicity between plants and animals. Therefore, it is important to test metabolites are photosynthesis inhibitors. To detect inhibition of photosynthesis to screen a potential of microbial culture broths, de novo starch synthesis in excised leaf segments were tested by a method developed by Kida et al. 1985.

All biologically active compounds of presence in culture filtrate were examined for inhibition of de novo starch synthesis in the excised leaf segments. They included: Herbicides that inhibit photosynthesis, Herbicides that do not inhibit photosynthesis. The de nova starch synthesis is closely associated with oxygen evolution in excised leaf photosynthesis. Therefore, several kinds of potent inhibitors as detected by the starch synthesis system were examined for their effects on oxygen evolution. Not only photosynthesis inhibitors but protein synthesis or respiratory system inhibited by de novo starch synthesis in leaf and indicated that its mechanism is coupled with de novo protein synthesis. The objective of the study is survey, isolation and identification weed pathogens, production of cell free broth and study of photosynthesis inhibitors activity of cell free broth of their host and conform their phytotoxicity. The isolates of fungi from naturally infected weeds may be useful to control weeds and they may act as potential biocontrol agents.

II. Materials and Methods

Bioprospecting: Field Survey and Collection of infected leaf parts

Detailed field surveys were undertaken between the months of July 2004 - November 2005 to find out disease on weed plants and collection of infected part to get potential strains. The weed plants were collected from field area. Weeds with various symptoms at field sites were carefully collected into sterile polythene bags. The weed parts infected with various fungal diseases were observed and collected and brought to Mycological Research Laboratory, Department of Bioscience, R D University, Jabalpur for preservation, observation and isolation of fungi. The collected weed parts were pressed, dried, preserved and properly identified with the help of available literature and monographs (Pullaiah & Chennaiah 1997, Rao & Kumari 2002; Venkaiah 2004).

Isolation of fungal pathogens

The diseased leaves were washed thoroughly under running tap water to remove soil particles and the infected portions of the leaves were cut into 1.0 to 1.5 cm. fragments. The pieces were surface sterilized by 70% ethyl alcohol for 1-2 minutes and then rinsed in sterile distilled water for three to four times. Finally, the leaf bits were rinsed in 0.01% mercuric chloride for 1 or 2 minutes fallowed by washing with sterile autoclaved double distilled water for 2 or 3 times. These fragments were transferred on to Potato dextrose agar (PDA) plates supplemented with 1.0% streptomycin sulphate (antibiotic solution) under completely sterile conditions in an inoculation chamber. After inoculation plates were incubated at 26 ±2°C for 21 days on 12 h light/dark photoperiod. The isolates were made using sterile needles when colonies formed on culture plates with sporulation. The small fragments or single conidia were used to prepare pure stock cultures under aseptic conditions. Stock cultures of the isolates were maintained to harvest spore production. Different agar media such as PDA, Czapak Dox Agar (CDA) and Sabouraud's Dextrose Agar (SDA) supplemented with 1% Streptomycin (1g of streptomycin was mixed thoroughly in 100 ml of sterilized distilled water) were used for the growth and optimum spore production. 1.0 % Streptomycin was used as an antibiotic for the control of bacterial growth in culture. Fungi were maintained on half strength PDA slants as stock cultures for further research in Fungal germ plasm collection centre (FGCC) of Mycological Research Laboratory, Dept of Bioscience, R D University, Jabalpur.

Identification of fungal pathogens

With the help of relevant fungal taxonomy manual and literature, we identified the fungal isolates by previous work of authors (Ellis 1976, Sutton 1980, Domsch et al. 2007). Fungal morphology was studied macroscopically by observing colony features (colony diameter, colour texture and pigmentation) and microscopically by staining with lactophenol cotton blue (mounting fluid). A small pinpoint mycelium of each isolate was examined under compound microscope for the observation of the conidia, conidiophores and arrangement of spores.

Liquid Fermentation

The growth of all phytopathogenic fungi isolated in the previous step was carried out in a liquid medium, aiming at the production of bioactive molecules with herbicidal action. For the pre-inoculum production, mycelium from each test tube containing one fungus was inoculated on PDA in a Petri dish and incubated for eight days at 28 °C, which was sufficient for the fungal growth to cover the entire surface of the agar. Afterwards, the agar surface in the Petri dish was washed with 5 mL of autoclaved water, and the suspension was transferred for fermentation. The fermentations were carried out in 250-mL Erlenmeyer flasks containing 125 mL of fermentation medium at 28 °C, 120 rpm for seven days. The medium was composed of (g L−1): glucose, 10.0; yeast extract, 7.5; peptone, 10.0; (NH4)2SO4, 2.0; FeSO4·7H2O, 1.0; MnSO4·H2O, 1.0; and MgSO4, 0.5, and the initial pH was adjusted to 6.

Preparation of culture filtrates

After the fermentation, the culture filtrate was individually filtrated and the pH of the culture filtrates were adjusted to 4.0 by adding 0.5 mol/L HCl. To obtain the cell free supernatants, the culture filtrates were set on a centrifuge at 10,000×g for 15 min and then passed through a 0.2 μm filter (Sartorius) separately. The 20% diluted culture filtrates were made by adding SDW. The filtered sample was used to evaluate its bioherbicidal activity in the bioassay. Each of the isolated fungi was considered a different bioherbicide.

Test Plants

The small seedlings of *Sida acuta* were collected from agricultural fields during the field study. The collected small seedlings were potted in plastic glass. The pots containing seedlings of weed plants were maintained in a greenhouse with a 12 h light/dark photoperiod. For studies, weed was maintained in replicates along with control plant. The plants in the greenhouse were watered daily and fertilized farmyard manure when required.

Seedling Bioassay: Screening of herbicidal metabolites generated from culture filtrates

To screen the herbicidal activities of the culture filtrates generated from all isolated and selected strain, Weeds leaf and stem parts sprayed using garden sprayer with 100 ml of cell free broth of each fungal isolates with 1 ml of Tween 20 Solution in one litre of water. The controlled plants were inoculated with 1 litre of sterilized double distilled water and 1 ml of Tween 20 solution. The phytotoxic symptoms were examined by visual observations of the symptoms on leaf surfaces as well as whole plants. The phytotoxic severity was recorded in terms of phytotoxic intensity by using a toxicity score chart. Phytotoxic intensity (DI), the intensity of infection was determined visually, based on the initiation of chlorosis and increase in necrotic area on the leaves, stems and roots of test plants every day. The necrotic spot of each fungal pathogen cell free broth on test plants was determined using a score chart (-,no symptoms, a healthy plant; +, mild symptoms, a plant showing slight symptoms on ≤15% of the leaf area; ++, moderate symptoms, a plant showing definitely bigger patches of necrotic areas on 16 to 59% of the leaf area; and +++, severe symptoms, enlarged lesions covering 60 to 80% of the leaf area) (Ray & Hill 2012).

Detection of de novo starch synthesis.

Seeds of *Sida actua* were sown in soil in plastic pots, watered, and allowed to germinate and grow in a greenhouse at 25 to 30°C. On the 10th day after planting (second leaf stage), the pots were transferred to a dark chamber and kept for 12hr at 25°C. Part of the second leaf was cut into segments of about 5 mm which were negative for starch when stained with iodine. Three segments were transferred to plastic Petri dishes (1 cm in diameter, Nunc) containing 1.8ml of the reaction medium and 0.2ml of a sample to be tested. The reaction medium consisted of 0.01 M potassium phosphate buffer (pH 6.5) and 0.01%(V/V) Tween 20 (A wetting agent). The petri dishes were illuminated at an intensity of 14 Klux for 16 hrs at 25^0C. the leaf segments were then removed, immersed in hot methanol for pigment extraction and stained with iodine (a solution of 0.2% iodine and 2% potassium iodide).

III. Results

Identification of foliar pathogenic fungi

The natural infection on weed plants was observed by disease symptoms on leaves, stems, roots and floral parts of various weed plants. The infected parts of the weeds were collected from field crops such as food crops, pulses, vegetable crops, oil crops and commercial crops where the weed community was competed with crops for their survival. Diseased leaves, stems, roots, flowers and whole plants of the weed flora were collected randomly from field. The common fungal symptoms on plants such as Leaf blights, leaf spots, root rot and anthracnose were observed on different parts of the weeds. Although all the stages of leaves showed infection, the mature leaves were more heavily affected. The parasitized fungi were isolated from some weeds infected with higher disease (Table 1). The fungal isolates such as *Alternaria alternata* (FGCCW#06), *Helminthosporium* (FGCCW#53), *Bipolaris* sp. (FGCCW#11), and *Curvularia lunata* (FGCCW#21) were screened from diseased leaf spots. *Colletotrichum* sp. (FGCCW#29) was isolated from diseased foliage, *Chaetomium globosum* (FGCCW#51) was isolated from diseased stem and *Fusarium oxysporum* (FGCCW#41) was isolated from infested root.

The identification of fungi was made by macro and microscopic observation. The microscopic characteristic of each isolate was studied using different structures of conidiophores, conidia and chlamydospores by slide culture technique.

Table 1 Fungal pathogens recorded on Sida acuta weed infested in fields

Pathogen	Symptoms
Alternaria alternata (FGCCW#06)	Leaf blight
Bipolaris sp. (FGCCW#11)	Leaf spot
Colletotrichum Sp. (FGCCW#29)	Diseased foliage
Chetomium globosum (FGCCW#51)	Leaf spot
Curvularia lunata (FGCCW#21)	Leaf spot
Fusarium oxysporum (FGCCW#41)	Root rot
Helminthosporium sp. (FGCCW#53)	Leaf spot

Alternaria alternata (FGCCW#06)

The fungus produced profuse mycelial growth on PDA. Initially the mycelium was hyaline that turned to grey brownish, multi-celled, septate and irregularly branched. In early growing stage hyphae were thin, narrow, and hyaline but became slightly thick as they grew old. Conidiophores arised singly or in clusters, usually 2-6 and were long or short. They were pale olivaceous to olivaceous-brown, straight or curved, geniculate, slightly swollen at apex having terminal scars indicating the point of attachment of conidia. Conidia were in chains, light olivaceous to dark brown, septate, muriform and measured 47.16 x 13.49 µm. Conidia were born in chains up to 10 or more on conidiophores. The chlamydospores were formed in the old culture of *A. alternata*. They were intercallary, thick walled, roundish to oval in shape, dark brown in colour.

Bipolaris sp. (FGCCW#11)

Colonies appeared black to greyish black in PDA; conidia relatively long and broad with dark brown colour, slender and slightly curved; Conidiophores brown, producing conidia through an apical pore and forming a new apex by growth of the subterminal region; conidia fusoid, straight or curved, germinating by one germ tube from each end; exosporium smooth, rigid, brown; endosporium hyaline, amorphous, separating cells of mature phragmospores; bipolaris has indeterminate conidiophores which extent sympodially producing a succession of dark, transversely septate, porospores. These are basically fusoid in shape and germinate only from the ends. The identification features of the isolates include the shape and colour of conidiophores and conidia. Conidiophores mid to dark brown in colour, medium to long, commonly long, slender, straight or curved, single or in groups of 2 or 3, pale near the apex, smooth, up to 700 µm long, and 5-10 µm thick, and bear conidia at wide intervals.

Colletotrichum sp. (FGCCW#29)

The white, gray-colored colonies with a dark and gray conidial mass in the center. Fruit body an acervulus; conidiophores produced in a dense, even stand on a thin or well-developed stroma; conidiophores simple, short, hyaline, producing abundant phialospores; phialospores produced in mucus, ovoid, non-septate, short-cylindric, falcate or crescent- shaped, hyaline, pinkish in mass, frequently producing dark setae; setae stout, septate, darkly pigmented, acutely pointed at the apex. The pink masses of amerospores with the dark setae standing out in sharp contrast are diagnostic for Colletotrichum.

***Chaetomium globosum* (FGCCW#51)**

Colonies grown on PSA media, aerial mycelium, pale brown, producing perithecia after five days; perithecia scattered or gregarious, globose to subglobose, broadly oval, olive green to greyish green, 160-390x 230-300 µm, thickly clothed with hairs; terminal hairs abundant, light coloured, finely roughened, obscurely septate, slender, about 3-4 µm in diameter, up to 77 µm long, tip blunt, undulate throughout, forming a dense inter-woven bushy head; lateral hairs light coloured, finely roughened, septate, 2.5-3.5 µm in diam, long, slender, straight to slightly flexed or undulate; asci oblong, clavate, 8-spored; ascosores dark, lemon shaped, broadly ovoid, apiculate at both ends, 8.5-10.5x8-9 µm.

Curvularia lunata (FGCCW#21)

Colonies blackish brown; stroma simple or branched; pesudothecia black, globose, usually forming on a columnar basal stroma or a flattened crust, 500 - 720 µm long, 400 – 490 µm wide, beaked with a conical trunkcate beak up to 300 µm high, 115-140 µm wide at the base, often hairy in the globose part, hairs, septate, simple, brown; aci cylindrical, short-stalked, wall not stained with lactophenol cotton blue, bitunicate,17-130x 12-13.5 µm, 2 – 8 spored; ascocarpes filiform, hyaline, helically coiled in the ascus and straightening at one or both ends, tapering at both ends, more at the base, sometimes with truncate base, mucilaginous, sheath up to 4 um thick. Conidiophores maco or mononematous, unbranched, terminal, often geniculate above, sympodial, cylindrical; conidia acropleurogenous, straight ovoid, obclavate or ellipsoidal, unequal sides or rarely with slight curvature, 3-5 mostly 3 -septate, middle calls darker, end cells subhyaline to pale or dark brown, mature conidia tuberculate, 23x-52x13-20 µm, young conidia subhyaline and smooth walled.

Fusarium oxysporum (FGCCW#41),

Colonies aerial mycelium, sparse to floccose, white or peach, but usually with a purple or violet tings; sporodochia discrete, erumpent, orange; reverse colourless, dark blue to dark purple; conidiophores unbranched or sparsely branched, monophilidic; stroma white, plectenchymatous, smooth, effuse; microconidia usually abundant, mostly 0 - septate, oval, ellipsoidal, kidney shaped or straight, produced on simple lateral phialides, solitary on free conidiophores never from in chains, 5-12x 2.3-3.5 µm; macroconidia 2-5 septa, spindle to fusiform, curved or almost straight, pointed at both ends, definitely or weakly pedicellate, 27-60x3-5 µm; chlamydospores mostly terminal, globose, smooth or roughened, 1-celled.

Helminthosporium (FGCCW#53)

Colonies effuse, dark, hairy; mycelium immersed in the substratum; stromata usually present conidiophores often fasciculate, erect, brown to dark brown; conidia develop laterally often in verticils, through pores beneath septa, while the tip of the conidiophores is actively growing and growth of the conidiophores ceases with the formation of terminal conidia. Colonies consist of conidiophores, loose or dense, regularly or irregularly velvety, brown to black, with strict or spreading margin. Conidiophores usually arise in groups, erect and straight, sometimes reclining, usually unbranched, only seldom with small side branches, septate, geniculate at pinpoints below the conidia, brown, green-brown to black, transparent or nontransparent. Conidia terminal or lateral on the geniculations, elongate, cylindrical, clavate or obclavate, smooth, mostly rounded at both ends, straight or bent, with more than four cross-walls, dark brown, green-brown to black, often with the end-cells lighter colour.

Screening of isolated strain for herbicidal activity:

Data recorded in tables 2 showed that toxicity of CFCF varied significantly amongst various fungal strains. CFCF obtained from Fusarium oxysporum FGCCW#43 showed strongest herbicidal potential against Sida acuta while CFCF from others ranked second and third respectively. CFCF of these strains showed herbicidal property.

Table 2: The phytotoxicity activity of Cell free culture filtrate of selected isolated fungi in seedling bioassay

Fungal isolate	**Inoculated parts**	**Phytotoxicity**
Alternaria alternata FGCCW#06	Leaves, Stems	++
Bipolaris sp. FGCCW#21	Leaves, Stems	+
Colletotrichum sp. FGCCW#11	Leaves, Stems	+
Curvularia lunata FGCC#21	Leaves, Stems	++
Fusarium oxysporum FGCCW#41	Leaves, Stems	++
Helminthosporium sp. FGCCW#53	Leaves, Stems	+++
Chetomium globosum FGCCW#51	Leaves, stems	+

DAT= Day after treatment

+= mild symptoms ++ = moderate symptoms +++ = severe symptoms

The results obtained by phytotoxicity chart confirmed that some of the test fungi were showed virulence on *Sida acuta* weeds (Table 2). The fungus *Helminthosporium* sp. FGCCW#53 was showing severe phytotoxicity on *Sida acuta* with dark brown-coloured spots on leaves and it caused necrosis of leaves. The fungus *Alternaria alternata* FGCCW#06, *Fusarium oxysporum* FGCCW#41, *Curvularia lunata* FGCCW#21 and *Colletotrichum* sp. FGCCW#11 were showed moderate symptoms of leaf chlorosis on weeds. The isolate *Chaetomium globosum* FGCCW#51 and *Bipolaris* sp. FGCCW#21 were considered as non-pathogenic fungi which was failed to produce any symptoms on host plant. The pathogenic fungi were reisolated from diseased leaves of inoculated plants and found similar to the original isolates in both macro and microscopic characteristics thus confirmed the pathogenicity of various test fungi on selected weeds.

Analysis of de novo Starch Synthesis in *Sida acuta* weed leaf Segments

Weed *Sida acuta* were examined for their starch accumulation in leaf tissue in order to determining de novo starch synthesis. After the weeds grown in a greenhouse their second and third leaves were cut into segments without dark incubation and tested for their starch accumulation as detected by iodine staining. Weeds were grown in plastic pots to the second leaf stage and then transferred to a dark chamber to remove starch in the leaves, indicated by a negative iodine stain. At least 8 hrs were needed to remove starch in every leaf tested. Therefore, all plants were kept in the dark for 12 hrs and then their leaves were cut into segments and subjected to starch synthesis. The synthesis was initiated by illumination in leaf segments floating on top of the medium in a petri dish. It took at least 5 hrs. before starch synthesized de novo could be detected with iodine staining. The starch synthesis was allowed to continue for 16 hrs for research on photosynthesis inhibitors. The most potent inhibitors of de novo starch synthesis were the photosynthesis inhibitors is *Helminthosporium* sp. FGCCW#53 metabolites. They inhibited starch synthesis at the concentration of 1 ppm. All isolate's metabolites tested as photosynthesis inhibitors, *Helminthosporium* sp. FGCCW#53 shown good results only(Singh 2007). Others were the weakest corresponding to its weaker starch synthesis inhibition.

Similar results were also confirmed by Kida et al. 1985 in the cotyledon disc bioassay using pumpkin as the experimental material. When pumpkin cotyledon discs were put on top of a buffer solution they continued to float as long as oxygen evolution occurred. When oxygen evolution was inhibited by adding photosynthesis inhibitors or shading from light, the cotyledon discs began to sink. Using this assay system, it was confirmed that the photosynthesis inhibitors restricted oxygen evolution while the other compound did not. In preliminary study, it was found that a number of culture filtrate inhibited starch synthesis because of cycloheximide produced in them. Fungal culture filtrates were tested in this bioassay system. Only Helminthosporium sp. FGCCW#53 culture filtrates were obtained as good candidates for further purification studies and mycoherbicide development for *Sida acuta*.

IV. Discussion

A total of 7 genera of fungal pathogens, namely Alternaria alternata, Colletotrichum sp., Helminthosporium sp., Bipolaris sp., Fusarium oxysporum, Curvularia lunata and Chaetomium globosum, were isolated from parasitized parts of the weed flora identified in crop fields at Jabalpur, MP. The characteristic features were studied using macro and microscopic methods and the final results were compared with early extensive study of various authors. The characteristic study on mycoflora was useful to understand the diagnostic features of the isolate. The in vitro studies were carried for the primary screening of host specific weeds which is the pioneer stage to develop mycoherbicide agents. Among the isolates, species of *Alternaria alternata, Fusarium oxysporum*, and *Colletotrichum* sp. were well developed as bio control agents for the management of various problematic weeds in crop fields in worldwide. Recently the isolates belonging to *Helminthosporium* sp., *Chaetomium* sp., *Bipolaris* sp., and *Curvularia* sp. were used as weed control agents by spraying their cell free culture filtrate into some serious weeds. Pathogenicity and host range tests of the study showed that a total of 7 isolates were primarily screened as bio control agents through in vitro studies and the genus of *Alternaria, Curvularia, Colletotrichum*, and *Fusarium* having the biological control potential and the remain genus belonging to *Helminthosporium, Chaetomium* and *Bipolaris* were under study.

Several microorganisms have been studied or are under development as potential sources for microbial herbicides. Biological herbicides represent a means to reduce dependence on synthetic herbicides; focus on ecologically grounded methods of management; reduce weed seed bank populations through environmentally friendly practices; and potentially reduce costs of weed control in crop production (Kennedy & Stubbs 2007). Recently the awareness of biological control methods was developed among the farmers and agronomists, researchers and plant breeders in India. The study on pathogenicity of fungal pathogens is more useful for the future steps and development of new agents in biological control of agricultural weeds by indigenous fungal pathogens. The heavy weed infestation was observed in various crop fields of study area during the extensive surveys conducted in agricultures fields. Some of these weeds may be controlled by the fungal species of *Alternaria alternata, Fusarium oxysporum, Curvularia lunata* and *Colletotrichum* sp. whose pathogenicity was confirmed using Koch's postulates. These biocontrol agents were showing more pathogenic nature and narrow

host range on some serious weeds of many countries and released into market as commercial mycoherbicides. In contrast, to this, Saxena and Pandey, 2001 and Thapar et al. 2002 have reported appreciable phytotoxicity of secondary metabolites from *Alternaria* isolate LC#110 and LC#104 against *Lantana camara* and *C. lunata* PH#38 against Parthenium respectively.

The above findings clearly indicate that among seven strains screened, CFCF of *Helminthosporium* sp. FGCCW#53 have significant herbicidal potential against the test weeds *Sida acuta*. Thus, it can be concluded that these strains could be used for production of herbicidal compounds. However, further investigations are needed to develop suitable formulations and production technologies before its large-scale application.

There are diverse microbial metabolites which can be the origin of many potent biologically active compounds. They are used as biopharmaceutical or bio agriculture importance compound. Currently there is only one bioherbicide i.e., bialaphos, others are synthetic chemicals have been employed as herbicides. The herbicidal activity and amount in culture broth is required to detect from fungal metabolites. The study suggested that the isolates may considered as desirable agents for the biological control of some weeds after the extensive work on the impact of the field environment and application technology on the virulence of these pathogens as mycoherbicides in India.

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