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Isolation and Characterization of Mycoflora from Infected Weeds

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A B S T R A C T

An epidemic study was conducted on weed plants for the primary investigation of natural enemies which may became promising biocontrol agents. A total of 7 weed plants include *Commelina benghalensis, Cyperus rotundus, Crotalaria verrucosa, Digera muricata, Sida cordifolia, Ipomoea pestigridis* and *Trianthema portulacastrum* were selected for *in vitro* studies. Fungal isolates namely *Ascochyta cypericola, Bipolaris* sp., *Chaetomium globosum, Colletotrichum capsici, Colletotrichum* sp., *Curvularia lunata, Curvularia tuberculata, Fusarium oxysporum, Helminthosporium* sp. and *Gibbago trianthemae* were isolated by inoculation of parasitized weed propagules like infected leaves, stems, root and floral parts on different fungal growth media. The diagnostic characteristic features of each isolate were critically studied using relevant literature. The pathogenicity of the isolates was examined to select biocontrol agent against host weed.

Keywords: Weed infestation, Biological Control, Mycoherbicides, *Gibbago Trianthemae Simmons*

Introduction

Weed infestation is one of the major constraints affecting the production of several crops. The common weed control strategies are water management, hand weeding, mechanical weeding and weeding by chemical herbicides. Water management can control certain weed species in irrigated lowlands. 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 weed 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. Chemical herbicides, on the other hand, not only more expensive but also contribute to environmental contamination. The continuous use of chemical herbicides can result in the development of herbicide-tolerant weed populations (Boyette 1994). Increasing awareness of herbicide influence on food crops and environment has encouraged researchers to develop alternative weed control approaches such as biological control (Charudattan 2001).

Weed control using this approach can complement and be integrated with traditional cultural and chemical methods of weed control. 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).

Fungi, the largest group of pathogens, can infect several kinds of plants and causes numerous diseases and they become in a wide variety and require a specific type of host. The study on 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. The investigation on fungal pathogens may useful to researchers, agronomists and formers to develop biocontrol agents (mycoherbicides) for weed management as well as sustainable agriculture. The method, biological control of weeds is eco-friendly and can enhance the soil fertility by avoiding herbicide pollution. The study includes the isolation of weed pathogens (natural enemies) from diseased weed plants, whose appearance were problematic in field. The hypothesis behind the research is that the fungal pathogens which control the population of several weeds by causing foliar diseases or root diseases on weed plants in nature may become effective biological control agents after the mass production of the agent and foliar application on weed plants.

The objective of the study is the isolation and identification of some weed pathogens from infected parts of their host and conform their pathogenicity. The isolates of fungi from naturally infected weeds may be useful to control some specific weeds and they may act as potential biocontrol agents.

Materials and Methods

Field study and sampling

Detailed field surveys were undertaken between the months of July 2011- November 2011 to find out the most common disease on weed plants. Throughout the study systematic observations were made in crop fields to identified infected weeds using epidemic studies. A total of 20 locations were selected for the observations and finally some of the weed plants highly infected with fungal disease symptoms ware noticed. A total of seven weeds with diseased symptoms were selected for the study of disease intensity of pathogens on their respective host species. The weed plants include *Commelina benghalensis* L. (Commelinaceae), *Cyperus rotundus* L. (Cyperaceae), *Crotalaria verrucosa* L. (Fabaceae), *Digera muricata* (L.) Mart. (Amaranthaceae), *Sida cordifolia* L. (Malvaceae), *Ipomoea pestigridis* L. (Convolvulaceae) and *Trianthema portulacastrum* L. (Aizoaceae) were collected from field area. 10 random samples of each plant were taken from each site for pathological studies.

All the weed plants filled with various symptoms at field sites were carefully collected into sterile polythene bags. The plant parts infected with various fungal diseases were observed and collected and brought to plant pathology laboratory at Department of Botany, Andhra University, Visakhapatnam for

preservation, observation and isolation of fungi. The collected weed plants were pressed, dried, preserved and properly identified with the help of available literature and monographs (Hooker 1872-1897; Gamble & Fischer 1915-1935; Bor 1960; Pullaiah & Chennaiah 1997; Rao & Sriramualu 1986; 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 sterilised 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 culture plates were incubated with artificial light supplied by fluorescent light. 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, Czapek,s 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. The pH of the culture media was standardised at 25^{o}C. 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.

Identification of fungal pathogens

Identification of the fungal isolates was made with help of the relevant literature (Gilman 1957; Barnett 1960; Barron 1968; Ellis 1971; Ellis 1976; Booth 1977; Sutton 1980; Sivanesan 1987; Holliday 1993; Domsch *et al.* 2007; Nagamani *et al.* 2006). The diagnostic characteristics of the each isolate were compared with previous work of the various authors. Fungal morphology was studied macroscopically by observing colony features (colony diameter, colour, texture and pigmentation) and microscopically by staining with lacto phenol 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. The macroscopic (colony) photographs of isolates were taken with digital camera (Nikon Coolpix S6700 20.1MP) and microscopic photographs were taken at Advanced Laboratory, Department of Botany, Andhra University, Visakhapatnam.

Preparation of spore inoculum

The fungal isolates were cultured on PDA in petridishes and incubated for 14 days at 26 ± 2°C with a 12 h light/dark photoperiod. After that, conidia and mycelium were harvested with a sterilized spatula by flooding the cultures with10 ml sterile distilled water and then scraping the mycelial mass slowly for conidial suspension. The suspension was then filtered through sterile muslin cloth folded in four layers and the final inoculum was taken into 100 ml conical flasks containing sterile distilled water mixed with 0.02% (v/v) Tween 20 (Merck). The inoculum concentration was adjusted to 10^4 - 10^6 spores/ml using Improved Neubauer haemocytometer (Depth = 0.1mm).

Test Plants

Seeds and seedlings of various weeds were collected from agricultural fields during the field study. The collected seeds were dried and maintained in healthy condition without any contamination. The plants for host range studies were grown by sowing the seeds in plastic pots (25 x 20 cm) containing sterilized, black soil. The pots containing seedlings of weed plants were maintained in a green house with a 12 h light/dark photoperiod. For host range studies, each weed was maintained in replicates along with control plant. The plants in the greenhouse were watered daily and fertilized farmyard manure when required.

Pathogenic tests on selected weeds

Pathogenic nature of the isolates was tested on some selected weeds and crop plants of various families to study the host rang of fungal pathogens. The test plants of *Commelina benghalensis* L. (Commelinaceae), *Cyperus rotundus* L. (Cyperaceae), *Crotalaria verrucosa* L. (Fabaceae), *Digera muricata* (L.) Mart. (Amaranthaceae), *Sida cordifolia* L. (Malvaceae), *Ipomoea pestigridis* L. (Convolvulaceae), and *Trianthema portulacastrum* L. (Aizoaceae) were inoculated with 5 x 10^4spores /ml of each isolate. Crop plants include *Oryza sativa* L. (Poaceae), *Zea mays* L. (Poaceae), *Cajanus cajan* (L.) Millsp. (Fabaceae), *Solanum melongena* L. (Solanaceae), *Abelmoschus esculentus* (L.) (Moench) (Malvaceae), *Lycopersicon esculentum* Miller. (Solanaceae), *Amaranthus viridis* L. (Amaranthaceae), and *Brassica oleraceae* L.(Brassicaceae) were used for host range studies of the different isolates. Crop plants also inoculated with same concentration (5x 10^4 spores /ml) of each fungal isolate. The controlled plants were inoculated with sterilized double distilled water + 0.02% Tween 20 solution. Inoculum was applied onto the plants within 2 hrs of sunset to avoid drying and to allow for a natural dew period shortly afterwards.

The replicates of both test plants and controlled plants were covered with polythene bags to maintain humidly for spore germination on the surface of the test plants for 24 h. Plants were observed three days after treatment (DAT) for disease symptoms. The disease severity was examined by visual observations of the disease symptoms on leaf surfaces as well as whole plants. The disease se-

verity was recorded in terms of disease intensity by using a disease score chart.

Disease intensity (DI)

The intensity of infection was determined visually, based on the initiation of disease and increase in disease area on the leaves, stems and roots of test plants every day. The disease intensity of each fungal pathogen 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 diseased 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).

Results

Screening of the 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 plant parts of the weeds (Fig.1). 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, Ascochyta* sp. were isolated from diseased leaves of *Commelina benghalensis* L. (Commelinaceae); *Ascochyta cypericola, Helminthosporium* sp. were screened from diseased leaves of *Cyperus rotundus* L. (Cyperaceae); *Chaetomium globosum* was isolated from diseased stem and leaves of *Cyperus rotundus* L. *and Fusarium oxysporum* was isolated from infested root of *Cyperus rotundus* L. a common weed of agricultural feilds. The pathogenic fungus *Alternaria alternata* was isolated from diseased leaves of *Crotalaria verrucosa* L. (Fabaceae). The fungi *Bipolaris* sp. *Curvularia lunata* and *Curvularia tuberculata* were screened from diseased leaf spots of *Digera muricata* (L.) Mart. (Amaranthaceae); *Colletotrichum capsici* was isolated from diseased leaves of *Sida cordifolia* L. (Malvaceae,)*; Colletotrichum* sp. was isolated from diseased foliage parts of *Ipomoea pestigridis* L. (Convolvulaceae) and *Gibbago trianthemae* was isolated from infested leaves and stems of *Trianthema portulacastrum* L. (Aizoaceae).The identification of fungi was made by macro and microscopic observation.

Diagnostic characteristics of fungal isolates

The diagnostic characteristics of the fungal isolates were examined under light microscopy (Fig.2, 3). The microscopic characteristic of each isolate was studied using different

structures of conidiophores, conidia and chlamydospores by slide culture technique.

Alternaria alternata (Fr.) Keissler

The fungus produced profuse mycelial growth on PDA. Initially the mycelium was hyaline that turned to grey- brownish, multicelled, 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. The length of the condium was 3-5 times more than its width. 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. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa. 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.

Ascochyta Lib.

Mycelium immersed, branched, septate, hyaline to pale brown; conidiomata pycnidial, amphigenous, septate, globose, brown, immersed, unilocular, thin walled of texture angularis, 1-3 cell thick; ostiole central, circular, slightly papillate; conidiophores absent; conidiogenous cells enteroblastic, phialidic, determinate, discrete, doliiform to lentiform, hyaline, smooth, formed from the inner cells of the pycnidial wall; conidia hyaline, meadianly 1- septate, continuous or constricted, thin walled, smooth, ovoid, oblong, cylindrical to irregular. Colonies spreading rapidly on PSA, down brown; hyphae brown; pycnidia gregarious, globose, subglobose, dark brown, ostiolate.

Ascochyta cypericola
Upadhyay, Kenfield, Strobel & W.M. Hess

Pycnidia on substrate immersed or semi-immersed; sometimes almost superficial or scattered or aggregated, varying from lentiform, globose -depressed to globose or globose - conical, with a circular pore, sometimes with ostioles of different shapes. Pycnidial wall variable in colour, pseudoparenchymatic, depending on substrate conditions – delicate, membranaceous, almost transparent or thick. Conidia variable, predominantly cylindrical, oblong-ovate, oblong-ellipsoidal, ovate, ellipsoidal, fusiform, subclavate, with rounded, sometimes slightly attenuated or acute ends, straight or slightly bent to flexuous, usually with a single median septum, sometimes initially one-celled, later with a single median or displaced septum, occasionally with two, rarely with three septa, hyaline to subhyaline (faintly tinged). Conidiophores usually absent. The diagnostic features of *Ascochyta cypericola* were conidia with a single consistently central septum, few conidia with two septa, very rarely with three septa.

Colletotrichum Corda

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, nonseptate, 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*.

Colletotrichum capsici (Syd.) E.J.Butler & Bisby.

The isolate was identified based on size and shape of conidia. Isolate produced cottony colonies on PDA with a colour of greyish - to dark grey on the ventral surface whereas the reverse of the colonies was mainly black. The colony diameter ranged from 65 to 70 mm after 10 days incubation. Conidia uniform with both their ends pointed. Colonies on PDA at first white and becoming greyish with age, reverse greyish – green, attaining 85 mm radial in14 days. Aerial mycelia white to grey. *Acervuli* dark brown to black; setae conspicuous and dark; Conidiophores unicellular, hyaline, cylindrical, phialidic, septate, sometimes branched, tapered towards the apex, 20 µm long and 3 µm wide. Conidia formed in white masses, one- celled, smooth walled, hyaline, falcate, tapering towards each end with acute apex and truncate base.

Helminthosporium Link ex Fries.

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; Diagnostic feature of the isolate was the large, septate prospores, produced apically or laterally in verticils from determinate conidiophores. 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.

Bipolaris maydis (Y.Nisik. & C.Miyake) Shoemaker.

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. Conidia are distinctly curved, broad in the middle, sharply tapering towards rounded ends, pale to mid-dark golden brown, smooth, 5-11 septate, mostly 70-160 µm long, 15-20 µm thick in the broadest part; and point of attachment is dark, often flat, and 3-5 µm wide. Pseudothecia contain asci with four slender, thread-like, 5-9 septate ascospores (6 - 7 x 130 - 340 µm) arranged in parallel coils. Pseudothecia rarely occur under natural conditions. The identical features of the isolates include colonies with fast-growing, fluffy, with concentric rings. Conidiophores single or often in groups from flat, dark brown to black stromata, straight to flexuous, septate, smooth, geniculate, mid to dark brown, paler towards the apex, up to 700 µm long, 5 to 10 µm thick . Conidiogenous nodes verruculose. Conidia distinctly curve, fusoid, pale to mid dark golden brown, smooth, 5-11 - distoseptate, 70 to 160 × 15 to 20 µm, hilum 3 to 4.5 µm wide.

Fusarium oxysporum Schlecht.

Colonies reaching 4.5 cm diam in 4 days at 25°C on PSA; 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.

Curvularia lunata (Wakker)Boedijin.

Colonies blakish 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. The diagnostic features of the isolate observed in culture include the colour and shape of the isolate.

The distinguished characteristics of the isolate : Conidiophores arise singly or in groups, simple or rarely branched, straight or sometimes geniculate near the apex, brown to dark brown, multiseptate, variable in length, up to 5-6 µm diameter. Conidia are mostly 3-distoseptate, ellipsoidal to fusiform, or often disproportionately enlarged in the third cell and markedly geniculate or hook-shaped, pale to somewhat colored, almost concolorous, 17-32 x 7-12.5 µm, and smooth.

Curvularia tuberculata Sivan.

Colonies on PDA dark gray, usually zonate; Colonies on natural substrat effused, brown to black, hairy; mycelium on natural substrate usually immersed; hyphae branched, septate, colorless or brown, smooth or verrucose; stromata often large, erect, black, cylindrical, sometimes branched. Conidia acropleurogenous, sometimes in whorls, arise through pores in the conidiophore wall, straight or curved, usually broadly fusiform, ellipsoidal, obovoid, clavate or pyriform, sometimes rounded at the base, sometimes with a distinctly protuberant hilum, septate, often with one or more cells larger and darker than the others, smooth or verrucose.

The diagnostic features of the isolate *:* Conidiophores arise singly or in groups, terminal or lateral on hyphae, stromata, and ascomata, simple or branched, straight or flexuous, smooth, pale to mid-brown, septate, up to 300 µm long, 2-7 µm thick. Conidia are straight, ovoid, obclavate or ellipsoidal, 3-5 (sometimes 8, but mostly 3) septate, intermediate cells brown to dark brown, end cells subhyaline to pale or dark brown, mature conidia tuberculate, 23-52 x 13-20 µm. Young conidia are smooth and subhyaline. First septum in the conidium is usually median, second septum often delimiting the basal cell but variations in septal formation may occur. Germination is both by bipolar and lateral germ tubes.

Chaetomium globosum Kunze

Colonies grown abundantly on PSA; 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.

Gibbago trianthemae E.G. Simmons (1986)

Subsurface mycelia growth was dense and

dark on PDA, and inconspicuous on TWA. Sporulation was excellent at agar surfaces of *Czapek* Dox Agar and the moderate amounts of sporulation appeared on PDA and TeDA with woolly aerial mycelium. Conidia produced in culture were characterized by means of secondary conidiophores. Conidiophores simple or 1-2 proliferated. 1-4 transeptate, pale straw- colored, up to 60-80 x 5-6 µm, very slightly swollen at apex, producing a solitary conidium at the apex of each proliferation, retaining a distinct umbilicate or crateriform depression at the conidiogenous locus after secession of conidium. Conidia initially solitary, almost perfectly ellipsoid; becoming broadly ellipsoid to broadly sub ovate -ellipsoid, with 1-4 complete or partial transverse septa (slightly constricted at initial median septum), 2 complete longitudinal septa intersecting at right angles in each conidium half, plus a few shorter ones in transverse sectors of the conidium; clear pale yellow-brown, smooth; with a minute basal pore-scar that is difficult to observe and that lacks any sort of complex surrounding structure or halo of pigmentation; commonly maturing at about 35-45 x 15-22 µm with 1-4 of the apical cells; enlarging slightly and each giving rise directly to a single secondary conidium morphologically identical with primary conidia; individual apical (sometimes basal) cells also sometimes giving rise to conidiophores that have the distinctive apically swollen and umbilicate appearance of hyphal conidiophores and that are about 7x 6 µm.

Pathogenicity of the isolates

The results obtained by disease score chart confirmed that some of the test fungi were showed virulence and the host specificity and causes serious infection on test plants (Table 2). The fungus *Alternaria alternata* was showed sever pathogenic nature on *Crotalaria verrucosa* L. with dark brown coloured lesions on leaves and stems caused necrosis on leaves at final stage of disease. *Alternaria alternata* also causes moderate pathogenic symptoms on *Commelina benghalensis* L. The isolate *Ascochyta cypericola* was pathogenic to *Cyperus rotundus L.* causes blight diseases on foliar and stem parts of the weed plant. The white mycelial isolate *Fusarium oxysporum* causes root rot on *Cyperus rotundus* L. The isolate *Curvularia lunata* was showing leaf spots on *Digera muricata* (L.) Mart. and the species *Colletotrichum capsici* causes anthracnose on leaves of *Sida cordifolia* L. and *Ipomoea pestigridis* L. also infested with *Colletotrichum* sp. which causes anthracnose. A phaeodictyoconidial hyphomycetes fungus identified as *Gibbago trianthemae* Simmons (1986) induced leaf spots and leaf blight of *Trianthema portulacastrum* L. (Aizoaceae) and mycoherbicide potential of the fungus was tested *in vitro* against its host weed (Fig. 4). Moderate symptoms of leaf spot disease caused by *Alternaria alternata* and *Bipolaris maydis* observed on horse purslane. No symptoms were observed on crop plants inoculated with spore concentrations and considered as resistant to the isolates. The isolate *Chaetomium globosum* Kunze was considered as non-pathogenic fungi which was failed to produce 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

Table 1.
Fungal pathogens recorded on weed plants infested in agricultural fields at Visakhapatnam District, Andhra Pradesh

Weed/Pathogen	Symptoms
Commelina benghalensis L. (Commelinaceae)	
Alternaria alternata (Fr.) Keissler.	Leaf blight
Ascochyta Lib	Leaf blight
Cyperus rotundus L. (Cyperaceae)	
Ascochyta cypericola sp.nov.,	Leaf blight
Ascochyta Lib	Leaf blight
Helminthosporium Link ex Fries.	Leaf spot
Fusarium oxysporum Schlecht.	Root rot
Crotalaria verrucosa L. (Fabaceae)	
Alternaria alternata (Fr.) Keissler.	Leaf blight
Digera muricata (L.) Mart. (Amaranthaceae)	
Bipolaris maydis (Y.Nisik. & C.Miyake) Shoemaker.	Leaf spot
Curvularia lunata (Wakker)Boedijin.	Leaf spot
Curvularia tuberculata Sivan.	Leaf spot
Sida cordifolia L. (Malvaceae)	
Colletotrichum capsici (Syd.)E.J.Butler & Bisby.	Anthracnose
Ipomoea pestigridis L. (Convolvulaceae)	
Colletotrichum Corda	Anthracnose
Trianthema portulacastrum L. (Aizoaceae)	
Gibbago trianthemae E.G. Simmons (1986)	Leaf spot & Blight

Table 2.
Pathogenicity of fungal isolates on different inoculated weed plants

Fungal isolate	Weed	Inoculated parts	Symptom appeared DAT	Disease intensity
Alternaria alternata	*Crotalaria verrucosa* L.	Leaves, stems	4	+++
	Commelina benghalensis L.	Leaves, stems	5	++
Ascochyta cypericola	*Cyprus rotundus* L.	Leaves, stems	5	+++
	Commelina benghalensis L.	Leaves, stems	5	+
Ascochyta sp.	*Cyprus rotundus* L.	Leaves, stems	5	+++
Bipolaris maydis	*Trianthema portulacastrum* L.	Leaves, stems	4	+
Colletotrichum capsici	*Sida cordifolia*	Leaves, stems	5	+++
	Ipomoea pestigridis	Leaves, stems	5	++
Colletotrichum sp.	*Sida cordifolia*	Leaves, stems	4	+++
Curvularia lunata	*Digera muricata* (L.) Mart.	Leaves, stems	5	++
Curvularia tuberculata	*Digera muricata* (L.) Mart.	Leaves, stems	5	++
Fusarium oxysporum	*Cyperus rotundus* L.	Roots	4	+++
Gibbago trianthemae	*Trianthema portulacastrum* L.	Leaves, stems	3	+++
Helminthosporium	*Digera muricata* L.	Leaves, stems	5	++

DAT= Days After Treatment; + = mild symptoms; ++ = moderate symptoms; +++ = severe symptoms

Discussion

A total of 9 genera include 12 species of fungal pathogens, namely *Alternaria alternata, Ascochyta* sp., *A. cypericola, Colletotrichum* sp., *C. capsici, Helminthosporium* sp**.,** *Bipolaris maydis, Fusarium oxysporum, Curvularia lunata***,** *C. tuberculata, Chaetomium globosum,* and *Gibbago trianthemae* were isolated from parasitized parts of the weed flora identified in crop fields at Visakhapatnam District, Andhra Pradesh. 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 phylogenetic study of the isolate and host-pathogen interactions. The in vitro studies were carried for the primary screening of host specific weeds which is the pioneer stage to develop mycoherbicide agents.

In the case of *Alternaria alternata* (Fr.) Keissler*,* our findings about conidial chains and colour of the isolate were agreed with results of Narian *et al.* (1985), Shinde (1995), Deshmukh (1998), Ghosh (1998), Shinde (2003), Akhtar *et al.* (2004) and Dipak *et al.* (2013). Furthermore the findings about shape and septation of conidia of the isolate were compared with the reports of Rao (1965), Sonawane (1983), Narain *et al.* (1985), Shinde (1995), Deshmukh (1998), Ghosh (1998), Shinde (2003) and Dipak *et al.* (2013). The morphological and microscopic observations *Ascochyta cypericola* were made with the help of relevant study on *Ascochyta* of Baker *et al*. (1949), Beaumont (1950), Brewer (1960), Alcorn (1968), Kaiser (1973), Yu (1947), Ellis (1950) and Ratna kumar (1998).

Our findings on morphological features of the isolate *Colletotrichum capsici* (Syd.) E. J. Butler & Bisby**.** agreed with the results of Hindorf (1973), Baxter *et al*. (1983), Freeman *et al.* (1998), Lardner *et al.* (1999), Shenoy *et al*. (2007), Than *et al*. (2008) and Sangdee *et al.* (2011) who observed the morphology of *Colletotrichum* populations. The characteristics of this isolate *Bipolaris maydis* (Y.Nisik. & C.Miyake) Shoemaker**.** corresponded with *B. maydis* (Nisikado and Miyake) Shoem (Ellis 1971; Sivanesan 1987; Reza & Motlagh 2011).

In the case of the isolate *Curvularia lunata* (Wakker) Boedijin. conidia are sparse in culture, and variable in shape and size among isolates (Ellis 1966) while conidia of *Curvularia tuberculata* Sivan. are straight, 3-septate, tuberculate (having tubercles) or rough-walled unlike other *Curvularia* species (Ellis 1966). *Chaetomium globosum* Kunze was identified by the presence of perithecia with many stiff dark terminal hairs with ornamentation was the diagnostic features of the isolate (Gilman, 1957, Navi *et al*. 1999, Prokhorov & Linnik 2011). Our findings about mycelium colour, texture and conidia of isolate *Gibbago trianthemae* were similar with results of Simmons (1986), Mitchell (1988), Aneja and Kaushal (1998), Aneja *et al. (*2000)

and Akhtar *et al. (*2013) who noticed the morphological characteristics of *Gibbago trianthemae* Simmons (1986).

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 world wide. Ratna Kumar (1998) reported the virulence of *Ascochyta cypericola* as pathogenic fungi on *Cyperus rotundus* L. and the additional studies are needed on the impact of field studies. Recently the isolates belonging to *Ascochyta* sp., *Helminthosporium* sp., *Chaetomium* sp., *Bipolaris* sp., and *Curvularia* sp. and *Gibbago trianthemae* Simmons were used as weed control agents by spraying their spore inocula onto some serious weeds. Pathogenicity and host-range tests of the study showed that a total of 11 isolates were primarily screened as bio control agents through *in vitro* studies and the genus of *Alternaria, Ascochyta, Curvularia, Colletotrichum, Fusarium* and *Gibbago* 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, Ascochyta cypericola, Fusarium oxysporum, Curvularia lunata, Colletotrichum capsici* and *Colletotrichum* sp. and *Gibbago trianthemae* whose pathogenicity was confirmed using Koch's postulates and host rang studies. 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. 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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Fig. 1 Fungal disease symptoms on weed plants A) Leaf blight on *Crotalaria verrucosa.* B) Anthracnose on *Sida cordifolia.* C) Leaf spot on *Trianthema portulacastrum.*

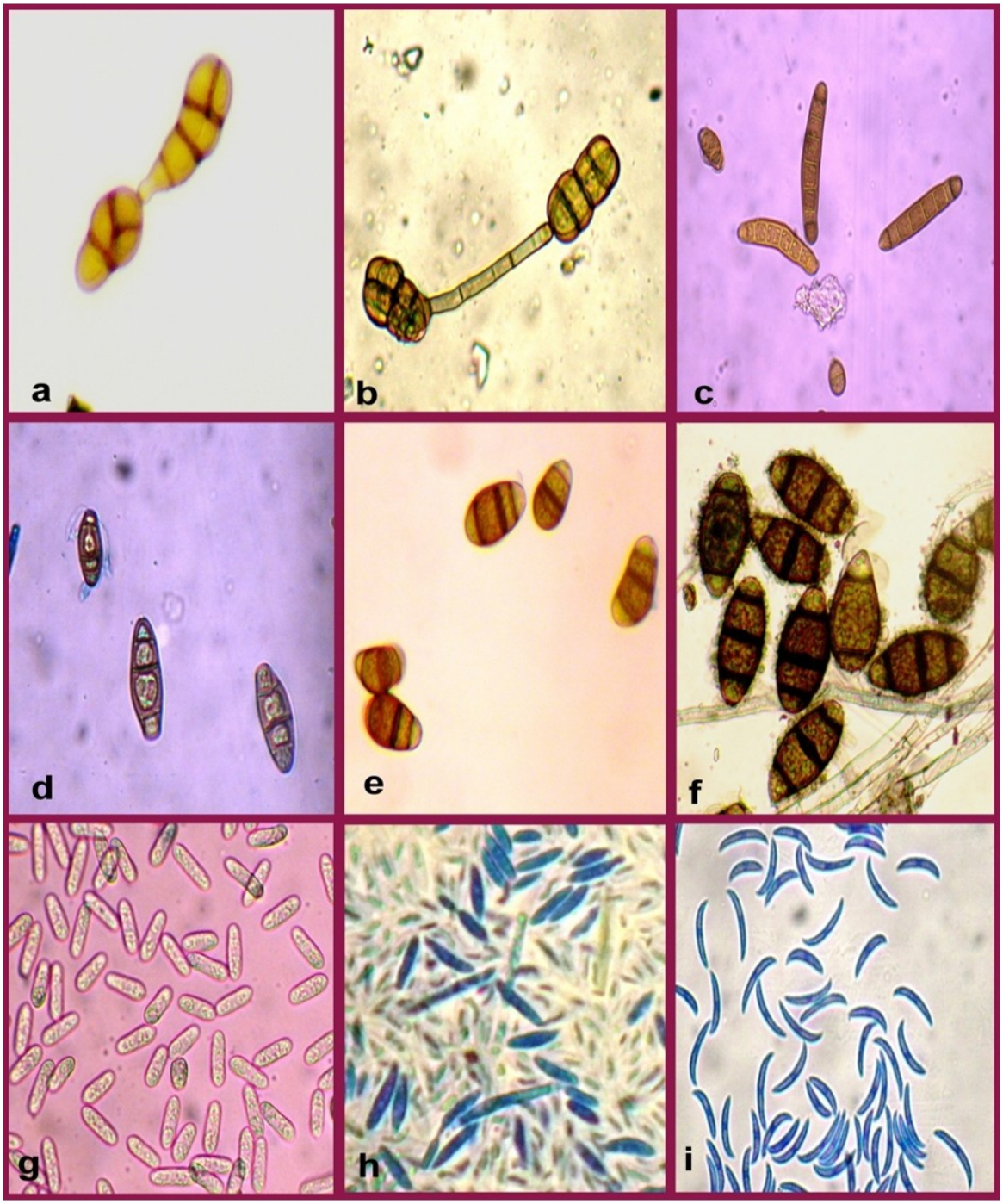

Fig. 2 Fungal pathogens isolated from weed flora infested at agricultural fields a) *Alternaria alternata* (Fr.) Keissler. b) *Gibbago trianthemae* E.G. Simmons (1986). c) *Bipolaris maydis* (Y.Nisik. & C.Miyake) Shoemaker. d) *Helminthosporium Link* ex Fries. e) *Curvularia lunata* (Wakker)Boedijin. f) *Curvularia tuberculata Sivan*. g) *Ascochyta cypericola* h) *Fusarium oxysporum* Schlecht. i) *Colletotrichum capsici* (Syd.)E.J.Butler & Bisby.

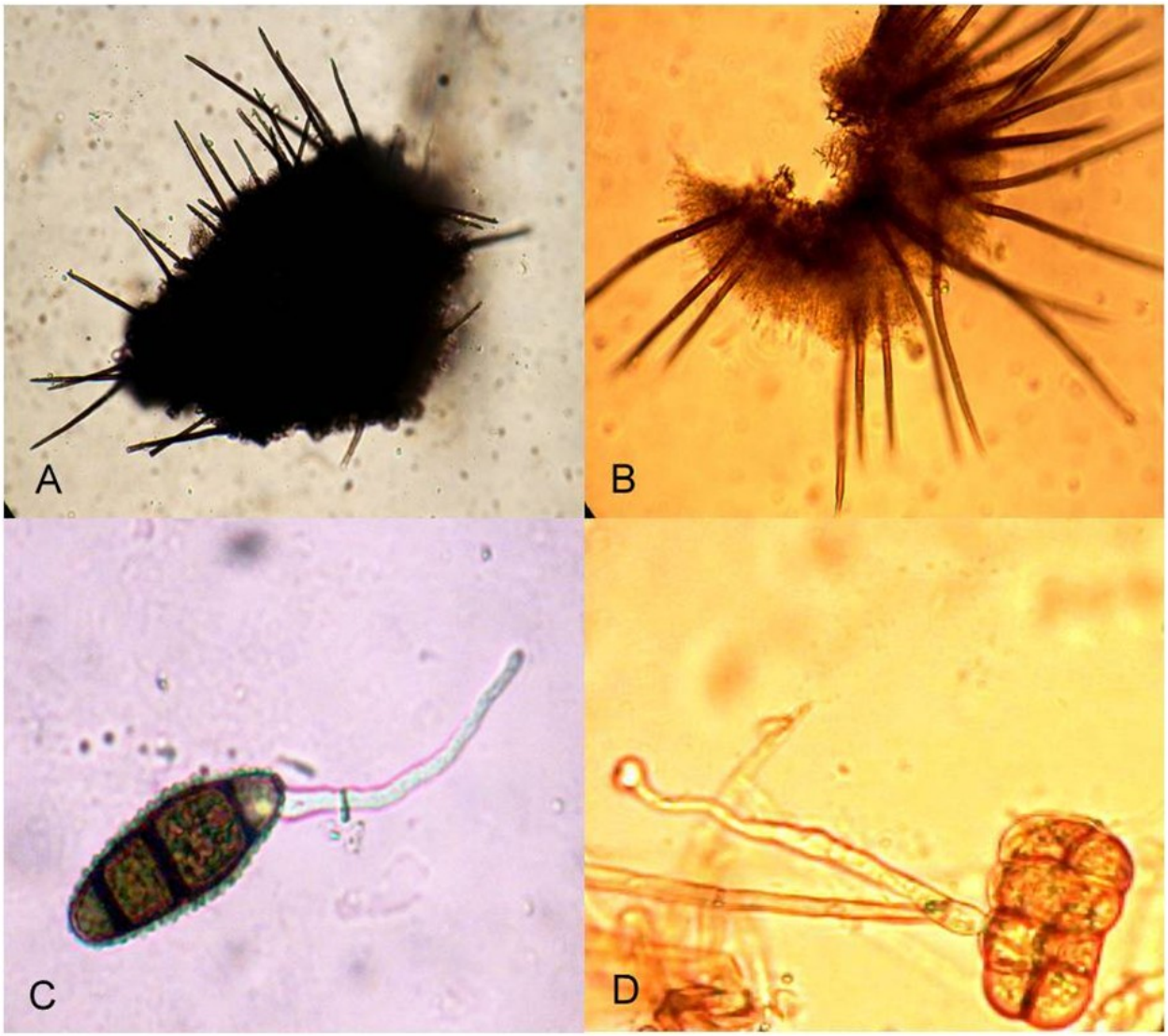

Fig 3 Diagnostic features of some fungal isolates A& B) *Acervuli of Colletotrichum capsici.* C) Spore germination and germ tube formation in *Curvularia tuberculata.* D) Formation of Appressorium on tip of the germ tube in conidial germination of *Gibbago trianthemae.*

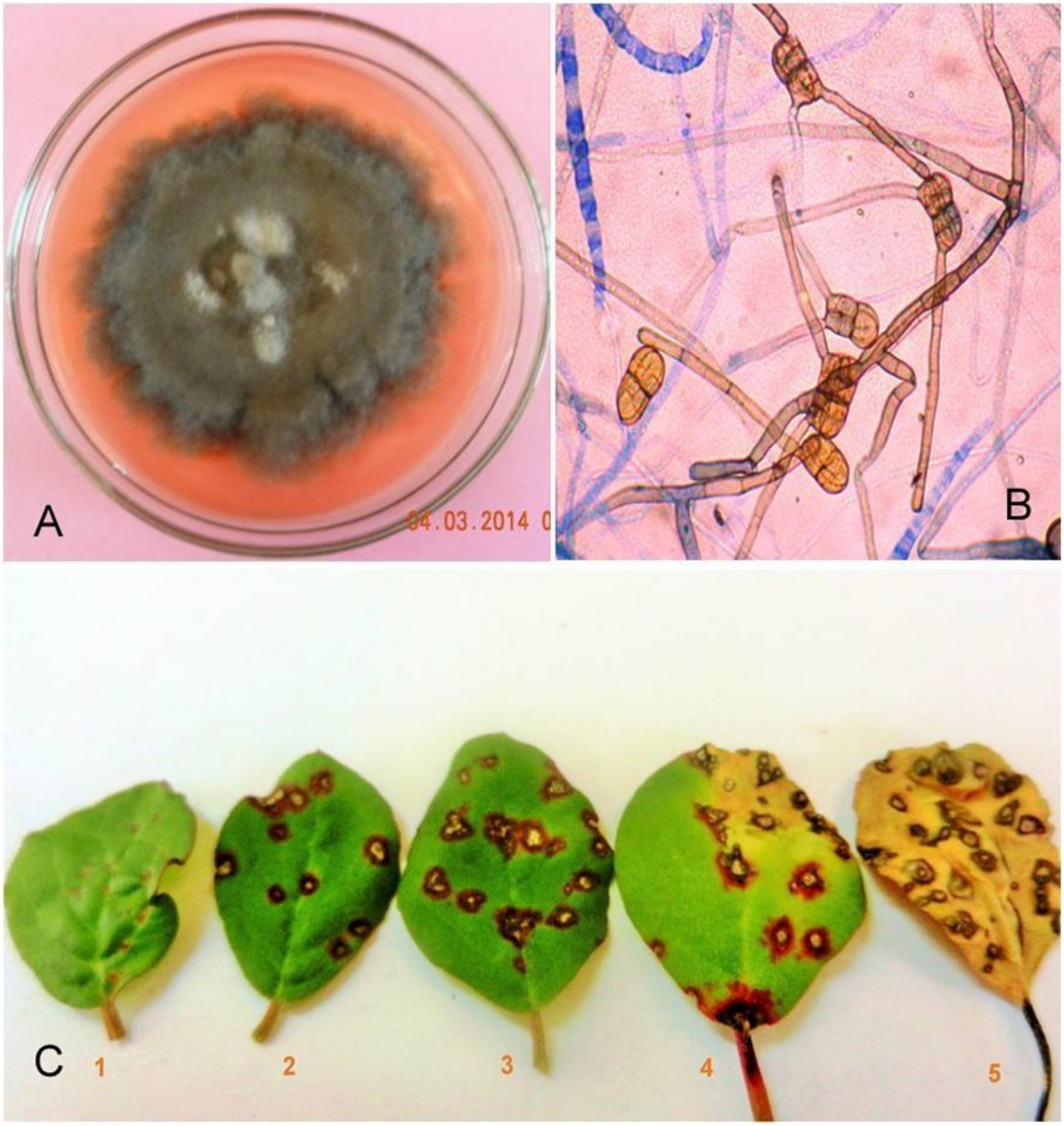

Fig. 4 Biocontrol potential of *Gibbago trianthemae* on *Trianthema portulacastrum* L. (Horse purslane), a noxious weed of Aizoaceae. A) Isolate of *Gibbago trianthemae* (colony on CDA medium). B) Conidia of *Gibbago trianthemae.* C) Leaf spot disease induced by *Gibbago trianthemae* on horse purslane weed *in vitro.*