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Isolation, Identification and *in vitro* Screening of Rhizospheric Fungi for Biological Control of *Macrophomina phaseolina*

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ABSTRACT

The present study was aimed to identify charcoal rot pathogen of groundnut (*Arachis hypogaea* L.) in Anantapur district, Andhra Pradesh and assess the interactions among the fungal isolates from rhizosphere. During field survey, a potential charcoal rot pathogen *Macrophomina phaseolina* was isolated and identified. About 10 different rhizosphere soils of healthy groundnut plants were selected for isolation of fungi. Among 25 isolates assayed for antagonism, ten different fungi significantly inhibited colony growth of *M. phaseolina* in dual culture plates and morphological identification was done at (400X). Antagonistic fungi inflicted 25.6-41% mycelial growth inhibition in *M. phaseolina.* Colony growth and sclerotia production of *M. phaseolina* ceased effectively by *Emericella nidulans* and *E. rugulosus*, respectively. Mutual inhibition between *Alternaria alteranata*, *Aspergillus flavus*, *A. terreus*, *Penicillium chrysogenum* and *M. phaseolina* was observed. *Penicillium oxalicum, Gliocladium roseum* and *Aspergillus versicolor* significantly reduces growth with <1 mm demarcation lineat 7 and 14 Days after Inoculation (DAI) on PDA medium. These antagonistic interactions influence the growth of the pathogen.

Key words: Biological control, charcoal rot, groundnut, *Macrophomina phaseolina*, rhizosphere fungi

INTRODUCTION

Peanut or groundnut (*Arachis hypogaea* L.) is mainly produced in the states of Andhra Pradesh, Tamil Nadu, Gujarat, Karnataka and Maharashtra in India (Talawar, 2004). In arid regions of India, many annual crops and weeds are susceptible to the charcoal rot pathogen *M. phaseolina* (Tassi) goid = *Rhizoctonia bataticola* (Taub.) Butler (Lodha *et al.*, 1986). In groundnut it is known to cause wilt as well as root, stem, peg and pod rots and leaf spots in seedlings and older plants (Pattee and Young, 1982). This pathogen also causes seedling blight, root rot and charcoal rot diseases on more than 500 plant species from more than 100 families (Singleton *et al.*, 1992) distributed worldwide. The disease appears in hot and dry weather when soil temperature is 80-95°F (27-35°C) for 2-3 weeks. The disease control is inefficient or difficult by using the chemical fungicides. The non-judicious use of a wide range of fungicides has led to harmful effects on to environment and increased resistant pathogen populations (Soytong *et al.*, 2001).

The search for effective alternative approaches to chemical control which have minimal deleterious effects, more eco-friendly and will contribute to the goal of sustainability in agriculture is needed (Jain *et al.*, 2009). Because of the increasing awareness about problems associated with the chemical based disease control, biological control has received considerable attention during

the last 40 years as a potential alternative (Cook and Baker, 1983). Biological control of plant disease by microorganisms, especially of soil-borne plant pathogens and nematodes, has been considered as a more natural and environmentally acceptable alternative to existing chemical treatment methods (Baker and Paulitz, 1996). Disease suppression by biocontrol agents is the sustained manifestation of interactions among plant, pathogen, bioagent, microbial community on and around the plant and the physical environment (Handelsman and Stabb, 1996). In their natural habitats, interactions between microbial cells can be as complex as they are dynamic. The outcome of each interaction is determined by factors such as the species, physiological condition and microbial load present; diverse physicochemical and biophysical parameters including temperature, water activity, chaotropicity and pH; motility of and/or distance between competing microbes and antimicrobials produced by the stress biology and other metabolic attributes of the microbial participants (Cray *et al.*, 2013). Fungal colonies growing in vicinity may effect important changes in one another or they may require close hyphal connection. Variations include trophic stimulation, morphogenetic alterations and various degrees of growth inhibition. Interactions between hyphae appear to be substantial in defining the pattern of colonization of dung. In the recent years, there has been a worldwide swing towards the use of eco-friendly methods for protecting crops from pests and diseases (Rao *et al.*, 1998). Although the number of biocontrol products is increasing, they still represent only about 1% of agricultural chemical sales (Fravel, 2005).

With this in view, the present investigation was carried out to isolate, identify and examine the efficacy of potential soil fungi from rhizosphere of peanut against *Macrophomina phaseolina*, a serious plant pathogen of economic importance under *in vitro* conditions.

MATERIALS AND METHODS

Soil sampling and isolation of fungi: Soil trials were taken from 10 different groundnut rising areas near Yogi Vemana University, Kadapa, Pendlimarri, Siddautavam (Lankamalleswara forest) and Kadiri during September 2012. Soil was taken at 10 cm depth. Samples were sieved and air dried for 3-5 days at 18°C. After drying, samples were kept at 10°C until used. Normal microflora were isolated from rhizosphere soil of healthy groundnut plants by serial dilution technique on Rose Bengal agar medium (Glucose-10 g, peptone-5 g, KH_2PO_4-1 g, $MgSO_4.7H_2O$-0.05 g, streptomycin-30 mg, RoseBengal-0.035 g, agar-15 g, distilled water-1000 mL) (Martin, 1950) and *Trichoderma* selective medium ($MgSO_4.7H_2O$-0.2 g, K_2HPO_4-0.9 g, KCl-0.15 g, NH_4NO_3-3.0 g, glucose-3.0 g, agar-15 g, Rose Bengal-0.15 g, chloramphenicol-0.25 g, distilled water-1000 mL, pH-6.5) (Elad *et al.*, 1981) and incubated at 27±3°C for a week (Rabeendran *et al.*, 1998). Resulting colonies were purified on PDA plates amended with streptomycin (20 mg L^{-1}) and identified using standard mycological keys.

Identification of soil fungi: The morphological characteristics used for identification of isolates were mycelial growth, pigmentation and presence of fungal structures such as conidia, pycnidia and cleistothecia. Mycelial disc from single spore culture was placed in the center of a 90 mm diameter PDA plate and incubated at room temperature for seven days. Isolates were grouped according to colony appearance and pigmentation. The mycelial growth rates of the fungal isolates were determined from PDA plates. Petri dishes containing PDA were each centrally inoculated with a 5 mm diameter agar plug from 7 day old cultures of each isolate, to determine their Average Linear Growth Rates (ALGR) (Table 1). Radial mycelial growth was recorded every 24 h during this period.

Table 1: Average linear growth rates of fungi isolates

Fungal culture	ALGR (mm per day)
Emericella nidulans	13.5±0.03
Emericella rugulosus	12.5±0.04
Alternaria alternata	10.0±0.02
Aspergillus flavus	13.5±0.04
Aspergillus terreus	8.3±0.04
Penicillium oxalicum	8.0±0.03
Emericella nidulans 2	12.9±0.02
Gliocladium roseum	8.8±0.03
Aspergillus versicolor	10.5±0.04
Penicillium chrysogenum	8.4±0.05
Rhizoctonia solani	20.0±0.02

ALGR: Average linear growth rates, Each value is an average of 3 replicate samples, ±Standard Error

The colony diameter was measured as the mean of two perpendicular diameters, based on the method of Elad *et al.* (1981). Average Linear Growth Rates (ALGR) were calculated using following equation:

$$ALGR \text{ (mm per day)} = (C7-C1)/C1$$

where, C7 is colony diameter in milliliter after seven days and C1 is colony diameter in milliliter after one day of incubation.

Three replicates were run for each treatment. Micromorphological features in fungi were studied by mounting fungal mycelium and examining under the light microscope (Olympus model BX-50F4). Images were captured using a camera (Canon model PC1737). Perennial structures that were formed in the agar medium, such as conidia were cut out from the medium and mounted in lactic acid. The sizes of the various structures such as conidia were determined by averaging the measurements of 5 samples of each structure. The morphological features of the conidia such as the shape and septation of conidia were recorded photographically; the size of conidia was measured and compared with the available literature.

Isolation of *Macrophomina phaseolina*: From June to January, charcoal rot-affected stems or roots of peanut (*Arachis hypogaea* L.) were identified, collected from different cropping regions of Anantapur and Kadapa. Two different methods were used for isolation of *M. phaseolina*.

Soil isolation: Isolates were obtained from soil using a soil assay technique based on the method of Alabouvette (1976). Soil samples were dried in an oven at 37°C, crushed through a 1 mm mesh sieve, mixed thoroughly and subsamples of 5 g were taken. Each subsample was submerged in 0.52% NaOCl for 10 min. The soil-NaOCl mixture was washed with distilled water through two sieves of mesh sizes, 180 and 45 μm, respectively. The residue retained on the 45 μm mesh sieve was incorporated into 100 mL of a Semi Selective medium (SS medium) for *M. phaseolina*. This consisted of Potato Dextrose Agar (PDA; 39 g L^{-1}) maintained at 55°C in a water bath, with the following ingredients added: 1.5 mL 0.52% NaOCl, 1 mL 0.5% chloramphenicol dissolved in 95% alcohol and 10 mL 2.25% quintozene (PCNB). The medium was poured into 90 mm petri dishes to solidify and then incubated at 27±3ºC.

Host tissue isolations: Uprooted plants were rinsed under running tap water and blotted dry with a sterile paper towel. Separate subsamples of roots and stems of each plant were treated by immersion in 0.5% NaOCl for 10 min to eliminate secondary invaders, followed by rinsing for

30 min in sterile distilled water. Tissues excised from dying rootlets, necrotic taproots and stems were cut into 2-3 mm long fragments and 18 of these were plated on three petri dishes containing the above described SS-medium and incubated at 27±3ºCfor 5-8 days. Colonies of *M. phaseolina* each appeared as a ring of fluffy white mycelium surrounding central area with black microsclerotia. Each colony was observed under the microscope for the presence of microsclerotia. Typical colonies from soil and host tissue isolations were propagated further onto PDA and stored at 5°C.

Dual culture technique: To study the antagonistic effects of the soil fungi isolates on *M. phaseolina*, dual culture plate technique (Dennis and Webster, 1971) was used. Different antagonists and pathogen combinations were examined on 20 mL of PDA in 90 mm petriplates, with three replicate plates per treatment. For dual culture technique, a mycelia plug (5 mm in diameter) taken from actively growing 7 day old culture of *M. phaseolina* and soil fungi isolates placed 80 mm apart from each other on the PDA. For control treatments, a plug of *M. phaseolina* was placed on the PDA medium against PDA plug. The plates were incubated at 27±3°C. Observations on the antagonistic activities of isolates on *M. phaseolina* were recorded after every 24 h for 7 days and inhibition percentage was calculated using the following formula (Edgington *et al.*, 1971):

$$\text{Inhibition percentage (\%)} = A1\text{-}A2/A1 \times 100$$

where, A1 is the colony area of uninhibited *M. phaseolina* in the control plate and A2 is the colony area of *M. phaseolina* in dual culture plate.

RESULTS
Isolation of soil fungi: About 25 different fungi were isolated from peanut fields. Among them, *Emericella nidulans*, *Emericella rugulosus*, *Alternaria alteranata*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus versicolor*, *Penicillium oxalicum*, *Penicillium chrysogenum*, *Rhizoctonia solani* and *Gliocladium roseum* were selected for further characterization based on inhibition of *Macrophomina phaseolina*.

Identification of soil fungi: Each isolate was cultured on PDA for identification work. The various filamentous fungi isolated were identified, based on the following characteristics in accordance with the literature available.

***Emericella nidulans*:** It is observed that an encountered isolate appear imperfect or anamorphic stage namely *Aspergillus nidulans*. The ALGR of culture observed as 13.5 mm per day at 27°C, conidia green from becoming brownish when mature to produce abundant. Cleistothecia, globose or oval shape, 130×350 µm in diameter and surrounded by hülle cells (Fig. 1). Hülle cells ellipsoidal to globose, 25 µm in diameter. Asci are globose to subglobose, 8-spored. Ascospores are red, smooth and 2×3.6 µm, with two narrow longitudinal furrows as similar described by (Domsch *et al.*, 1993).

***Emericella rugulosus*:** Colonies on PDA attains 80 mm after seven days at 27°C and its ALGR is 12.5 mm per day. Colony color dark green and on reverse brown orange to dark purple brown
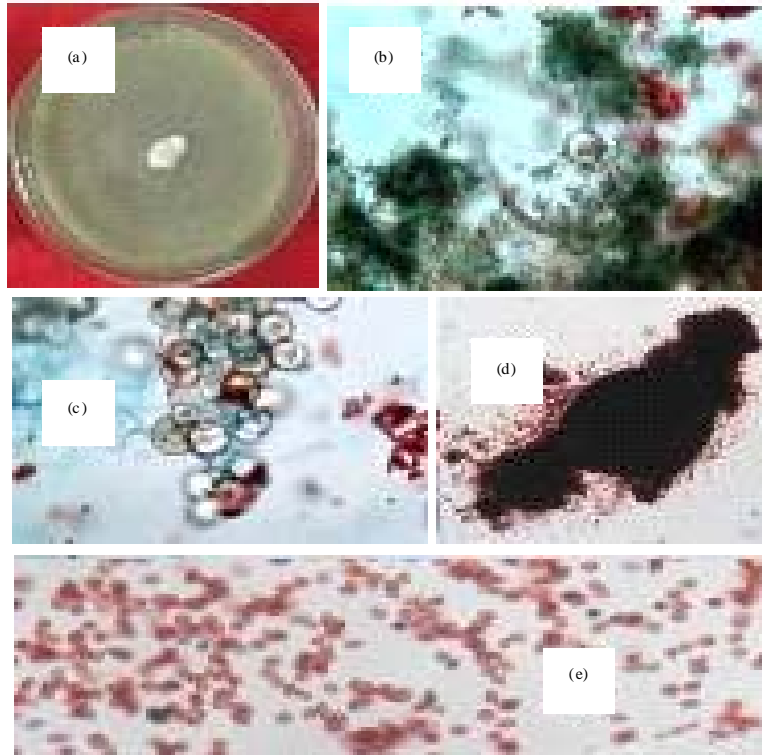
Fig. 1(a-e): (a) *Emericella nidulans*, (b) Cleistothecia, (c) Hülle cells, (d) Ascospores and (e) Asci (400X)
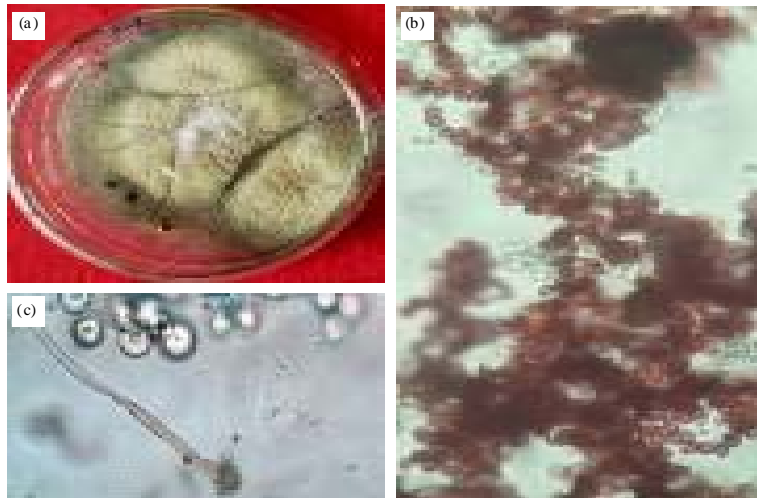

Fig. 2(a-c): (a) *Emericella rugulosus*, (b) Ascospores and (c) Hülle cells (400X)

(Fig. 2). Conidial heads are short columnar, 60 µ long by 60 µ wide. Conidiophore smooth, brown in color, 60 by 4 µ in diameter. Vesicle flask shaped 10 µ in diameter. Sterigmata biseriate, matulae 5 µ long by 6 µ wide. Conidia globose, small 4 µ in diameter. Cleistothecia 325 µ in diameter.
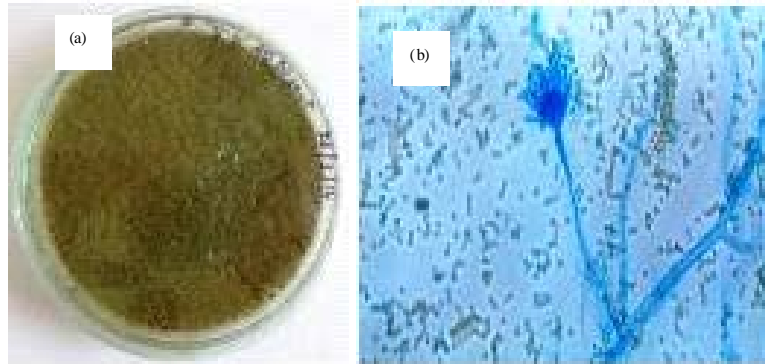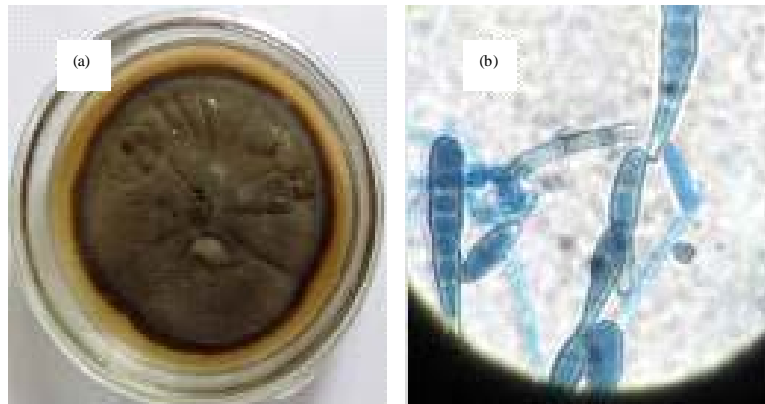
Fig. 3(a-b): (a) *Aspergillus flavus* and (b) Conidia

Fig. 4(a-b): (a) *Alternaria alternata* and (b) Muriformconidia

Ascospores purple brown in color, having two 0.8 μ thick equatorial crests, rugulose surface, spores body having double crests 6 μ long, excluding crest 4.4 μ long by 3.8 μ wide, Hülle cells globose, 20 μ in diameter (Afzal *et al.*, 2013).

***Aspergillus flavus*:** Colonies on potato dextrose agar widely spreading, growing rapidly, with ALGR 13.5 mm per day: Conidial heads pale yellow when young, becoming dark green in age, in older cultures deep grey-green, reverse colourless to pale yellow brown (Fig. 3). Conidiophores across separately from the substratum, 800 of 1200 μm long, 18 μm in diameter, broadening upward. Walls were pitted as to appear rough of spiny with low magnification, gradually enlarging upward to form a vesicle 30 and 60 μm in diameter (Xalxo *et al.*, 2013).

***Alternaria alternata*:** Colonies grew on PDA reach 70 to 80 mm in 7 days and ALGR is 10 mm per day. Olive black colony without aerial mycelium (Fig. 4). Conidiophores short, simple, straight, branched or unbranched, up to 40 μm long conidia often in long branched chains of 10 or, uniform with 6 transverse septa, longitudinal or obligue septa present. The conidia were muriform shape and light brown colour. The length of their conidia was varied from 42 μm (Watanabe, 2010).

***Aspergillus terreus*:** Colonies on PDA media appeared as rapidly growing powdery colonies with a characteristic cinnamon-brown colour on the surface and a yellow to beige-brown color on the
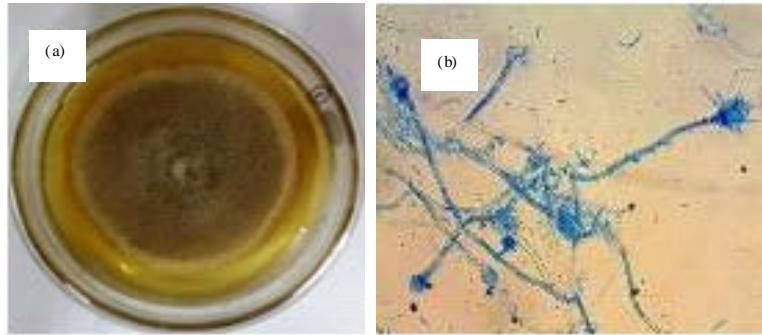
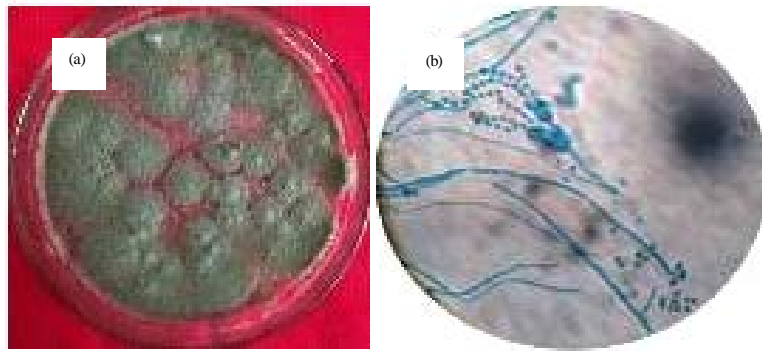Fig. 5(a-b): (a) *Aspergillus terreus* and (b) Conidia

Fig. 6(a-b): (a) *Penicillium oxalicum* and (b) Phialides

reverse. The ALGR of colony calculated as 8.3 mm per day, exudates amber colored (Fig. 5). Conidial heads long columnar, compact with uniform diameter, cinnamon brown, 550×30 μm, conidiophores smooth, colorless, 250×6 μm, vesicles hemispherical, dome-like ,16 μm in diameter, phialides biseriate; conidia globose to subglobose and 2.4 μm in diameter (Xalxo *et al.*, 2013).

Penicillium oxalicum: Colony attains 54 mm diameter in 7 days and ALGR is 8 mm per day. Colonies are radially sulcate, sometimes convolute, centrally depressed, moderately deep to deep, surface texture velutinous to floccose, mycelium white, conidiogenesis light to moderate, greyish olive, clear brown exudate sometimes present and reverse beige to reddish brown (Fig. 6). Conidiophores arising from surface mycelium; stipes 290 μm in length with thin, smooth walls, characteristically terminating in verticils of 3 metulae; metulae 20 μm long; phialides were ampulliform. Conidia measured 3.02 μm ellipsoidal, smooth and in columns (Molar *et al.*, 2013).

Gliocladium roseum: Colony appears as green in color with white edged margins reaches 50 mm in 7 days on PDA and ALGR is 8.8 mm per day, at maturity color of the colony turns into powdery green (Fig. 7). Conidiophores hyaline, erect, branched with penicillate phialides bearing single spore masses. Conidia hyaline, long-ellipsoidal, subglobose. Perithecia 250×300 μm. Asci 40×7.5 μm, ascospores 11.3×3.8 μm (Watanabe, 2010).

Penicillium chrysogenum: Colonies on PDA appear to grow 8.4 mm per day. Green-blue velvety with a heap of white mycelium at the centre and reverse is yellow-white (Fig. 8). The penicillia are
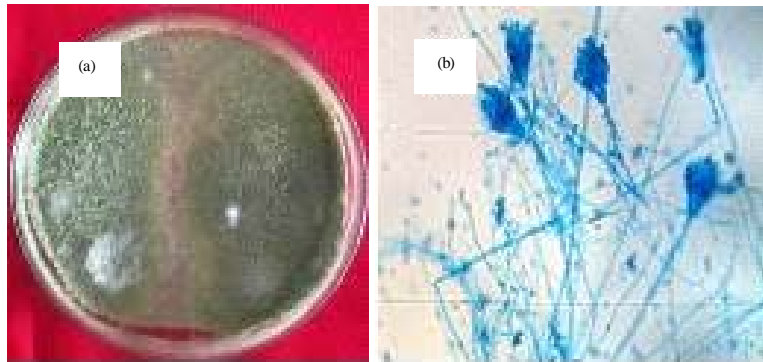
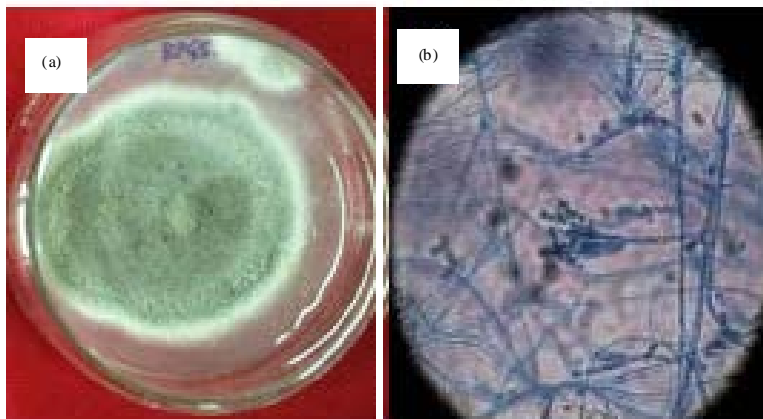Fig. 7(a-b): (a) *Gliocladium roseum* and (b) Conidia

Fig. 8(a-b): (a) *Penicillium chrysogenum* and (b) Phialides

asymmetrical and often complex with diverging branching patterns. The conidiophores are smooth-walled with size 1000 μm. There are 3 to 5 metulae of 10×2 μm. The phialides are aggregated in clusters of 4-6; they are ampulliform and are 9×2 μm. The conidia are subglobose to elliptical, smooth-walled arranged in long irregular chains. They are 4×3.5 μm.

Aspergillus versicolor: Colonies on PDA at 27°C grew slowly, reaching 65 mm in diameter after 7 days its ALGR is 10.5 mm per day. Colonies centrally rising, velvety to rather floccose, colour blue-green, reverse pale yellowish and exudates absent (Fig. 9). It was interesting to find that a large number of heads were fragmentary and like penicillium. Conidiophores monoverticillate, hyaline, smooth-walled, 8×3 μm. Metulae 9×4 μm long. Phialides, 8×3 μm long (Xalxo *et al.*, 2013).

Rhizoctonia solani: Colonies on Potato Dextrose Agar (PDA) grow at a rate of 20 mm per day at 27°C. The colony range in color from buff to black (Fig. 10). Sclerotia are produced on the surface of cultures after 4 to 6 weeks and are irregularly shaped, light tan to black and usually >1 mm (0.04 in.) in width (Watanabe, 2010).

Identification of *Macrophomina phaseolina*: *Macrophomina phaseolina* was isolated by both soil and host tissue methods were identified following (Watanabe, 2010). Observations were made
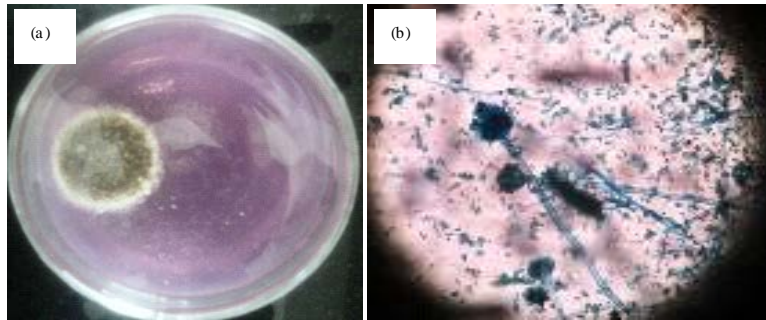
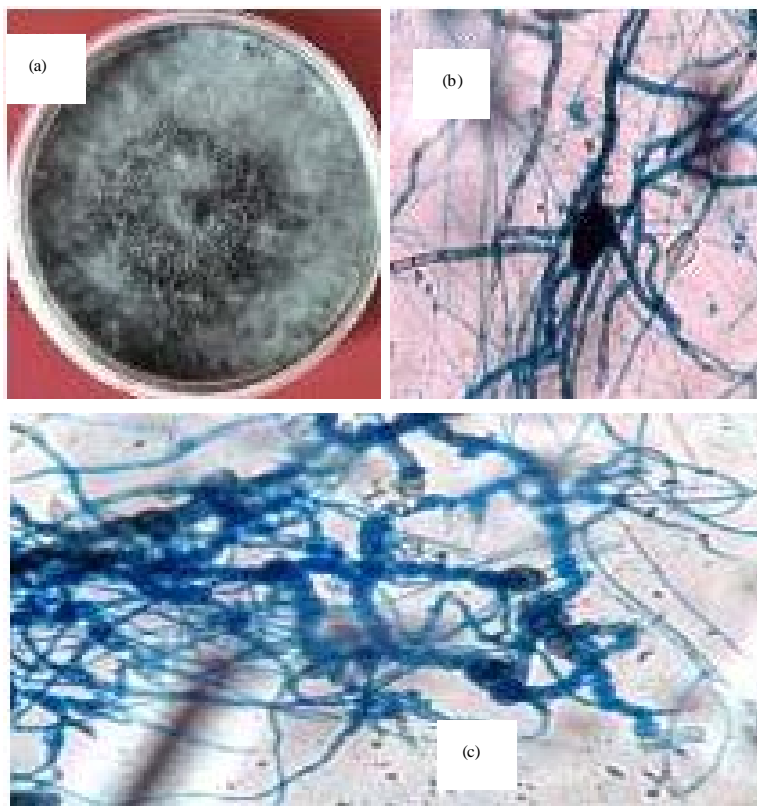Fig. 9(a-b): (a) *Aspergillus versicolor* and (b) Conidia

Fig. 10(a-c): (a) *Rhizoctonia solani*, (b) Microsclerotia and (c) Mycelia (400x)

with a light microscope (Olympus model BX-50F4) and using a camera (Canon model PC1737). Colony characters and pigment production were noted after 3 day of growth on PDA at 27±3°C (Fig. 11). Pycnidia black, globose, ostiolate apically. Conidiophores hyaline, simple, cylindrical, narrowing apically. Conidia hyaline, cylindrical, 1-celled. Microsclerotia black, homogenous in size. Pycnidia 200 µm in diameter; ostioles 30 µm in diameter. Conidiophores 20×6 µm. Conidia 30×6 µm. Sclerotia 120 µm in diameter. Hyphae 7.5 µm wide (Watanabe, 2010).

Effect of antagonistic fungi on mycelial growth of *Macrophomina phaseolina in vitro*: All the ten soil isolates exhibited antagonistic potential against *M. phaseolina* by inhibiting its mycelial
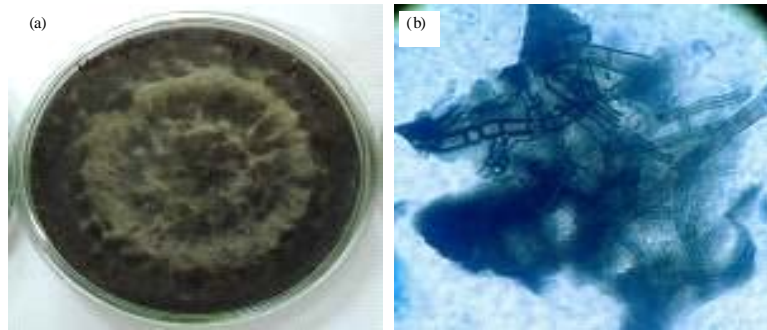
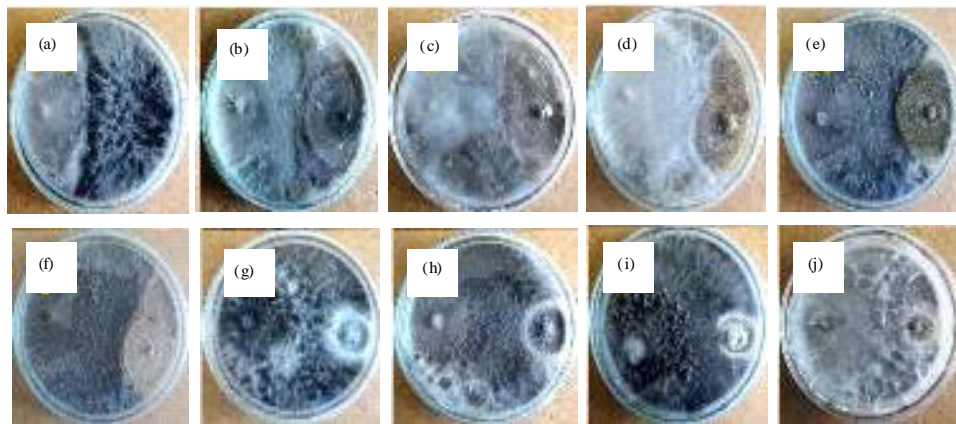Fig. 11(a-b): (a) *Macrophomina phaseolina* and (b) Microsclerotia (400x)

Fig. 12(a-j): Dual culture and colony interactions of rhizosphere fungi and *M. phaseolina*, (a) *M. phaseolina+R. solani*, (b) *M. phaseolina+E. nidulans*, (c) *M. phaseolina +E. rugulosus*, (d) *M. phaseolina+A. flavus*, (e) *M. phaseolina+A. alternata*, (f) *M. phaseolina+A. terreus*, (g) *M. phaseolina+P. oxalicum*, (h) *M. phaseolina +G. roseum*, (i) *M. phaseolina+P. chrysogenum* and (j) *M. phaseolina+A. versicolor*

growth. The mycelial inhibition on seventh day ranged from 26.9 to 51.9% for *M. phaseolina*. After inoculation, growth of *M. phaseolina* was found to be inhibited by isolates and attained a growth of 47.3, 41.2, 36.8, 35.1, 35, 33.4, 32.4, 30.6, 27.4, 26.4 and 25.6 mm (Table 2). This indicates that isolate *E. nidulans* (40.9%) proving more effective against the pathogen, followed by *E. rugulosus* (39.0%) had maximum antifungal activity against *M. phaseolina* compared to the other isolated fungi (Fig. 12). *Rhizoctonia solani* inhibits the growth of *M. phaseolina* by 51%. *E. nidulans* was more antagonistic at the day of 14 than at 7dai and significantly suppresses the growth of pathogen and decreases sclerotia production. Mutually inhibitory interactions of *M. phaseolina* with *E. rugulosus*, *A. flavus* and *P. oxalicum* were observed and rated 4 according to colony interactions chart proposed by Skidmore and Dickinson (1976). Intermingling growth where the *M. phaseolina* has ceased growth and is being overgrown by *R. solani* and rated as 3 as given in Table 3. Mutually slight inhibition of hyphal growth was observed between *A. alternata* and *M. phaseolina* rated as 3. Initially, *A. terreus* shows inhibition >2 mm over *M. phaseolina* and later it comes to 1 mm of inhibition. Slight inhibition of *M. phaseolina* with *P. oxalicum*, *G. roseum*, *A. versicolor* and *P. chrysogenum* where <1 mm demarcation line was clearly visible as shown in (Fig. 12).

Table 2: Dual culture technique

Treatments	Radial growth of bioagents (mm)	Growth of pathogen (mm)	Inhibition (%)
Rhizoctonia solani +M. phaseolina	47.3±0.3	43.2±0.09	51.9
Emericella nidulans +M. phaseolina	41.2±0.5	43.1±0.09	40.9
Emericella rugulosus +M. phaseolina	36.8±0.3	54.9±0.03	39.0
Alternaria alternate +M. phaseolina	35.1±0.2	56.1±0.02	37.5
Aspergillus flavus +M. phaseolina	35.0±0.2	57.2±0.09	35.6
Aspergillus terreus +M. phaseolina	33.4±0.2	59.3±0.07	35.1
Penicillium oxalicum +M. phaseolina	32.4±0.2	60.2±0.05	32.4
Emericella nidulans 2 +M. phaseolina	30.6±0.3	61.2±0.02	32.0
Gliocladium roseum +M. phaseolina	27.4±0.2	65.7±0.02	29.5
Penicillium chrysogenum +M. phaseolina	26.4±0.2	66.1±0.03	27.1
Aspergillus versicolor +M. phaseolina	25.6±0.08	67.5±0.03	26.9
M. phaseolina control	-	90.0±0.04	-

Each value is an average of 3 replicate samples, ± Standard Error

Table 3: Five separate modes of interacting colony growth

	DAI	
Treatments	7	14
Rhizoctonia solani+M. phaseolina	3	3
Emericella nidulans+M. phaseolina	4	4
Emericella nidulans 2+M. phaseolina	4	4
Alternaria alternate+M. phaseolina	4	4
Aspergillus flavus+M. phaseolina	4	3
Aspergillus terreus+M. phaseolina	5	4
Penicillium oxalicum+M. phaseolina	4	4
Emericella nidulans+M. phaseolina	4	4
Gliocladium roseum+M. phaseolina	4	4
Penicillium chrysogenum+M. phaseolina	4	4
Aspergillus versicolor+M. phaseolina	4	3

DAI: Days after inoculation, (1) Mutually intermingling growth where both fungi grew into one another without any macroscopic signs of interaction, (2) Intermingling growth where the fungus being observed is growing into the opposed fungus either above or below or above and below its colony and its corollary, (3) Intermingling growth where the fungus under observation has ceased growth and is being overgrown by another colony, (4) Slight inhibition where the fungi approached each other until almost in contact and a narrow demarcation line, *c.* 1-1. mm, between the two colonies was clearly visible and (5) Mutual inhibition at a distance of > 2 mm

DISCUSSION

Macrophomina phaseolina was isolated from infected groundnut fields in Anantapur region and proved to be pathogenic isolate causing charcoal rot and seedling diseases of groundnut plants. Among 10 different isolated fungal species, *A. flavus, A. terreus* are dominant, *E. nidulans* is subdominant and *E. rugulosus, A. alternata, P. oxalicum, R. solani* are extant in rhizosphere as reported by Subrahmanyam and Rao (1977). Though *R. solani* shows antagonistic activity, it intermingles and overgrown on pathogen. The tested *E. nidulans* and *E. rugulosus* showed antibiotic mechanism to inhibit growth of *M. phaseolina* which was supported by Sibounnavong (2012) that antibiotic substance from *E. nidulans* and *E. rugulosus* could inhibit the growth and break the cells of *F. oxysporum* f. sp. *lycopersici. Alternaria alternata* checks the growth of *M. phaseolina* was held by Babu *et al.* (2003), who identified *A. alternata* produces a red metabolite in potato dextrose broth and found to be toxic to waterhyacinth. Inhibition of *M. phaseolina* by *A. flavus* was supported by Cotty and Bayman (1993) indicated that atoxigenic strains of *A. flavus* will competitively exclude the aflatoxin producing strains of same fungal species. *A. terreus* as a biocontrol agent against *M. phaseolina* was similarly reported by Melo *et al.* (2006) specified sclerotial viability of the pathogen was found to be affected by treatment of sclerotia, with conidia

of the hyperparasite *A. terreus*. *Penicillium oxalicum* slightly inhibits *M. phaseolina* supported by De Cal *et al.* (1997) identified disease reduction was observed when *P. oxalicum* was applied before or after inoculation with the pathogen. *Penicillium chrysogenum* constrains the growth of *M. phaseolina* (Dong *et al.,* 2006) reported that dry mycelium of *P. chrysogenum* contributed to induced resistance against *Verticillium dahliae* in cotton. *Gliocladium roseum* slightly obliges the growth of *M. phaseolina* supported by Rodriguez-Kabana and Morgan-Jones (1988) indicated that *G. roseum* produce a diffusible yellow pigment in PDA cultures and its correlation to biocontrol efficacy. *Aspergillus versicolor* slightly hinders *M. phaseolina* strengthen by (Bhattacharyya *et al.*, 1985) identified *A. versicolor* as a Biological Control Agent (BCA) against *M. phaseolina.*

CONCLUSION

It is concluded that this research finding *E. nidulans*, *E. rugulosus*, *A. alternata*, *A. flavus*, *A. terreus*, *P. oxalicum*, *P. chrysogenum*, *G. roseum* and *A. versicolor* developed as bioagents to control *M. phaseolina* under *in vitro* conditions. Further research has to be done to characterize the volatile and non-volatile metabolites from potential antagonistic fungi to assess their effect on *M. phaseolina* and *in vivo* control of charcoal rot of groundnut caused by *M. phaseolina.*

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