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Medicinal plants as alternative biocontrol agents in the control of seed borne pathogen *Macrophomina phaseolina*

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ABSTRACT

In a search for plant extracts with potent antifungal activity against plant pathogenic fungi, the methanol extracts of different medical plants effectively controlled the development of plant diseases caused by *Macrophomina phaseolina*. The methanolic extracts of forty nine plants hexane, chloroform and methanolic extracts were used for the study, among them methanolic extracts of forty eight medicinal plants exhibited varying degrees of inhibition activity against *M. phaseolina*, extracts of *Tephrosia pumila* have no activity. Extracts of *Avicenia officinales, Cleome viscose, Grewia arborea, Hyptis sueolences, Ocimum sanctum, Peltophorum pterophorus, Sesbanian grandiflora, Terminalia chebula, Tephrosia pumila, Tephrosia villosa, Withania somnifera* showed high activity and *Terminalia chebula* having strong antifungal activity against *M. phaseolina* even at low concentrations. Chloroform extracts has very low antimicrobial activity where as hexane has no antimicrobial properties.

Keywords: **:** *Macrophomina phaseolina,* Crude methanolic extracts, Antifungal activity, Potato Dextrose Agar (PDA), Well diffusion assay.

INTRODUCTION

Plants that possess therapeutic properties on the animal or plant body are generally designated as Medicinal plants, with the development of microorganisms resistant to chemicals applied indiscriminately to crops, research has been done with the goal to search for alternative and safe forms of agrochemical pest control without causing any damage to environment and to humans, maintaining the crop qualitatively and quantitatively. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [1, 2]. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [3, 4]. Much work has been done on ethno medicinal plants in India [5, 6]. Interest in a large number of traditional natural products has increased [7]. Traditionally plants have been well exploited by man for the treatment of human diseases, Ayurveda is a good example, but not much information is available on the exploitation of plant wealth for the management of plant diseases, especially against phytopathogenic fungi [8].

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) is a prevalent stalk rot of sorghum (*Sorghum bicolor* [L.] Moench) and corn *(Zea mays* L.). The disease occurs most often when sorghum plants are subjected to drought stress as grain approaches the soft dough stage. The fungus can infect the root and lower stem of over 500 plant species and is widely distributed [9]. Charcoal rot is an important disease during hot, dry weather or when unfavorable environmental conditions stress the plant. *M. phaseolina* causes disease on sorghum, soybean, peanut, and corn. In sorghum, it causes seed and seedling rots, wilt, root and stem rots, leaf spot, and rotting of developing pods and seed. Charcoal rot on soybean leads to early maturation, chlorosis and incomplete pod filling. While in corn the fungus causes a stalk rot during hot, dry conditions.

The objectives of this research were to evaluate the fungi toxic effect of different organic solvent extracts of medicinal plants used in Traditional Indian medicine were studied against the sorghum seed borne mycoflora.

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MATERIALS AND METHODS

Plant material and extracts preparation:

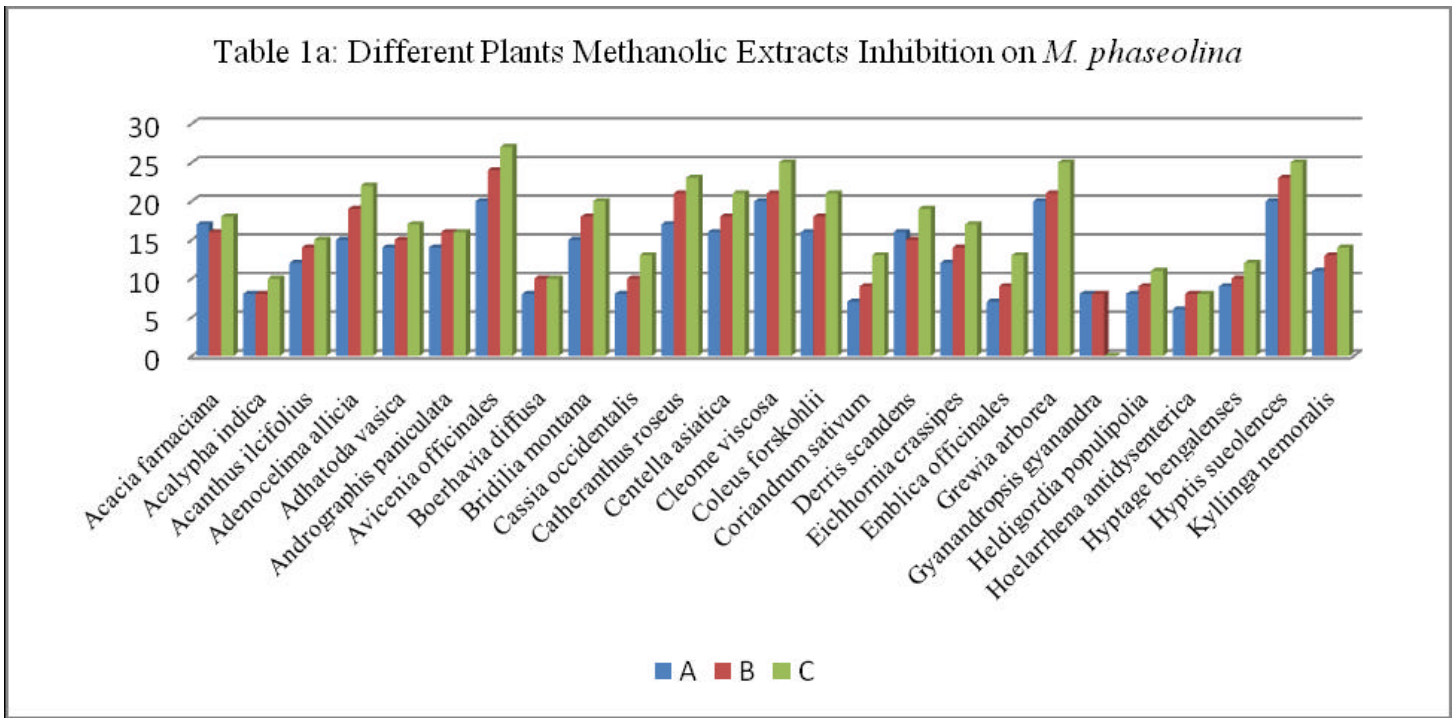
The plant materials of forty nine plant species were collected from different places in Visakhapatnam district, Andhrapradesh (Table-4). The selected parts of different medicinal plants were cut into small pieces and shade dried at room temperature for fifteen days, finely powdered plant materials were successively extracted with organic solvent methanol basing on order of polarity using soxhlet apparatus. The different extracts obtained were subsequently concentrated under reduced pressure to get their corresponding residues. Methanolic extracts in different concentrations (100mg/ml, 300mg/ml, and 500mg/ml) to get the final drug concentration 5mg/well, 15mg/well, and 25mg/well respectively, control (DMSO) and standard (Bavistin5µg/ml),were transferred to the cups of each agar plate, incubated at room temperature (28ºC) and examined for inhibition zones after 36 hours of incubation. The extracts were screened for antifungal activity.

Microbial cultures and growth conditions:

The plant extracts were assayed for antifungal activity against the fungal strain *Macrophomina phaseolina (Tassi) Goidanich* (F 2165) obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh. This fungus was grown on PDA plate at 28ºC and maintained with periodic sub-culturing at 4ºC.

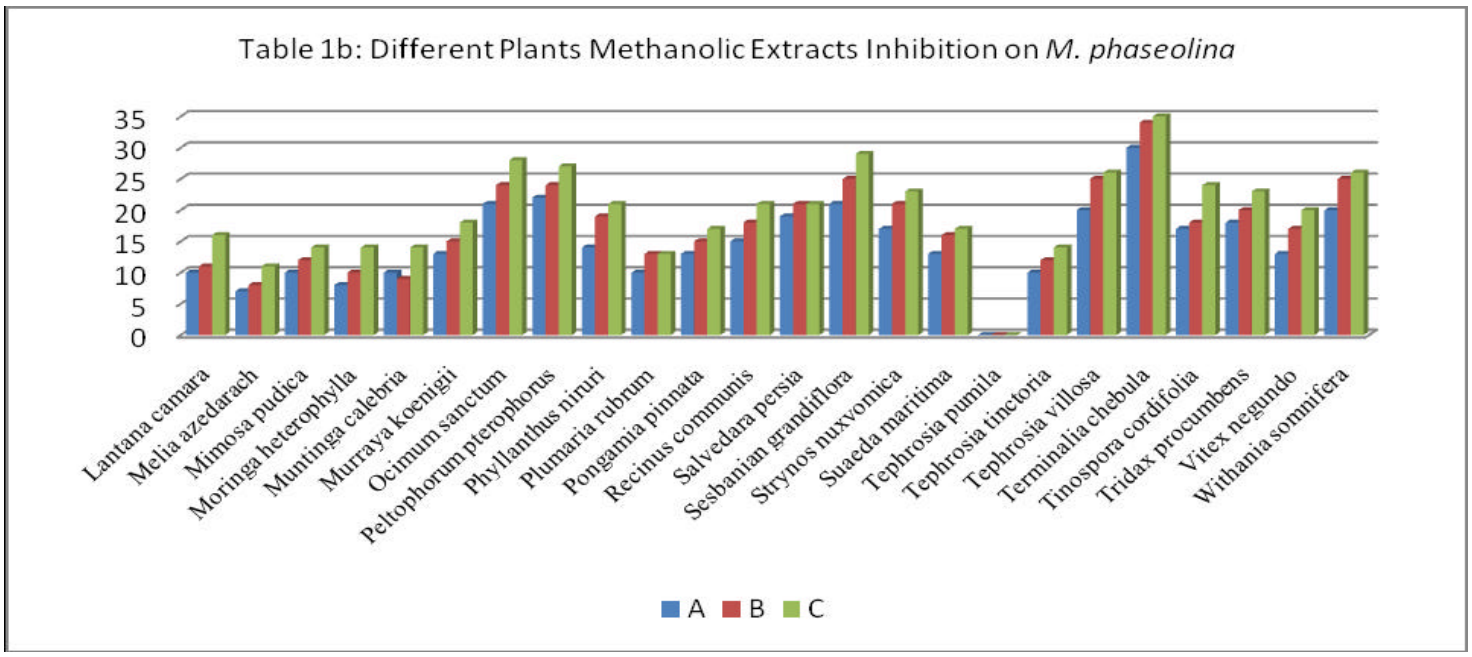
Antifungal activity:

The methanolic extracts of forty nine different plant extracts were screened for antifungal activity (Table. 1 and 2) by agar well diffusion method [10] with sterile cork borer of size 6.0mm. The cultures of



Volume per well: 50µl, A: 100 mg/ml = 5 mg/well, B: 300 mg/ml =15 mg/well, C: 500 mg/ml= 25 mg/well, Borer size used: 6mm.

48hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of methanolic extracts of forty nine different plant extracts were introduced serially after successful completion of one plant analysis. Incubation period of 24- 48hours at 28^{0}C was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions.

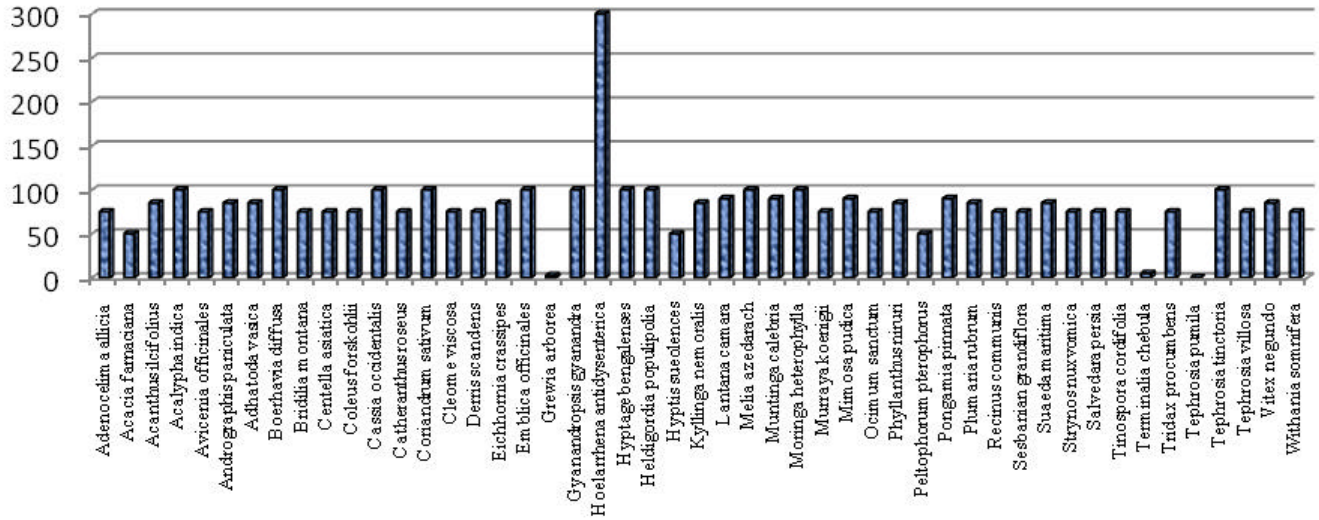


Volume per well: 50µl, A: 100 mg/ml = 5 mg/well, B: 300 mg/ml =15 mg/well, C: 500 mg/ml= 25 mg/well, Borer size used: 6mm

Table-1. List of Investigated Medicinal Plants

Botanical Name	Parts used	Uses / Ailments treated
Acacia farnesiana (L.) Willd	Bark, roots	Astringent, Demulcent, Poultice, Stomachic.
Acalypha indica Linn.	Aerial parts	Skin diseases, Ulcers Bronchitis, Head ache, Snake bite
Acanthus ilicifolius Linn.	Leaf extract	Relieve rheumatism
Adenocalymma alliaceum (Lam.)	Leaves	Astringent,
Adhatoda vasica Nees.	Leaves,whole plant	Cough, chronic bronchitis, rheumatism, asthma and asthma.
Andrographis paniculata Nees.	Whole plant	Anti-biotic, anti-viral, anti-parasitic and immune system stimulant.
Avicennia officinalis L.	Seed	Relieving ulcers
Boerhaavia diffusa Linn.	Whole plant	Scabies, myalgia, aphrodisiac
Bridelia montana (Roxb.) Willd	Leaf	Stomach pains, sore eyes and headaches.
Cassia occidentalis Linn.	Whole plant	Boils, Spasm. Hysteria, Whooping cough
Catharanthus roseus Linn.	Leaves and roots	Anti-mitotic and Anti-microtubule agents
Centella asiatica Linn.	Whole Plant	Diuretic, treatment of leprosy, use as brain tonic and stimulates hair growth.
Cleome viscosa Linn.	Leaves and seeds	Anthelmintic, carminative, diaphoretic and rubefacient.
Coleus forskohlii (Willd.).	Roots	Treat heart and lung diseases, intestinal spasms, insomnia and convulsions. Antispasmodic.
Coriandrum sativum Linn.	Fruits	Colic, Laxative, Blood purifier, Indigestion, sore throat
Derris scandens (Roxb.) Benth	Stem	Arthritis, Anti-inflammatory
Eichhornia crassipes (C.Mart.)	Whole plant	Biomass, soil reclamation
Emblica officinalis Gaertn.	Fruit	Aperient, Carminative, Diuretic, Aphrodasiac, Laxative, Astringent and Refrigerant.
Gmelina arborea Linn.	Roots	Gonorrhea, catarrh of bladder, cough, cleaning the ulcers, insanity, epilepsy, fevers, indigestion, nerve tonic.
Gynandropsis gynandra (L.)	Leaf	Anti-irritant
Hildegardia populifolia (Roxb.)	Stem bark	Dog bite, Malaria.
Holarrhenaantidysenterica Foxh.	Bark and seeds	Ddysentery, piles, leprosy, colic, dyspepsia, chronic chest complaints, , spleen diseases, jaundice, bilious, calculi
Hiptage benghalensis (L.) Kurz.	Leaves and bark	Insecticidal, cough, inflammation; skin diseases and leprosy
Hyptis suaveolens (L.) Poit.	Leaves	Antispasmodic,antirheumatic and antisoporific
Kyllinga nemaralis Rottb.	Whole Plant	Promotes action of liver, and relief prunitus
Lantana camara Linn.	Whole Plant	Antidote to snake venam, Malaria, wounds cuts ulcers, Eczema, Tumours
Melia azedarach L.	Leaves, Seed Flower, oils	Vermifuge, Insecticide, Astringent, Tonic and Antispetic. It posseses anti diabitic, anti bacterial and anti viral
Mimosa pudica Linn.	Whole Plant	Menorrhagia, piles, Skin wounds Diarrhoea, Hydrocele, Whooping caugh, Filiriasis
Moringa heterophylla L.	Roots,Seeds,	Antibiotic Anti-inflammatory and Diabetes
Muntinga calabria Linn.	Leaves	Antiseptic
Marraya Koenigii (L.) Spreng.	Leaves	Skin diseases, Heminthiasis, Hyperdipsia, Pruritus, etc.
Ocimum sanctum Linn.	Leaves, Seeds	Malaria, bronchitis, colds, fevers, absorption, arthritis.
Peltophorum pterocarpum (DC.)	Whole plant	Reclamation
Phyllanthus niruri L.	Leaves	Jaundice, Diabetes
Plumeria rubra Linn.	Leaves	Ulcers, leprosy, inflammations, rubefacient.
Pongamia pinnata (L.) Pierre.	Bark, seeds	Anti maleria , skin disease, rheumatic and leprous sores
Ricinus communis Linn.	Leaves	Jaundice, sores,
Salvadora persic, Linn.	Roots	Antimicrobial and dental diseases
Sesbania grandiflora (L.)	Flowers	treat gonorrhoea and for curing infection of the cornea.
Strychnos - nux - vomica Linn.	Seeds	Cholera, chronic wounds, Ulcers, paralysis, Diabetes
Suaeda maritima (L.) Dumort.	Whole plant	Bioremediation
Tephrosia pumila (Lamk.) *Persoon.*	Root	Rheumatism, fevers, pulmonary problems, bladder disorders, Coughing, hair loss, and reproduc tive disorders
Tephrosia tinctoria Pers.	Root	Antisyphilitic
Tephrosia villosa (L.) Pers.	Root,Leaves,Bark	Anthelmintic, alexiteric, leprosy, ulcers, antipyretic, cures diseases of liver, spleen, heart, blood, asthma etc.
Terminalia chebula Retz.	Fruit	Antimicrobial, digestive problems, mouthwash/gargle, astringent, and douche for vaginitis.
Tinospora cordifolia (Willd.)	Stem	Analgesic and anti-inflammatory.
Tridax procumbens Linn.	Whole plant	Antimicrobial, Anti-oxidant and Anti-inflammatory,
Vitex pentaphyllal Linn.	Aerial parts	Foetid discharges, Febrifuge Rheumatism affections, catarrhal
Withania somnifera (L.) Dunal	Leaves	Sore eyes, Febrifuge, ulcers Cure sterility of women sedative

Table-3: Minimum Inhibitory Concentration (MIC)



Volume per well: 50µl, Borer size used: 6mm

Minimum inhibitory concentration (MIC) assays:

Based on the preliminary screening chloroform and methanolic extracts were found to have potent antimicrobial activity and Minimum Inhibitory Concentrations (MIC) of the extracts was determined according to Elizabeth [12]. A final concentration of 0.5% (v/v) Tween-20 (Sigma) was used to enhance crude extract solubility. A series of two fold dilution of each extract, ranging from 0.2 to 100 mg/ml, was prepared. After sterilization, the medium was inoculated with 3µl aliquots of culture containing approximately 105 CFU/ml of each organism of 24hours slant culture in aseptic condition and transferred into sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes. After the media solidified a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/ wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. Different plant crude extracts ranging from 0.2 to 100 mg/ml were added to the cups/wells of each petri dish and the control plates without plant extract. Inhibition of organism growth in the plates containing test crude extracts was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of extracts inhibiting visible growth of each organism on the agar plate. Similarly the MICs of methanolic extracts were determined against all other microorganisms. The results were given in Table-3.

RESULTS AND DISCUSSION

Antifungal activity of forty nine botanical extracts was assayed and data on effect of plant extracts on the growth of *M. phaseolina* presented in table 1. The data revealed that significant reduction in growth of *M. phaseolina* was observed with extracts of forty nine plants. It was observed that extracts showed significant differences in their efficacy to inhibit the growth of *M. phaseolina.* All the extracts tested gave significant inhibition of mycelial growth of *M. phaseolina* over control. The methanolic extracts of forty nine plants were used for the study; among them forty eight medicinal plants exhibited varying degrees of inhibition activity against *M. phaseolina* extract of *Tephrosia pumila* have no activity. The following plants, viz, as *Avicenia officinales, Cleome viscose, Grewia arborea, Hyptis sueolences, Ocimum sanctum, Peltophorum pterophorus, Sesbanian grandiflora, Terminalia chebula, Tephrosia pumila, Tephrosia villosa, Withania somnifera* showed high activity among them *T. chebula* having strong antifungal activity against *M. phaseolina* even at low concentrations.

In particular, the authors may recommend that the methanolic extract of *Terminalia chebula* to be used as potent biocide to treat diseases in plants caused by *M. phaseolina* as it showed maximum activity even at lower concentrations. It was revealed in this study, that increase in the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. Extensive bioprocess parameter studies should under taken the methanolic extract of *Terminalia chebula* as a strong antifungal agent against *M. phaseolina* causing plant diseases.

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