Northeast Journal of Contemporary Research, December 2018. Vol. 5 No. 1, pp. 36-42
ISSN 2349-3089

Antifungal Activity of Some Ethnomedicinal Plants Against *Sclerotinia Sclerotiorum* (Lib.) DE BARY

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ABSTRACT

In the present study ten different locally available plants *Mimosa pudica, Polygonum hydropiper, Leucas aspera, Solanum torvum, Mirabilis jalapa, Xanthium strumarium, Solanum nigram, Nyctanthes arbor-tristis, Mikania micrantha* and Citus leaves were taken for study of antifungal activities. Plants extract were prepared by smashing fresh plant material and mixed with the concentrated ethanol in a waring blender for 10 min. Fungal pathogen *Sclerotinia sclerotiorum* was used in this study. Among the ten plants taken *Mimosa pudica, Mikania micrantha, Mirabilis jalapa, Polygonum hydropiper* and *Solanum nigram* was most effective against *Sclerotinia sclerotiorum.*

Key words: *Mimosa pudica, Polygonum hydropiper, Leucas aspera, Solanum torvum, Mirabilis jalapa, Xanthium strumarium, Solanum nigram, Nyctanthes arbor-tristis, Mikania micrantha* and Citus leaves Antifungal activity.

INTRODUCTION

Plant disease causes significant damage and economic losses in agriculture and horticulture crops every year. Global losses caused by plant pathogens are estimated to be 12% of the potential crop production, despite the continuous release of new resistant cultivars. As a consequence, management strategies including the use of chemical pesticides are often employed inappropriately and indiscriminately. Furthermore, fungi are continuously becoming resistant to fungicides and they are at risk of being withdrawn from the market. In addition to reducing crop yield, fungal pathogens often lower crop quality by producing toxin that affect human health. (Duraisamy Saravankumar *et al.,* 2015). For disease management, several strategies have been applied against the soil□borne pathogens to reduce the survival of the resting fungal structures such as sclerotia. Fungicide sprays can prevent infection by ascospores; however, due to difficulty in achieving spray penetration of the crop canopy, disease can still occur. Once the pathogen has become established in the soil, steam sterilization or fumigation with methyl bromide can be used to kill the sclerotia. The high cost of steam sterilization and pesticides, development of fungicides resistance pathogen isolates, governmental restriction on the use of fumigants with environmental concerns over regular use of fungicides and the difficulty in finding suitable rotation crops to reduce pathogen inoculum have led to increase in the search for efficient alternative to chemical fungicide management of *S. sclerotiorum* (Staub 1991;

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Köhl and Fokkema 1998; Zhou and Boland 1998). Based on the knowledge that plants develop their own defense against fungal pathogens (Gurgel *et al.* 2005), they appear as an interesting source for antifungal compounds. In a study, Fabricant and Farnsworth (2001) reported that 94 species of plants are utilized for the production of 122 single-agent natural products that are being used as singleagent drugs around the world. Thus, even with this very incomplete database of global ethnomedical information, there is abundant opportunity for the discovery of new medicinal age.

MATERIALS AND METHODS

Study area: Tezpur is the headquarter of the centrally located Sonitpur district in Assam. Mythologically known as Sonitpur. Tezpur is located on the northern bank of the river Brahmaputra. The average temperature in summer is around 36 C while the average winter temperature is around 13 C. Latitude and longitude of tezpur is 26.6528 N, 92.7926 E. There are many rare and endemic plant species available in tezpur. It is also rich in biodiversity hence it is include in one of the biodiversity hotspot of the world. Among these plant some medicinal plant are also available,in some high medicinal value used for ages by the tribes there in ethnobotany.

Plant meterials: Plant meterials collected were-*Mimosa pudica, Polygonum hydropiper, citrus leaves, Leucus aspera, Mirabilis jalapa, Mikania micrantha, Solanum nigrum, Nyctanthes arbor-tristis, Solanum torvum, Xanthium strumarium.*

Chemicals: Chemicals used in the study were potato dextose agar(PDA), dextrose, starch, distilled water, demethyl sulphoxide(DMSO) and ethanol.

Instruments: Instruments used in the study were Borosilicate glasswares- petridishes, Beaker, conical flask, Glass rod, Hot air oven, Balance, Heating mantel, Autoclave, Laminar air flow, Forceps, Punching maching for making uniform circular discs, Inoculating loop, Spatula, Scale, Bunsen burner and Incubator**.**

Identification and collection of plant specimen: The leaves of the selected plant specimens were collected from Tezpur, Sonitpur district in the month of january,2019 after that the selected plant speciman were properly indentified and certified by Botany Department of Darrang college, Tezpur. The fresh leaves of selected plant species were than washed 2-3 times in running tap water to remove any dust perticule on the surface of the leaves and air dried at room temperature.

Preparation of plant extract: Thoroughly washed fresh plant material (50 g) was smash with 50 ml sterile 100% concentrated ethanol in a warring blender for 10 min. The plants material was first filtered through double-layered muslin cloth, and then filtered through Whatman No. 1 filter paper. The extract was preserved aseptically in a brown bottle at 5 ^{o}C until further use. The plants that showed antifungal activity were only selected for further work in solvent extraction.

Preparation of media:

Potato dextrose agar media: The media prepared was prepared by suspending 39 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing.

Fungal stains: The phytopathogenic fungal strains were collected from the Defense Research Laboratory (DRL), Solmara, Tezpur.

Sub-culturing of fungal strain: To sub-culture fungi on new petriplates, freshly prepared agar plate is used. With the help of an aseptic needle or an inoculating loop prepared by dipping it in 90% ethanol and then putting it in flame of a spirit lamp, a loopful of inoculum from the culture plate is taken under an aseptic condition in

laminar air flow chamber and then inoculated on the fresh media plate. The freshly inoculated media is then incubated in BOD incubator at a temperature of 26_+ 2c for the culture to grow.

Disk Diffusion Method (Kirby-Bauer Test)

: For determining the zone of inhibition on plates containing PDA media, disc diffusion method carried out. The discs of uniform diameter of 4mm are cut out of filter paper by punching machine and sterilized for several time. The discs were then dipped in 100% concentrated of ethanolic plant extract diluted with DMSO and left some time before being used. On freshly prepared PDA media on petridishes, a drop of fungal inoculum from PDB have been placed on each of the petridishes and spread with a spreader uniformly throughout the media. After that the discs left few time in the absolute concentration are then placed in the inoculated media and incubated in a BOD incubator for 48 hours for the respective fungi to grow. If the discs containing the extracts stop the fungus from growing or kill the fungus, there will be an area around the disc where the fungus have not grown enough to be visible. Zone of inhibition will be produced. Observe it carefully and measure the diameter of the zone to evaluate the effectiveness of that antibiotic against particular organism.

RESULTS AND DISCUSSION

The present study tested the antifungal activity of ethanolic leaf extracts of *Mimosa pudica, Polygonum hydropiper, Leucas aspera, Solanum torvum, Mirabilis jalapa, Xanthium strumarium, Solanum nigram, Nyctanthes arbor-tristis, Mikania micrantha* and Citrus leaves against *S. sclerotiorum.* The antifungal activities of ethanolic plant extract were carried out for different concentration of plant extract i.e. concentration 1, 2, and 3. The result showed that the antifungal activity increases with the increase in concentration of the extract.

It has been seen that in concentration 1 antifungal activities against *S. sclerotiorum*, *Citrus leaves, Leucus aspera, Nyctanthus, Xanthium strumarium,* and *Solanum nigrum and Solanum torvum* did not show any inhibition zone. *Mirabilis jalapa* showed the highest inhibition zone of 8mm.Whereas *Polygonum hydropiper* showed the lowest inhibition zone of 4.3mm against *Sclerotinia sclerotiorum.*

In concentration 2 antifungal activities against *S. sclerotiorum, Leucus aspera* and *Nyctanthus* did not show any inhibition zone. Mimosa pudica showed the highest inhibition zone of 12.6mm.Whereas *Citrus* and *Solanum torvum* showed the lowest inhibition zone of 5mm against *Sclerotinia sclerotiorum.*

In concentration3 antifungal activities against *S. sclerotiorum, Leucus aspera* and *Nyctanthus* did not show any inhibition zone. *Mikania micrantha* showed the highest inhibition zone of 19.6mm.Whereas *X. strumarium* showed the lowest inhibition zone of 8mm against *Sclerotinia sclerotiorum.* Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. 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. This finding agrees with the report of Banso *et al.* (1999) that higher concentration of antimicrobial substance showed appreciation in growth inhibition.

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Ogundipe *et al.*, 1998 and Ibrahim *et al.*, 1997). It may be concluded that keeping aside the environmentally hazardous commercial fungicides, these leaf extracts could be a suitable substitute for controlling the fungal pathogens. However, further evaluation in field conditions is needed.

Table 1. Zone of inhibition against *sclerotinia sclerotiorum* of concentration 1 ethanolic plant extracts.

PLANT EXTRACT	1st ZONE OF INHIBITION [mm]	2nd ZONE OF INHIBITION [mm]	3rd ZONE OF INHIBITION [mm]	AVARAGE [mm]
M. pudica	6mm	_	_	6
P. hydropiper	4mm	3mm	6mm	4.3
Citrus leaves	_	_	_	_
L. aspera	_	_	_	_
M. jalapa	8mm	_	_	8
N. arbor-tristis	_	_	_	_
S. nigrum	_	_		_
M. micrantha	7mm	_	5mm	6
S. torvum	_	_	_	_
X.strumarium	_	_	_	_

Table 2. Zone of inhibition against *S. sclerotiorum* for concentration 2 of ethanolic plant extracts.

PLANT EXTRACT	1ST ZONE OF INHIBITION [mm]	2nd ZONE OF INHIBITION [mm]	3rd ZONE OF INHIBITION [mm]	AVARAGE [mm]
M. pudica	7mm	9mm	8mm	8
P. hydropiper	9mm	5mm	_	7
Citrus leaves	_	6mm	3mm	4.5
L. aspera	_	_	_	_
M. jalapa	_	_	_	
N. arbor-tristis	_	_	_	_
S. nigrum	_	7mm	_	7
M. micrantha	10mm	4mm	7mm	7
S. torvum	4mm	_	5mm	4.5
X. strumarium	_	_	7mm	7

Table 3. Zone of inhibition against *S. sclerotiorum* for concentration 3 of ethanolic plant extracts.

PLANT EXTRACT	1ST ZONE OF INHIBITION [mm]	2nd ZONE OF INHIBITION [mm]	3rd ZONE OF INHIBITION [mm]	AVARAGE [mm]
M. pudica	10mm	7mm	8mm	8.3
P. hydropiper	12mm	10mm	18mm	13.3
Citrus leaves	9mm	5mm	_	7
L. aspera	_	_	_	_
M. jalapa		10mm	4mm	7
N. arbor-tristis	_	_	_	_
S. nigrum	_	8		8
M. micrantha	10mm	13mm	19mm	14
S. torvum	8mm	_	10mm	9
X. strumarium		6mm	10mm	8

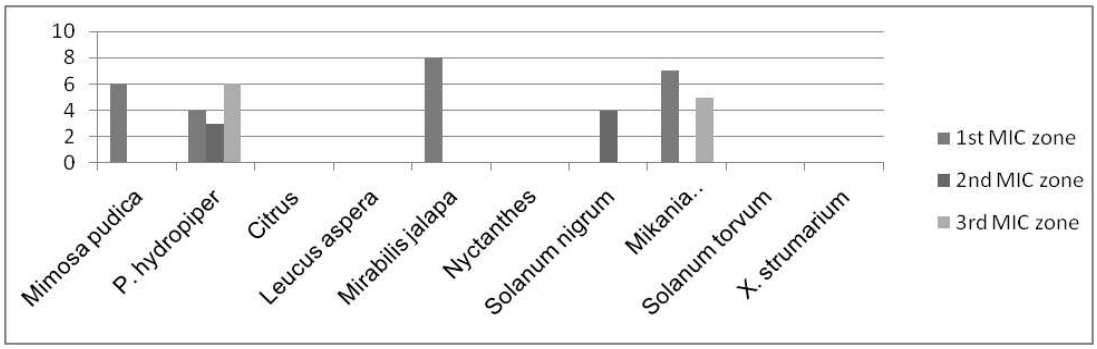

Figure 1. For concentration 1 of plant extract:

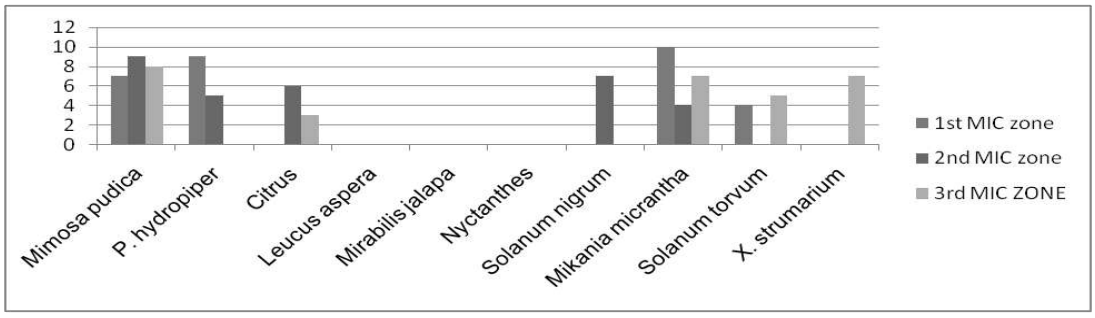

Figure 2. For concentration 2 of plant extract:

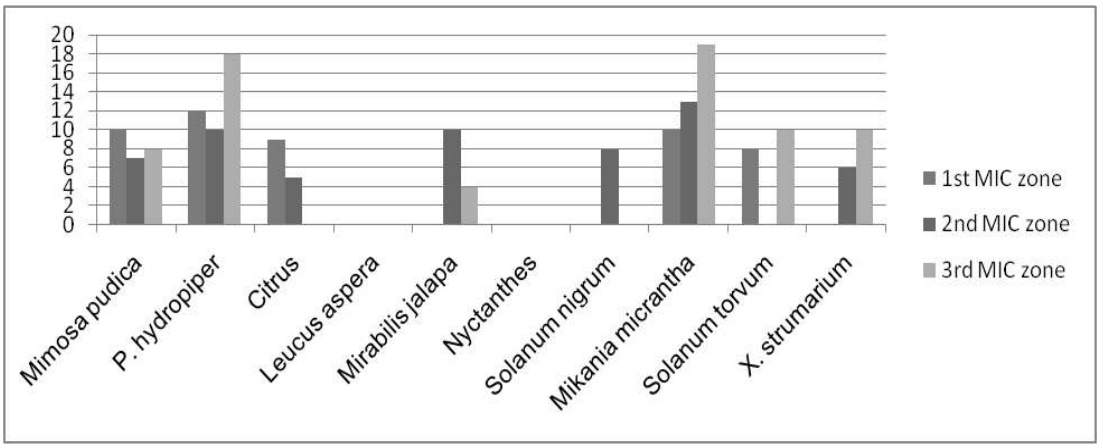

Figure 3. For concentration 3 of plant extract :

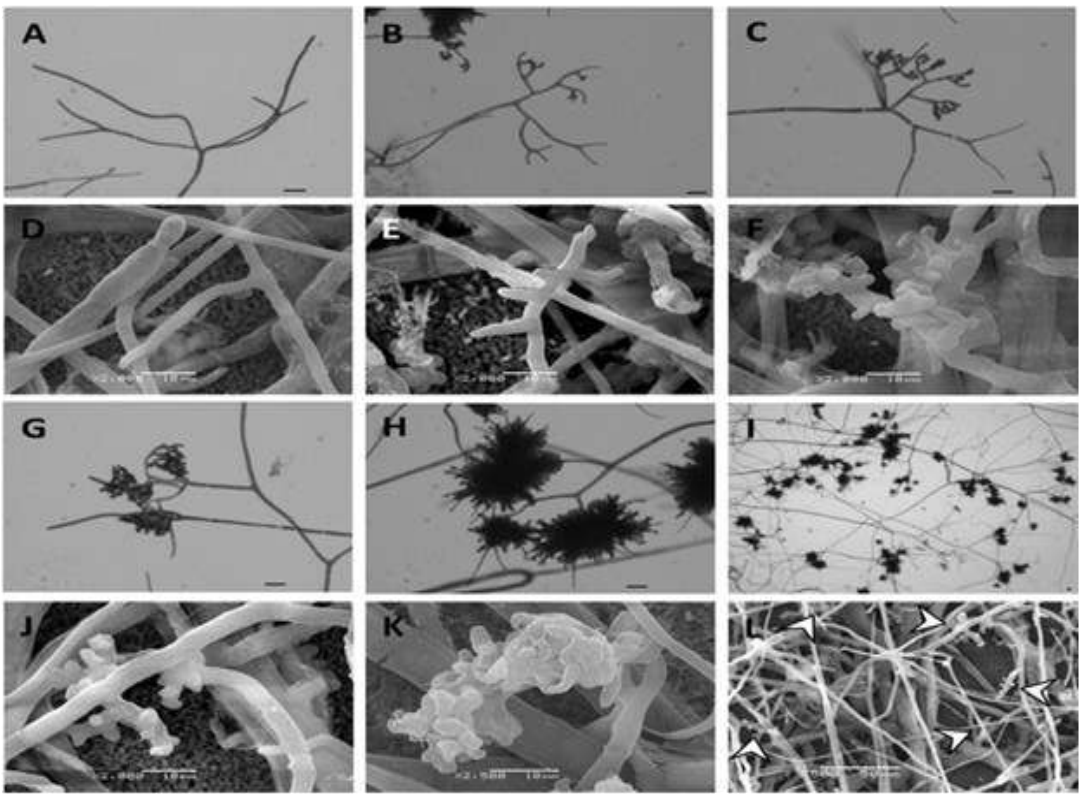

Plate 1. Microscopic views of Fungi

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