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Evaluation of inhibitory effects of medicinal plants extract against *Bipolaris oryzae* of rice

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ABSTRACT

Paddy crops suffer from many diseases called by fungi, bacteria, viruses and nematodes. *Bipolaris oryzae* is a soil borne plant pathogenic fungus which causes brown spot disease in paddy. Plants are reservoir of biological active compounds to control various pathogens. Medicinal Plants such as *Bergia capensis* L., *Marselia quadrifolia* L., *Parthenium hysterophorus* L., *Lippia nodiflora* L., *Azadirachta indica* A. Juss., *Eclipta prostrata* L., *Datura metel* L., *Cyperus compressus* L., *Ocimum sanctum* L., and *Adhatoda vasica* Nees. Totally four solvents were used for the present work. Out of four solvents used (Methanol, Acetone, Petroleum ether and Aqueous) the methanolic extract showed good inhibitory activity. The antifungal potential of medicinal plants as alternative chemical fungicides for controlling brown spot of rice.

Key words: *Bipolaris oryzae*, Rice, Medicinal plants extract, antifungal activity

INTRODUCTION

Rice is one of the major cereals used all over the world (Salim *et al.*, 2003). Tropical and sub-tropical regions of the world are the major rice-producers, with 90% of production occurring in Asia. This important crop suffers from different microbial diseases, with brown spot the most devastating and harmful of the paddy. The *Bipolaris oryzae* strain has been the most serious in South East Asia, particularly since the widespread cultivation of dwarf high-yielding cultivars. It has caused huge yield losses during recent years. In Japan the yield losses reported ranged between 20-30%, occasionally increasing up to 50% (Ou, 1985). The pathogen is known to cause damage at different stages like, storage, seed germination and seedling establishment, vegetable growth and reproductive phase. Datnoff and Lentini (1994) also reported that this disease caused yield loss ranging from 16 -40% in Florida. Seed borne plant pathogenic fungi a major economic loss, which is a major problem among the agricultural community.

However, certain plants have been known for their medicinal and antimicrobial properties since ancient times. Their products can offer advantages because they are relatively safe and easily biodegradable. Biologically active compounds that effectively control various pests and pathogens are known from approximately 2400 plant species (Saleem, 1988). The antifungal activities of different plant extracts against plants disease have been previously investigated (Miah, 1990; Ganguly, 1994; Okigbo & Nmeke, 2005; Leksomboon *et al.*, 2001).

Plant extracts are screened to detect secondary metabolites with biological activities, including antifungal activity. Hence a detailed systematic investigation was conducted to test *in vitro* antifungal activity against important seed borne pathogens *Bipolaris oryzae* of paddy.

MATERIALS AND METHODS

Plant Collection

The Medicinal Plants were collected from the localities in and around Thanjavur (Dt), Tamilnadu. Medicinal Plants such as *Bergia capensis* L., *Marselia quadrifolia* L., *Parthenium hysterophorus* L., *Lippia nodiflora* L., *Azadirachta indica* A. Juss., *Eclipta prostrata* L., *Datura metal* .L., *Cyperus compressus*. L., *Ocimum santcum* L., and *Adhatoda vasica* Nees. brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags and used for the further study

Sterilization of Plant Materials

The disease free and fresh plants were selected for this investigation. About 2gm fresh and healthy leaves were taken for each solvent including aqueous. Then, surface sterilized with 0.1% mercuric chloride and alcohol for few seconds. Again the plant materials were washed thoroughly with distilled water (Three times).

Preparation of Plant Extracts

Two gram of sterilized plant leaves were kept in the 10 ml organic solvents such as methanol, petroleum ether, acetone and aqueous. Then these are grind with the help of mortar and pestle. The grind plant material was subjected to centrifugation, for 10-15min (at 10,000rpm). Again, it was filtered through whatmann No. 1 filter paper. The supernatant was collected and made to known volume by, adding sterile distilled water and stored for further antifungal screening purposes.

Test pathogen

Bipolaris oryzae (Breda de Haan) is a soil-borne pathogenic fungus which causes Brown spot disease leaves of in paddy. The pathogen was isolated from paddy field soil, Thanjavur (Dt), Tamilnadu.

Preparation of Microbial Inoculum

Composition of Potato Dextrose Agar Medium

Potato (Peeled) - 200g
Dextrose - 20g
Agar - 18g
Distilled water - 1000ml
pH - 5.6

The young microbial inoculum culture was prepared and used during the research period. The Potato Dextrose Broth (PDB) was prepared.. The pure microbial cultures were collected inoculated into potato dextrose broth tubes by using inoculation loop. The tubes were incubated at 27°C for 48-72 hours. The developed cultures were used for the experiments.

Screening for Antifungal Activity assay

Antifungal activity assay using Agar-well diffusion method (Perez *et al* .,1990). In the freshly prepared and sterilized potato dextrose agar medium, a pinch amount of streptomycin was added and mixed well. Then these 20 ml of medium was poured into each petriplate and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swab. Then a well 0.5cm was made in the medium by using sterile cork borer, 150µl of the each aqueous, methanol, acetone and petroleum ether plant extracts were transferred into separate wells. Then these plates were incubated at 27°C for 8-72 hours. After incubation period the results were observed and measure the diameter of inhibitor zone around the each well.

RESULTS AND DISCUSSION

In the present investigation, the antifungal properties of methanol, acetone, petroleum ether and aqueous of medicinal plant viz *Bergia capensis* L., *Marselia quadrifolia* L., *Parthenium hysterophorus* L., *Lippia nodiflora* L.,

Azadirachta indica A. Juss., *Eclipta prostrata* L., *Datura metal* .L., *Cyperus compressus*. L., *Ocimum santcum* L., and *Adhatoda vasica* Nees were tested against soil borne pathogenic fungus *Bipolaris oryzae* .

Deepika Srivastava *et al.*, (2011) investigated that antifungal activity of two common weeds *Lantana camara* (Lantana) and *Parthenium hysterophorus* (Congress grass) against *Alternaria* sps causing different plant diseases of tomato, potato and brinjal .Maximum inhibition was reported at 20mg/ml concentration of *Parthenium hysterophorus*. Madhanraj *et al.*, (2010) studied that antifungal ability of some plant extracts against *Fusarium solani* pathogen causing wilt disease of banana.The leaves of medicinal plant extract such as *Adathoda vasica*, *Azadirachta indica* and *Vitex negundo* was more effective at 20% concentration against the pathogen was reported. Antimicrobial and antifungal potency of *Parthenium* has also been reported by Bajwa *et al* 2003 and Patel 2007. Prince *et al.*, (2011) tested the antifungal activity of eight different medicinal plants namely *Aloe vera*, *Ocimum sanctum*, *Cenetella asiatica*, *Piper betle*, *Calotropis gigantea*, *Vitex negundo*, *Ocimum basilicum* and *Azadirachta indica* were tested against plant pathogenic fungus (red rot disease causing agent) *Colletotrichum falcatum* by agar well –diffusion method.The best inhibitory effect of neem extract against *Bipolaris oryzae* was also observed that Bisht and Khulbe (1995). Ganguly (1994) obtained that good inhibitory effect of *Azadirachta indica* against *Helmithosporium oryzae*. Deka Bhuyan,*et al* ., (2010) studied brown spot disease of rice caused by *Bipolaris oryzae* to examined the effect of two essential oils (EOs) from *Lippia geminata* and *Cymbopogon jwarancusa* on in vitro growth and sporulation of these two pathogens.

In the present investigation, the extract of ten medicinal plants showed significant reduction in growth of *Bipolaris oryzae*. The Most of the medicinal plants were recored that the maximum zone of inhibition as a methanol extract, expect *Datura metal*. Methanolic extract of *Parthenium hysterophorus* and *Azadirachta indica* exhibited maximum zone of inhibition as 28mm and 25mm against soil borne pathogenic fungus *Bipolaris oryzae*. Methanolic extract showed better antifungal activity when compared to all other solvent extracts. The results of the antifugal activity of medicinal plants were given in table-1. It has been revealed that the methanol extracts exhibited moderate activity against *Bipolaris oryzae*.

Table:1 Antifungal activity of some medicinal plants against *Bipolaris oryzae*

S. N.	Plant extract	Zone of inhibition (diameter in mm)			
		Methanol	Petroleum ether	Acetone	Aqueous
1.	<i>Bergia capensis</i> .L	8	5	-	-
2.	<i>Marselia quadrifolia</i> .L	15	8	5	-
3.	<i>Parthenium hysterophorus</i> .L	28	25	22	-
4.	<i>Lippia nodiflora</i> . L	12	7	-	-
5.	<i>Azadirachta indica</i> . A,Juss	25	21	20	0.5
6.	<i>Eclipta prostrata</i> . L	15	8	-	-
7.	<i>Datura metal</i> .L	18	20	15	-
8.	<i>Cyperus compressus</i> .L	8	5	-	-
9.	<i>Adhatoda vasica</i> Nees	20	15	18	-
10.	<i>Ocimum sanctum</i> . L	25	20	18	-

CONCLUSION

The present investigation concluded that antifungal activity of medicinal plants showed better activity to control seed borne pathogenic fungi. The results also indicated the necessity for further investigation to isolate and

characterize active principle responsible for the activity and its subsequent exploitation for paddy disease management, using locally available medicinal plants.

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