ISSN: 2349-9141

IJIRR

International Journal of Information Research and Review
Vol. 1, Issue, 3, pp. 068-072, September, 2014

Full Length Research Article

BIOEFFICACY OF PLANT EXTRACTS ON INHIBITION OF PYTHIUM MYRIOTYLUM

***Tahira Parveen and Kanika Sharma**

Department of Botany, University College of Science, MLS University, Udaipur, Rajasthan, 313001, India

ARTICLE INFO

Article History:
Received 02nd June, 2014
Received in revised form 10th July, 2014
Accepted 13th August, 2014
Published online 30th September, 2014

Keywords:
Antifungal Activity,
Plant Extracts,
Pythium Myriotylum,
Poisoned food Technique.

ABSTRACT

Crude alcohol extract, 50% hydro-alcohol and aqueous extracts of 10 plants species belonging to 9 families were screened *in vitro* for antifungal activity against economically important phytopathogenic fungus, *Pythium myriotylum* which was isolated from infected ginger. Bioassays of the extracts were conducted by "Poisoned food technique" on agar plate culture with triplicates. Nine of ten (90%) plant species showed inhibitory activity against mycelial growth of the tested fungi. Among the 10 plants taken, *Jacaranda mimosifolia* showed best activity with 22.0% inhibition by 50% hydro-alcohol extract and 19.66% inhibition by its aqueous extract followed by *Moringa olifera* and *Lawsonia inermis* which exhibited 18.3% and 16.0% growth inhibition respectively. 13.0% and 12.6% inhibition was observed with *Terminallia arjuna* and *Polyalthia longifolia* respectively. All other selected plants exhibited inhibitory activity ranging from 5.3% to 10.0% against *Pythium myriotylum* .On the basis of these results, we conclude that the plants selected for this study can be regarded as a rich source of metabolites with significant antifungal activity, since 10mg/ml concentrations of crude extracts of these plants are showing significant activity. Partially purified fractions with high concentrations and active molecules may have enhanced activity against the test pathogen, as well as different species of *Pythium* and other fungi also.

INTRODUCTION

Zingiber officinale Rosc. (Ginger), is a perennial rhizomatous herb belonging to the family Zingiberaceae (Hayden *et al.,* 2004) also an important commercial crop grown for its aromatic rhizomes which are used as a spice and medicine (Sharma *et al*., 2010). It is a distinct family of aromatic tropical plants that yield spices, dyes, perfumes and medicines as well an important crop that earns a sizeable amount of foreign exchange for the country (Tarafdar and Saha, 2007). Ginger is a high return but also a high risk crop. Rhizome rot (also known as soft rot) is one of the most destructive diseases of ginger worldwide (Dohroo 2005). It reduces the potential yield of ginger to a great extent in the field, storage, and market and may cause more than 50 percent losses (Joshi and Sharma, 1980). 50–90% loss has been reported by Nirmal *et al*. (1992). Several practices have been used for the management of rhizome rot disease. Among them rhizome treatment with chemicals is one of the effective method which often provides some protection, against rhizome rot. But it has residual effect and it is non-economical also. Hence biological control of this pathogen is a promising approach, seeing that it is comparatively benign towards the environment (Paulitz and Bélanger, 2001; Rattink, 1992). So the present study was conducted to investigate the inhibitory effect of crude alcohol, hydro-alcohol (50%) and aqueous extracts of plants given in the table no. 1 against *Pythium myriotylum.* The test pathogen was isolated from infected ginger rhizome.

****Corresponding author: Tahira Parveen***
Department of Botany, University College of Science, MLS University, Udaipur, Rajasthan, 313001, India

MATERIALS AND METHODS

Collection, isolation and identification of the pathogen Diseased samples of ginger rhizomes were collected in sterilized polybags from various ginger farms in Jhadol, Udaipur, (Rajasthan) in the month of July –August. Plant samples were rinsed thoroughly under running tap water. Specimens were cut into 0.5-cm long segments, blotted dry on paper towels, and placed onto 2% water agar (Plaats-Niterink 1981). Cultures were incubated at room temperature (20-24℃) and observed daily for the emergence of fungal mycelium from the tissue. After 1–3 days, hyphal tips were removed from the colonies, transferred to V8 agar (Guo and Ko 1993), and identified according to the descriptions and key suggested by Plaats-Niterink (1981). Pure culture was maintained on PDA at 4 °C. Pure culture was also identified by Dr. Anila Doshi (Head,

Department of Plant pathology, Rajasthan College of Agriculture Udaipur Rajasthan, India) as *Pythium myriotylum*.

Pathogenecity Test

5 days old culture of test pathogen growing on PDA plate was mixed in Sand-maize meal medium (9:1, 90gm of soil and 10gm of grinded maize). The mixture was kept for 10 days, then this inoculum was mixed with the top soil in the pot containing one month old plant of ginger. After 4 weeks of inoculum addition in the pot, disease severity was assayed by inoculating small pieces of leaves and rhizomes on WA. (Ghosh and Purkayastha 2003).

Preparation of Plant Extracts

Ten plants (Table No. 1) belonging to 9 different families were collected from the Botany Garden of University College of Science, Rajasthan college of Agriculture and from Fisheries Department, Udaipur. These botanicals were selected on the basis of presence of antimicrobial properties as given in the literature (Bobbarala *et al.,* 2010, Pattnaik *et al*., 2012, Dileep *et al*., 2013, Garampalli and Rajkumar 2013). All the plants were identified by Dr. Maina, Head, BSI (Botanical Survey of India) Jodhpur, Rajasthan, India. Mature leaves of all the selected test plants were washed thoroughly with tap water, air dried in the shade on separate paper sheets then they were ground to a fine powder with the help of an electric blender. For extract preparation, 10gm of each powdered materials were added individually to 100ml of distilled water, 50% hydro-alcohol and 100% alcohol respectively and after 24 hours, the contents were filtered through four -fold muslin cloth followed by Whatman filter paper No.1 (Kekuda *et al*., 2010) and used for antifungal studies.

Table No. 1 List of Plants Screened for Antifungal Activity

S.No.	Name of the Plant	Vernacular Name	Family
1	Azadiracta indica	Neem	Meliaceae
2.	Aegle marmelos	Beel patrak	Rutaceae
3.	Cassia fistula	Amaltas	Fabaceae
4.	Jacaranda mimosifolia	Blue gulmohar	Bignoniaceae
5.	Lawsonia inermis	Mehandi	Lythraceae
6.	Moringa olifera	Sehjana	Moringaceae
7.	Murraya koenigii	Meetha neem	Rutaceae
8.	Polyalthia longifolia	Ashapal	Annonaceae
9.	Terminallia arjuna	Safeda	Combretaceae
10	Ziziphus jujuba	Jhadi ber	Rhamnaceae

Assay of in Vitro Antifungal Activity of Plant Extracts

In vitro antifungal efficacy of crude alcohol, 50% hydro-alcohol and aqueous, leaf extract against *Pythium myriotylum* was determined by Poisoned food technique (Groover and Moore 1962). 9 ml of PDA (Potato Dextrose Agar) media was mixed with 1ml (10mg/ml) of extract and sterilized in autoclave then poured into the sterilized Petri plates. A 5mm diameter fungal disc taken from actively growing 5 days-old culture of *Pythium myriotylum* on PDA, was placed in an inverted position in the centre of the Petri plates containing PDA amended with leaf extracts respectively. Plates containing medium with fungicide Mancozeb 0.2% (Indofil® mancozeb 75% WP) served as a positive control and plates with medium and 1ml of the solvents/water used to dissolve the extracts served as negative control. All plates were incubated at 28 °C and three replicates were maintained for each treatment. Radial growth of mycelium was measured 5 days after inoculation. The results were compared with negative control. Experiment was repeated twice and mean of the readings were taken for calculations. The percent inhibition of the fungus in treatments was calculated using the following formula:

Inhibition of mycelial growth (%) = (C–T/C) ×100

Where 'C' is average diameter of fungal colony in control plates. 'T' is average diameter of fungal colony in poisoned plates (Gupta and Tripathi, 2011).

RESULTS

In the present study soft rot causing pathogen *Pythium myriotylum* was isolated from infected ginger rhizomes which were collected from Jhadol. The leaves of all the 10 selected plants were exracted in aqueous, 50% hydroalcohol and in 100% alcohol and their % extractive values are ranging from 1.0% to 21.15%. The highest % extractive value was found to be 21.15% followed by 17.55% which were from the hydro-alcohol and aqueous extracts of *Lawsonia inermis* respectively. The % extractive values of all the selected plant extracts are given in the table no. 2. Crude extracts of ten plants of the 9 species tested, showed 5.3% to 22.0% inhibitory activity against mycelial growth of *Pythium myriotylum* (Graph.no.1). Maximum inhibition of fungal growth was recorded by 50% hydro-alcohol extract of *Jacaranda mimosifolia* and it was found to be 22.0%. It was followed by 19.66% growth inhibition by aqueous extract of the same plant. 50% hydro-alcohol extract *of Moringa olifera* leaf also showed significant antifungal activity with 18.3% growth inhibition of *P. myriotylum*. Other plant extracts showed varying level of growth inhibition ranging from 5.3% to 16.0% (Table no.2).

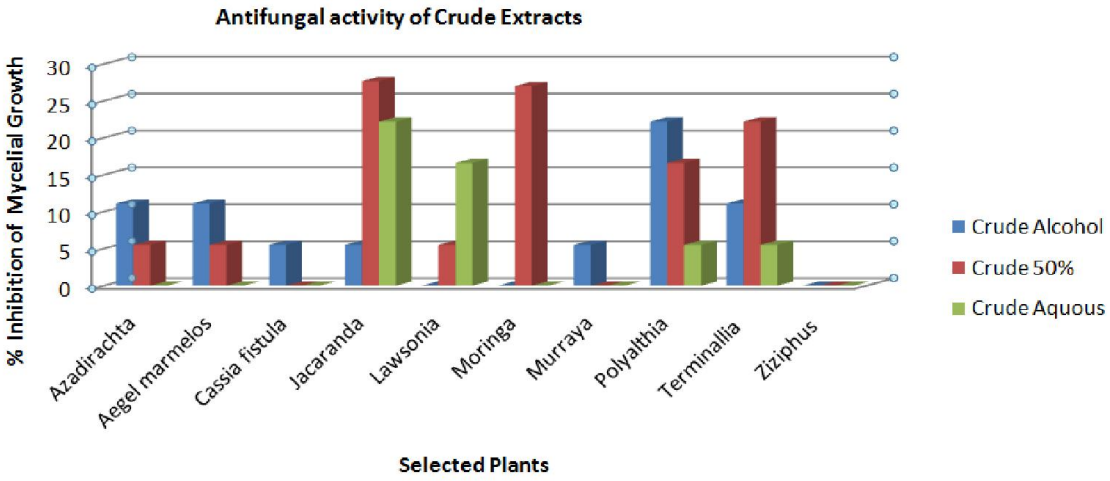
DISCUSSION

The genus *Pythium* is a complex genus containing over 200 described species that occupy a variety of terrestrial and aquatic ecological habitats (Dick, 2001). Perhaps the most economically important members of this genus are plant pathogens (Hendrix and campbell, 1973). Being very generalistic and unspecific in their host range, it is a major problem for a wide range of horticultural crops also. (Owen- Going 2002, Chaube and Pundhir 2005). *Pythium* species cause soft rot in ginger, Butler (1907) recorded the incidence of this disease for the first time from Surat (Gujarat, India). In India, at least six pathogenic species of *Pythium* have been reported to cause soft rot in ginger, and these include *P. myriotylum, P. aphanidermatum, P. deliense, P. perilium, P. vexans, P. ultimum* and *P. butleri* (Shahare and Asthana 1962, Haware and Joshi 1974, Dohroo 1987). *Pythium* species cause soft rot of ginger in Rajasthan, Himachal Pradesh, Orissa, Maharashtra, Tamil Nadu, Andhra Pradesh, and Sikkim (Singh *et al.,* 2012). Lodha (2012) reported that *Pythium myriotylum* is the main species of *Pythium*, associated with the rhizome rot of ginger in Udaipur district. Around 90% of ginger produced in Rajasthan comes from Jhadol, a tribal-dominated block in Udaipur district. However, during the last 10 years both the area under cultivation and average productivity of ginger have shown a declining trend due to severe rot attack, a large number of farmers cultivated ginger in the region, but many gave up its cultivation owing to the frequent ginger rot disease that destroys the crops (ACCESS 2008).

Table No. 2 % Extractive Value of Extracts and % Inhibition of Pythium myriotylum

S.No.	Name of Plant	Extract Type	% Extractive value	% Inhibition ± SD
1.	Azadiracta indica	Alcohol	2.65	12.59±0.6409
		50%hydro-alcohol	1.25	5.92±0.6409
		Aqueous	4.4	NA
2	Aegle marmelos	Alcohol	5.25	10.74±0.6409
		50%hydro-alcohol	6.25	5.92±0.6409
		Aqueous	17.3	NA
3	Cassia fistula	Alcohol	1.75	6.29±0.6409
		50%hydro-alcohol	1.00	NA
		Aqueous	2.25	NA
4	Jacarandas mimosifolia	Alcohol	8.50	5.92±0.6409
		50%hydro-alcohol	8.80	22.59±0.6409
		Aqueous	9.25	18.51±0.6409
5	Lawsonia inermis	Alcohol	9.95	NA
		50%hydro-alcohol	21.15	5.92±0.6409
		Aqueous	17.55	17.03±0.6409
6	Moringa olifera	Alcohol	6.50	NA
		50%hydro-alcohol	5.30	18.51±0.6409
		Aqueous	3.4	NA
7	Murraya koenigii	Alcohol	7.21	8.14±0.6409
		50%hydro-alcohol	4.30	NA
		Aqueous	3.85	NA
8.	Polyalthia longifolia	Alcohol	4.21	9.99±0.0058
		50%hydro-alcohol	2.82	11.85±0.6409
		Aqueous	2.73	6.29±0.6409
9.	Terminallia arjuna	Alcohol	3.25	5.92±0.6409
		50%hydro-alcohol	2.89	12.96±0.6409
		Aqueous	2.19	NA
10.	Zyzyphus zuzube	Alcohol	3.25	NA
		50%hydro-alcohol	2.89	NA
		Aqueous	2.19	NA
21	Mancozeb		100%	
22	Control C1		0%	
23	Control C2		0%	

NA: No Activity, C1: Negative control, C 2: Positive control



Graph 1. Efficacy of Various Extracts on % Inhibition of Pythium myriotylum

No single method is available to provide adequate control of the disease caused by *Pythium* (Babadoost 2004). Nowadays, synthetic pesticides are known to be the most effective method of the pest and disease control. However, they are not considered as a long-term solution due to the concerns associated with pesticides application such as problems of public health, environmental pollution, reduction in crop quality, toxic effect on non-target organisms and causing resistance in pest and disease agents, (Kagale 2004, Rai *et al.,* 2006, Rahhman *et al.,* 2010). WHO banned many agriculturally important pesticides due to wide range of toxicity against non target organisms including humans which are known to cause pollution problem (Barnard *et al*., 1997). Besides this recent studies have indicated that some isolates of *Pythium* are becoming less sensitive even to Metalaxyl (Daughtrey, 1998) which is a commonly used fungicide. This has necessitated search for alternatives for controlling the rhizome rot of ginger (Pandey *et al*., 2010). In recent years, natural plant products as environmentally safe option have received attention for controlling phytopathogenic diseases. Many studies have shown that plant extracts effectively controlled various plant pathogens *in vitro* (Sankarasubramanian *et al*., 2008, Mishra *et al*., 2009, Yanar *et al*., 2011, Talibi *et al.,* 2012). The fungicidal activity of some plant extracts in controlling different plant pathogens have been reported by several workers (Tewarri *et al.,* 1991, Amadioha 2000, Okigbo and Emoghene 2004, Okigbo and

Nmeka 2005). (Sagar *et al.,* 2007), (Haouala *et al*., 2008) and (Suleiman and Emua 2009) also reported antifungal activity of plant extracts against *Pythium*. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandrin *et al.,* 1985). The present study clearly demonstrates the significant inhibitory activity of various extracts of selected plants on rot causing pathogen *P. myriotylum* in *in vitro* condition. These results and the encouraging percentage of plants (90% in this research) with antifungal activity indicate that the plants selected can be regarded as rich sources of plants with antifungal activity. They could form the basis for further investigation of fractionation for finding active fractions. The present investigation was attempted to evaluate ten plants belonging to 9 different families of the plant kingdom to show the fact the plants are still a reservoir of many pharmaceuticals which can be isolated and used in plant disease management. It provide environmental friendly alternative to chemical fungicides for managing the pathogens.

Conclusion

From the results of the present study, it can be concluded that the crude extracts of selected plants are effective against the *Pythium myriotylum* and can be regarded as a rich source of metabolites with antifungal activity, the plant extracts which are showing inhibition for pathogen may have potential to be developed as potent fungicides in organic farming against rot causing pathogen .The plant world, the rich storehouse of natural chemicals could be exploited for the use as pesticides. Many species of higher plants have not been yet explored, much less surveyed for biologically active constituent and new sources of commercially valuable pesticides. This is mainly due to lack of information on the screening and evaluation of diverse plants for their antimicrobial potential. Systematic and scientific evaluation of plant derived bio-active molecules for using their potential for the effective management of fungal plant diseases to maintain high level of bio-safety and non-adverse effects on the environment will open up many revenues for the betterment of environment and mankind. The botanicals are cost effective, non hazardous, easily available and do not pollute the environment. Also, biologically active plant derived pesticides are expected to play a significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the growth of disease causing pathogens, would be a more realistic and ecologically sound method for development of future commercial pesticides for crop protection strategies.

Acknowledgement

We would like to sincerely thank Dr. Vinod Maina (Head, BSI Jodhpur), Dr. Kusum Mathur (Retd. Head Dept. of Plant Pathology, RCA Udaipur), Dr. Anila Doshi (Head, Dept. of Plant Pathology, RCA Udaipur) for their cooperation in identification of plants and isolation and identification of pathogen respectively for the research. Financial assistance received from the UGC, Government of India is also gratefully acknowledged.

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