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Antifungal effect of *Terminalia arjuna* leaf extracts on *Pythium myriotylum*

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ABSTRACT

Crude alcohol extract, 50% hydro-alcohol, aqueous extract as well as partially purified leaf extracts of Terminalia arjuna were screened in vitro for antifungal activity against economically important phytopathogenic fungus, Pythium myriotylum which is economically important as it causes Soft-Rot in ginger. It was isolated from infected ginger. Bioassays of the extracts were conducted by "Poisoned food technique" on agar plate culture with triplicates. Test fungus showed inhibitory activity as mycelial growth inhibition. 13.0% inhibition was observed with crude extract of leaf of Terminallia arjuna. Among the partially purified fractions, Acetone fraction gave the best inhibition. MIC and MFC were found to be 5mg/ml and 10mg/ml respectively.
On the basis of these results, we conclude that the plant selected for this study can be regarded as a rich source of metabolites with significant antifungal activity.

1. Introduction

Zingiber officinale Rosc. (Ginger), is a perennial rhizomatous herb belonging to the family Zingiberaceae (Hayden *et al.*,2004). It is 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. 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. So the present study was conducted to investigate the inhibitory effect of crude alcohol, hydro-alcohol (50%) and aqueous extracts as well as partially purified leaf extracts of *Terminalia arjuna* against *Pythium myriotylum.* The test pathogen was isolated from infected ginger rhizome.

***Pythium myriotylum* (Test Fungus):**

P. myriotylum is also distributed worldwide in warm regions with a very large host range. The classification is according to Agrios, (2005).

Kingdom : Chromista
Phylum : Oomycota
Class : Oomycetes
Order : Peronosporales
Family : Pythiaceae
Genus : *Pythium*
Species : *myriotylum*

Binomial name is *Pythium myriotylum* Drechsler (1930).According to Farr *et al.* (1989) the pathogen has been reported in 79 plant hosts in genera such as *Aponogeton, Arachis, Caladium, Citrullus, Coronilla, Cucumis, Glycine, Ligustrum, Lolium, Peperomia, Phaseolus, Robinia, Solanum, Triticum* etc.

2. Materials & Methods:-

A. 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.*

B. 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).

C. *Preparation of Plant Extracts*: Leaves of T. arjuna were collected from the Botany Garden, University College of Science, Udaipur, Rajasthan. Plant was identified by Dr. Maina, Head, BSI (Botanical Survey of India) Jodhpur, Rajasthan, India, where a specimen voucher no.

T.A. 021 was deposited. Collected matured leaves were washed thoroughly with tap water, air dried in the shade on 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% hydroalcohol 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. Partially purified extracts were also prepared through soxhlet assembly using organic solvents in series from petroleum ether to water (given in the table no. 2).

C. Assay of *In-vitro* Antifungal Activity of Plant Extracts:

In vitro antifungal efficacy of crude alcohol, 50% hydro-alcohol, aqueous as well as partially purified leaf extracts 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).

3. Result:

In the present study soft rot causing pathogen *Pythium myriotylum* was isolated from infected ginger rhizomes which were collected from Jhadol. The leaves of selected plant were extracted in aqueous, 50% hydroalcohol and in 100% . Partially purified fractions were obtained by using organic solvents shown in table no. 2

Table No. 1
(*In-vitro* Antifungal Activity of Crude Extracts of Leaf of *Terminalia arjuna* Against *Pythium myriotylum*)

S.No.	Extract type	% Growth inhibition			Average Mean ± S.D
1	Aqueous	NA	NA	NA	NA
2	50% Alcohol	12.3	12.0	11.5	11.93±0.40
3	100% Alcohol	5.0	4.9	5.2	5.03±0.15
4	Control	NA	NA	NA	NA

(Control: Without Any Treatment)

Table No.2
(Antifungal Activity of Various Fractions of *Terminalia arjuna* Leaf Extract Against *Pythium myriotylum)*

S.No.	Fraction	% Growth Inhibition			Average Mean ± S.D
		R1	R2	R3	
1	Petroleum Ether	11.2	10.8	11.1	11.0±0.21
2	Benzene	10.0	11.2	10.5	10.5±0.60
3	Chloroform	8.0	8.5	8.9	8.6±0.45
4	Acetone	35.0	33.3	34.0	34.1±0.85
5	Methanol	8.2	8.8	7.5	8.1 ±0.65
6	Aqueous	NA	NA	NA	NA
7	Control (C1)	NA	NA	NA	NA
8	Control (C2)	NA	NA	NA	NA
9	Mancozeb	50.0	53.33	50.0	51.1 ±1.92
10	Thiram	33.33	35.55	32.22	33.7±1.69

NA: No Activity; C1: 20% Methanol, C2: PDA

4. 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).

The present study may be a mile stone for the formation of herbal biocontrol agents against P. myriotylum as well as other species of *Pythium.*

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