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Management of "Soft Rot" of Ginger by Botanicals

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Abstract

Crude alcohol extract, 50% hydro-alcohol and aqueous extracts of 20 plants species belonging to13 families were screened *in vitro* for antifungal activity against economically important phytopathogenic fungus, *Pythium aphanidermatum* which was isolated from infected ginger. Bioassays of the extracts were conducted by "Poisoned food technique" on agar plate culture with triplicates. Sixteen of 20 (80%) plant species showed inhibitory activity against mycelial growth of the tested fungi. Among the 20 plants taken, *Jacaranda mimosifolia*, *Moringa olifera*, were giving the best activity with 27.7% inhibition. *Polyalthia longifolia* and *Terminallia arjuna* showed 22.2% inhibition. Besides these, *Lawsonia inermis*, *Aegle marmelos*, *Nigella sativa*, *Azadirachta indica*, also exhibited good inhibitory activity against *Pythium aphanidermatum.* According to these results, we can conclude that these plants can be regarded as a rich source of metabolites with significant antifungal activity against *Pythium*. As their crude extracts are giving good results, their purified fractions may have enhanced antifungal activity.

Key-Words: Antifungal activity, Plant extracts, *Pythium aphanidermatum*, Poisoned food technique

Introduction

Zingiber officinale Rosc. (Ginger) belonging to the family Zingiberaceae (Hayden *et al.* 2004) is an important commercial crop grown for its aromatic rhizomes which are used as a spice and medicine (Sharma *et al*., 2010). It is an important crop that earns a sizeable amount of foreign exchange for the country (Tarafdar and Saha, 2007). Rhizome rot (also known as soft rot) is one of the most destructive diseases of ginger worldwide (Dohroo 2005), with losses of 50–90% (Nirmal 1992). The species most commonly associated with the disease is *Pythium aphanidermatum* (Stirling *et al* 2009), which is a soil as well as seed borne pathogen. In spite of destructive effect of *Pythium aphanidermatum*, chemical (Folman *et al*., 2004) and physical control (Benhamou *et al*., 1997) of this fungal pathogen are very difficult to realize. Biological control of this pathogen is a promising approach, seeing that it is comparatively benign towards the environment (Paulitz and Bélanger, 2001; Rattink, 1992).

Hence 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 aphanidermatum.* The test pathogen was isolated from infected ginger rhizome.

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Material 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. The rhizomes (3-5 mm in length) were cleaned and surface sterilized by washing with running tap water followed by 0.5% sodium hypochlorite for 3 minutes, blotted dry on sterile filter paper and placed onto different media like water agar (WA), PPP agar (0.10 g of pimaricin, 0.05 g of penicillin and 0.05 g of polymyxin per liter in corn meal agar) and PARP agar (0.005 g of pimaricin, 0.25 mg of ampicillin, 0.01 g of rifampicin, and 0.10 g of pentachloronitrobenzene per liter in corn meal agar) respectively for isolation of *Pythium* species (Jeffers and Martin 2010). Plates were incubated in the dark at 25±1°C for 2 days, then actively growing hyphal tips from the periphery of the plates were transferred to fresh PDA plates. Pure cultures were maintained on PDA at 4 °C and identified by standard keys on the basis of sexual as well as asexual structures as suggested by Waterhouse (1967,1968) . Pure cultures were also identified by Dr. Anila Doshi (Head, Department of Plant pathology, Rajasthan College of Agriculture Udaipur Rajasthan.) as *Pythium aphanidermatum.*

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). It was kept for 10 days, then this inoculum was mixed with the top soil in the pot. The pot was 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, pseudostem and rhizomes. (Ghosh and Purkayastha 2003).

Preparation of plant extracts

Twenty plants (table no. 1) belonging to 13 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 (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.

Table 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.	*Citrus limona*	Neembu	Rutaceae
5.	*Clitoria ternatae*	Butterfly pea	Fabaceae
6.	*Delonix regia*	Orange gulmohar	Fabaceae
7.	*Eucalyptus globules*	Nilgiri	Mytraceae
8.	*Jacarandas imosifolia*	Blue gulmohar	Bignoniaceae
9.	*Justicia gendarusa*	Gendarusa	Acanthaceae
10.	*Lawsonia inermis*	Mehandi	Lythraceae
11.	*Moringa olifera*	Sehjana	Moringaceae
12.	*Murraya koenigii*	Meetha neem	Rutaceae
13.	*Nigella sativa*	Kalonji	Apiaceae
14.	*Pithecelobium dulse*	Jungle jalebi	Mimosaceae
15.	*Pongamia pinnata*	Karanj	Fabaceae
16.	*Polyalthia longifolia*	Ashapal	Annonaceae
17.	*Prosopis juliflora*	Babul	Fabaceae
18.	*Tecomella undulate*	Rohida	Bignoniaceae
19.	*Terminallia arjuna*	Safeda	Combretaceae
20	*Ziziphus zuzube*	Jhadi ber	Rhamnaceae

Mature leaves of all the selected test plants and seeds of *Nigella sativa* 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.

Assay of *in vitro* antifungal activity of Plant Extracts

In vitro antifungal efficacy of crude alcohol, 50% hydro-alcohol and aqueous, leaf / seed extracts against *Pythium aphanidermatum* 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 aphanidermatum* on PDA, was placed in an inverted position in the centre of the Petri plates containing PDA amended with leaf/seed extracts respectively. Plates containing medium with fungicide Mancozeb 0.2% (Indofil® mancozeb 75% WP) served as a positive control and plates with medium 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 and Discussion

In the present study soft rot causing pathogen *Pythium aphanidermatum* was isolated from infected ginger rhizomes which were collected from Jhadol. The leaves of all the 19 selected plants and seeds of *Nigella sativa* 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 sixteen plants of the 20 species tested, showed 5.5% to 27.7% inhibitory activity against mycelia growth of *Pythium aphanidermatum* for (Table no.-2, Fig.no.1).

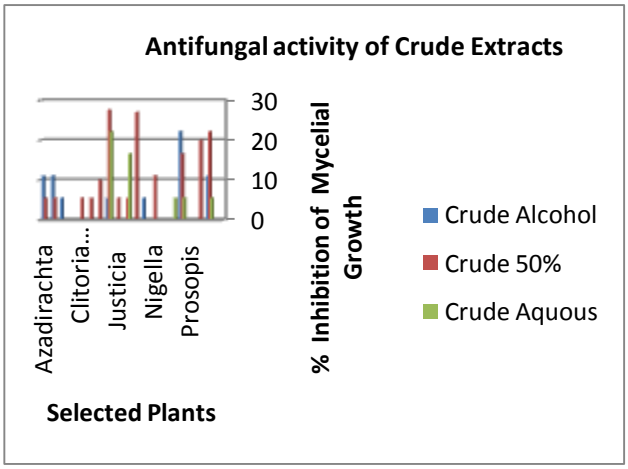
Maximum inhibition (27.7%) of fungal growth was recorded with 50% hydro-alcohol extracts of *Jacaranda mimosifolia*, and *Moringa olifera*.

Table 2: % Extractive Value of Extracts and % Inhibition of *Pythium aphanidermatum*

S. N	Name of Plant	Extract Type	% Extractive value	% Inhibition ± SD
1.	*Azadiracta indica*	Alcohol	2.65	11.11±0.583
		50%hydro-alcohol	1.25	5.5±0.635
		Aqueous	4.4	NA
2	*Aegle marmelos*	Alcohol	5.25	11.11±0.583
		50%hydro-alcohol	6.25	5.5±0.635
		Aqueous	17.3	NA
3	*Cassia fistula*	Alcohol	1.75	5.5±0.635
		50%hydro-alcohol	1.00	NA
		Aqueous	2.25	NA
4	*Citrus limona*	Alcohol	6.81	NA
		50%hydro-alcohol	5.35	NA
		Aqueous	13.4	NA
5	*Clitoria ternatae*	Alcohol	2.45	NA
		50%hydro-alcohol	1.50	5.5±0.635
		Aqueous	1.10	NA
6	*Delonix regia*	Alcohol	5.80	NA
		50%hydro-alcohol	11.55	5.5±0.635
		Aqueous	5.0	NA
7	*Eucalyptus globules*	Alcohol	4.50	NA
		50%hydro-alcohol	3.60	5.5±0.635
		Aqueous	7.25	NA
8	*Jacarandas mimosifolia*	Alcohol	8.50	5.5±0.635
		50%hydro-alcohol	8.80	27.7±0.635
		Aqueous	9.25	22.2±0.635
9	*Justicia gendarusa*	Alcohol	4.20	NA
		50%hydro-alcohol	3.15	5.5±0.635
		Aqueous	1.75	NA
10	*Lawsonia inermis*	Alcohol	9.95	NA
		50%hydro-alcohol	21.15	5.5±0.635
		Aqueous	17.55	16.6±0.635
11	*Moringa olifera*	Alcohol	6.50	NA
		50%hydro-alcohol	5.30	27.7±0.635
		Aqueous	3.4	NA
12	*Murraya koenigii*	Alcohol	7.21	5.5±0.635
		50%hydro-alcohol	4.30	NA
		Aqueous	3.85	NA
13	*Nigella sativa*	Alcohol	17.20	NA
		50%hydro-alcohol	8.15	11.11±0.635
		Aqueous	7.80	NA
14	*Pithecelobium dulse*	Alcohol	4.82	NA
		50%hydro-alcohol	2.15	NA
		Aqueous	2.00	NA
15	*Pongamia pinnata*	Alcohol	5.25	NA
		50%hydro-alcohol	7.15	NA
		Aqueous	3.85	5.5±0.635
16	*Polyalthia longifolia*	Alcohol	8.25	22.22±0.635
		50%hydro-alcohol	12.7	16.6±0.635
		Aqueous	3.25	5.5±0.635
17	*Prosopis juliflora*	Alcohol	3.15	NA
		50%hydro-alcohol	2.10	NA
		Aqueous	2.15	NA
18	*Tecomella undulate*	Alcohol	4.50	NA
		50%hydro-alcohol	12.0	20.20±0.693
		Aqueous	3.80	NA
19	*Terminallia arjuna*	Alcohol	4.21	11.11±0.583

		50%hydro-alcohol	2.82	22.22±0.635
		Aqueous	2.73	5.5±0.635
20	*Ziziphus 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: Showing Efficacy of Various Extracts on % Inhibition of *Pythium aphanidermatum*

Pythium aphanidermatum is a major cause of ginger soft rot. Being very generalistic and unspecific in their host range, it is also a major problem for a wide range of horticultural crops (Owen 2002, Chaube and Pundhir 2005). No single method is available to provide adequate control of the disease caused by it (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). 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) . Evaluation of the effect of plant species against rot causing fungi, *P. aphanidermatum*, has also been earlier investigated under laboratory and greenhouse conditions in different parts of the world (Sagar *et al*. 2007). Haouala *et al*. 2008, Suleiman and Emua 2009 reported the fungitoxic efficacy of some plant extracts against *P. aphanidermatum* isolated from rhizome rot specimen of ginger.

Here in the present investigation some of the taken plants have antifungal activity on other fungi and some of them have inhibitory effect on *Pythium* spp. The present study clearly demonstrates the significant inhibitory activity of various extracts of selected plants on rot causing pathogen *P. aphanidermatum* in *in vitro* condition. These results and the encouraging percentage of plants with antifungal activity (80% in this research) 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 twenty plants belonging to 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

Finding new wide spectrum biological antifungal agents is still a priority today because of many adverse effects of the synthetic chemicals, like resurgence of resistant pathogens and disturbance of ecological balance as well as the ill effects of synthetic fungicides on human beings.

The use of crude plant extracts to control plant diseases is an old practice in many parts of the

World, the plant products require a systematic study in order to search for better fungicides for management of fungal diseases. 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, with special reference to the management of plant diseases.

From the results of the present study, it is concluded that the crude extracts of selected plants are effective against the *Pythium aphanidermatum* which is an economically important plant pathogen, the plant extracts which are showing inhibition for pathogen may have potential to be developed as potent fungicides in organic farming.

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