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RESEARCH PAPER

Use of plant products (Extracts) as a natural fungicide against *Rhizoctonia solani* Kuhn.

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Eleven commonly available plant species *Acalypha indica* L., *Achyranthes aspera* L., *Anisomeles indica* L., *Curcuma malabarica* Velay., *Dendrophthoe falcata* L., *Hedychium spicatum* Buch., *Lantana indica* Roxb., *Lantana camara* L., *Leucas aspera* Willd., *Oxalis corniculata* L., *Psidium guajava* L. were tested for *in vitro* fungicidal activity on *Rhizoctonia solani* Kuhn., a causative agent of Black Scurf disease of potato. Out of eleven, the products of four plants showed significant fungicidal activity against the test pathogen by poisoned food technique. *Curcuma malabarica* Velay. and *Hedychium spicatum* Buch. showed 100 per cent inhibition of mycelial growth at 0.5 ml concentration. Results of the present investigation indicate that the selected plant species possess fungicidal activity and can be exploited as natural fungitoxicants to control the growth of *Rhizoctonia solani* Kuhn.

Key words : Fungicidal activity, Plant extract, *Rhizoctonia solani*, Black Scurf disease, Poisoned food technique

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INTRODUCTION

The use of chemical fungicides is a very popular practice to control various plant diseases as compared to natural one which is prepared from plants or plant parts. The widespread use of synthetic fungicides (chemical fungicides) in agriculture is a relatively recent phenomenon and most of the major developments have taken place during the last 60 years. It has been the major way of fungal disease control in the world during the past decades and now-a-days it plays an important role in crop protection. However, now-a-days the use of synthetic fungicides is limited due to their non-biodegradability, pollutive nature and residual toxicity. The chemical residues are liable to remain on the plant or within its tissues following fungicidal treatment. Fungicide residues in plants and their fruits pose a great health risk to the consumer, led to the search for safe alternatives to synthetic fungicides.

Plants are reservoir of biological active compounds to combat various pathogens. Several studies showed that the plant extract can be a source of natural fungicide (Arokiyaraj *et al*., 2008; Gangadevi *et al*., 2008 and Brindha *et al*., 2009). The natural plant products obtained from plants efficiently fulfill this criterion and have vast potential to influence modern agrochemical research. When extracted from plants, these chemicals are referred to as natural. The use of natural fungicides is now emerging as one of the primary means to protect crops and their products and the environment from synthetic or chemical fungicide. Natural fungicide degrade more speedily than most synthetic or chemical fungicides and, therefore, are considered to be eco-friendly and less likely to kill favourable micro-organism. Most of the natural

fungicide generally degrade within a few days and sometimes even within a few hours.

Black scurf, caused by *Rhizoctonia solani* Kuhn., is becoming more common and serious disease in the potato fields. It inflict enormous losses to the production of potato crop with the occurrence of disease symptoms in the fields as black sclerotial masses on tuber surface and aerial tubers on foliar parts (Wharton *et al.*, 2007).

The objective of the present study was to evaluate the use of Natural Fungicide which is extracted from natural products (extracts) of selected plant species and study their fungicidal activity against *Rhizoctonia solani* Kuhn.

RESEARCH METHODOLOGY

Plant materials:

In the present study, eleven plant species of different families have been selected from different areas of Gorakhpur, to analyze their fungicidal activity against *Rhizoctonia solani* Kuhn. (Table A). The presented work was done in the year 2015-2016.

Preparation of extract:

Crude extract:

20 g of freshly collected disease-free leaves and rhizome of plants were surface sterilized with sodium hypochlorite solution (4%) for 2 min followed by washing with sterilized distilled water to remove all the traces of sodium hypochlorite. The sample was then chopped into small pieces and macerated to pulp using a sterilized pestle and mortar. The pulp was squeezed by double layered sterilized muslin cloth and filtered through Wattman's No. 1 filter paper. The crude extract thus, obtained was subjected to fungicidal testing against the test fungus *Rhizoctonia solani* Kuhn.

Table A : List of plant species tested for fungicidal activity

Sr.No.	Name of plant	Family	Parts used
1.	*Acalypha indica* L.	Euphorbiaceae	Leaves
2.	*Achyranthes aspera* L.	Amaranthaceae	Leaves
3.	*Anisomeles indica* L.	Lamiaceae	Leaves
4.	*Curcuma malabarica* Velay.	Zingiberaceae	Rhizome
5.	*Dendrophthoe falcate* L.	Loranthaceae	Leaves
6.	*Hedychium spicatum* Buch.	Zingiberaceae	Rhizome
7.	*Lantana indica* Roxb.	Verbenaceae	Leaves
8.	*Lantana camara* L.	Verbenaceae	Leaves
9.	*Leucas aspera* Willd.	Lamiaceae	Leaves
10.	*Oxalis corniculata* L.	Oxalidaceae	Leaves
11.	*Psidium guajava* L.	Myrtaceae	Leaves

Table B : Fungitoxicity of plant extracts against *Rhizoctonia solani* Kuhn

Sr.No.	Name of plant	Family	% inhibition on mycelial growth
1.	*Acalypha indica* L.	Euphorbiaceae	32
2.	*Achyranthes aspera* L.	Amaranthaceae	29
3.	*Anisomeles indica* L.	Lamiaceae	100
4.	*Curcuma malabarica* Velay.,	Zingiberaceae	100
5.	*Dendrophthoe falcate* L.	Loranthaceae	53
6.	*Lantana indica* Roxb.	Verbenaceae	42
7.	*Hedychium spicatum* Buch.	Zingiberaceae	100
8.	*Lantana camara* L.	Verbenaceae	42
9.	*Leucas aspera* Willd.	Lamiaceae	24
10.	*Oxalis corniculata* L.	Oxalidaceae	80
11.	*Psidium guajava* L.	Myrtaceae	41

Aqueous extract:

For the preparation of aqueous extracts, 10g of each dried sample was grinded into a fine powder with 100 ml sterile distilled water and left for overnight at room temperature (30 ± 2°C). The content of the flask was then filtered through filter paper to obtain clear infusion in laminar air flow (Chaudhry and Tariq, 2006). Poisoned food technique was used for the evaluation of fungicidal potential (New, 1971).

Microbial cultures and growth conditions:

The plant extracts were assayed for fungicidal activity against the fungal strain *Rhizoctonia solani*. Kuhn. (MTCC No. 4633) obtained from Microbial Type Culture (MTCC), Chandigarh. This fungus was grown on PDA plate at 27^0C ± 2°C and maintained with periodic sub – culturing at 4^oC.

***In vitro* screening – Effect of plant extracts on mycelium growth of *Rhizoctonia solani* Kuhn.:**

The screening of the plants was done by poisoned food technique (New, 1971). For treatment sets, 1 ml of the prepared extract of each plant was mixed with 9 ml of molten PDA medium in a pre-sterilised Petri plate and the contents were agitated in a circular mode in order to mix the extract homogeneously. Media without plant extract served as control. Chloramphenicol (250 mg^{-1} per Petri dish) was added to the medium to prevent bacterial growth (Francisco *et al.*, 2010).

A fungal disc (5 mm in diameter) cut from the periphery of 7 days old culture of *Rhizoctonia solani* Kuhn. with the help of flame sterilized cork borer, served as inoculums was placed at the centre of each Petri plate. The plates were incubated for 7 days at 27 ± 2°C. The experiment was performed under aseptic laminar conditions and replicated 3 times.

Colony diameters in mutual perpendicular directions were measured on the seventh day in assay plates. Fungitoxicity was recorded in terms of the per cent inhibition of mycelial growth and calculated using the following formula (Singh and Tripathi, 1999).

$$\text{Per cent inhibition of mycelial growth} = \frac{dc - dt}{dc} x100$$

where,

dc = Average diameter of fungal colony in control sets.

dt = Average diameter of fungal colony in treatment sets.

The experiments were repeated twice and each set contained five replications. The results presented in (Table B) are based on the mean values of all replications.

Determination of minimum inhibitory concentration (MIC):

The MIC of the extract required for absolute inhibition of mycelial growth of the test fungus, *Rhizoctonia solani* Kuhn., was determined by the poisoned food technique (New, 1971). Requisite amounts of the prepared extract were added to pre-sterilize Petri plates containing 10 ml of molten PDA medium. Now the different concentrations of the extract were added to the medium. The contents of the plates were agitated in a circular mode to mix the extract in the medium evenly. In control sets, the same amount of sterilized distilled

Table C : Determination of minimum inhibitory concentration (MIC)

Determination of minimum inhibitory concentration (MIC)			
Concentration of extract (ml)	Inhibition of mycelial growth (%)		
	Anisomeles indica	*Curcuma malabarica*	*Hedychium spicatum*
1.0	100	100	100
0.9	100	100	100
0.8	100	100	100
0.7	100	100	100
0.6	85	100	100
0.5	72	100	100
0.4	65	81	90
0.3	30	52	76
0.2	0	32	53
0.1	0	16	37

Fig. A: *Rhizoctonia solani* Kuhn. (Control)

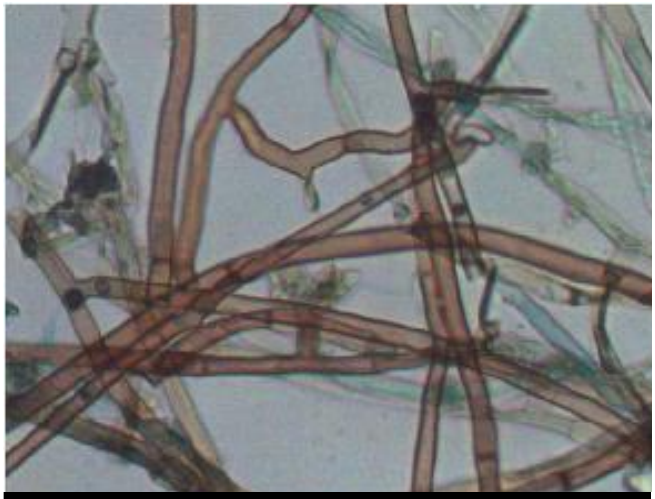

Fig. D : Microscopic view of septate *Rhizoctonia solani* Kuhn. on PDA medium (Control Set).

Fig. B : Treatment (*Curcuma malabarica*) on *Rhizoctonia solani* Kuhn.

Fig. E: Plant material (Rhizome) of *Hedichium spicatum* Buch.

Fig. C: Plant material (Rhizome) of *Curcuma malabarica* Velay.

Fig. F: Treatment (*Hedichium spicatum* Buch. Ham.) on *Rhizoctonia solani* Kuhn.

water was used in place of the extract. The assay plates were incubated for six days at 27 ± 2°C. The observations were recorded on the seventh day in terms of the per cent inhibition of mycelial growth and data presented in Table (C) are based on the averages of all the replications.

RESEARCH FINDINGS AND ANALYSIS

Eleven commonly used plant were screened in the present investigation for their fungicidal activities against the *Rhizoctonia solani* using poision food method. A significant variability was observed in fungicidal efficacy of all extracts against growth of *Rhizoctonia solani*. The fungicidal activity of extracts of all plant extracts is depicted in Fig. 1. The growth reduction in percentage was taken into consideration and fungicidal effect was evaluated. An enhancement in fungicidal activity against *Rhizoctonia solani* was observed, with increase in concentration of extracts. Maximum percentage inhibition was detected at 0.5 ml concentration.

Although eleven plant species were screened in present investigation for their fungicidal activity, the extracts of *Anisomeles indica* L., *Curcuma malabarica* Velay., *Hedichium spicatum* Buch., had inhibitory effect against *Rhizoctonia solani*. The extract of *Curcuma malabarica* Velay. and *Hedychium spicatum* Buch. showed 100 per cent inhibition at 0.5 ml concentration (Fig. 2) whereas the extract of *Anisomeles indica* L. there is 85 per cent inhibition at 0.5 ml concentration. This extract showed 100 per cent inhibition at 0.7 ml concentration. The extract of *Oxalis corniculata* L. showed 80 per cent inhibition at 0.5 ml concentration.

This difference in fungicidal potential of plant extracts may be due to the difference in the chemical compositions of active biomolecules. (Qasem and Abu-Blan, 1996 and Amadioha, 2000). The ineffectiveness of plant extracts of *Acalypha indica* L., *Achyranthes aspera* L., *Dendrophthoe falcate* L., *Lantana indica* Roxb., *Lantana camara*, *Leucas aspera* Willd., *Psidium guajava* L. on *Rhizoctonia solani* Kuhn. is might be due to inactiveness of their compounds in extracts (Amadioha, 2000).

Plant extracts is a group of substances extracted from different parts of plants which contain a great many of compounds with antimicrobial properties (Sasidharan *et al.*,2011). These compounds can be obtained from roots, barks, seeds, buds, leaves and fruits. The higher plants contain a wide spectrum of secondary substances having antimicrobial activity.

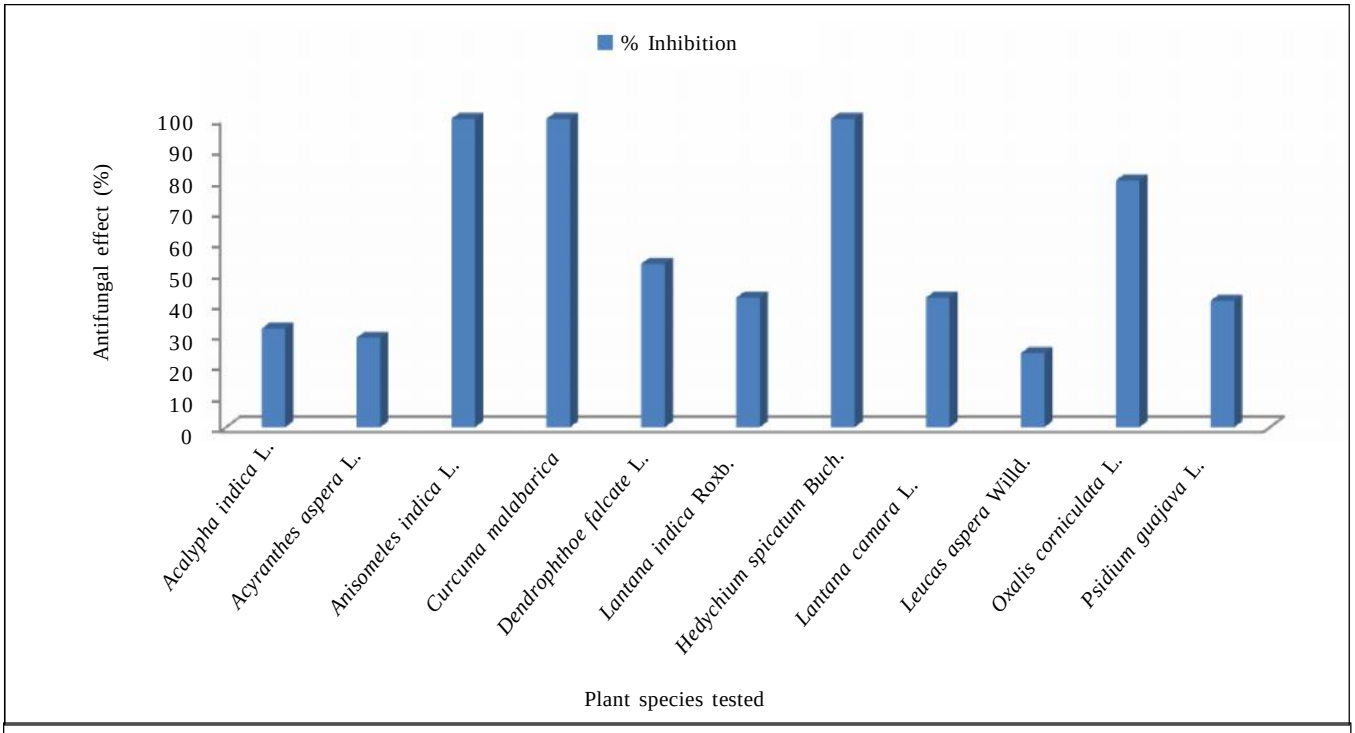

Fig. 1 : Fungicidal effect of plant extracts on mycelial inhibition of *Rhizoctonia solani* Kuhn

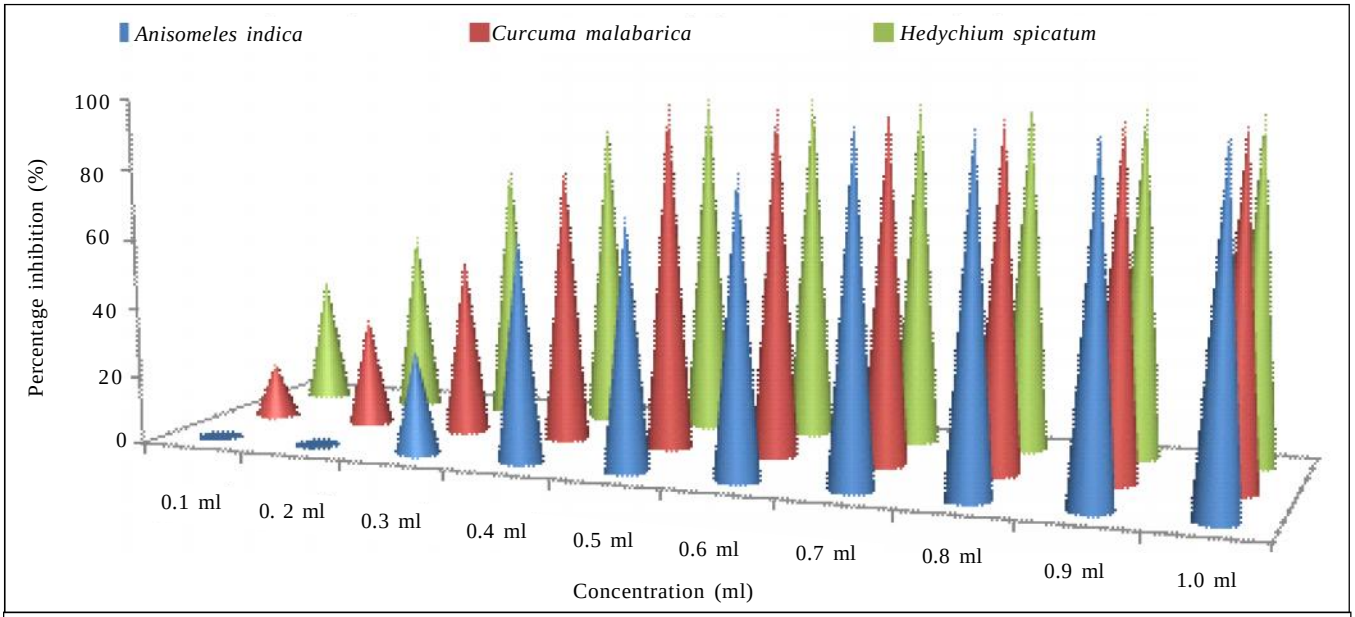

Fig. 2 : Per cent of inhibition on mycelial growth of *Rhizoctonia solani* at minimum concentration

Plant biochemicals and crude extracts have also been reported to have antimicrobial properties against plant pathogens *in vitro* as well as *in vivo*. Further, the use of natural products is regarded as the best suited ecofriendly measure as they are easily biodegradable and safer. The replacement of synthetic fungicides by natural products that are nontoxic and specific in their action is gaining considerable attention (Bashar and Baharat, 1992).

The finding of this experiment reveals that the extracts of *Curcuma malabarica* Velay., *Hedychium spicatum* Buch., were found effective against the test organism *Rhizoctonia solani* Kuhn. Therefore, these extracts can be used as natural fungicides (as an alternative to chemical fungicides) to control the growth of *Rhizoctonia solani* Kuhn.

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