

Original Research Article

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**In Vitro Screening of Antifungal Potency of Plant Products
against *Rhizoctonia solani***

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The antifungal potency of plant products (extracts) of ten different Angiospermic plants namely *Amaranthus viridis* Linn., *Coccinia grandis* Linn., *Eclipta alba* Linn., *Euphorbia hirta* Linn., *Melia japonica* Linn., *Nicotiana plumbaginifolia*, *Physallis minima* Linn., *Phyllanthus niruri* Linn., *Solanum torvum* Sw., *Vernonia cinerea* Schreb. were tested against *Rhizoctonia solani* Kuhn. by Food Poison Technique. The leaf extract of selected plants was tested for its antifungal potency against *Rhizoctonia solani* Kuhn. Among the different plants tested, the leaf extract of *Solanum torvum* Sw. show maximum antifungal potency against the *Rhizoctonia solani* Kuhn.

Introduction

Rhizoctonia solani Kuhn. a fungal pathogen, causes black scurf disease on tuber of potato, and occurs annually to some degree in most production areas. This disease is an economically important disease of potatoes around the world, which reduces the quality and yield of potatoes and has become an important impediment for export of seed potatoes.

The most important method of protecting the plants against the fungal attack is the use of fungicides. However, many fungicidal agents available in the market are toxic and

have undesirable effects on other organisms present in the environment. The synthetic fungicides are mostly non-biodegradable, they accumulate in the soil, plants and water, and consequently effect the humans through the food chain. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some eco-friendly measures for the management of diseases.

Many of the earlier pesticides were the extracts of plants, and several plants have

been exploited more widely as sources of commercial insecticides. But, from 1940s, synthetic agrochemicals largely replaced the plant-derived products as the key commercial pesticides. However this trend is now reversed as it becomes evident that plant natural products still have enormous potential (Choi *et al.*, 2004). Natural products derived from plants seem to be a viable solution to the environmental hazards caused by the synthetic pesticides and therefore research is going on to identify the effective natural products to replace the synthetic pesticides (Kim *et al.*, 2005). The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Singh and Dwivedi, 1987).

The present study was conducted to find plant products to control black scurf disease on potato caused by *Rhizoctonia solani* Kuhn. Our studies involved *in vitro* screening of antifungal potency of plant products against *Rhizoctonia solani* Kuhn.

Material and Methods

Plant materials

Ten plant species belonging to different families of angiosperms, collected from different parts of Gorakhpur and adjacent areas, were identified with the help of identification keys of flora (Srivastava 1976).

The identity of each collected plant was confirmed against their authentic herbarium specimens lodged in the herbarium of the Department of Botany, St. Andrew's College, Gorakhpur. A voucher specimen of all plants has also been deposited in the herbarium.

Preparation of crude leaf extract

20 grams of freshly collected disease-free leaves 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 antifungal testing against the test fungus *Rhizoctonia solani* Kuhn.

Microbial Cultures and Growth Conditions

The plant extracts were assayed for antifungal 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 $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and maintained with periodic sub – culturing at 4°C .

The potato tubers were peeled off and weighed for about 250g tubers were chopped in to small pieces in to the sterile conical flask. After boiling the supernatant were collected and dextrose (15g) with agar (18g) to dissolve the ingredients. Finally the medium was sterilized in autoclave for 20min.

Screening of crude plant extracts against *Rhizoctonia solani* Kuhn

The screening of the plants was done by Poisoned Food Technique for treatment sets, 10 ml of the prepared crude extract of each plant was mixed with 10 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. In control sets, a requisite amount of sterilized double distilled water was added in place of the extract.

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. The plates were incubated for 7 days at $27 \pm 2^\circ\text{C}$.

Colony diameters in mutual perpendicular directions were measured on the seventh day in assay plates. Fungitoxicity was recorded in terms of the % inhibition of mycelial growth and calculated using the following formula (Vincent 1947)

$$\text{Percent inhibition of mycelial growth} = \frac{dc - dt}{dc} \times 100$$

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 : (A) are based on the mean values of all replications.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the crude leaf extract required for absolute inhibition of mycelial growth of the test fungus, *Rhizoctonia solani* Kuhn., was determined by the Poisoned Food Technique. The crude leaf extract of *Solanum torvum* Sw. was prepared as described previously.

Requisite amounts of the prepared crude leaf extract were added to pre-sterilized Petri plates containing 10 ml of molten PDA medium. Now 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 ml of the crude leaf extract is

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 water was used in place of the extract. The assay plates were incubated for six days at $27 \pm 2^\circ\text{C}$. The observations were recorded on the seventh day in terms of the percent inhibition of mycelial growth and data presented in Table: (B) are based on the averages of all the replications.

Results and Discussion

Antifungal potency of ten selected plant extracts was assayed by Food Poison Technique. The result revealed that the extracts of two plants viz., *Solanum torvum* Sw. and *Phyllanthus niruri* Linn. show significant reduction in growth of *Rhizoctonia solani* Kuhn., (Table.1 & 2). However, the crude extract of *Solanum torvum* Sw. exhibited maximum antifungal potency against *Rhizoctonia solani* Kuhn.

Production of Potato crop plants has been the endeavor of human race since the dawn of civilization, from nomadic agriculture to colonized civilization, human emancipation has been continually encouraged to produced and conserve more and more potato crops for continuous utilization. Potato is the staple food of more than half of world population.

One of the big threats of potato crop is the Black Scurf of disease, caused by *Rhizoctonia solani* Kuhn. Several synthetic fungicides have been developed to control the disease. However most of them have been proved to be phytotoxic, non-biodegradable, pollutive and producing undesirable effects on the organism. To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant diseases, the interest on plant products such as plant extract has been increased.

Table.1 Fungitoxicity of crude plant extracts against *Rhizoctonia solani* Kuhn.

Sl. No.	Name of Plant	Family	% Inhibition on mycelial growth
1	<i>Amaranthus viridis</i> Linn.	<i>Amaranthaceae</i>	49
2	<i>Coccinia grandis</i> Linn.	<i>Cucurbitaceae</i>	53
3	<i>Eclipta alba</i> Linn.	<i>Asteraceae</i>	56
4	<i>Euphorbia hirta</i> Linn.	<i>Euphorbiaceae</i>	61
5	<i>Melia japonica</i> Linn.	<i>Meliaceae</i>	16
6	<i>Nicotiana plumbaginifolia</i> Viv.	<i>Solanaceae</i>	19
7	<i>Physallis minima</i> Linn.	<i>Solanaceae</i>	32
8	<i>Phyllanthus niruri</i> Linn	<i>Phyllanthaceae</i>	81
9	<i>Solanum torvum</i> Sw.	<i>Solanaceae</i>	100
10	<i>Vernonia cinerea</i> Schreb.	<i>Asteraceae</i>	32

Table.2 Determination of Minimum Inhibitory Concentration (MIC)

Concentration of extract (ml)	Inhibition of Mycelial growth (%)
1.0	100.00
0.9	100.00
0.8	100.00
0.7	100.00
0.6	100.00
0.5	97.00
0.4	46.00
0.3	20.00
0.2	00.00
0.1	00.00



A. Control set of *Rhizoctonia solani* Kuhn.



B. Treatment set of *Solanum torvum* Sw. on *Rhizoctonia solani* kuhn.



C. Microscopic view of septate *Rhizoctonia solani* Kuhn. on PDA medium.



D. Plant Material
(*Solanum torvum* Sw.)

Crude Extracts from aromatic and medicinal plants were used as alternative fungicides to control *Rhizoctonia solani* Kuhn. The result shows that the crude extract of *Solanum torvum* Sw. possessed antifungal potency against *Rhizoctonia solani* Kuhn. Therefore the extract of *Solanum torvum* Sw. can be used as fungicide against *Rhizoctonia solani* Kuhn. to control Black Scurf disease of Potato. However, for the development of plant extracts as alternatives of synthetic fungicides, further studies are required.

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