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Evaluation of Plant Extracts as Antifungal Agents Against *Fusarium solani* (Mart.) Sacc.

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Abstract: Experiments were carried out to test the aqueous extracts of twenty plants for their antifungal activity against *Fusarium solani* the causal of dry rot disease of potato. The obtained results showed a differential activity of the plant extracts against the mycelium growth. The combined leaf extracts of *Lawsonia alba* and stem extracts of *Acacia catechu* in general showed a strong enhancement in activities over the individual extract of each against the mycelium growth. The seed extracts of *Dedonia viscosa* also showed strong inhibitory effect against the test fungi. The petal extracts of *Mimosa hamata*, leaf extracts of *Acacia arabicae*, *Jacranda mimosaefolia* and *Ocimum sanctum* showed appreciable good inhibitory effect against the test fungi.

Key words: *Fusarium solani* · Antifungal · Plant-extracts · Phytochemicals

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's fourth important food crop after wheat, rice and maize because of its great yield potential and high nutritive value for human. FAO data show that in 2005, for the first time, the developing world's potato production exceeded that of the developed world and India ranks third contributing around 7.5% to the world's production. Ever increasing world population requires the production of huge quantity of potato but our efforts are hampered due to various biotic as well as abiotic factors. Among the biotic factors, *Fusarium solani* (Mart.) Sacc. was first described by C.F.P. Von Martius in 1842 from rotted tubers of potato [1]. Dry rot of potato caused by *Fusarium solani* is an internationally important disease of potato resulting in about 25 to 60% loss in yield in different countries and attempts have been made to manage the disease by treating with chemical compounds, biological agents as reported by Wharton and Kirk [2]. In the present study, efficacy of twenty plant extracts including combined plants extracts for antifungal activity against dry rot pathogen was tested.

MATERIALS AND METHODS

Plant materials *viz*. leaf, petal, pod, seed and stem were collected from various parts of Haryana and the neighboring states on the basis of traditional values (Table 1). The collected plant materials were thoroughly washed with tap water, followed by distilled water and kept in dark between the filter papers at room temperature till completely dry. Each plant sample was individually grounded into powder for preparation of extract. The fungus *Fusarium solani* (IARI 5155 F) used for the study was obtained from the Division of Plant Pathology, IARI, New Delhi. The fungal cultures were maintained at 4°C on Yeast Glucose Agar medium with periodic sub-culturing.

Antifungal Activity Test: The plant part extract (15% w/v) was prepared by brewing in hot water. Fifteen g dry powder of each plant sample was weighed and put in a cheesecloth bag and suspended in 100ml of boiling distilled water for 20 minutes. The extract was allowed to stand for some time and decanted off into the flask and final volume was raised to 100ml by adding boiled distilled water. The supernatant was used for assay. The antifungal activity of each plant part extract was determined by measuring the mycelia growth inhibition of tested fungi as described by Bragulat *et al*. [3]. A known volume of 15% plant sample extract was supplemented with yeast extract, glucose and agar. The medium was sterilized by autoclaving at 15 lb. pressure for 15 minutes. Yeast Glucose Agar plates, without any plant extract supplementation, was run as control. The disc of (0.65cm) in diameter test inoculum was cut out from the edge of a growing fungal colony on glucose agar medium using a sterilized cork borer and placed at the centre of the agar

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Table 1: Common Names and Families of Plants used in Experiment

Sr. No.	*Botanical Name*	*Common Name*	*Name of Family*	*Distribution*	*Traditional Uses of Plants*
1.	*Acacia arabicae* Willd.	Kikar	*Mimosaceae*	India and Tropical Africa	Used for making furniture's, tanning, dyeing fabrics yellow, stem yields gum while seeds are fermented with dates to give beverages [4].
2.	*Acacia catechu* Willd.	Katha	*Mimosaceae*	East India	Used in the treatment of diarrhea and throat infections [4].
3.	*Adhatoda vasica* Nees.	Adusa	*Acanthaceae*	Tropical India	A decoction of the leaves is expectorant and is used to relieve bronchitis [4].
4.	*Aegle marmelos* (L.)	Bael Patter	*Rutaceae*	India	A decoction of the leaves is a febrifuge and expectorant and is particularly used for asthmatic complaints. Also used to treat acute bronchitis, fever and dysentery [4].
5.	*Albizia stipulata* Benthm.	Siris	*Mimosaceae*	Tropical Asia to Australia	The bark is used to treat boils and the leaves and seeds to treat diseases of the eyes [4].
6.	*Anthocephalus cadamba* (Mig.)	Kadam	*Rubiaceae*	Tropical Asia The bark is used as a tonic and reduces fever [4].	
7.	*Azadirachta indica*(A.) Juss.	Neem	*Meliaceae*	East India, Ceylon	Non-drying oil is extracted from the seeds. It is used for soap-making and to treat skin diseases, locally. The bark and leaf extracts are used as a tonic and to reduce fevers [4].
8.	*Lantana camera(L.)*	Ghaneri	Verbenaceae	Tropical America	A decoction of the leaves is used locally as a tonic and stimulant [4].
9.	*Lantana macrophylae(Mart.)*	Ghaneri	Verbenaceae	South America	A decoction of leaves is used in Brazil to treat rheumatism and the fruits are used to make a tonic [4].
10.	*Lawsonia alba(L.)*	Mahendi	Lythraceae	Old World Tropics, N. Africa, Arabia to India.	The bark used to treat jaundice and nervous complaints, flowers yield a scented oil, dried leaves yield a green powder used to dye hair, palm and nails orange brown (Henna) and to dye horses coats and fabric [4].
11.	*Melia azadirachta(L.)*	Neem	Meliaceae	East India, Ceylon	Non-drying oil is extracted from the seeds. It is used for soap-making and to treat skin diseases, locally. The bark and leaf extracts are used as a tonic and to reduce fevers [4].
12.	*Mimosa hamata(Willd.)*	Aill	Mimosaceae	Tropical Asia	Tonic, in urinary complaints, glandular swellings, blood-purifier [4].
13.	*Murraya koenigii(Kurz.)*	Kadi Pata	Rutaceae	East Asia,, Pacific Islands, Himalayas.	A decoction of the bark leaves and root is used locally as a tonic [4].
14.	*Musa paradisiaca(L.)*	Kela	Musaceae	Tropical Asia	The high starch content of the fruits, flour from the fruit is an excellent invalid food [4].
15.	*Nerium indicum(Mill.)*	Red Kaner	Apocynaceae	Tropical Asia	A poultice of the root is used against ringworm, to induce abortion and for suicide, flowers are used for perfume and produce good honey [4].
16.	*Nicotiana tabocum(L.)*	Tamakhu	Solanaceae	Tropical America	The cured and dried leaves are used to make tobacco, snuff ans a source of nicotine for the manufacture of insecticides and nicotine sulphate [4].
17.	*Nychtenthus arbor-tristis(L.)*	Har Sringar	Verbenaceae	India	The leaves yield a bright yellow dye [4].
18.	*Ocimum basilicum(L.)*	Ban Tulsi	Labiatae	India, S.E. Asia, N. E. Africa	The plant is cultivated for the essential oil used in perfumery, soap making, to flavour liqueurs and sauces [4].
19.	*Ocimum sanctum(L.)*	Tulsi	Labiatae	Old World Tropics	The plant is sacred to the Hindus and is grown in front of temples; the leaves are used as a condiment [4].
20.	*Onosoma echinoids(L.)*	Inderjo	Boraginaceae	Central Europe To Himalayas	The roots yield a red dye (Orsanette) used in India to dye fats and wool, in place of Alkanna [4].

edium under sterilized conditions. The experiments were conducted in triplicates along with equal number of controls. The fungus was incubated at 27 ± 1°C and their growth diameters were measured after five days. The percentage inhibition was calculated by the formula as:

$$\% \text{ Inhibition} = [(C-T) \times 100/C]$$

Where
C = Diameter of test fungus (control),
T = Diameter of test fungus.

Assay for Antifungal Activity of Combined Plant Samples: The sample of each plant was prepared as explained earlier. The selected plants extracts were combined in the ratio 1:1. Assay for the antifungal activity of the combined extracts was carried out by the food poisoning method described by Bragulat *et al.* [3].

RESULTS

The activity of the plant extracts against the mycelial growth of *Fusarium solani* is presented in Table 2. It was observed that out of twenty plants parts extracts tested, leaf extracts of *Lawsonia alba* (62.07 %) showed maximum inhibitory effect against the mycelium growth of *Fusarium solani* followed by stem extracts of *Acacia catechu* (54.69%). The seed extracts of *Dedonia viscosa* (40.16%) was observed to show also strong inhibitory effect against the mycelium growth of *Fusarium solani*. Four plants showed moderate inhibitory effect against the mycelium growth of test fungus *i.e.* petal extracts of *Mimosa hamata* (34.68%), leaf extracts of *Acacia arabicae* (34.24%), seed extracts of *Jacranda mimosaefolia* (30.42%) and leaf extracts of *Ocimum sanctum* (30.12%). Meanwhile nine plants have shown insignificant inhibition of mycelium growth against the test fungus 6.25-26.80% and four plants samples

Table 2: Anti-fungal activities of plants-extracts against *Fusarium solani* (Mean ± SD)

Sr. No.	Plant species	Part Used	Percentage Inhibition of Mycelium Growth
1.	*Acacia arabicae* Willd.	Leaf	34.24 ± 0.38
2.	*Acacia catechu* Willd.	Stem	54.69 ± 1.47
3.	*Anthocephalus cadamba* (Mig.)	Stem	7.82 ± 1.75
4.	*Cassia nodusa* (Ham.)	Seed	21.35 ± 1.77
5.	*Dedonia viscosa* (L.)	Seed	40.16 ± 1.12
6.	*Jacranda mimosaefolia* (D.Don.)	Seed	30.42 ± 1.82
7.	*Lagerstroemia flos-reginae* (Retz.)	Seed	---
8.	*Lantana camera* (L.)	Petal	19.16 ± 2.12
9.	*Lantana macrophylae* (Mart.)	Leaf	16.78 ± 2.22
10.	*Lawsonia alba* (L.)	Leaf	62.07 ± 1.24
11.	*Melia azadirachta* (L.)	Seed	26.80 ± 2.82
12.	*Mimosa hamata* (Willd.)	Petal	34.68 ± 1.34
13.	*Murraya koenigii* (Kurz.)	Leaf	---
14.	*Musa paradisiaca* (L.)	Leaf	6.25 ± 1.44
15.	*Nerium indicum* (Mill.)	Leaf	18.34 ± 1.44
16.	*Nicotiana tabocum* (L.)	Leaf	13.23 ± 1.44
17.	*Nychtenthus arbor-tristis* (L.)	Leaf	---
18.	*Ocimum basilicum* (L.)	Leaf	22.73 ± 1.14
19.	*Ocimum sanctum* (L.)	Leaf	30.12 ± 0.82
20.	*Onosoma echinoids* (L.)	Pod	---
21.	*Lawsonia alba* (Leaf) + *Acacia catechu* (Stem)		78.64 ± 0.42

did not show any inhibitory activity. The mixtures of leaf extracts of *Lawsonia alba* + stem extracts of *Acacia catechu* (78.64%) showed an enhancement in activities over the individual extracts (Table 2).

DISCUSSION

Considering the need for an alternative eco-friendly approach to control the phytopathogens, it was believed to be worthwhile to screen the antifungal effects of locally available flora. The results of this study are indicating the differential activities of the plant extracts on the mycelium growth of *Fusarium solani* because many of these extracts have shown very strong inhibition against the mycelium growth of test fungi and a definite potential for new effective fungicides exists. Among the different plants screened, the leaf extracts of *Lawsonia alba* shown marvelous inhibitory effect against the mycelium growth of test fungus, which might be due to the presence of some antimicrobial secondary metabolites in the plant sample, some phytochemicals have also been reported in literature and possess various medicinal properties [4, 5, 6, 7,8], hence, the spray of the leaf extracts of *Lawsonia alba* could be used for protecting plants against pathogenic organisms instead of synthetic chemicals.

The stem extracts of *Acacia catechu* showed inhibitory activity against the growth of *Fusarium solani*, which might be due to the presence of some antimicrobial phytochemicals in the plant such as catechin and catechutannic acid, taxifolin, tannins, gambrine and fisetin and reported in literature to possess various medicinal [4, 5, 6] as well as antimicrobial properties [9]. The seed extracts of *Dedonia viscosa* showed appreciable good inhibitory activity against the mycelium growth of test fungus, which could be due to the presence of some antimicrobial phytochemicals [4, 10]. The mixtures of leaf extracts of *Lawsonia alba* + stem extracts of *Acacia catechu* showed an enhancement in activities over the individual extracts of leaf extracts of *Lawsonia alba* and stem extracts of *Acacia catechu* respectively. Possible reasons for enhancement may be due to: (a) Greater concentration of the various groups of botano-chemicals than in case of individual extracts due to additive effect of the extracts. (b) Greater diversity of the various groups of botano-chemicals due to supplementation by one or the other plant extracts. (c) The possibility of synergistic effect of the botano-chemicals in the cocktail cannot be ignored. Therefore, the spray of the combined leaf extracts of *Lawsonia alba* and stem extracts of *Acacia catechu* could be used for protecting potato crops against pathogenic organisms *Fusarium solani* and a strong substitute of synthetic chemicals.

The antimicrobial activities of plants studied have also been found registered in various literature *i.e. Ocimum basilicum* [11], *Ocimum sanctum* (Chitra and Kannabiran [12], *Lantana macrophylae* [13], *Melia azadirachta* [14], *Cassia nodusa* [15], *Anthocephalus cadamba* [16].

Since the extracts of *Acacia arabicae*, *Mimosa hamata*, *Musa paradisiaca*, *Jacranda mimosaefolia*, *Nerium indicum* and *Nicotiana tabocum* including the combined extracts of leaf extracts of *Lawsonia alba* + *Acacia arabicae* used in this study have not been tested before as inhibitor of phytopathogenic fungi, therefore, they are the new addition to this field of study. The presence of various secondary metabolites such as alkaloids, quaternary alkaloids, coumarins, flavanoids, steroids/terpenoids, phenols etc. have been reported in the various plants extracts [5, 10, 17], which may be responsible for the antifungal properties of the plant studied.

CONCLUSION

The study has shown that some plants namely *Lawsonia alba*, *Acacia catechu*, *Dedonia viscosa* are very effective in inhibiting the mycelium growth of *Fusarium solani*. These plants could be further subjected to field trials to access their effectiveness in field conditions and can subsequently be explored for the possibilities towards the identification of the key bioactive agents, through implying modern Microbiology and Biochemical techniques.

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