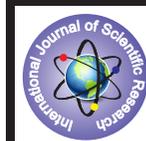


Antifungal Activity of Plant Extracts Against *Phytophthora in festans*



Botany

KEYWORDS : *Terminalia bellerica*, *Psoralea corylifolia*, *Phytophthora in festans*, plant extracts, antifungal activity.

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ABSTRACT

Acetone, methanol and hexane extracts of 10 plant species viz., *Azadirachta indica*(seed), *Cassia angustifoli*(leaf), *Terminalia bellerica*(seed), *Psoralea corylifolia*(seed), *Tamarindus indica*(leaf), *Annona squamosa*(leaf), *Ricinuscommuni*(seed), *Pongamiapinnata*(leaf), *Ricinuscommuni*(seed) and *Phyllanthusasperlatus*(seed) were tested against the mycelial growth of *P. infestans* at 500 ppm. Acetone, methanol and hexane extracts of *Terminalia bellerica* completely inhibited the mycelial growth. Similarly, acetone extracts of *Psoralea corylifolia* also showed complete inhibition and were significantly superior to others but acetone, methanol and hexane extracts of *Cassia tora* showed no inhibition.

I. INTRODUCTION:

Some plants are capable to kill pests that make excellent leads for new pesticide development [1,2,3,4]. The efficacy of 35 plant extracts against *P. infestans* on tomato plant under greenhouse conditions, reported greatest antifungal effects were produced by the extracts of *Potentilla erecta* and *Salvia officinalis* [5]. Extract of *Reynoutria sachalinensis* was highly effective against *P. infestans* [6]. L-Carvone showed moderate to high inhibitory activity on the colony growth of *Helminthosporium solani*, *Fusarium sambucinum*, *F. solani* and *P. infestans* [7]. The methanol leaf extracts of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifer* and *Ziziphus mauritiana* showed significant antifungal activity against *Aspergillus flavus*, *Dreschlera turcica* and *Fusarium verticillioides*. There are many medicinal plants which represent a rich source of antimicrobial activity [2].

The main objective of this study was to determine antimicrobial activity of some selected plants against mycelial growth of *Phytophthora infestans*.

II. MATERIALS AND METHODS:

Collection of botanicals and preparation of mother extract

Ten plant species viz., *Azadirachta indica* (seed), *Cassia angustifoli* (leaf), *Terminalia bellerica* (seed), *Psoralea corylifolia* (seed), *Tamarindus indica* (leaf), *Annona squamosa* (leaf), *Ricinus communi* (seed), *Pongamia pinnata* (leaf), *Ricinus communis* (seed) and *Phyllanthus asperlatus* (seed), were collected from Meerut and Haridwar region, brought to the laboratory, shade dried and kept in oven at 60 °C overnight for uniform drying. Dried plant material was powdered in a mechanical grinder. Powdered plant material was then subjected to hot solvent extraction using solvents of increasing polarity i.e. hexane, methanol and acetone. Extraction was carried out for 18 hours at specified boiling temperature of 69, 65 and 56 °C for hexane, methanol and acetone respectively. The extracts received in the receiving flask were subjected to vacuum flash evaporator to retrieve solvents. The concentrated extract was collected in pre-weighed labeled plastic vials and the original weight of the extract was determined. Mother extracts were prepared in acetone by adding required

amount of acetone to each of the vials so as to get the final concentration of 100 %. Wherever required for proper mixing of extracts with the solvents, a little quantity of methanol or hexane were added to respective vials and the remaining amount required to attain 100 % concentration was made up with acetone. Each of the vials containing the extracts were properly labeled, parafilm was wrapped around the cap of the vial to prevent evaporation and stored in refrigerator till further use. Care was taken to see that the amount of stock solution added to rye media did not exceed 100 µL/100 mL media, above which acetone has detrimental effect on *P. infestans* growth itself.

Testing of plant extract against *P. infestans*

Solvent extracts of various plant species were tested against the mycelial growth of *P. infestans* by food poison technique [8] at 500 ppm. For each treatment, 100 mL of Rye B agar was prepared in conical flask. Just before pouring the medium at molten state, calculated amount of the plant extract was added to the medium using an auto pipette so as to get the final concentration of 500 ppm. The medium containing the plant extract was poured into the Petridish and allowed to solidify. At the center of each Petridish, 5 mm disk of actively growing mycelial bit of *P. infestans* was placed and incubated and 18°C. For each treatment, three replications were maintained along with one suitable control. Radial growth of the mycelium in each treatment was compared with the growth of *P. infestans* in untreated control and based on these observations inhibition percentage (%) was calculated as:

C-T

$$I = 100 \times \frac{C - T}{C}$$

Where, I = Inhibition % of fungal growth, C = Growth (mm) in control and T = Growth (mm) in treatment

Composition of Rye medium

Rye seed	60 gm
Sucrose	15 gm
Agar	15 gm

Penta chloro nitrobenzene (PCNB) 65 mg
 Distilled water 1000 mL

Rye A medium [9]

About 60 gm rye grains were soaked in distilled water at room temperature. After 36 hours, supernatant was decanted and retained. The soaked grains were macerated with little amount of distilled water and incubated at 50°C for 3 hours. After incubation, the extract was filtered through two layers of muslin cloth. The filtrate was collected in an Erlenmeyer flask and the sediment was discarded. The filtrate was added to the original supernatant and was mixed with 20 gm sucrose, 15 gm agar, 65 mg PCNB and the volume was adjusted to one liter by adding distilled water. The medium was autoclaved at 15 psi for 20 min and stored at 4°C for subsequent use.

III. RESULT AND DISCUSSION

Acetone, methanol and hexane extracts of 10 plant species were tested against the mycelial growth of *P. infestans* at 500 ppm. Acetone, methanol and hexane extracts of *Terminalia bellerica* completely inhibited the mycelia growth (Figure1). Similarly, acetone extracts of *Psoralea corylifolia* also showed complete inhibition and were significantly superior to others (Table 1). However, acetone and methanol extracts of *Cassia tora* did not show inhibition. Rest of the plant species showed little to moderate effect. Hexane extract of *Cassia tora* and *Phyllanthus asperlatus* showed minimum inhibition while the rest of the extracts showed moderate effect.

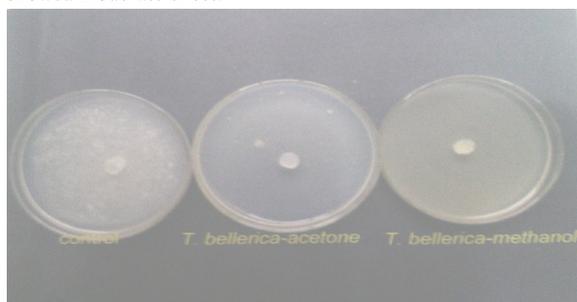


Figure 1: Effect of acetone, methanol and hexane extracts on Terminalia bellerica culture.

Table 1: Effect of plant extracts on mycelia growth of Phytophthora infestans.

S. No.	Plant Species	Plant Part used	% Mycelial Inhibition		
			Solvents		
			Acetone	Methanol	Hexane
1.	<i>Azadirachta indica</i>	Seed	38.9(38.8)	24.1(23.4)	20.4(26.8)
2.	<i>Psoralea corylifolia</i>	Seed	100.0(88.4)	88.9(70.9)	94.4(78.8)
3.	<i>Cassia angustifoli</i>	Leaf	16.7(24.1)	24.1(23.4)	16.7(24.1)
4.	<i>Terminalia bellerica</i>	Seed	100.0(88.4)	100.0(88.4)	100.0(88.4)
5.	<i>Tamarindus indica</i>	Leaf	27.8(31.8)	14.8(22.8)	16.7(24.1)
6.	<i>Annona squamosa</i>	Leaf	24.1(29.4)	14.8(22.8)	20.4(26.8)
7.	<i>Pongamia pinata</i>	Leaf	31.5(34.1)	29.6(32.9)	18.5(26.4)
8.	<i>Ricinus communis</i>	Seed	64.8(53.6)	31.5(34.1)	38.3(35.2)
9.	<i>Phyllanthus asperlatus</i>	Seed	29.6(32.9)	27.8(31.8)	13.0(21.1)
10.	<i>Cassia tora</i>	Leaf	0.0(1.7)	0.0(1.7)	13.0(21.1)

Under the present study, acetone, methanol and hexane extracts

of 10 plant species were tested *in vitro* against *P. infestans*. Results revealed that acetone, methanol and hexane extracts of *Terminalia bellerica* completely inhibited the mycelial growth *in vitro*. Acetone extracts of *Psoralea corylifolia* also showed similar result whereas *Cassia tora* did not show inhibition in acetone and methanol extracts.

The inhibitory effect of fruit extracts of *T. bellerica* was attributed to gallic acid and ethyle gallate that were present in the fruits [10]. Reports on the inhibitory effect of *Terminalia* are available mainly against human pathogens. Elizabeth (2005) tested the antimicrobial activity of *T. bellerica* against 9 human microbial pathogens and reported that crude extract showed zone of inhibition ranging from 15.5-28.0 mm [11]. They also reported that *T. bellerica* is highly inhibitory to *Staphylococcus aureus*. Khilare and Gangawane (1997) reported that *Terminalia chebula* extracts was highly effective against *Penicillium digitatum* causing green mold of Mosambi [12]. Presence of tannins in the fruit extract of *Terminalia bellerica* might have prevented the development of microorganisms by precipitating the microbial protein and making nutritional proteins unavailable for them [13].

Psoralea corylifolia have been evaluated for the presence of bioactive constituents using various polarity solvent including hexane, butanol, ethanol and water [14]. The seeds of *Psoralea corylifolia* have been used as an ancient Hindu remedy for leucoderma, psoriasis and inflammatory diseases of the skin [15]. Azadirachtin is secondary metabolite [16] which also show antimicrobial activity and it is a potent natural biocide that protect the plants from various biotic and some abiotic stresses [17]. Kiran and Raveesha (2005) demonstrated the strong fungicidal activity of seed extracts of *P. corylifolia* against *Fusarium* and *Aspergillus sp* which are known to cause pre and post harvest diseases of maize, sorghum, groundnut and vegetable crops [18].

IV. CONCLUSION:

Late blight caused by *Phytophthora infestans* is the most dreaded diseases of potato world over including India. It causes losses to an extent of three billion US \$ in developing countries alone and In India, over 60 billion rupees annually. In India, work on various aspects including pathogen population, sexuality, host parasite interaction, development of late blight resistant varieties and forecasting for the initial occurrence of late blight has been carried out during the last five decades. However, these research works were mainly carried out for the hills and much needs to be done in the subtropical plains particularly in western UP.

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