

## In vitro evaluation of antifungal potential of selected plant species against *Fusarium solani*

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**Abstract.** Pant D, Tapwal A, Tiwari B, Thakur JS. 2023. In vitro evaluation of antifungal potential of selected plant species against *Fusarium solani*. *Asian J Nat Prod Biochem* 21 (2): 51-57. The hazardous effect of synthetic fungicides is a major concern. Therefore, the development of safe and eco-friendly fungicides is momentarily imperative. The Himalaya is considered a rich repository of floristic diversity, endowed with several medicinally important plant species exhibiting antimicrobial potential. The aim of this study was to evaluate the anti-mycotic potential of five selected Himalayan plant species viz. *Berberis lycium* Royle, *Zanthoxylum armatum* DC., *Valeriana jatamansi* Jones, *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. and *Polystichum squarrosus* (D. Don) Fee by poisoned food technique against *Fusarium solani* which is considered as one of the major causes of Shisham. Crude extract of *B. lycium* exhibited maximum growth inhibition (51.58%) of *F. solani*, followed by *Z. armatum* (21.94%) and *V. jatamansi* (21.45%). However, the inhibitory effect of *A. adenophora* and *P. squarrosus* extracts on fungal growth was insignificant. Furthermore, the potentials of three solvents (aqueous, ethanol and acetone) were evaluated for the extraction of antimycotic phytochemicals from the leaf of *B. lycium* and ethanol was found to be comparatively more effective. Phytochemical analysis of a crude extract of *B. lycium* revealed the presence of different groups of bioactive compounds, such as alkaloids, flavonoids, terpenoids, steroids, phenolics and tannins. Thus, the presence of diverse groups of phytochemicals in *B. lycium* makes it more effective in controlling the growth of *F. solani* and can be used in the development of eco-friendly fungicides.

**Keywords:** Antifungal, *Berberis lycium*, *Fusarium solani*, Himalayan plant species, phytochemicals

### INTRODUCTION

Fungi are the most common pathogens that cause severe damage to agricultural crops and forest tree species. Forests endure serious harm from fungal pathogens, which cause prominent diseases like wilting, scab, moldy coatings, rusts, blotches, rotting tissue, anthracnose, leaf spot, blight, mildew, coils, gall, canker, damping-off, root rot, dieback, etc. (Iqbal et al. 2018). Most fungal pathogens are opportunistic in nature, invading the host plant during physiological stress or through isolated regions of damaged or diseased tissue (Lilja et al. 2010). On a global scale, *Fusarium* is one of the top ten most common genera of plant pathogenic fungi (Dean et al. 2012). *Fusarium solani* is one of such destructive pathogens that cause wilt disease in *Dalbergia sissoo* Roxb. ex DC. (Shisham). *Fusarium* wilt disease has been linked to Shisham population declines in Uttar Pradesh, Bihar, Punjab, and Himachal Pradesh. The *F. solani* is usually found on roots, and the disease appears during the humid months of July to September. Pathogen hyphae and jelly-like substances plug the vessels, causing wilt symptoms (Bakshi and Singh 1959). The *D. sissoo* is an important timber tree species of India, and it is especially susceptible to root pathogens, which cause wilting and root rot (Bhandari et al. 2014). Dieback due to wilting is a serious problem in this species. In severe conditions, the dieback is identified by thinning leaves and crowns, drying of branch tips, table roofing, and a stag-

head (Khan and Khan 2000). Infected trees show signs of wilting, and as the leaves fall off, the branches become clear, and the plants die within a few months (Kumar and Khurana 2016). The *F. solani* is a soil-dwelling fungus that can survive in the soil for several years without active contact with the host. Mycotoxins, which are secondary metabolites produced by *F. solani*, pose a serious threat to plants and animals (Prasad et al. 2018). The management wilt (caused by *F. solani*) by applying fungicides is cumbersome and can pollute underground water resources. Therefore, eco-friendly strategies like applying biocontrol agents or botanicals in disease management are much preferred.

Biological control refers to disease management by eco-friendly means like plant extracts, plant products, and microorganisms such as bacteria, viruses, fungi, or other living creatures that suppress pathogens (Dawood 2015). Due to a lack of mobility, plants have evolved alternate defensive systems, compromising an extensive range of chemical metabolites as instruments to handle stress circumstances (Munuswamy et al. 2013). The basic role of plant secondary metabolites is in defense against various diseases and herbivores. In other words, secondary metabolites are released by plants as a defense strategy (Netala et al. 2015). The secondary metabolites of plants, such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins, are quite effective against various phytopathogens. These

metabolites are known to exhibit antimicrobial properties and are used by plants to defend themselves against harmful microorganisms (Das et al. 2010). Tapwal et al. (2011) evaluated the phyto-extract of five plants for antimicrobial potential against phytopathogens under laboratory conditions and observed that the extract of *Cannabis sativa* L. was most effective against *Curvularia lunata* (Wakker) Boedijn. Singh et al. (2018) screened nine plant extracts at varied concentrations on the growth of *F. solani*, and *Curcuma longa* L. rhizome extract was the most effective. Similarly, Seepe et al. (2020) evaluated the antifungal activity of eight plants against *Fusarium* species inoculated on maize seeds and the maximum inhibitory effect was shown by acetone extract of *Melia azedarach* L. against *F. proliferatum*, followed by mixed acetone extracts from *Combretum erythrophyllum* (Burch.) Sond. and *Quercus acutissima* Carruth. Exhibited antifungal activity on *F. verticilloides*, *F. proliferatum* and *F. solani*. Sheel et al. (2022) tested five weed extracts on the mycelial growth of *Botryodiplodia palmarum* (Cooke) Petr. & Syd. and observed growth inhibition ranging from 2.77 to 85.71%. Wilt has been associated with *F. solani* (Bajwa et al. 2003) and threatens the production and post-harvest storage of numerous fruits and vegetables (Meenu and Kaushal 2017), causing large economic losses due to stem rotting and softness in bananas (Prihatna et al. 2018; Pegg et al. 2019), tomato (Ravikumara et al. 2022), pigeon pea, and nearly any plant species. Bhardwaj (2012) evaluated the antifungal efficacy of aqueous extracts from 20 different plants against *F. solani*, the fungus that causes dry rot disease in potatoes. Aznabakieva et al. (2023) diagnosed the disease as root and stem rot of pepper plants caused by *F. solani* by comparing the symptoms on wilted pepper plants with those of *Fusarium* wilt. Furthermore, it has been noted that *F. solani*, which causes dry root rot, can affect the most prevalent citrus rootstocks (Menge and Nemeč 1997). In many nations where soybeans are cultivated, *F. solani* damages soybean roots in the early stages of the growing season (Roy et al. 1997) and produces Sudden Death Syndrome (SDS) (Wrather et al. 2010). Plant extracts in disease management are environment-friendly, inexpensive, safer than synthetic pesticides and fungicides, and are currently classified as “green agrochemicals” with their growing use (Karunamoorthi 2012). Himalaya is considered a rich resource of medicinally important plants. Traditionally, the Himalayan flora has been utilized to cure various diseases of bacterial and fungal origin in humans and animals. The antimicrobial property of these medicinal plants could also be used to control the plant pathogenic fungi. Therefore, this study aimed to evaluate the antimycotic potential of five plants (three medicinal plants, one fern and one weed) against *F. solani*.

## MATERIALS AND METHODS

### Study area

The leaves of five plant species viz., *Berberis lycium* Royle, *Zanthoxylum armatum* DC., *Valeriana jatamansi*

Jones, *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. and *Polystichum squarrosus* (D. Don) Fee were collected from Conifer Forest near Himalayan Forest Research Institute, Shimla, Himachal Pradesh, India (N 31° 04.066' E 077° 10.493' E, 1898 masl). The leaves of these plants were picked, washed and shade-dried for 2-3 weeks. The dried leaves were ground to powder, stored in airtight containers at 4°C and utilized within two weeks.

### Fungal culture

A pure culture of *F. solani* (FRI NTCC-1146) was procured from National Type Culture Collection (NTCC), Forest Protection Division, Forest Research Institute (FRI), Dehradun, Uttarakhand, India.

### Preparation of crude extracts

Powdered dried leaves were mixed with sterile distilled water in 1:16 (w/v) and kept in a water bath shaker at 180 rpm for three days. The extract was filtered through Whatman filter No. 1 paper and stored under refrigeration (Pandey and Tripathi 2014).

### Assessment of antifungal activity of crude extracts

Plant extracts were evaluated for their antimycotic efficacy against *F. solani* following the poisoned food technique (Nene and Thapliyal 1993). Potato Dextrose Agar (PDA) was autoclaved for 15 minutes at 15 psi, 121°C and amended with the requisite quantity of crude extracts before pouring to get desired concentrations (10%, 20% and 30%). The crude extracts of selected plant species were passed through syringe filters (pore size: 0.45 µm) to avoid contamination. After the solidification of the media, the 5 mm diameter mycelial disc of the pathogen was cut from the actively growing culture and inoculated. PDA plate without amendment served as control. The radial growth of the pathogen was recorded after 5 days of incubation at 25±1°C, and percent growth inhibition was determined by the following formula:

$$\text{Percent growth inhibition} = \frac{\text{Colony diameter in control} - \text{Colony diameter in treatment}}{\text{Colony diameter in control}} \times 100$$

### Preparation of solvent extracts

Dried powdered leaves were mixed separately in water, ethanol and acetone, and then metabolites were extracted for about five hours in a soxhlet extraction unit. After that, extracts were filtered through Whatman No. 1 paper and solvents were evaporated at temperature (40°C) using a rotatory evaporator to get the final volume of 30 mL. The extracts were then stored at 4°C for further use (Sheel et al. 2022).

### Assessment of antifungal activity of solvent extracts

Each extract (0.5 mL) was pipetted into a previously weighed watch glass, kept in a hot air oven at 80°C overnight and weighed again to determine the dry content of extracts. Following the poisoned food technique (Nene and Thapliyal 1993), PDA media was mixed with aqueous, ethanol and acetone extracts to make different concentrations, i.e., 500 ppm, 1000 ppm and 1500 ppm and

PDA plates without extracts served as control. A 5 mm disc of the pathogen was inoculated, and growth was recorded on the fifth day of incubation and compared to determine growth inhibition.

#### Phytochemical screening of plants extracts

The shade-dried leaves of selected plant species were evaluated for the presence or absence of phytochemical constituents, such as alkaloids, flavonoids, steroids, terpenoids, carbohydrates, amino acids, saponins (Morsy 2014), proteins, phenolics and tannins (Shah et al. 2014).

##### *Test for alkaloids*

The presence of alkaloids in the extract was analyzed by Mayer's reagent Test, in which 2 mL of filtrate was taken in a test tube, and 1-2 drops of Mayer's reagent were added. The presence of alkaloids was confirmed by the appearance of a yellow-colored precipitate.

##### *Test for flavonoids*

The presence of flavonoids in the extract was detected by a lead acetate test. Two (2) mL of extract and three (3) drops of 10% lead acetate solution were added to a test tube. The presence of flavonoids was indicated by an intense yellow color.

##### *Test for steroids and terpenoids*

The Salkowski test was performed to detect the presence of steroids and terpenoids. Five (5) mL of extract was taken, and 2 mL of chloroform was added. After that, 3 mL of concentrated sulphuric acid was added from the sides of the test tube. The presence of steroids was indicated by a cherry red color in the chloroform layer, while the formation of a reddish-brown color at the interface confirms the presence of terpenoids.

##### *Test for carbohydrates*

To confirm the presence of carbohydrates in the extract, Fehling's test was performed. An equal volume of Fehling's solution A and B were mixed, and 2 drops of the extract were added and gently heated, a brick-red precipitate of cuprous oxide confirms the presence of both carbohydrates and reducing sugars.

##### *Test for amino acids*

A sulphur test was performed to detect the presence of amino acids. Five (5) mL of extract was taken in a test tube, and 2 mL of 40% sodium hydroxide solution was added. This was followed by adding 2 drops of 10% lead acetate solution, and the preparation was gently heated. Brown or black color indicates the presence of cysteine amino acid.

##### *Test for proteins*

The Presence of proteins in the extract was analyzed by the biuret test. 2 mL of extract was taken, 1 drop of 2% copper sulphate solution was added, followed by 1 mL ethanol (95%) and potassium hydroxide pellets. The presence of proteins was indicated by the appearance of pink color in an ethanolic solution (Shah et al. 2014).

##### *Test for phenolics and tannins*

The presence of phenolics and tannins in the extract was determined by following the ferric chloride Test. Two (2) mL of extract were taken in a test tube, and a few drops of 5% ferric chloride solution were added. The appearance of dark green color confirms the presence of phenolics and tannins (Shah et al. 2014).

##### *Test for saponins*

The presence of saponins was confirmed by the Froth Test. The extract powder was blended with 2 mL of distilled water and left to stand for 10 minutes. The presence of saponins was indicated by the appearance of stable froth (Morsy 2014).

#### Statistical analysis

All the experiments were carried out in triplicates, and data was analyzed by Analysis of Variance (ANOVA) using Blue Sky Statistic software to determine significant differences. Post-hoc test was applied at the level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

#### Morphology of *Fusarium solani*

On PDA, the colony of *F. solani* appeared as fluffy or cottony with abundant aerial mycelium, whitish-yellow to pale pink-reddish color. The average colony diameter on the fifth day of incubation was  $6.9 \pm 1.07$  cm. Hyphae were septate, branched, and hyaline,  $2.32$ - $3.87$   $\mu\text{m}$  in diameter, and produced conidia in 3-6 days (Figure 1).

#### *In vitro* antimycotic activity of crude extracts

The crude extracts of selected five botanicals inhibited the growth of *F. solani* in the range of 3.32% to 51.58%. The inhibitory effect shown by the plant extracts was found to be dose-dependent and maximum inhibition was observed at 30% concentration. At 30% concentration of crude extracts, maximum growth inhibition was exhibited by *B. lycium* (51.58%), followed by *Z. armatum* (21.94%), *V. jatamansi* (21.45%), *A. adenophora* (11.21%) and minimum by *P. squarrosom* (10.12%) (Figure 2). The inhibitory effect of *A. adenophora* and *P. squarrosom* extracts on *F. Solani* was insignificant at  $p < 0.05$  level. In the case of *Z. armatum* and *V. jatamansi*, substantial inhibition of fungal growth was observed at 10 and 20% concentration, but statistically significant inhibition was only noticed at 30% concentration. Nevertheless, of the tested plant species, the highest antimycotic activity against *F. solani* was observed in the crude extract of *B. lycium*, followed by *V. jatamansi* and *Z. armatum*. Inhibition of fungal growth by 10.7%, 50% and 53.57%, respectively, was observed at 10, 20 and 30% concentrations of *B. lycium* compared to control. In a similar study, Raturi et al. (2014) recorded growth inhibition of *F. oxysporum* by ethanol extract of *Z. armatum* from 10.88% to 17.55%. Likewise, Guleria et al. (2013) evaluated the efficacy of essential oil extracted from the leaves of *Z. armatum*, which exhibited extraordinary

antifungal activities against *Alternaria alternata* (Fr.) Keissl. (35.6±1.49%), *A. brassicae* (Berk.) Sacc. (14.5±0.36%), *C. lunata* (42.0±1.63%), respectively. Similarly, Joseph et al. (2008) evaluated antifungal efficacy of various plant extracts viz., *Azadirachta indica* A. Juss., *Artemisia annua* L., *Eucalyptus globulus* Labill., *Ocimum sanctum* L. and *Rheum emodi* Wall. at different concentrations (5, 10, 15 and 20%) against *F. solani* and observed maximum growth inhibition by the leaf extract of *A. indica*, followed by *R. emodi*, *E. globulus*, *A. annua* and *O. sanctum*.

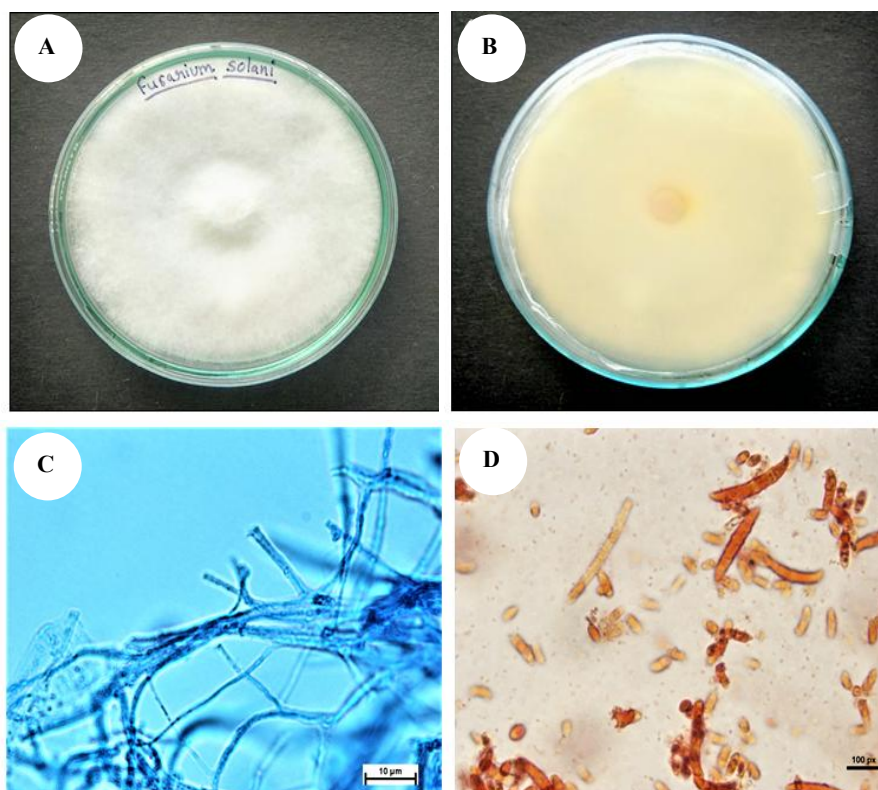
#### In vitro antimycotic activity of solvent extracts

Of the tested plant species, *B. lycium* was further selected to evaluate the efficacy of selected solvents for extracting antimycotic compounds from leaf samples. This was due to the significantly higher growth inhibitory potential associated with *B. lycium*.

The growth of *F. solani* was inhibited in the range of 2.35-73.17% by all solvent extracts at all the tested concentrations. Maximum growth inhibition was recorded by the aqueous extract of *B. lycium* at 1500 ppm concentration (37.03%), followed by 1000 ppm (23.54%) and minimum inhibition at 500 ppm (21.97%) (Figure 3 and Table 1). Likewise, the maximum growth inhibition by ethanol extract of *B. lycium* was also recorded at 1500 ppm concentration (73.17%), followed by 1000 ppm (18.00%) and minimum inhibition was recorded at 500 ppm (2.35%).

Furthermore, acetone extract showed 18.82% growth inhibition at 1500 ppm concentration, but the colony diameter reduction was insignificant compared to the control. However, the highest statistically significant inhibition was observed only in 1500 ppm ethanol extract, followed by 1500 ppm aqueous extract.

Ethanol has been used to extract metabolites like phenolic derivatives and several antioxidant compounds from plants (Hikmawanti et al. 2021). Abu-Taleb et al. (2011) used ethanol as a solvent to extract metabolites of *Alhagi maurorum* Medik., *Calotropis procera* (Aiton) Aiton fil. and *Datura innoxia* Mill. And recorded 35-85% growth inhibition of *F. solani*. Shukla and Dwivedi (2012) evaluated the antimicrobial efficacy of ethanolic extracts of four plants against *F. udum* and *F. oxysporum*. Their results revealed that at a 15% concentration of extract, maximum growth inhibition of *F. udum* was exhibited by turmeric (89.2%), followed by garlic (88.26%) and black pepper (82.22%) and at the same concentration. Garlic, turmeric and black pepper reduced the growth of *F. oxysporum* up to 94.63%, 87.96% and 77.74%. Respectively. Hussain et al. (2011) investigated the antibacterial activity of aqueous, petroleum ether, and ethanolic extracts of the root of *B. lycium* against *Candida albicans*. The results revealed that ethanolic extract exhibited maximum growth inhibition (27.00±0.65), followed by aqueous extract (27.00±0.31), whereas petroleum ether extract had no significant effect against *C. albicans*.

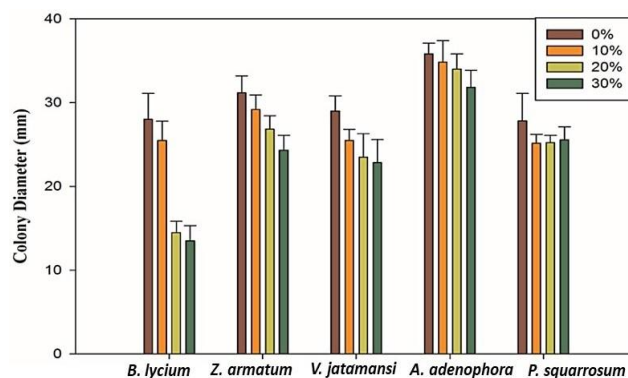
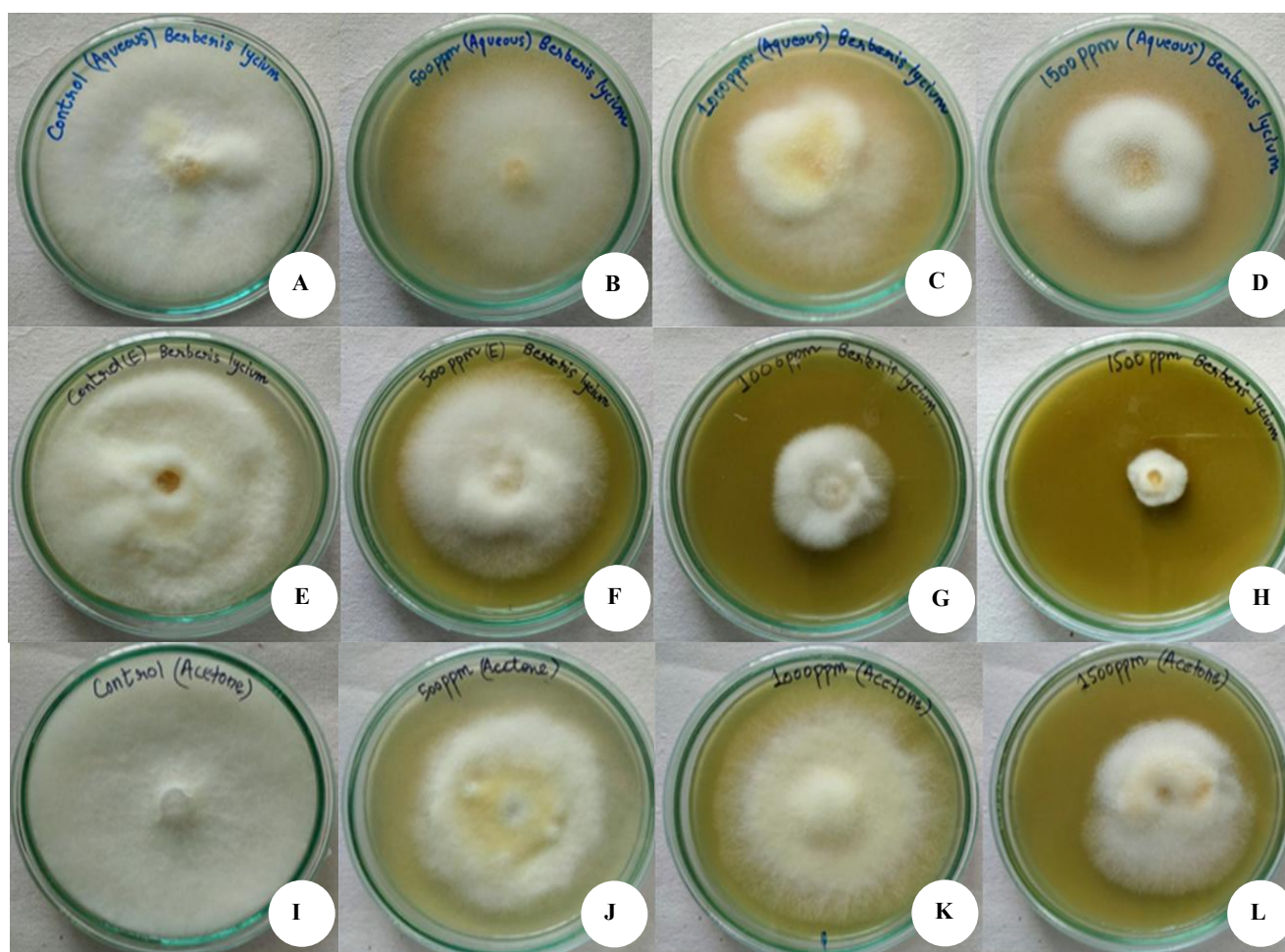


**Figure 1.** A. Top view of five days old culture of *F. solani*, B. Reverse view of *F. solani*, C. Hyphae of *F. solani* (at scale 10 µm), D. Macro and micro conidia of *F. solani* (at scale 10 µm)

**Table 1.** Growth inhibition of *F. solani* by the extracts of *B. lycium* at different concentrations

Type of extracts	Growth inhibition (%) of <i>F. solani</i> at different concentrations of extracts		
	500 ppm	1000 ppm	1500ppm
Aqueous	21.97±3.27 <sup>a</sup>	23.54±3.87 <sup>a</sup>	37.03±13.42 <sup>a</sup>
Ethanolic	2.35±1.54 <sup>a</sup>	18.00±9.60 <sup>a</sup>	73.17±8.08 <sup>b</sup>
Acetone	4.31±1.33 <sup>a</sup>	10.71±2.85 <sup>a</sup>	18.82±4.56 <sup>a</sup>

Note: At  $\alpha = 0.01$  and  $0.001$  (Post Hoc Tukey's HSD), the mean sharing common alphabet in a row are not significantly different for each other

**Figure 2.** Colony diameter of *F. solani* at 0 (control), 10, 20 and 30% concentration of crude extracts of selected plant species**Figure 3.** A. Control of *F. solani* in *B. lycium* (Aqueous extract), B-D. Growth inhibition of *F. solani* by aqueous extracts of *B. lycium* at different concentrations, E. Control of *F. solani* in *B. lycium* (Ethanol extract), F-H. Growth inhibition of *F. solani* by ethanol extracts of *B. lycium* at different concentrations, I. Control of *F. solani* in *B. lycium* (Acetone extract), J-L. Growth inhibition of *F. solani* acetone extracts of *B. lycium* at different concentrations

### Phytochemical screening

Many phytopathogens are believed to be inhibited by phytochemicals belonging to different groups, such as alkaloids, flavonoids, terpenoids, steroids, phenolics and tannins. The need for eco-friendly and cost-effective means of controlling plant diseases was the driving force behind assessing the antifungal activity of native plant species. In the present study, crude extracts of five plant species were screened against the plant pathogenic fungus *F. solani*, and *B. lycium* extract was found to be relatively more effective in inhibiting fungal growth. *B. lycium* is a shrubby species belonging to the Berberidaceae family that has traditionally been used to cure various ailments in the Indian Himalayan region. It is used to cure jaundice, diabetes, internal wounds, rheumatism, diarrhea, stomachache, eye infections and fractured bones (Ali et al. 2015).

Extracts of this species effectively limit microbial growth, including *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteum*, *Escherichia coli*, *Enterobacter aerogenus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Salmonella typhimurium*, *C. albicans* etc. (Malik et al. 2017). Inhibitory effect of fruit and root extracts of *Berberis orthobotrys* Bien. ex Aitch. Against *F. solani* has been documented by Dad et al. (2017).

Selecting a suitable solvent is critical for extracting bioactive components from plants with the desired activity. The chemical character (polarity) of the intended bioactive molecule heavily influences solvent selection. Based on the polarity, three major solvent systems, i.e., polar, intermediate polar and nonpolar solvents, are generally used to extract the plant metabolites (Abubakar and Haque 2020). In earlier studies, bioactivity activity against pathogenic microorganisms was reported in extracts of *B. lycium* using polar solvents like water and alcohol (Malik et al. 2017). Therefore, this study observed maximum growth inhibitory activity with ethanol. Among most organic solvents, ethanol is considered safer and widely used, either in pure form or combined with water for extraction.

The present study evaluated the presence of phytochemicals like alkaloids, flavonoids, steroids, terpenoids, carbohydrates, reducing sugars, phenolics, tannins, amino acids, and proteins in the crude extract of *B. lycium*. All tested phytochemical groups were present in the extracts of five plants, except for protein, which was present only in the extract of *V. jatamansi* (Table 2).

Plants harbor a variety of phytochemicals, which play an important role in defense and stress management. Phytochemicals are categorized as primary or secondary metabolites based on their involvement in plant metabolism (Wadood et al. 2013). Pathogenic fungi growth is inhibited by a variety of secondary metabolites derived from plants and other natural sources (Elgharbawy et al. 2020). Based on their chemical structures, these phytochemicals belong to different groups such as terpenes, phenolics, polyketides, and alkaloids.

**Table 2.** Phytochemical screening of selected plant species

Phytochemicals	Presence or absence of phytochemicals in selected botanicals				
	<i>B. lycium</i>	<i>Z. armatum</i>	<i>V. jatamansi</i>	<i>A. adenophora</i>	<i>P. squarrosus</i>
Alkaloids	+	+	+	+	+
Phenolics and tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Steroids and terpenoids	+	+	+	+	+
Reducing sugars	+	+	+	+	+
Saponins	+	+	+	+	+
Amino acids	+	+	+	+	+
Proteins	-	-	+	-	-

Several phenolics, including flavonoids, are generally produced by plants to defense against pathogenic microbes (Cushnie and Lamb 2005). Terpenoids are involved in the breakdown of membranous tissue and the weakening of microorganism's cell walls (Hernandez et al. 2000). Saponins are specialized terpenoids that possess a wide range of bioactivities. Many have anti-herbivore and/or antimicrobial plants use in their defense mechanisms (da Silva Magedans et al. 2021).

In conclusion, the antimycotic activity of crude extracts of 5 plants species was investigated against *F. solani*. Out of five, the highest inhibition activity was observed in the ethanolic extract of *B. lycium*. Therefore, *B. lycium* could be used to develop potential botanical fungicides to manage virulent and destructive plant pathogenic fungi like *F. solani*. Among the tested solvents, ethanol was found to be most suitable for extracting antifungal phytochemicals from *B. lycium*, and it is essential to isolate and characterize these phytochemicals to utilize their potential in pest management.

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