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# Phytochemical Composition and Fungicidal Potential of Moss Philonotis Revoluta against Spore Germination Process of Fungus Helminthosporium Turcicum

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***Abstract:*** *Information about the occurrence of phytochemicals which act as antibiotic substances in bryophytes and the medicinal use of bryophytes are sporadic. Despite of a long history of medicinal use of bryophytes by various tribal and local communities across the world, their significant utilization in medicines is still a dream due to lack of concise ethnobotanical information, scarcity of material degrading habitats etc. Bryophytes have emerged as potential plants for many bioactive phytochemicals with antimicrobial efficacies. Antifungal activity of a moss Philonotis revoluta has been assessed using various extracts. For this assessment hanging drop technique is used against spore germination of fungus Helminthosporium turcicum. The methanolic extract of moss exhibited a superior effect than the extract prepared in acetone. Malformation such as stunting of growth, curling and dying of tip of fungal hyphae was also reported which affects the growth of fungus. This study projects Philonotis revoluta as an eco-friendly antifungal agent.*

***Keywords:*** *Phytochemical, Fungicidal potential, Moss, Philonotis revoluta, Helminthosporium turcicum, Hanging drop technique.*

## I. Introduction

Plants are treasured source of natural products for upholding normal health of human beings. Conventional therapeutic systems of Ayurveda, Unani and Sidha are the prevalent verification of their use in therapy. In the last few decades, with more rigorous researches for therapies based on natural systems, plants are used extensively to cure different diseases especially dermal ailments [1].

The search for unexplored plants or plant group with substantial antimicrobial action has attained massive importance these days, due to a growing concern about the attainment of antibiotic- resistance by the pathogenic microorganism.

According to a report of World Health Organization (WHO) approximately 80% of folks from developing nations use conservative systems of natural medicine, which are dependent on the compounds present in medicinal plants. Thus, there is need to investigate more and more plants for their phytochemical and antioxidant possessions, protection and effectiveness with experimental rationale [2].

Liverworts and mosses have been used customarily in Indian culture curing many skin related diseases due to their remarkable antimicrobial activity [3]. In Chinese medicine system the use of these miniature plants dates back to 400 years ago. Species of *Fissidens* and *Polytrichum* were used as hair growth stimulating drugs and diuretic [4]. Furthermore, North American Indians used moss taxa like, *Polytrichum juniperinum*, *Bryum* spp., *Mnium* spp. and *Philonotis* spp. to cure bruises, burns and injuries [5]. Many species of bryophytes are known that are not affected by bacteria, fungi, larvae of insect, etc. [6] because they possess compounds like phenylquinone, aromatic and phenolic substances, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, and few aliphatic compounds that offer defense against these pathogenic organisms, therefore, along with other large plants, bryophytes are also considered as potential medicinal plants [7]. Pathogenic fungi alone causes nearly 20 per cent reductions in the yield of major food and cash crops [8]. Present study reveals the antifungal activity of a moss against *Helminthosporium turcicum*-a causal organism of main food crops. This was done with a view to exploring their potentials as natural fungicides.

## II. Materials And Methods

**Collection of plant material**

Plants selected for evaluating the antifungal activity against test fungi was *Philonotis revoluta* (Bosch & Lac) a moss order Eubryales family Bartramiaceae**.** The plant material was collected in rainy Season from Mt.Abu, Distt. Sirohi (Raj.) The collected plant material was identified with the help of moss flora of Rajasthan, India [9].

**Extract preparation**

Two types of plant extracts were prepared methanolic and acetonic. For methanolic extract preparation, plant material weighted was grinded in mortar and pestle with equal amount of methanol till the formation of fine paste, then it was Centrifuged and filtered. This filtrate was used as (100%) crude extract then it was serially Diluted by double distilled water to prepare various concentrations from 10-100 per cent. The same method was adopted for acetone extract preparation, except grinding the plant material with acetone instead of methanol.

**Test organisms**

*Helminthosporium turcicum* (Pass). Leonard and Suggs, was used for the evaluation of antifungal potential of plant extracts of bryophyte. *Helminthosporium turcicum* was brought from the Department of Pathology, Maharana Pratap Agriculture College, Udaipur.

**Bioassay for Antifungal Activity**

Fungal spores of the test fungi were bioassayed against the extracts on cavity slides
By hanging drop method [10]. Hyphal length was measured after 8 hrs. Of inoculation using Ocular-micrometer [11] under Compound Microscope. Percentage of spore germination was counted under light microscope after 12 Hrs of incubation.

**Phytochemical Analysis**

Qualitative phytochemical analysis of a moss *Philonotis revoluta* extract was done by different
Methods [12] to detect the Presence or absence of certain bioactive compounds.

**Table 1.**Phytochemical screening of the bryophyte extracts

| **Phytochemicals** | *P.revoluta* | | |
|---|---|---|---|
| | **Aqueous Extract** | **Acetonic extract** | **Methanolic extract** |
| 1.Flavanoids | + | + | + |
| 2.Saponins | - | - | - |
| 3.Alkaloids | - | - | - |
| 4.Terpenoids | + | + | + |
| 5.Sterols | + | + | + |
| 6.Anthroquiones | - | - | - |
| 7.Phenols | + | + | + |
| 8.Cardiac glycosides | + | + | + |

+ = Phytoconstituent present
- = Phytoconstituent absent

## III. Results And Discussion

Different concentrations of plant extracts showed gradual reduction for the growth of *H. Turcicum* and significant reduction was observed from 10 to 100 per cent concentration. Investigation of the antifungal properties of acetonic crude extract of *P. Revoluta* on the growth parameters of *H. turcicum* showed significant reduction in spore germination and hyphal length.

Percentage of spore germination was decreased from 75.55 to 30 from 10 to 100 per cent extract concentration. Hyphal length was maximum (102.29µm) in 10 per cent and minimum (29.22µm) in 100 per cent extract concentration **(Table1.1, Graph 1 and Fig.1).**

In methanolic extract highest (95.55) percentage of spore germination was observed in the control whereas, 71.11 per cent was observed at 10 per cent. Lowest (16.66) percentage was found in 100 per cent concentration of the extract. Hyphal length was measured 84.02µm at 10 per cent while 18.26µm at 100 per cent, highest (142.48µm) hyphal length was measured in the control **(Table 1.2, Graph 2 and Fig 2).**

Acetonic and methanolic extracts were made individually to evaluate their antifungal effectiveness. Even though both the extracts showed different levels of antagonistic activity in opposition to the fungal strain, the methanolic extract was assessed best in comparison to other extract. The possible reason behind this might be the varying solubility of various plant metabolites in different solvents, in this manner differential antifungal activity was observed.

Alcoholic extract of moss was active against phytopathogenic fungi [13]. Extract of certain Bryophytes such as *Plagiochasma articulatum, Anthoceros longii, Fissidens bryoides* showed antibiotic property against *Agrobacterium tumifacians* [14]. Antifungal activity of a moss was determined against certain Phytopathogenic fungi [15]. Effect of liverwort *R.gangetica* against *F.moniliforme* and found cold water extract more effective than boiled water extract [16] Antibiotic activity of 52 species of the Bryophytes tested against 12 microorganisms. Solubility data and antibiotic spectra of the active plants indicated the occurrence of the variety of antibiotic substances among bryophytes [17] . Bryophytes extract consist bioflavonoids as the antimicrobial

substances [18]. Phytotochemical analysis and antimicrobial Activity of moss *Bryum celluiare* (Hook.)(Bryales: Bryaceae) against test fungi *Drechslersa maydis* (Drech.) and *Curvularia lunata* (Wakker) Boedijn) the causal organisms of leaf Blight of *Zea mays* L. (Poales: Poaceae) and Leaf spot of wheat respectively and reported that *B. cellulare* is a store house of various bioactive compounds [19]. Some of the antifungal compounds showed antifungal property against selected test fungi Crude aqueous and ethanolic extract of *B. cellulare* strongly inhibited spore germination and mycelia growth of fungus *C. lunata* [20]. Malformation such as stunting of growth, curling and dying of tip of fungal hyphae was also reported which affects the growth of fungus

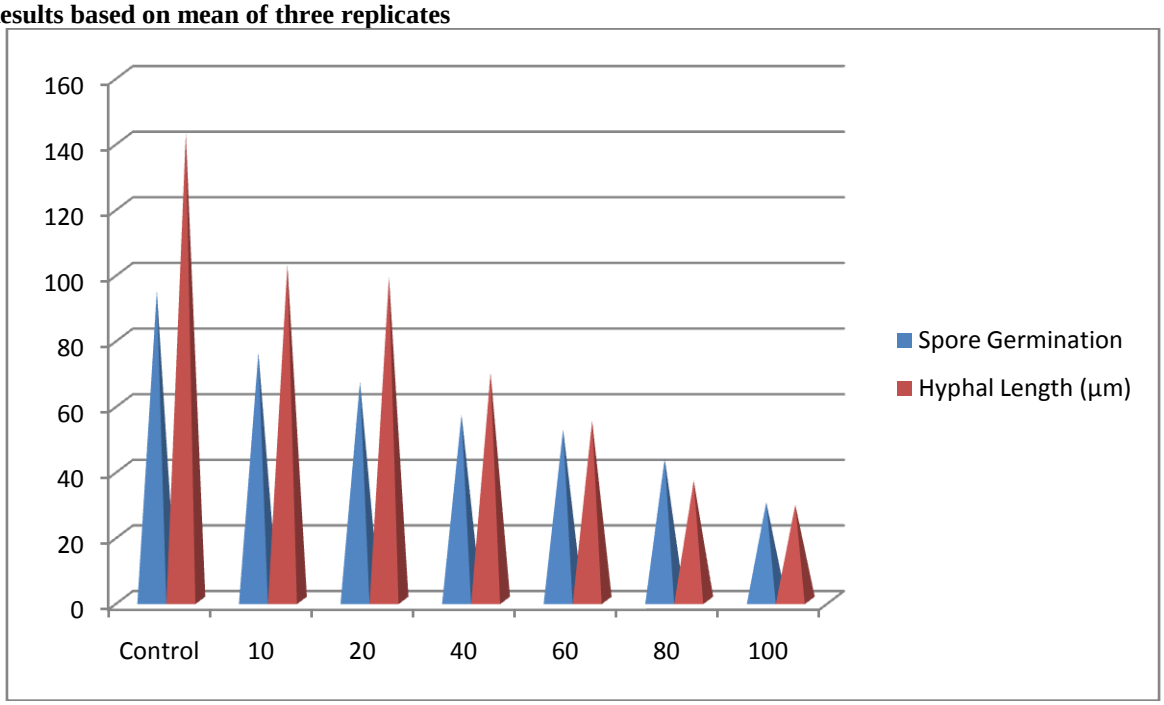
## IV. Conclusion

The present study concluded that at higher concentrations of extract spore hyphal length and germination percent was found minimum suggest that some antifungal potent chemicals are present in *Philonotis revoluta* which have inhibited the growth of test fungi. Further this study will also unlock the naturally occurring antifungal phytochemicals in this moss which can be further used as biocontrol agents or natural fungicides against plant pathogens.

**Table 1.1:** Showing the effect of different concentrations of P. revoluta acetonic extract on H. turcicum

| S.No. | Extract concentrations (%) | Spore germination Percent | | Hyphal length (µm) | |
|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD |
| 1. | Control | 94.4433 | 1.9280 | 142.4800 | 10.9600 |
| 2. | 10 | 75.5567 | 1.9283 | 102.2933 | 6.3277 |
| 3. | 20 | 66.6667 | 3.3350 | 98.6400 | 10.9601 |
| 4. | 40 | 56.6667 | 3.3350 | 69.4133 | 6.3278 |
| 5. | 60 | 52.2233 | 3.8509 | 54.8000 | 10.9600 |
| 6. | 80 | 43.3333 | 3.3350 | 36.5333 | 6.3277 |
| 7. | 100 | 30.0000 | 3.3300 | 29.2267 | 6.3278 |
| | GM | 59.8414 | 20.3708 | 76.1981 | 39.1314 |
| | Se | 1.7825 | | 4.9787 | |
| | CD5% | 5.4066 | | 15.1011 | |
| | CV | 5.16 | | 11.32 | |

**Results based on mean of three replicates**




**Graph 1**. Showing the effect of different concentrations of *P. revoluta* acetonic extract on *H. turcicum*

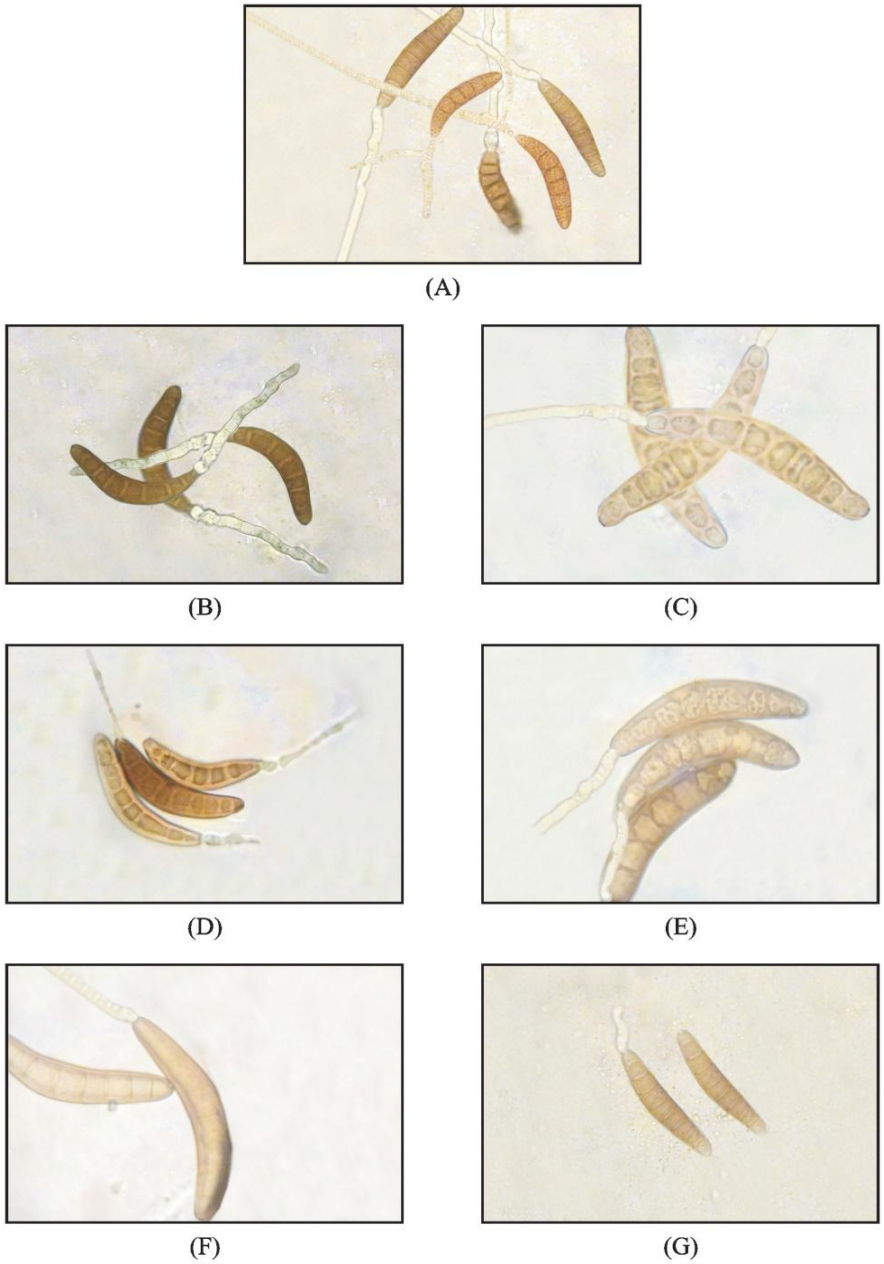
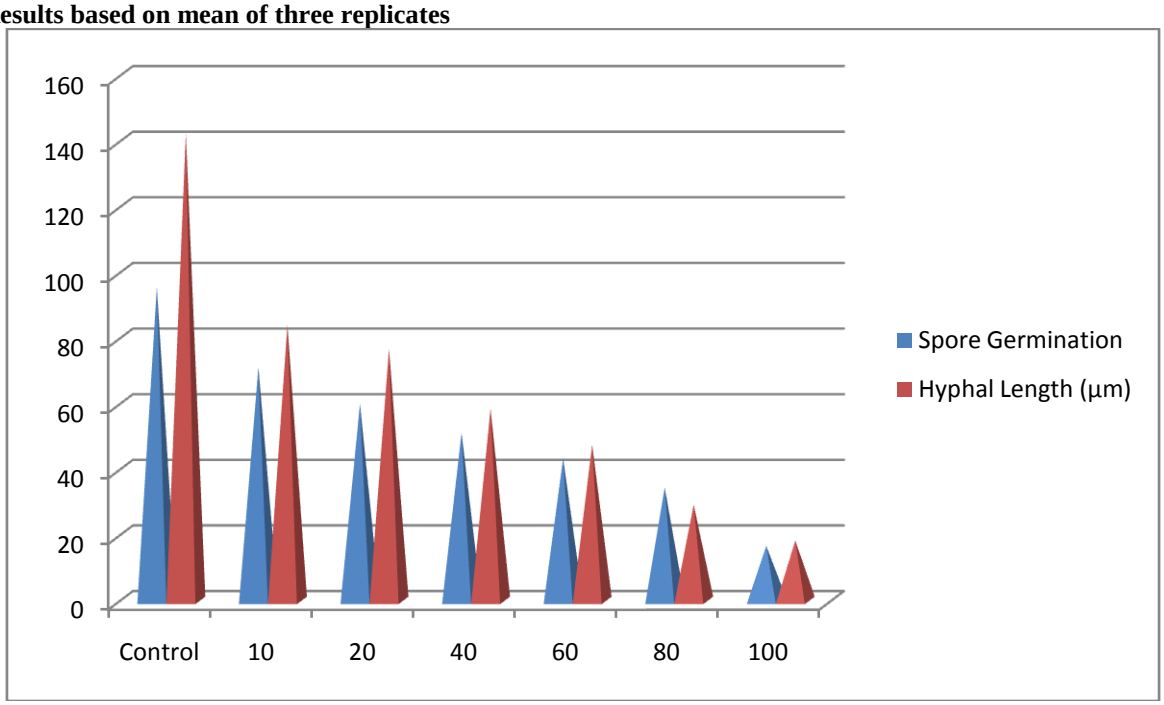



**Fig. 1.** Spore germination and hyphal length of *Helminthosporium turcicum* In the control (A); 10 (B), 20(C), 40 (D), 60 (E), 80 (F) and 100 (G) per cent Concentration of *Philonotis revoluta* acetonic extract.

**Table 1.2:** Showing the effect of different concentrations of *P. revoluta* methanolic extract on *H. turcicum*

| S.No. | Extract concentrations (%) | Spore germination Percent | | Hyphal length (µm) | |
|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD |
| 1. | Control | 95.5567 | 1.9287 | 142.4800 | 10.9600 |
| 2. | 10 | 71.1100 | 1.9226 | 84.0267 | 6.3278 |
| 3. | 20 | 60.0000 | 3.3300 | 76.7200 | 10.9600 |
| 4. | 40 | 51.1100 | 1.9226 | 58.4533 | 6.3278 |
| 5. | 60 | 43.3333 | 3.3350 | 47.4933 | 6.3278 |
| 6. | 80 | 34.4433 | 1.9283 | 29.2267 | 6.3278 |
| 7. | 100 | 16.6667 | 3.3350 | 18.2667 | 6.3278 |
| | GM | 53.1743 | 24.4379 | 65.2381 | 39.8914 |
| | Se | 1.5144 | | 4.5797 | |
| | CD5% | 4.5935 | | 13.8910 | |
| | CV | 4.93 | | 12.16 | |

**Results based on mean of three replicates**




**Graph 2.** Showing the effect of different concentrations of *P. revoluta* methanolic extract on *H. turcicum*

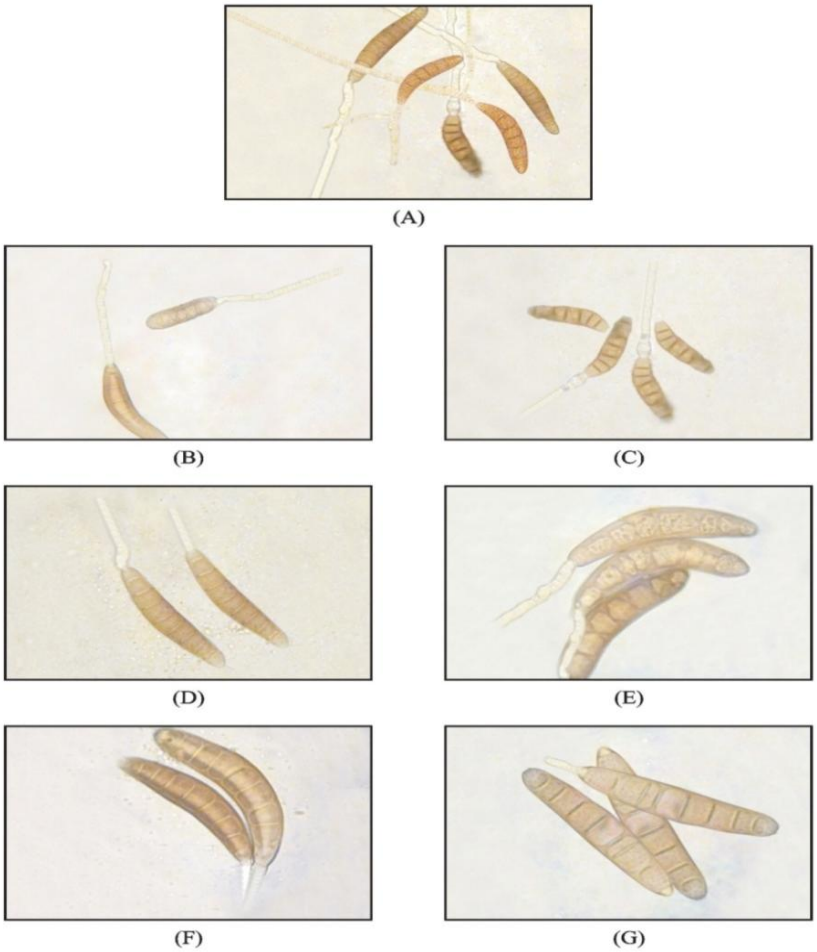



**Fig. 2.** Spore germination and hyphal length of *Helminthosporium turcicum* In the control (A); 10 (B), 20(C), 40 (D), 60 (E), 80 (F) and 100 (G) per cent Concentration of *Philonotis revoluta* methanolic extract.

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