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STUDIES ON ANTIFUNGAL POTENTIAL OF *BRYUM CELLULARE* (A MOSS) CRUDE EXTRACTS AGAINST SPORE GERMINATION OF FUNGUS *CURVULARIA LUNATA*

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ABSTRACT: Chemical protection plays a decisive role in the resistance of plants against various pathogens and their spore germination. The so-called secondary metabolites which are a characteristic feature of plants and are important to protect plants against a wide variety of microorganisms including fungi. In order to this, the present study was carried out to evaluate the antifungal potential of *Bryum cellulare* aqueous and ethanolic crude extracts against percentage inhibition of spore germination and hyphal length of test fungi *Curvularia lunata*, the causal organism of leaf spot of *Zea mays*. The preliminary tests showed that flavanoid, sterols and terpenoids etc are present in the test plant. Further various concentrations of crude extracts of aqueous and ethanolic form were tested against spore germination and hyphal length of *C.lunata* through hanging drop method. The results showed that all the extracts posses a significant antifungal potential but highest inhibition of percentage in spore germination was observed in ethanolic extract of *B.cellulare*. The percentage of spore germination was 60.43 and 15.20 in 10 and 100 per cent concentration of the extract respectively in comparison to control i.e. 110.46 per cent. In aqueous it was 85.13 and 33.26 in 10 and 100 per cent concentration in comparison to its respective control.

INTRODUCTION: Bryophytes are considered as a 'remarkable reservoir' of novel natural products or secondary metabolites, which have shown interesting biological activity and could be used in medicine. Bryophytes especially mosses and liverworts are the source of man biologically active novel compounds pertaining to pharmaceutical uses [1-4]. The occurrence of antibiotic substances in bryophytes have been well documented by botanists and microbiologists [5]. They possess compounds such as alkaloids (clavatoxine, clavatine, nicotine, lycopodine) polyphenolic acids (dihydrocaffeic) and flavonoids (apigenin, triterpenes etc.) but only few of the species have yet been thoroughly studied.

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Recently, public demand of plant base medicine and rise of antibiotic resistant bacteria have motivated biologists to look for new plant based natural products. Therefore, bryophytes can be a promising source of many new biologically active compounds in nature. Apart from the medicinal properties, bryophytes are important component of ecosystem diversity and adds up to the species richness [6, 7, 8] and also increases the plant biomass in forests in some cases [9]. Further, bryophytes are important environmental indicators and have been used as predictors of past climate change to validate climate models and potential indicators of global warming [10]. They play a chief role in ecosystem functions, such as soil development, nutrient biogeochemical cycling, water retention, plant colonization seed germination, seedling growth, and forest renovation [11].

Deora *et al.*[12] carried out the studies on antibacterial effect of aqueous crude extract of Plagiochasma articulatun, *Anthoceros longii*,

Fissidens bryoides; liverwort, hornwort and moss respectively for their antibiotic effect on *Agrobacterium tumifacians* and reported that mosses are highly antibiotic in nature followed by hornwort and liverworts. The aim of the present study was to find out the antifungal acitivity of *Bryum cellulare* against spore germination of phytopathogenic fungi *Curvularia lunata*. Phytochemical screening of different extract will also carried out to detect the presence of secondary metabolites.

MATERIAL AND METHODS:

Collection of Plant material and extract preparation:

The moss *Bryum cellulare* was collected in rainy season (2013) from Mt. Abu, Distt. Sirohi (Raj.) around Nakki Lake, Guru Shikhar and Sunset point in both vegetative and sporophytic phases. Both plants were washed with distilled water to remove soil particles, attached litter, dead material. For ethanolic extract preparation, plant material weighted was grinded in mortar and pestle with equal amount of ethanol 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 aqueous extract preparation except grinding the plant material with water instead of ethanol.

Test Organism:

The test fungi *Curvularia lunata* (MTCC No.283) was obtained from the Institute of Microbial Technology, Chandigarh, India and sub-cultured in laboratory at 30ºC temperature to obtain its pure isolate.

Screening of antifungal activity:

Fungal spores of the test fungi were bioassayed against the extracts on cavity slides by hanging drop method[13]. Hyphal length was measured after 8 hrs. of inoculation using Ocular-micrometer[14] under Compound Microscope. Percentage of spore germination was counted under light microscope after 24 hrs of incubation.

Phytochemical Analysis: Phytochemical analysis of moss *Bryum cellulare* extract was done by the methods of Trease and Evans [15] to detect the presence or absence of certain bioactive compounds.

TABLE 1: PHYTOCHEMICAL SCREENING OF THE *BRYUM CELLULARE* EXTRACTS

Compound	Ethanolic extract	Aqueous extract
Alkaloids	-	-
Anthoquinones	-	-
Cardic Glycosides	-	-
Flavanoids	+	+
Saponins	-	-
Sterols	+	+
Terpenoids	+	+

(+) = phytoconstituents present
(-)= phytoconstituents absent

RESULTS AND DISCUSSION:

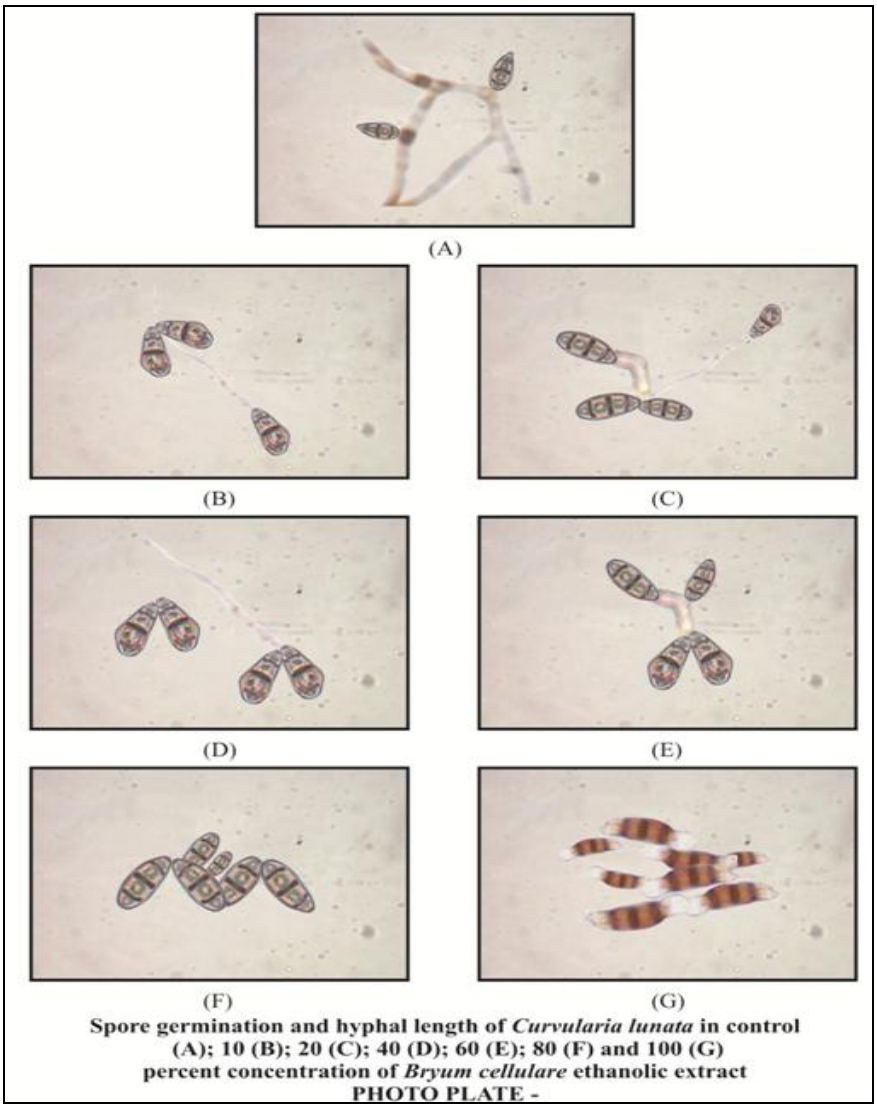
Antimicrobial activity of selected bryophyte extracts in different solvents on test microorganism *Curvularia lunata* are represented in **Table 2, 3.** The percentage of spore germination and hyphal length of *Curvularia lunata* was examined using different concentrations of ethanolic and aqueous extracts of *Bryum cellulare.* The observations on the spore germination indicated the adverse effect of ethanolic extract. The spore germination was decreased from lower to higher concentrations, only 15.20 per cent spore germination reported at 100 per cent concentration while it was 60.43 per cent at 10 per cent. Hyphal length also decreased from 60.40 µm to 0.00 at 10 to 100 per cent concentration (**Table 2, Photoplate 2**). Percentage of spore germination was 85.13 and 33.26 at 10 to 100 per cent concentration and 121.30 µm hyphal length were observed at 10 per cent while 38.13 µm were noticed in 100 per cent concentration of aqueous extract (**Table 3, Photoplate 3**). Various extract were tested for the presence of flavanoids, terpenoids, sterols, alkaloids etc. however terpenoids, sterols, flavanoids were detected in extracts of *Bryum cellulare* (**Table 1**). The bryophyte extracts prepared in different solvents were found effective in reducing fungal growth as they possess various secondary metabolities which acts as antifungal agent.

The activity of different solvent extracts was in order of ethanolic> aqueous as the bioactive compounds are more soluble in organic solvents. The present results showed similarity with the results of Deora *et al.,*[16] who determined the

antifungal activity of a moss against certain phytopathogenic fungi. Deora and Suhalka [17] studied the effect of liverwort *R.gangetica* against *F.moniliforme* and found cold water extract was more effective than boiled water extract. Bodade et al, [18] evaluate the antimicrobial efffect *of Plagiochasma appendiculatum, Thuidium cymbifolium, Bryum cellulare, Bryum argentium* and *Racomitrium crispulum* on 12 microorganism.

TABLE 2: EFFECT OF ETHANOLIC CRUDE EXTRACT OF *BRYUM CELLULARE* ON CURVULARIA LUNATA

SN	Extract Concentration (%)	Spore Germination (%)		Hyphal Length (µm)	
		Mean	SD	Mean	SD
1	Control	110.4667	0.4041	128.4667	0.3484
2	10	60.4333	0.3211	60.4000	0.3597
3	20	55.2667	0.4507	53.9667	1.1015
4	40	48.1333	0.3514	41.4667	0.5859
5	60	39.7333	0.4511	30.8333	0.6806
6	80	22.6000	0.2644	18.9667	0.4725
7	100	15.2000	0.4359	0.0000	0.0000
	GM	50.2619	29.6574	53.5857	40.1832
	Se	0.2243		0.3448	
	CD5%	0.6804		1.0458	
	CD1%	0.9450		1.4525	
	CV	0.77		1.11	



Spore germination and hyphal length of *Curvularia lunata* in control (A); 10 (B); 20 (C); 40 (D); 60 (E); 80 (F) and 100 (G) percent concentration of *Bryum cellulare* ethanolic extract
PHOTO PLATE -

FIG.1: *BRYUM CELLULAR* ETHANOLIC EXTRACT

TABLE 3: EFFECT OF AQUEOUS CRUDE EXTRACT OF *BRYUM CELLULARE* ON *CURVULARIA LUNATA*

SN	Extract Concentration (%)	Spore Germination (%)		Hyphal Length (µm)	
		Mean	SD	Mean	SD
1	Control	124.4667	0.1558	143.1333	0.1454
2	10	85.1333	0.3521	121.3000	0.5292
3	20	75.1000	0.3000	83.6667	0.2082
4	40	62.5667	0.3059	78.3000	0.6083
5	60	59.6000	0.1994	69.0667	0.1528
6	80	48.0000	0.7810	54.2000	0.2997
7	100	33.2667	0.4162	38.1333	0.6658
	GM	69.7333	27.9786	87.6714	36.3467
	Se	0.2344		0.2313	
	CD5%	0.7108		0.7015	
	CD1%	0.9872		0.9743	
	CV	0.58		0.46	

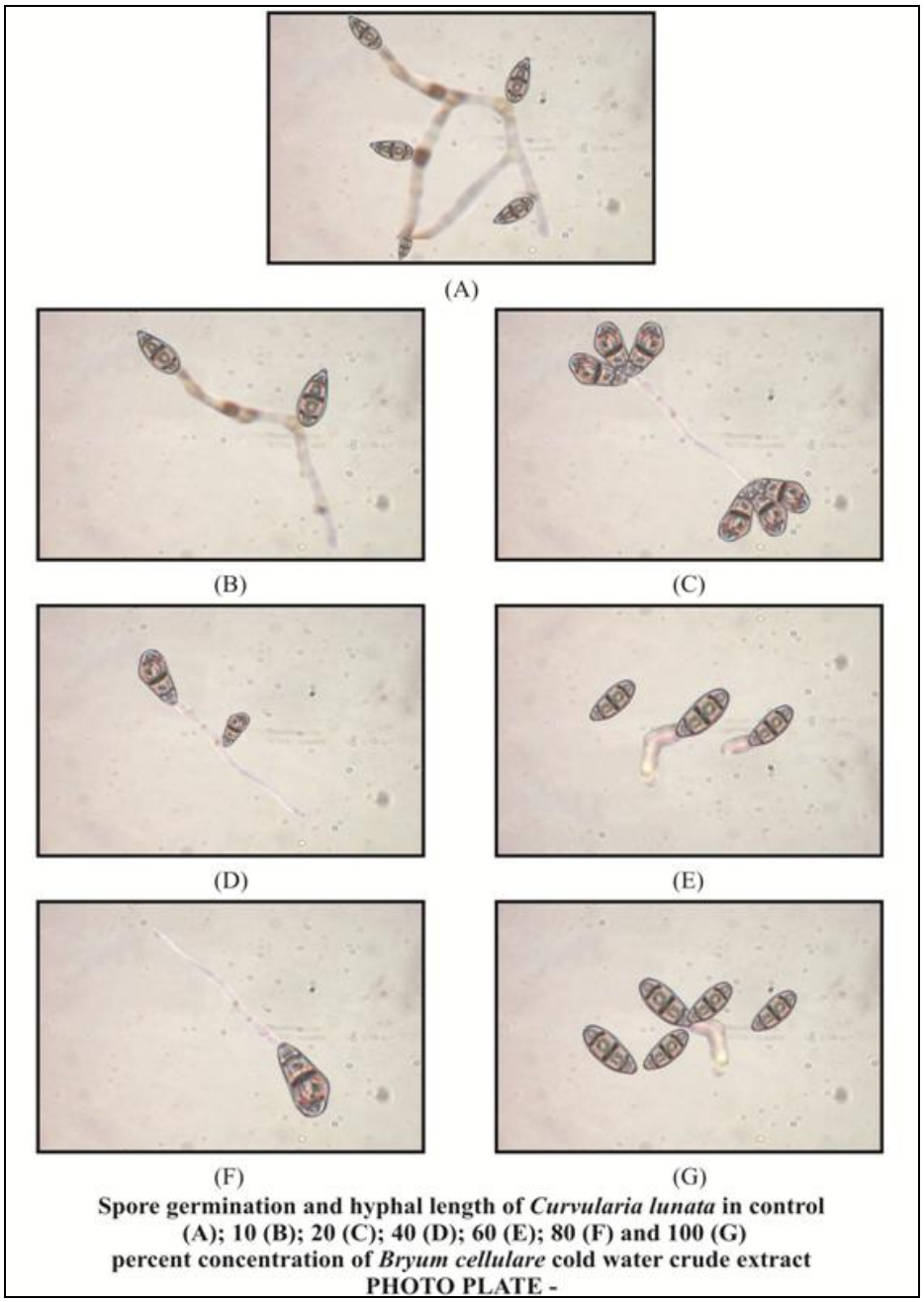


FIG.2: *BRYUM CELLULAR* AQUEOUS EXTRACT

CONCLUSION: From the present study it was concluded that spore germination and hyphal length at higher concentration suggests that some antifungal potent chemicals are present in *Bryum cellulare* which have inhibited the growth. Further this study will also unlock the naturally occurring antifungal phytochemicals in this moss which can be further used as biocontrol agents against plant diseases.

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