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Bryophytes: A Potent Tool for Controlling Some Fungal Diseases of Crop Plants.

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ABSTRACT*: The aim of this study was to evaluate the antifungal potential of moss Bryum argentium, and Bryum cellulare against the phytopathogenic fungi Curvularia lunata, the causal organism of leaf spot of Zea mays using pour plate method. A phytochemical screening of the extracts was also carried out to determine the antifungal substances present in both the bryophytes. The results revealed that the radial growth and fresh weight of test fungi was drastically reduced in response to all concentrations ranged from 10-100 per cent. Especially the plant material (Bryum argentium) extracted with ethanol showed strong antifungal activity with the significant inhibition on the growth of test fungi. Further it is concluded that at all concentrations B.argentium is most efficacious followed by the extract of B.cellulare. Therefore, after further biochemical assay the extracts of this plant can be used as mycoherbicide to control pathogens.*

KEY WORDS: *Phytopathogenic fungi, Bryum argentium, Bryum cellulare , Bryophytes, Curvularia lunata, Zea mays.*

I. INTRODUCTION

Maize, *Zea mays* L. is one of the most important cereals in the world after wheat and rice with regards to cultivation area and total production. Maize is high yielding, easy to process, readily digested and cheaper than other cereals. It is also a versatile crop, growing across a range of agro ecological zones. Every part of the maize plant has economical value: the grain, leaves, stalks, tassel and cob can all be used to produce a large variety of food and non-food products [1]. With this importance of maize, it is being plagued by an array of diseases which include the leaf spot of maize which is caused by *Curvularia lunata*. This disease is seed and soil borne disease prevalent in the hot, humid maize areas. The disease produces small necrotic or chlorotic spot with a light colored halo; lesions are about 0.5 cm per spot when fully developed and this cause significant damage to maize up to 60 per cent due to great loss of photosynthetic region of the crop.

Attempts have been made to develop maize cultivars that are resistant to leaf spot, and many other control measures have also been used to check this fungal disease. These include improved cultural practices on the farm and chemical control using fungicides and were then found to be effective against leaf spot when tested. But, most of these fungicides are not available to peasant farmers because most of the fungicides are expensive, require skilled labour and add to the cost of production while the yield obtained by their use may not be sufficient to justify cost of production. Also, most of these fungicides are toxic to humans and with the dwindling foreign exchange and prohibitive cost; most of the useful fungicides are usually out of reach of peasant farmers who are regarded as the producers of food. The pathogen on its own, also build up resistance to the fungicides and even when resistant varieties are planted in endemic areas. Warhurst [2] observed that the decoction of the leaves of *Cinchona officinalis* were used to treat a amebiasis while it's dry bark and active against *Pseudomonas falciparum* and herpes. Smith and Reynard [3] revealed that lower plants possess antimicrobial properties which could be attributed to chemicals including polygodial, norpiquisone and lunularin which constitute the phytochemical component of the lower plants. The results showed that the organisms were susceptible to different concentrations of extracts of the lower plants which was a function of their antibiotic activity. Toyota and Asakawa [4] screened the extract of *Plagiochasma appendiculatum* and evaluated that it possesses antimicrobial activity which was due to presence of terpenoids in it. Deora *et al*. [5] carried out the studies on antibacterial effect of aqueous crude extract of *Plagiochasma articulatum, Anthoceros longii* and *Fissidens bryoides* against test organism *Xanthomonas citri in vitro*. The number of colonies and inhibition zone of this bacteria enriched with Nutrient agar medium were examined after 24 and 48 hours. It was observed that *P. articulatum* extract was more active than *A. longii* and *F.bryoides.* The objective of the present study was to get the most effective plant extract within farmers' reach to control *C.lunata* causing leaf spot of maize.

II. MATERIAL AND METHODS

Plant material and extract preparation

The plant material 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 the plants were washed with tap water to remove soil particles, air dried and then packed in envelope and kept in the oven at 80°C for 24 h, separately. The dried material was then blended into powder. 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. The fractions obtained were centrifuged at 6,000 rpm for 10 min and were used for studying antifungal activity.

Test Organism

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

Screening of Antifungal Activity

Antifungal activity of bryophyte fraction was determined by using pour plate method. The plant extracts of 10 ml each were first poured into petri dishes. Then, 10 ml molten PDA was poured aseptically on the plant extract in the petri dishes and swirled round for even dispersion of the extract into the agar. The extracts were incorporated at different concentrations of 100, 80, 60, 40, 20 and control. A 5 mm mycelium agar disc of *C. lunata* was released into the poisoned agar/ extracts incorporated into PDA. The treatments were replicated three times, incubation period for antifungal activity was 72 hrs. The average diameter of resultant colony was measured after incubation. The growth of *C. lunata* mycelium on PDA without any amendment was used as control. The percent inhibition of mycelial growth by plant extract was calculated by using the formula given by Vincent (1927).

Phytochemical Analysis

Qualitative phytochemical analysis of a moss *Bryum argenteum* and *Bryum cellulare* extract was done by the methods of Trease and Evans [6] to detect the presence or absence of certain bioactive compounds.

III. RESULTS AND DISCUSSION

Antifungal activity of *both* bryophyte extracts in different solvent on *Curvularia lunata* are represented in Table 1 and Table 2. Although all the extracts of both bryophytes *Bryum agrenteum* and *Bryum cellulare* showed varying levels of antifungal activity against the test fungi, the ethanolic extract of *Bryum argenteum* was found to be more active than other fractionated extracts. The colony diameter was decreased from lower to higher concentration, only 2.83 mm colony diameter was reported at 100 per cent concentration while it was 17.73 mm at 10 per cent. Per cent inhibition was 38.13 to 90.11 at 10 to 100 per cent concentration. While the colony diameter of ethanolic extract of *Bryum cellulare* was 3.86 mm at 100 per cent concentration and 19.26 mm at 10 per cent concentration. Percent inhibition was 34.09 to 86.77 at 10 to 100 per cent concentration. The aqueous extract of *Bryum argenteum* showed colony diameter of 30.46 mm to 7.93 mm at 10 to 100 per cent concentration while percent inhibition was observed 24.14 to 80.24 at 10 to 100 per cent concentration. Colony diameter was 35.46 and 10.13 at 10 to 100 per cent concentration and percent inhibition was 16.15 to 76.04 at 10 to 100 per cent concentration in aqueous extract of *Bryum cellulare*. Extract were tested for the presence of flavanoids, terenoids, sterols, alkaloids etc. however terpenoids, sterols, flavanoids, cardic glycosides were detected in varying degree in extracts of *Bryum argenteum* and *Bryum cellulare* (table 3). The bryophyte extracts prepared in different solvents were found effective in reducing fungal growth as they posses various secondary metabolities which acts as antifungal agent. The present results

showed similarity with the results of Deora and Bhati [7] who find out that extract of certain bryophytes such as *Plagiochasma articulatum, Anthoceros longii, Fissidens bryoides* showed antibiotic property against *Agrobacterium tumifacians.* Deora *et al.*, [8] determined the antifungal activity of a moss against certain phytopathogenic fungi. Deora and Suhalka [9] 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.*, [10] evaluate the antimicrobial efffect *of Plagiochasma appendiculatum,Thuidium cymbifolium, Bryum cellulare, Bryum argentium* and *Racomitrium crispulum* on 12 microorganism. Solubility data and antibiotic spectra of th active plants indicated the occurrence of the variety of antibiotic substances among bryophytes.

IV. CONCLUSION

Results of the present study concludes that all the extract of *Bryum argenteum* and *Bryum cellulare* exhibited varying degrees of antimicrobial activities against *C.lunata* but ethanolic extract of *Bryum argenteum* was found in this study to be most effective against the test fungi. Growth assessment carried out showed that growth was hindered by the extract in the test fungi. It is obvious that anything that can hinder growth in organisms will also check disease caused by this organism.

Table1: Phytochemical screening of the *Bryum argenteum* and *Bryum cellulare* extracts

	Bryum argentium		***Bryum cellulare***	
Compound	**Ethanolic extract**	**Aqueous extract**	**Ethanolic extract**	**Aqueous extract**
Alkaloids	-	-	-	-
Anthoquinones	-	-	-	-
Cardic Glycosides	+ ++	+	+	+
Flavanoids	+++	+	+	+
Saponins	-	-	-	-
Sterols	+++	+	+	+
Terpenoids	+++	+	+	+

Table.2 Effect of ethanolic extract of *Bryum argenteum* on *Curvularia lunata*

SN	Extract Concentration (%)	Colony Diameter (mm)	Fresh weight (gm)	Percent Inhibition (%)
		Mean	Mean	Mean
1	Control	28.6667	84.3333	0
2	10	17.7333	47.3000	38.1397231
3	20	14.3667	40.4333	49.8836629
4	40	10.8333	28.4333	62.2094625
5	60	8.5667	24.7000	70.1161975
6	80	5.6000	21.9667	80.465139
7	100	2.8333	15.4667	90.1164068

Fig.2 Effect of ethanolic extract of *Bryum argenteum* on *Curvularia lunata*

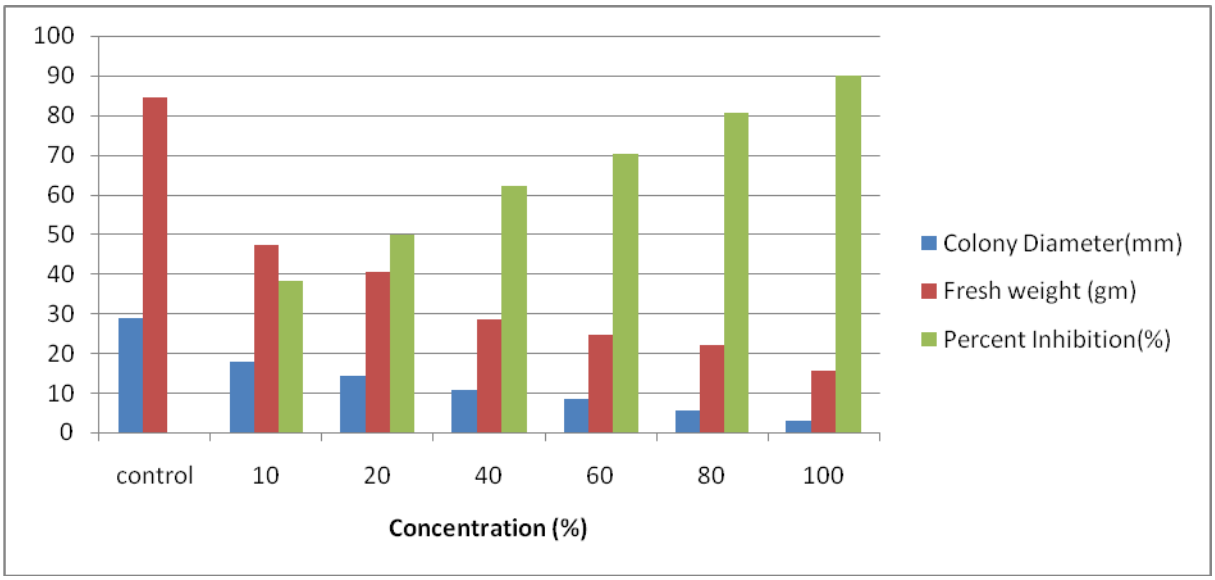


Table.3 Effect of aqueous crude extract of *Bryum argenteum* on *Curvularia lunata*

S.N	Extract Concentration (%)	Colony diameter (mm)	Fresh weight (gm)	Percent Inhibition (%)
		Mean	Mean	Mean
1	Control	40.1667	99.7333	0
2	10	30.4667	75.5333	24.1493576
3	20	27.4333	69.4667	31.7013845
4	40	22.5333	56.4000	43.9005445
5	60	15.2667	42.5333	61.9916498
6	80	11.7667	32.3667	70.7053355
7	100	7.9333	29.3333	80.249062

Fig.3 Effect of aqueous crude extract of *Bryum argenteum* on *Curvularia lunata*

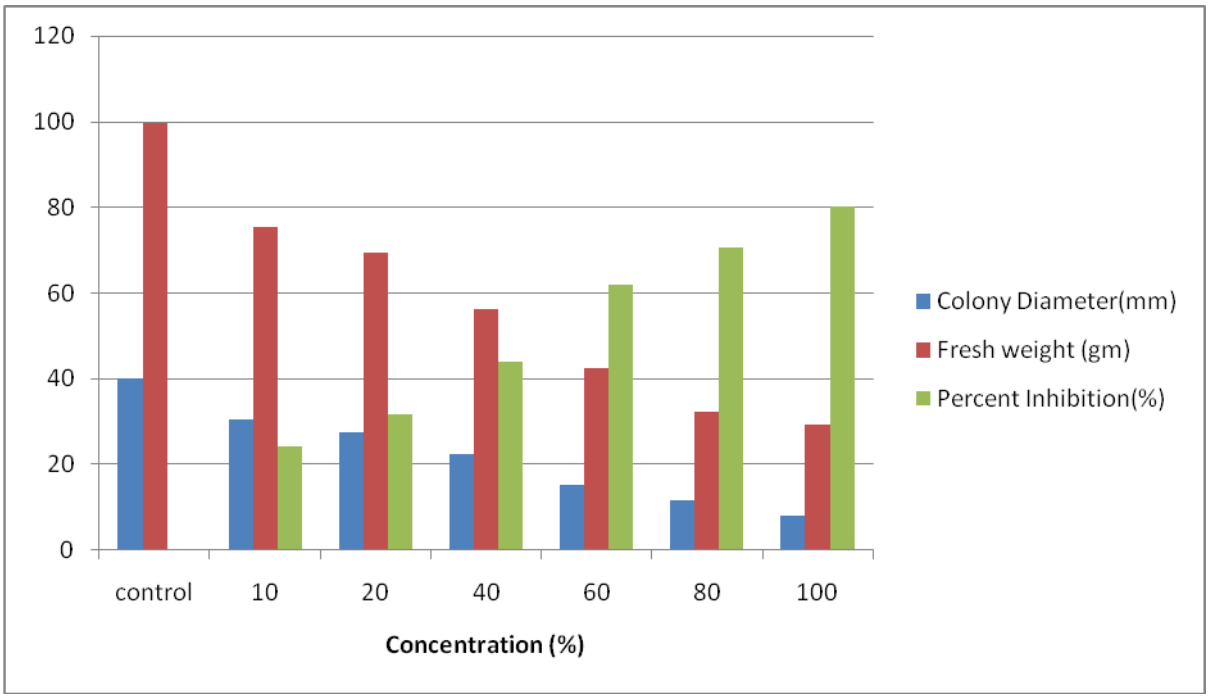


Table.4 Effect of ethanolic extract of *Bryum cellulare* on *Curvularia lunata*

S.N	Extract Concentration (%)	Colony Diameter (mm)	Fresh weight (gm)	Percent Inhibition (%)
		Mean	Mean	Mean
1	Control	29.2333	84.7667	0
2	10	19.2667	56.0000	34.0933114
3	20	17.1667	43.0000	41.2769
4	40	12.9000	36.9333	55.8722416
5	60	10.5667	30.2333	63.8538926
6	80	6.7000	26.9333	77.0809317
7	100	3.8667	19.1667	86.772961

Fig.4 Effect of ethanolic extract of *Bryum cellulare* on *Curvularia lunata*

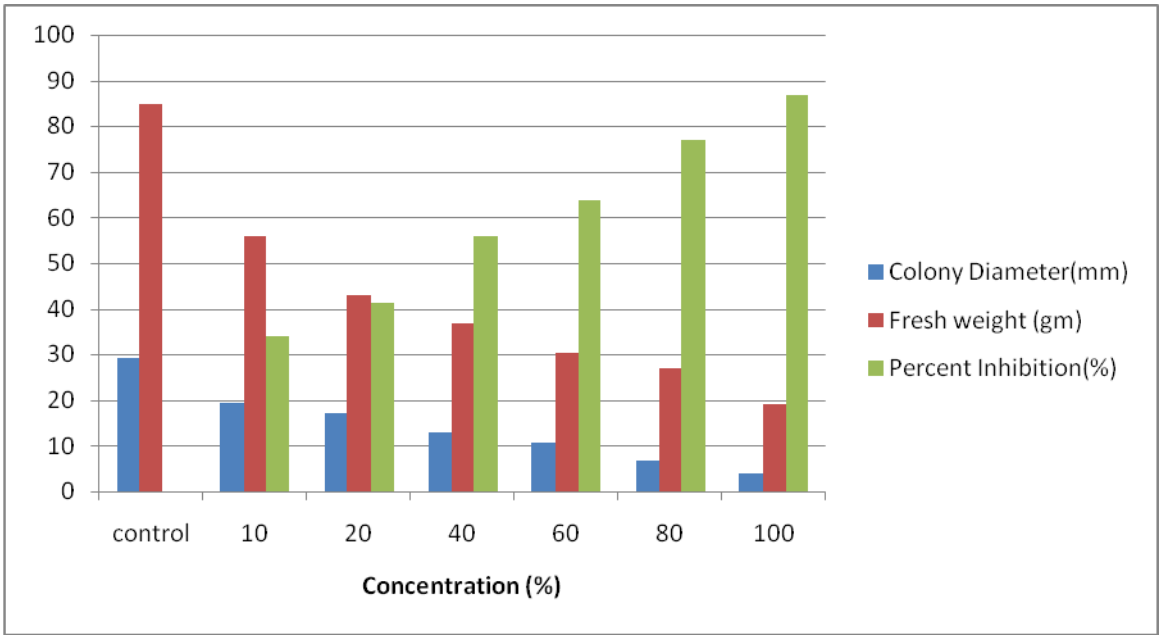
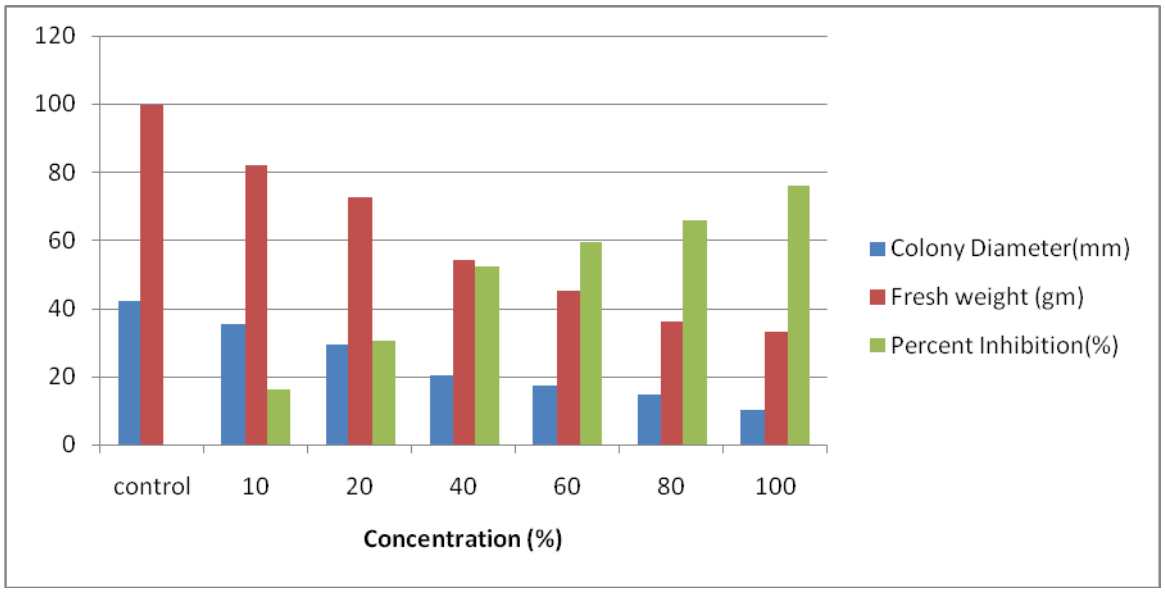


Table.5 Effect of aqueous crude extract of *Bryum cellulare* on *Curvularia lunata*

S.N	Extract Concentration (%)	Colony Diameter (mm)	Fresh weight (gm)	Percent Inhibition (%)
		Mean	Mean	
1	Control	42.3000	99.5000	0
2	10	35.4667	82.0000	16.1543735
3	20	29.3667	72.7667	30.5751773
4	40	20.2000	54.1000	52.2458629
5	60	17.1667	45.0000	59.4167849
6	80	14.5000	36.2667	65.7210402
7	100	10.1333	33.2000	76.044208

Table.5 Effect of aqueous crude extract of *Bryum cellulare* on *Curvularia lunata*



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