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Research Article

Evaluation of Bryophyte for Green Fungicides as Alternative Treatment to Control Plant Pathogen

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Abstract
The aqueous, acetonic and methanolic extract of *Riccia gangetica*, a bryophyte, was found to be effective against phytopathogen. The fungitoxicity of the extract was measured by percent spore germination inhibition and hyphal length using hanging drop technique. Extract of plant were prepared in aqueous solution, acetone and methanol from 10-100 concentration. The solvent concentration with highest antifungal activity was recorded in 100 per cent methanolic extract. Rest of the plant extracts exhibited moderate to minimal antifungal activity. Distinct morphological changes were observed in treated fungal spores in comparison to control . The treatment clearly showed anomalies in the spores which becomes shrinked and malformed.

Keywords: Phytopathogen, Fungitoxicity, *Riccia gangetica,* Spore germination, Hanging drop technique, Extract.

INTRODUCTION

With the growing concern about the environment and human health, matters such as fungicide and pesticide pollution, negative impacts on our environment and the poisoning of non-targeted species have been paid wide attention. Post-harvest diseases destroy 10-30% of the total yield of crops and the loss is considerable in a few perishable crops especially in developing countries[1]. In addition to the target pathogen fungicides may also kill various beneficial organisms and their toxic form persist in the soil. Biologicals, because of their natural origin are biodegradable and mostly do not leave toxic residue or by products to contaminate the environment[2]. Thirteen botanicals, eight bio-agents and twenty-three fungicides against *H. turcium* leaf blight of maize. Neem and Kernal extract caused maximum inhibition of growth followed by *Aloe-vera*. Among bio-agents *Trichodeema* was effective. Of the fungicides tested mancozeb highly inhibited the growth. Plant extracts were found equally effective which can be used as alternative to mancozeb[3]. Bryophytes contain numerous potentially compounds, including oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, amino acids, fatty acids, aliphatic compounds, phenylquinones and aromatic and phenolic substances[4]. Bryophytes extract consist bioflavonoids as the antimicrobial substances[5]. Three species of mosses (*Anomodon rostratus*, *Plagiomnium cuspidatum*, *Orthotrichum rupestre*) produce substances that inhibit bacteria and fungi[6]. Antimicrobial substances are present in the bryophytes, which can control some plant diseases caused by fungi and bacteria and may be substitute for common synthetic plant pesticides[7]. The search of more new antifungal natural plant products could be more useful in controlling plant pathogens. The present piece of work reports the antifungal efficacy of *Riccia gangetica* against pathogen *Curvularia lunata* tested for spore germination inhibition. The microscopic studies dealing with pathogen gives an idea about the mode of action of Liverwort extract.

MATERIALS AND METHODS

Collection of plant material

Plant selected for evaluating the antifungal activity against test fungi was *Riccia gangetica* (Ahmed). 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[8].

Extract preparation

Three types of plant extracts were prepared methanolic, acetonic and aqueous. 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 and aqueous extract preparation, except grinding the Plant material with acetone and water instead of methanol.

Test organisms

Pure culture of *Curvularia lunata* (MTCC No 2030) was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Several diseases caused by *C. lunata* on plants such as stem blight disease of cassava,

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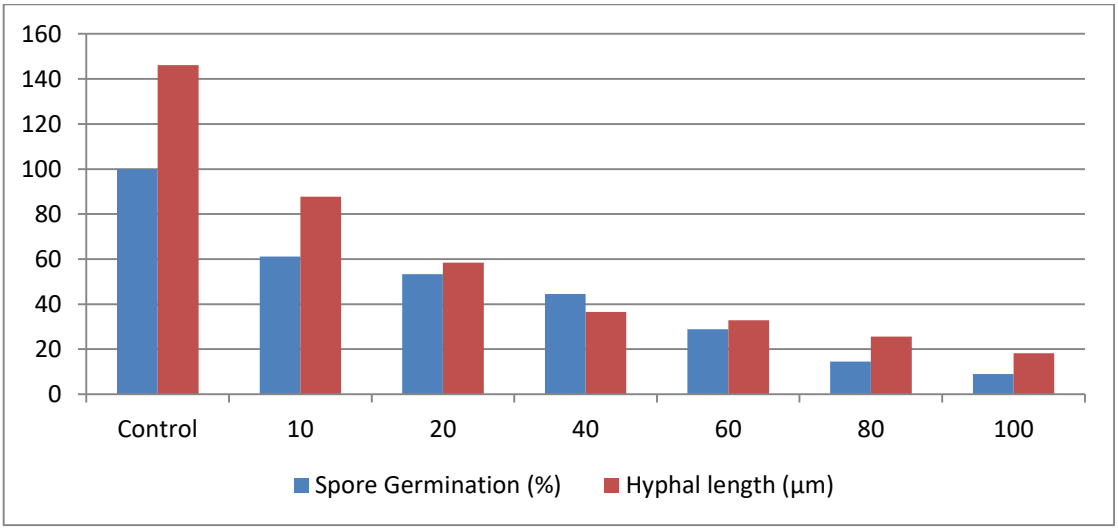
Table 1: Effect of *R. gangetica* aqueous extract on *C. lunata.*

S.No.	Extract concentrations (%)	Spore germination Per cent (%)		Hyphal length (µm)	
		Mean	SD	Mean	SD
1.	Control	100.0000	0.0000	146.1333	6.3278
2.	10	61.1100	1.9226	87.6800	10.9600
3.	20	53.3333	3.3350	58.4533	6.3278
4.	40	44.4433	1.9283	36.5333	6.3277
5.	60	28.8900	3.8452	32.8800	10.9600
6.	80	14.4433	1.9283	25.5733	6.3278
7.	100	8.8900	1.9226	18.2667	6.3278
	GM	44.4443	29.6895	57.9314	43.5848
	Se	1.3928		4.5797	
	CD5%	4.2246		13.8910	
	CD1%	5.8673		19.2927	
	CV	5.43		13.69	



Graph 1

Table 2: Effect of *R. gangetica* acetonic extract on *C. lunata.*

S.No.	Extract concentrations (%)	Spore germination (%)		Hyphal length (µm)	
		Mean	SD	Mean	SD
1.	Control	98.8900	1.9230	157.0933	6.3278
2.	10	50.0000	3.3300	76.7200	10.9600
3.	20	37.7767	3.8509	43.8400	10.9600
4.	40	23.3333	3.3350	29.2267	6.3278
5.	60	13.3333	3.3350	18.2667	6.3278
6.	80	4.4433	1.9283	10.9600	0.0000
7.	100	0.0000	0.0000	0.0000	0.0000
	GM	32.5395	32.5736	48.0152	51.8115
	Se	1.6268		4.1425	
	CD5%	4.9345		12.5649	
	CD1%	6.8533		17.4509	
	CV	8.66		14.94	

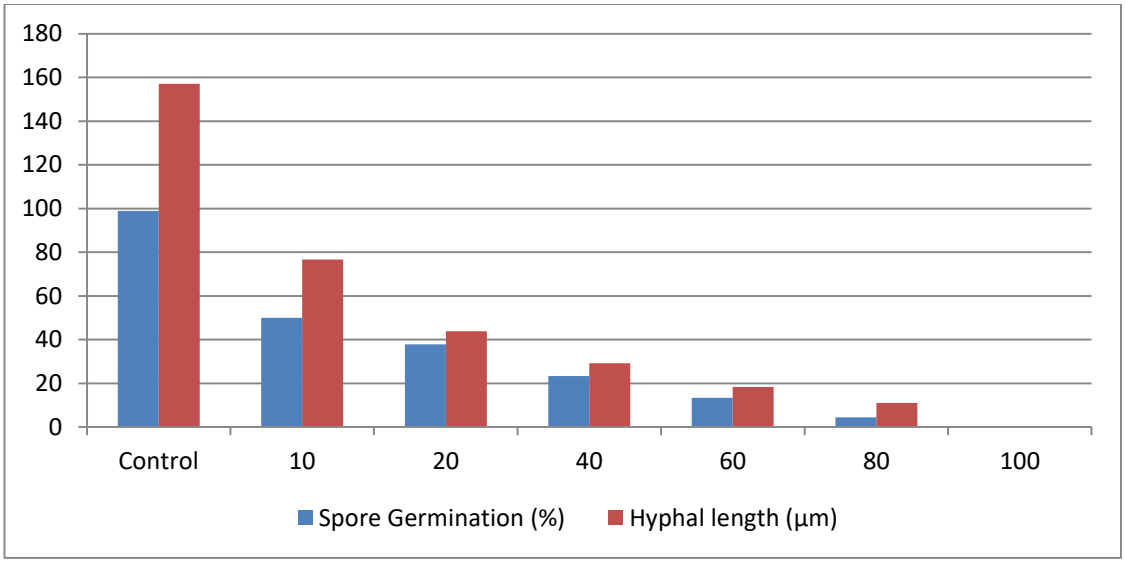
leaf spot of maize and ergot of *Sorghum.*

Bioassay for Antifungal Activity

Fungal spores of the test fungi were bioassayed against the extracts on cavity slides By hanging drop method[9]. Hyphal length was measured after 8 hrs. Of inoculation using Ocular-micrometer[10] under Compound Microscope. Percentage of spore germination was counted under light microscope after 12 Hrs of incubation.

RESULTS

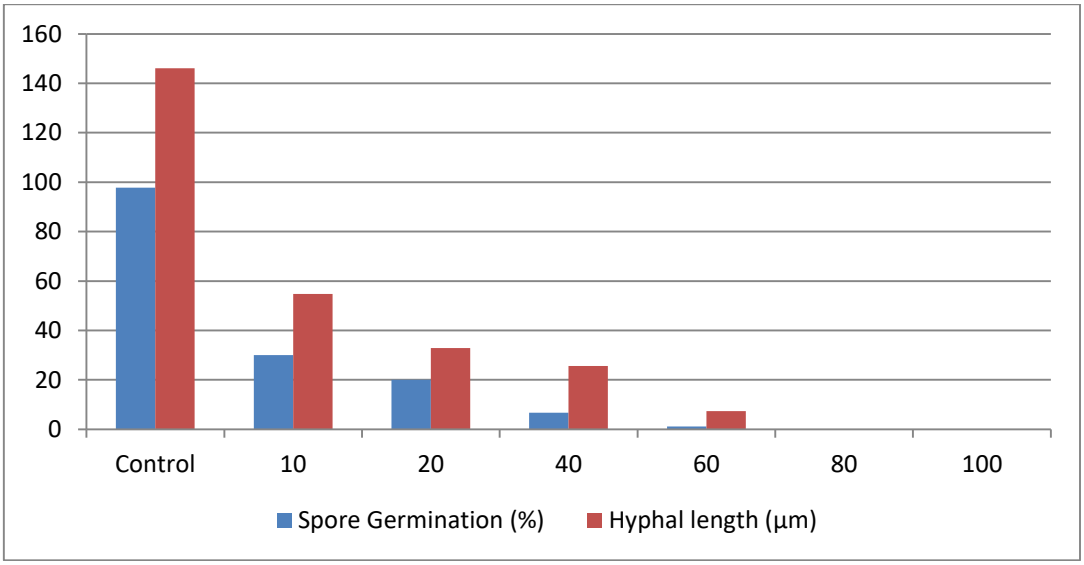
Antifungal activity of aqueous, acetonic and methanolic extracts of different concentrations of *R. gangetica* showed significant reduction in spore germination and hyphal length of *C.lunata* from 10 to 100 per cent concentration. In aqueous extract the percentage of spore germination ranged between 61.11 and 8.89 at 10 and 100 per cent



Graph 2

Table 3: Effect of *R. gangetica* methanolic extract on *C. lunata*.

S.No.	Extract concentrations (%)	Spore germination (%)		Hyphal length (µm)	
		Mean	SD	Mean	SD
1.	Control	97.7767	3.8507	146.1333	6.3278
2.	10	30.0000	3.3300	54.8000	10.9600
3.	20	20.0000	3.3300	32.8800	10.9600
4.	40	6.6667	3.3350	25.5733	6.3278
5.	60	1.1100	1.9226	7.3067	6.3278
6.	80	0.0000	0.0000	0.0000	0.0000
7.	100	0.0000	0.0000	0.0000	0.0000
	GM	22.2219	33.5048	38.0990	49.3345
	Se	1.5710		4.1425	
	CD5%	4.7650		12.5649	
	CD1%	6.6179		17.4509	
	CV	12.24		18.83	



Graph 3

concentration corresponding with 100 per cent germination in the control. Hyphal length was measured 87.68µm at 10 per cent and 18.26µm at 100 per cent (Table 1, Graph 1, and Fig 1). The acetonic extract exhibited variable efficacy against fungi and complete inhibition of spore germination was observed at highest concentration.

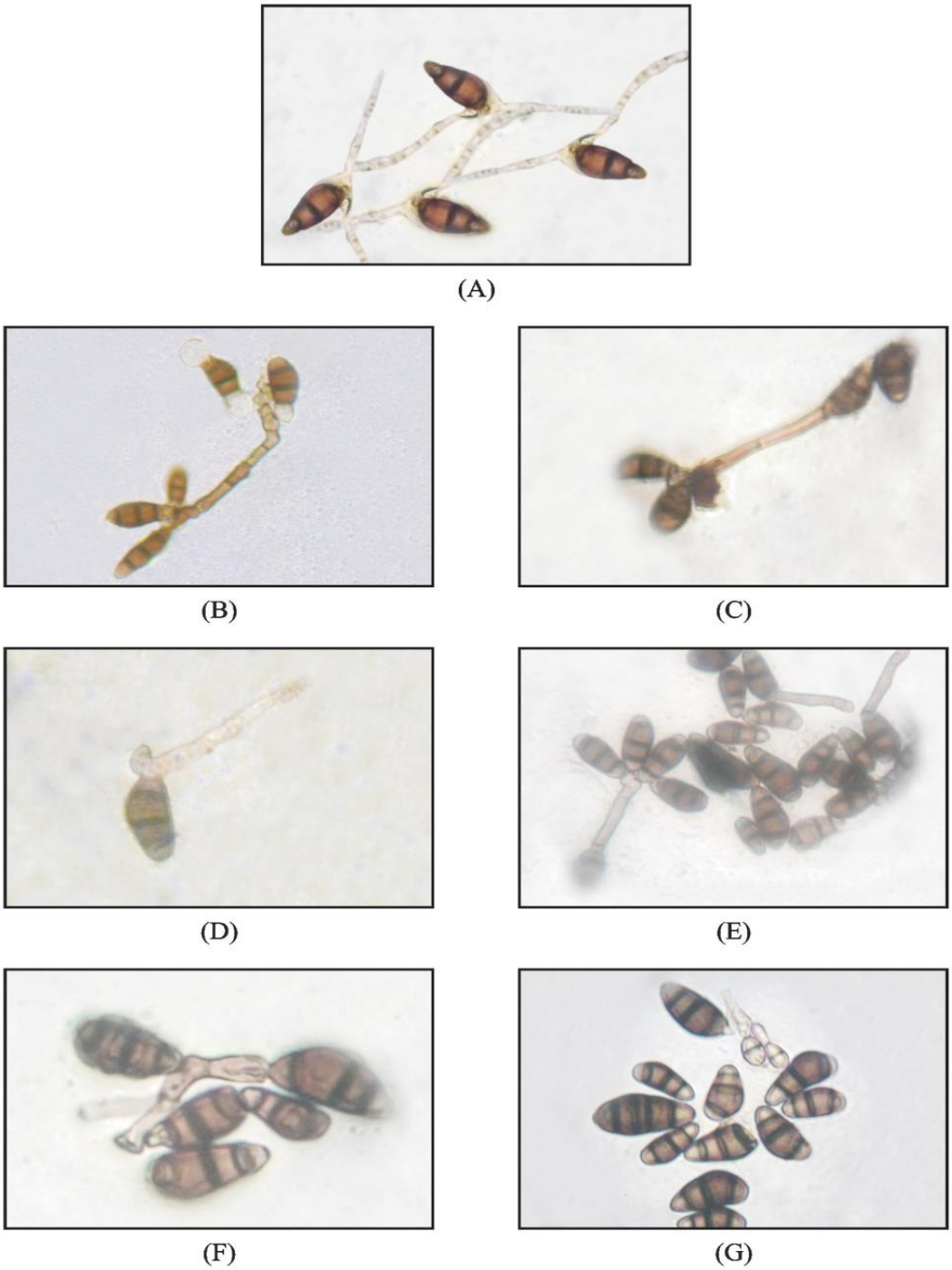


Figure 1: Spore germination and hyphal length of *Curvularia lunata* in the control (A); 10 (B), 20 (C), 40 (D), 60 (E), 80 (F) and 100 (G) per cent concentrations of *Riccia gangetica* aqueous water extract.

50 per cent spore germination and 76.72 µm hyphal length was reported at 10 per cent which decreased to zero at 100 per cent concentration (Table 2, Graph 2 and Fig 2). In methanolic extract under increasing extract concentrations, process of spore germination slowed down. 30 per cent spore germination and 54.80 µm hyphal lengths were recorded at 10 per cent while germination was completely inhibited at 100 per cent concentration and caused malformation of spores (Table 3, Graph 3 and Fig 3).

DISCUSSION

The overall results indicated that methanolic extract of *R. gangetica* impart maximum antifungal activity and these extracts could be of practical use as antifungal compound with low impact on the environment. Spore germination has taken place up to 60 per cent concentration of the extract only. However, malformation of spores was noticed at 100 per cent concentration Moss *Homalia trichomonoides* exhibit antifungal activity against *Candida albicans*[11]. Antifungal properties of *Dumortiera hirsuta*, *Sphagnum portarecense* were found active against

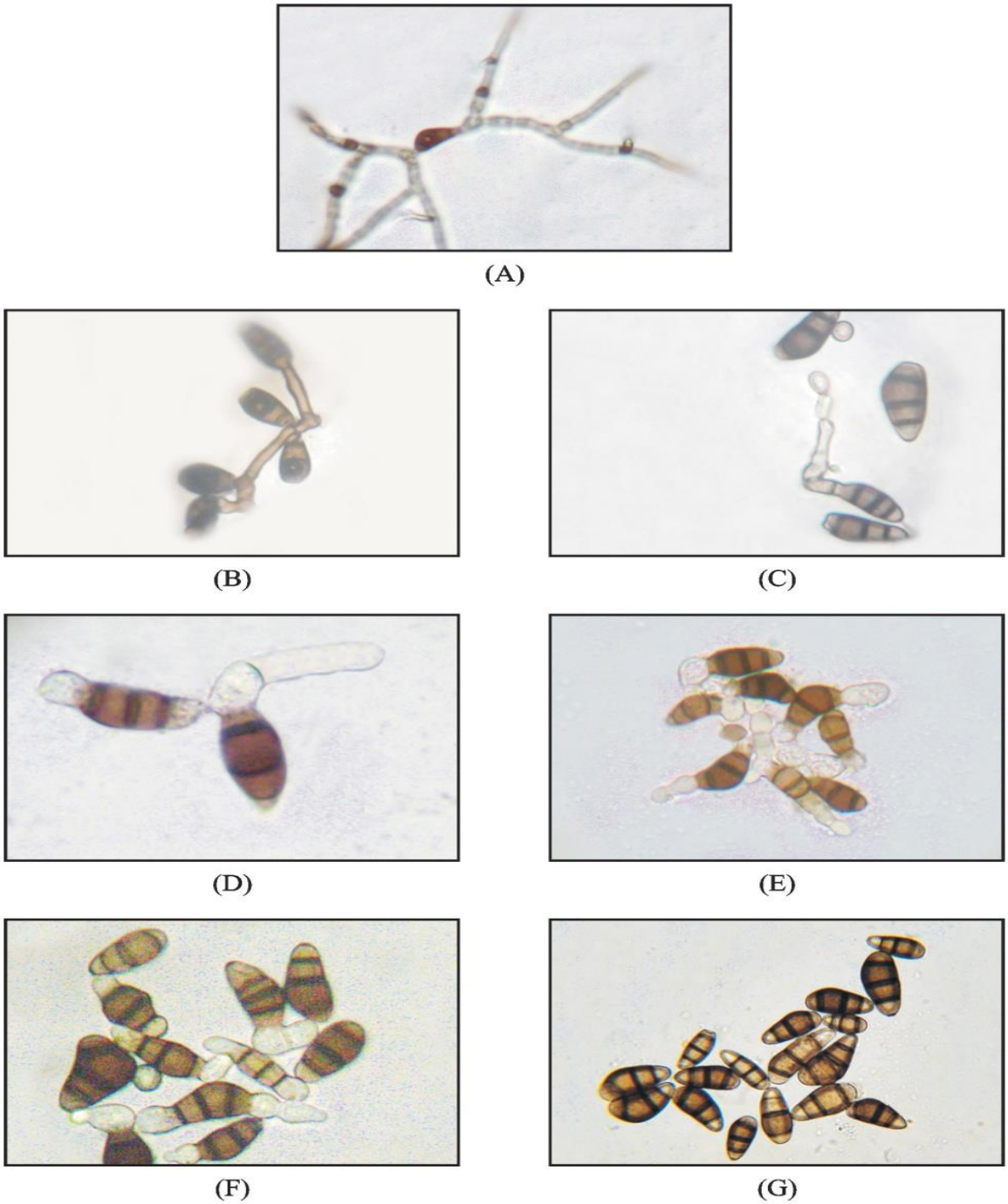


Figure 2: Spore germination and hyphal length of *Curvularia lunata* in the control (A); 10 (B), 20 (C), 40 (D), 60 (E), 80 (F) and 100 (G) per cent concentrations of *Riccia gangetica* acetonic extract.

Candida albicans[12]. 18 species of bryophytes showed antifungal activity[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

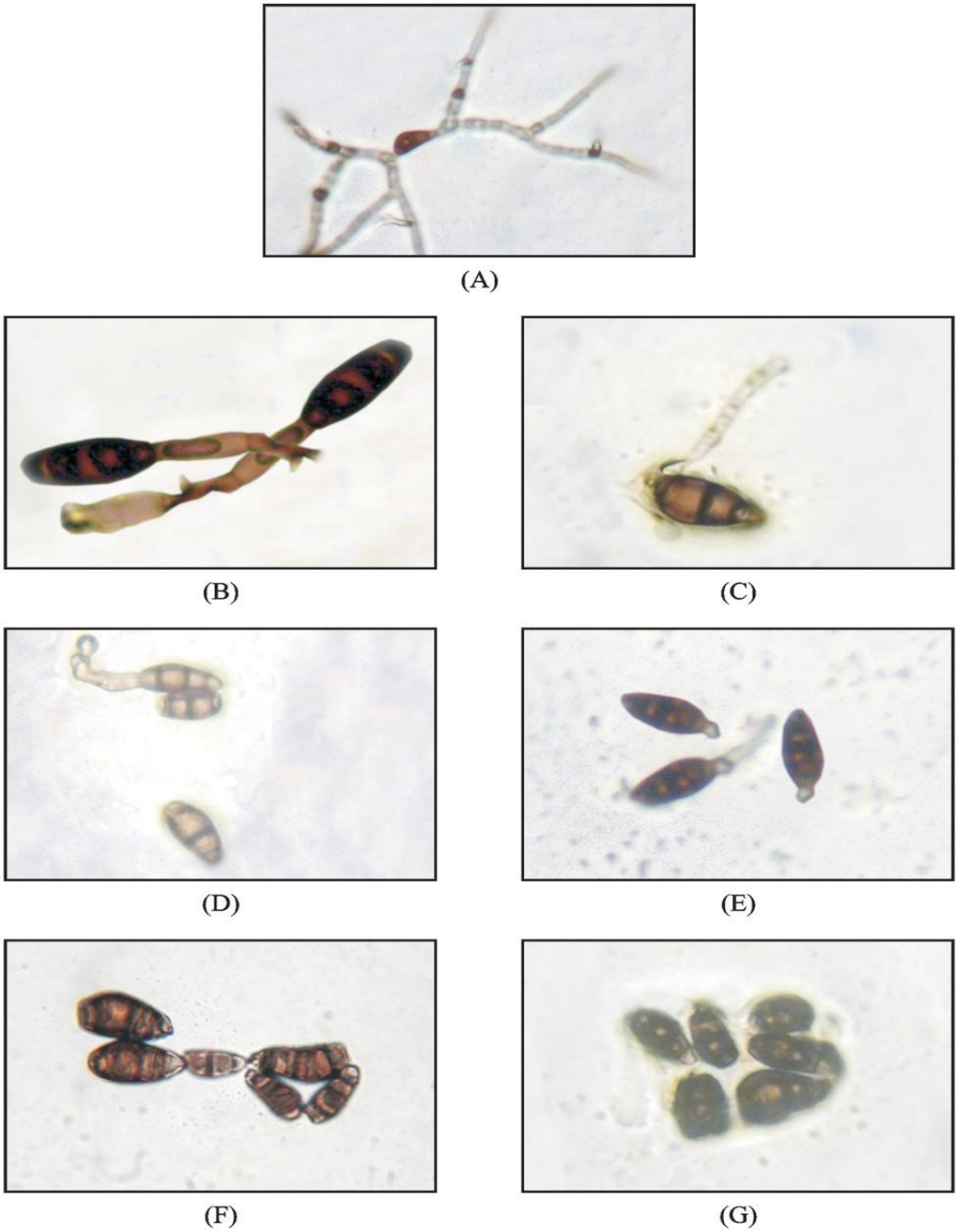


Figure 3: Spore germination and hyphal length of *Curvularia lunata* in the control (A); 10 (B), 20 (C), 40 (D), 60 (E), 80 (F) and 100 (G) per cent concentrations of *Riccia gangetica* methanolic extract.

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 fungi.

CONCLUSION

The present study revealed that at higher concentrations of extract spore hyphal length and germination percent was found minimum suggest that some antifungal potent chemicals are present in *Riccia gangetica* which have inhibited the growth of test fungi and higher concentrations of extracts inhibit germination process and caused malformation or shrinkage of spores.

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