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Evaluation of Fungicidal Potential of Some Moss Extracts on Phytopathogenic Fungus “*Fusarium Solani*”

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Abstract

Mosses are a group of non-vascular plants containing sources of secondary metabolites with anti-cancer, anti-microbial, and antifungal properties. This study aimed to evaluate the antifungal potential of the different extracts (ethanolic, methanolic and acetonic extracts) of mosses, collected from Iran against the phytopathogenic fungi *Fusarium solani* (IRAN 11C), by the twofold serial dilution method. The used extract concentrations percentage were: 100, 50, 25, 12.5, 6.25 and 3.125%. The results indicated that, by increasing the concentration of extracts, the antifungal activities also increased, and the radial growth of test fungi was reduced. Ethanolic extracts in particular showed the best antifungal activities with significant inhibition on the growth of test fungi at the highest and lowest concentrations.

Keywords: Mosses, Extract, Antifungal effects, Minimum inhibitory concentration.

Bazı Karayosunu Ekstraktlarının Fitopatogenik Mantar “*Fusarium Solani*” Üzerindeki Mantar Öldürücü Potansiyelinin Değerlendirilmesi

Öz

Karayosunları, anti-kanser, anti-mikrobiyal ve antifungal özelliklere sahip ikincil metabolizma kaynakları içeren bir grup vasküler olmayan bitkidir. Bu çalışmanın amacı, İran'dan toplanan karayosunlarının farklı ekstraktların (etanolik, metanolik ve asetonik ekstraktlar) antifungal potansiyelini, fitopatogenik mantarlar *Fusarium solani*'ye (IRAN 11C), Hastalığa yol açan ve buğdayın kök çürümesine karşı değerlendirmektir. İki katlı seri seyreltme yönteminde kullanılan ekstrakt konsantrasyonları yüzdesi: %100, 50, 25, 12.5, 6.25, 3.125 idi. Sonuçlar, ekstraktların konsantrasyonunu arttırarak antifungal aktivitelerin de arttığını ve test mantarlarının radyal büyümesinin azaldığını gösterdi. Özellikle etanolik ekstraktlar, en yüksek ve en düşük konsantrasyonlarda test mantarlarının büyümesinde önemli bir inhibisyon ile en iyi antifungal aktiviteyi gösterdi.

Anahtar kelimeler: Karayosunları, Ekstrakt, Antifungal etkiler, Minimum inhibitör konsantrasyonu.

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1. Introduction

Bryophytes are the largest group of land plants after the Angiosperms and are placed between algae and ferns (Asakawa, 2007). This group comprises three categories: Bryophyta (mosses), Marchantiophyta (liverworts), and Anthoceroophyta (hornworts) varies between 15000 and 20000 species known in the world (Goffinet and Shaw, 2009). The small size and biomass of these plants have made them ignored for extensive uses. The most important properties that helped the bryophytes to maintain their position in today's flora, is their content of bioactive compositions. They are used for pharmaceutical, horticultural, household purposes (Glime and Saxena, 1991). Due to the growing concern about the infection increase caused by antibiotic-resistant microorganisms, attention to these plants with antimicrobial activity has become increasingly important in recent years. The antimicrobial activity of various bryophytes was studied in detail by Banerjee (2001). Deora et al. (2007) studied three bryophytes such *Plagiochasma articulatum* Kashyap, *Anthoceros longii* Steph., *Fissiden bryoides* Hedw. for their antibiotic effect on *Agrobacterium tumefaciens*.

Shirzadian et al. (2009) investigated different extracts of 23 species of bryophytes including 21 species of mosses and two species of liverworts on seven pathogenic fungi and observed that, ethanolic extract of six species of mosses namely *Grimmia pulvinata* (Hedw.) Smith, *Philontis marchica* (Hedw.) Brid., *Drepanocladus aduncus* (Hedw.) Warnst., *Bryum pallens* (Brid.) Sw., *Haplocladium* sp., *Plagimnium rugicum*, and two species of liverworts, namely *Pellia epiphylla* (L.) Corda and

Dumortiera hirsuta (Sw.) Nees had the most spectrum of antifungal effects.

Alam (2013) examined the effects of the ethanol, methanol, and chloroform extracts of *Hyophila rosea* Williams against three fungal species, namely, *Aspergillus flavus*, *Alternaria alternate*, and *Phytophthora infestans*, and observed that, all the three extracts showed significant inhibition against tested microorganism in comparison with the commercial fungicide Bifonazol.

Deora and Suhalka (2016) also evaluated the fungicidal potential of moss *Philonotis revolute* Bosch. & Lac. against a fungus *Helminthosporium turcicum*.

The main purpose of the present study is to evaluate the antifungal effects of ethanolic, methanolic and acetic extracts of some mosses from Khuzestan province of Iran and determine their minimum inhibitory concentration (MIC) on the phytopathogenic fungus *Fusarium solani* (Mart.) Sacc.

2. Materials and Methods

2.1. Plant material

Samples of regarding plants were collected from their locations [Chelo Andika and Sheyvand (Khuzestan, Iran)] in spring (2018) and identified by the help of some taxonomic monographs such Smith (2004) and Kürschner (2006, 2007, 2008) (Table 1). After collection of the plant samples, they were kept in the refrigerator (+4 °C) and processed to obtain their extracts.

Table 1. List of collected samples of mosses along with their related data

No.	Taxa	Locality	Coordinates	Altitude (m)
1	<i>Cinclidotus fontinaloides</i>	Chelo, Andika	49 68 70 E 32 40 47 N	600
2	<i>Cinclidotus riparius</i> .	Chelo, Andika	49 68 70 E 32 40 47 N	600
3	<i>Oxyrrhynchium hians</i>	Sheyvand waterfall, Izeh	50 19 31 E 31 36 32 N	550
4	<i>Oxystegus tenuirostris</i>	Chelo, Andika	49 68 70 E 32 40 47 N	600
5	<i>Palustriella commutata</i>	Sheyvand waterfall, Izeh	50 19 31 E 31 36 32 N	550
6	<i>Platyhypnidium riparioides</i>	Sheyvand waterfall, Izeh	50 19 31 E 31 36 32 N	550
7	<i>Syntrichia ruralis</i>	Sheyvand waterfall, Izeh	50 19 31 E 31 36 32 N	550
8	<i>Tortula muralis</i>	Sheyvand waterfall, Izeh	50 19 31 E 31 36 32 N	550

2.2. Preparation of the extract

Moss specimens were transferred to the lab and washed with water to remove soil particles. One gram of plant material per repetition was finely ground with a pestle and mortar. The extract was prepared using 10 ml of ethanol 96 % (Merck), methanol 80 % (Merck), and acetone 80 % (Merck). The suspensions were kept in a refrigerator for 24 hours and then centrifuged (2500 rpm, 30 min) (Banerjee and Sen, 1979) These extracts were used as 100 per cent crude extract, then serially diluted by distilled water to provide different concentrations from 100 – 3.125 %.

2.3. Test organism

The pure culture of test fungi *Fusarium solani* (Mart.) Sacc (IRAN 11C) was obtained from the Department of Pathology, Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran.

2.4. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the moss extracts was examined on Potato Dextrose Agar (PDA). The used extract concentrations (100, 50, 25, 12.5, 6.25 and 3.125 %) were added to PDA (at 45 °C) and then the resulting media were poured in petri dishes (8 cm in diameter). Ethanol 96 %, methanol 80 % and acetone 80 % were added to the medium in control plates. Then, inoculum discs (5 mm in diameter) from seven days growing cultures of *F. solani* placed in the middle of petri plates containing PDA and extracts. Each treatment was examined on three plates as repetition which were incubated in 27 °C. After seven days (when the fungus overgrows on control plates), the radial growth of *F. solani* was recorded for each plate. The percentage of fungal growth inhibition was calculated as in Pandey formula (Pandey et al., 1982).

Growth inhibition % = [(growth in control – growth in sample)/growth in control] × 100

The values reported for MIC were the lowest concentrations of extracts on which the fungus grew a little after seven days.

2.5. Statistical analysis

The data of the two parameters (concentration and moss extract) were statistically analyzed using SAS 9.2 program with Completely Randomized Block (CRB). Inhibition of radial growth was examined using analysis of variance (ANOVA) and the means were compared by Duncan test.

3. Findings

Different doses (100, 50, 25, 12.5, 6.25 and 3.125 %) of the extracts from eight mosses were tested against *F. solani* to determine their antifungal activity *in vitro* tests (Figures 1–2). The results of analysis of variance showed that all tested extracts caused inhibition in the growth of fungi. Furthermore, the data analysis revealed the differences between the extracts and dosages, and their interaction was significant ($p < 0.01$). The results indicated that, by increasing the concentration of extracts, the antifungal activities also increase (Tables 2–3). The comparison of means showed that, maximum inhibition of *F. solani* growth was found at the highest doses which were followed by other concentrations 50, 25, 12.5, 6.25, and 3.125 % of the moss extracts, as compared to control, showed the least inhibition on fungus growth. The ethanolic extract of *Oxystegus tenuirostris* at highest dose (100%) was the most effective in decreasing fungus growth (49.66 %) followed by acetic extract of *Syntrichia ruralis* (32.66 %), methanolic extract of *Tortula muralis* (30.33 %) and ethanolic extract of *Cinclidotus riparius* (29.66 %). The growth inhibition of *O. tenuirostris* varies from 49.66 to 8 % in various concentrations. The lowest effect of inhibitory fungus growth at highest concentration (100 %) is related to *Oxyrrhynchium hians* (10.33 %). In the lowest concentration (3.125 %) of various extracts, the maximum inhibition of fungus growth was obtained by *Cinclidotus fontinaloides* (10.33 %) and *O. tenuirostris* (8 %). Among methanolic and acetic extracts, *T. muralis* and *S. ruralis*, indicate the highest effect on the tested fungus respectively 30.33 % and 32.66 % in high concentration (100 %). The MIC for all the extracts against the microorganisms in the study was in the range of 3.125 % to 6.25 % except for *S. ruralis* which was 0.25 %.

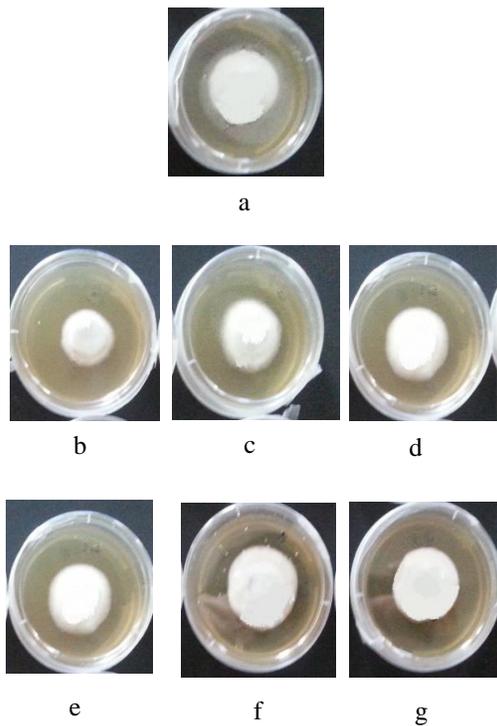


Figure 1. Mycelium growth of *F. solani* on PDA in the control (a), 100 (b), 50 (c), 25 (d), 12.5 (e), 6.25 (f), 3.125 (g) percent concentration of ethanolic extract of *Cinclidotus riparius*.

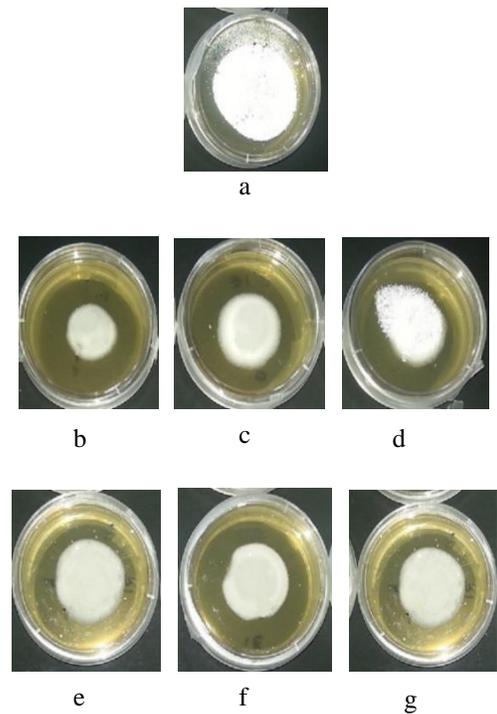


Figure 2. Mycelium growth of *F. solani* on PDA in the control (a), 100 (b), 50 (c), 25 (d), 12.5 (e), 6.25 (f), 3.125 (g) percent concentration of ethanolic extract of *Oxystegus tenuirostris*.

Table 2. ANOVA table for the effect of different concentrations of moss extracts on inhibition of *F. solani* growth.

Source	DF	Sum of Squares	Mean Squares	F value	Pr>F
Extract	9	3633.646181	403.738465	1313.86	<.0001
Concentration	5	7887.647569	1577.529514	5133.66	<.0001
Extract * Concentration	45	3203.585069	71.19.779	231.67	<.0001
Error	120	36.87500	0.30729		
Total	179	14761.75382			
CV		4.997171			

Table 3. Percent of inhibition growth of *F. solani* by different concentrations of moss extracts on PDA and MIC.

Extract	Mosses	Concentration %						MIC
		100%	50%	25%	12.5%	6.25%	3.125%	
Ethanolic	<i>Cinclidotus fontinaloides</i>	14.66 f	10.33 d	10.33 c	10.33 b	10.33 a	10.33 a	3.125%
	<i>Cinclidotus riparius</i>	29.66 c	24.05 a	20.33 a	14.66 a	10.33 a	4.66 bcd	3.125%
	<i>Oxystegus tenuirostris</i>	49.66 a	24.66 a	20.33 a	14.66 a	10.33 a	8.00 ab	3.125%
	<i>Palustriella commutate</i>	14.66 f	10.33 d	66 d4.	4.66 d	4.66 c	0.00 d	6.25%

	<i>Platyhypnidium riparioides</i>	24.66 d	14.66 b	10.33 c	5.00 cd	5.00 bc	5.00 bc	3.125%
Methanolic	<i>Cinclidotus riparius</i>	20.66 e	14.33 b	14.33 b	10.33 b	10.33 a	3.33 cd	3.125%
	<i>Oxyrrhynchium hians</i>	10.33 g	04.66 e	4.66 d	4.66 d	4.66 c	4.66 bcd	3.125%
	<i>Tortula muralis</i>	30.33 c	14.66 b	10.00 c	4.66 d	4.66 c	4.66 bcd	3.125%
Acetonic	<i>Cinclidotus riparius</i>	15.66 f	10.66 d	5.66 d	5.66 c	5.66 b	5.66 bc	3.125%
	<i>Syntrichia ruralis</i>	32.66 b	12.66 c	5.33 d	0.00 e	0.00 d	0.00 d	25%

*Within each column having the same letters are not significantly different (Duncan $p < 0.01$).

4. Results and Discussion

The observations showed that, ethanolic extract was more effective on fungus growth. This finding was similar to the one obtained Deora and Guhil (2014). They assessed the antifungal potential of moss *Bryum argenteum* and *B. cellulare* in various concentrations from 10-100 % against the phytopathogenic fungi *Curvularia lunata*, the causal organism of leaf spot of *Zea mays*. They reported that, the ethanolic extract of *B. argenteum* had a strong antifungal activity with significant inhibition on the growth of *Curvularia lunata*. They also found that, the radial growth and fresh weight of test fungi was significantly decreased in response to all concentrations ranging between 10–100 %.

Veljic et al. (2008) showed that, the methanol extract of *P. commutata* possessed an acceptable antifungal activity with a MIC 2.5–5.0 mg/ml against *Aspergillus niger*, *A. ochraceus*, *A. versicolor*, *A. flavus*, *Penicillium funiculosum*, *Trichoderma viride*, and *Candida albicans*.

Latinovic et al. (2019) evaluated antifungal activity of *C. fontinaloides* in three dosages 5 μ L, 10 μ L and 15 μ L to suppress mycelial growth of certain fungal plant pathogens *Botryosphaeria dothidea* and *Calosphaeria* sp. and indicated that extract of *C. fontinaloides* exhibited no inhibitory activity no matter the dosage applied. Also, in the present study, ethanolic extract of *C. fontinaloides* showed no significant different inhibition to different concentrations.

Colak et al. (2011) used four different extracts (ethyl alcohol, methyl alcohol, chloroform and acetone) of *P. riparioides*, *Leucodon sciuroides*, *Hypnum cupressiforme*, *Homalothecium sericeum*, and *Anomodon viticulosus* against eight bacterial and

fungus strains. They observed that, ethanol extracts of *P. riparioides* had inhibition effect against *Saccharomyces cerevisiae*.

Tedela et al. (2014) looked into the antimicrobial effects of acetonic, ethanolic, methanolic and hexanic extracts of *Calymperes erosum* and *Bryum coronatum* at the concentrations of 0.625, 1.25, 2.50 and 5.00 mg/ml against 20 clinically important bacteria pathogens. The MIC of the extracts of *C. erosum* were between <0.625 and >5.0 mg/ml. They also reported that, ethanolic extract had relatively higher activity among the extracts following acetone and *Bryum coronatum* extracts. Alam et al. (2011) investigated the fungi toxicity and growth inhibition of the aqueous extract of *Dumortiera hirsuta* in 13 concentrations (50–700 ppm) against seven postharvest phytopathogens and found that spore germination of all phytopathogens completely inhibited by the *Dumortiera* extract within the ranging between 400–550 ppm concentrations.

In this study acetonic extract of *S. ruralis* had the lowest antifungal activity with a MIC 25 %. The literature data about the antimicrobial effects of some mosses are negligible and need more attention. Elibol et al. (2011) indicated that, acetonic extract of *S. ruralis* had not antifungal effect against *Saccharomyces cerevisiae*.

The above-mentioned study indicates that, although all tested concentrations may have a deterrent effect, the lowest tested concentration does not necessarily mean the minimum inhibitory concentration and upward intermediate concentrations often have a more acceptable inhibitory effect. The bryophyte extracts made ready in various solvents were effective in the reduction of the fungal growth as they possess different secondary metabolites that

acting as antifungal agents. The activity of various solvent extracts was in the order of ethanolic>methanolic>acetic as the bioactive compounds are more soluble in organic solvents.

The possible reason behind this might be the multitudinous solubility of various plant metabolites in different solvents, in which the differential antifungal activity was observed. The manner of action of these plant extracts probably include some cellular modifications (e.g. destruction of cytoplasm and malformations in cell wall structure) and finally affects all over growth of hyphae and subsequent mycelia (Sharma, 2008).

Numerous environmental troubles are resulted in the intensive use of commercial fungicides in agriculture, whereas the natural plant-derived products for agriculture have less impact on the environment. These findings can conform the infrastructure for latter research to plan an optimized preparation of moss extracts to further evaluation them against a wider range of fungal strains. The possible antifungal components can be extracted at mass scale by using the advanced techniques (e.g. tissue culture) so that they can be eco-friendly used for controlling of phytopathogens.

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