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Research Article

Growth inhibition effects of extracts of eight mosses on the phytopathogenic fungus *Fusarium solani*

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Abstract: Mosses have proved to have antifungal properties due to their specific chemical compounds. In this study, the different extracts of some mosses collected from Khuzestan province were tested on a phytopathogenic fungus *Fusarium solani*, an important pathogen of crops, especially wheat, and compared to the commercial fungicide Benomyl. For this purpose, the dried mosses samples of ethanol, methanol, and acetone were extracted. The antifungal activity was tested by the disc diffusion method, and the growth inhibition zone was measured. Wheat seeds of the "Chamran" cultivar were implanted into moss extract and then transferred into pots containing 1: 10 mixture of soil and soil contaminated with *F. solani*. After 35 days, the root and crown of wheat plants were examined based on the Wallwork scale. Ethanolic and methanolic extracts caused an inhibitory of 90% and 81% relative to Benomyl, while acetonic extract had fewer effects (76%) in the *in vitro* tests. *In vivo* observations had also indicated that ethanolic extracts can significantly control root and crown rot 63.8%.

Keywords: Benomyl, antifungal effect, Mosses, Disk–diffusion method

Introduction

Bryophytes are a group of land plants, which include liverworts, hornworts, and mosses. More than 22000 species of mosses are known throughout the world (Crosby & Magill 1978). One of the reasons that helped bryophytes to survive is their content of biologically active compounds. They are used in pharmaceutical products, horticulture, and household purposes. Also, they are ecologically important as good indicators of environmental conditions (Glime & Saxena 1991). However, many studies have been carried out in the world on the antimicrobial properties of bryophytes. The antimicrobial activities of various bryophytes were first reported in 1952 (Madsen & Pates 1952). Several years later, Banerjee & Sen (1979) reported that bryophytes also possess antimicrobial activity due to their unique chemical constituents. Mekuria *et al*. (1999) studied the effect of moss extracts against plant pathogenic fungi and indicated that alcoholic extract of mosses was active against *Candida albicans* (Robin) Berkhout. Subhisha & Subramonian (2005) screened the extracts of *Pallavicinia lyelli* (Hook.) Carruth. and evaluated that it possesses some antifungal compounds. Iwashina (2003) showed that

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flavonoid compounds in bryophytes hold many biological activities against plants, fungi, and other microorganisms. Deora *et al.* (2007) studied three bryophytes *Plagiochasma articulalum* Kash. (a liverwort), *Anthoceros longii* Steph. (a hornwort), and *Fissidens bryoides* Hedw. (a moss) for their antibiotic effect on *Agrobacterium tumifacians* (Smith & Town.) Conn. Deora & Suhalka (2016) also evaluated the fungicidal potential of moss, *Philonotis revolute* Bosch. & Lac., against fungus *Helminthosporium turcicum* (Pass). Leonard & Suggs. Alam *et al*. (2013) investigated the fungitoxicity, and growth inhibition of the aqueous extract of *Dumortiera hirsute* (Sw.) Nees against seven postharvest phytopathogens found that spore germination of all phytopathogens completely inhibited by the *Dumortiera* extract within a range of 400–550 ppm concentrations. Therefore, in this study, antifungal effects of methanolic, ethanolic, and acetonic extracts of some mosses collected from different regions of Khuzestan province, Iran, were tested on the growth of *Fusarium solani* (Mart.) Sacc., one of the most important pathogens of crops, especially wheat, and compared to the commercial fungicide, Benomyl.

Materials and Methods

Plant materials

In the present study, samples of all tested mosses were collected from their native habitats, such as Chelo Andica and Sheyvand region (Khouzestan province, Iran). After collecting moss samples, they were immediately placed in the refrigerator (+4 °C) and processed to obtain extracts.

Preparation of the extracts

Moss samples were transferred to the lab and washed with sterile water to remove soil particles, dead material, and fragments of epiphytic hosts. One gram of plant material per repetition was finely ground with a pestle and mortar, and then extract was obtained using 10 ml of ethanol, methanol, and acetone. The suspensions were kept in the refrigerator for 24 hrs and then centrifuged (2500 rpm, 30 min) (Banerjee & Sen, 1979).

Test organism

The pure culture of test fungus, *Fusarium solani* (Mart.) Sacc. (IRAN 11C) was obtained from the Iranian Research Institute of Plant Protection (Tehran, Iran). This test organism was sub-cultured in the laboratory at 25 °C temperature to obtain its pure isolates.

Screening of antifungal activity

A. *In vitro*:

The antifungal activity was tested by the disc diffusion method. Plates of Potato Dextrose Agar (PDA) were inoculated with six mm diam. Plugs of seven days old fungus culture margins. The filter paper discs (six mm diam.) impregnated with different extracts (0.1 g/ml) and Benomyl (0.1g/ml) were placed at a certain distance from the edge of fungus grown and kept in the incubator for 48 hrs at 25 °C. Ethanol, methanol, and acetone were used in control plates. The experiment was conducted as a completely randomized design with three replications. The diameter of the inhibition zone was measured using a millimeter ruler. Finally, data were processed using SAS 9.2. Statistical analysis of results was based on Duncan's multiple range test. Differences of $p < 0.01$ were considered significant.

B. *In vivo*:

To investigate the effect of mosses extracts *in vivo*, sterile wheat seeds of "Chamran" cultivar were implanted into extracts of different species of mosses and Benomyl, then planted in pots containing 1: 10 mixture of soil and soil contaminated with fungus. The pots were kept for 35 days in the Khuzestan Agriculture and Natural Resource Research and Education Center (AREEO) Ahvaz greenhouse in 25 °C and natural light. Considering that the fungus species *F. solani* causes crown and root rot of wheat, the root and crown of wheat plants were examined after 35 days, based on the Wallwork scale (Wallwork *et al*. 2004). Finally, data were processed using SAS 9.3. Statistical

analysis of results was based on the Duncan significance test. Differences of $p < 0.01$ were considered significant.

Results

Taxonomy of Mosses

Samples of all tested mosses were collected from their native habitats in Khouzestan province, Iran. All specimens were identified according to Smith (2004) and Kürschner (2006, 2007, 2008). Finally, Eight species belong to seven genus, and five families were identified (Table 1). The voucher specimens are preserved in the herbarium of the Ministry of Jihad-e Agriculture ("IRAN") at the Iranian Research Institute of Plant Protection (Tehran, Iran).

In vitro findings

Antifungal activity of selected moss extracts in different solvents on *Fusarium solani* are represented in Fig. 1.

Table 1 List of collected moss samples and their related data.

No.	Taxa	Family	Locality	Coordinates	Altitude (m)
1	*Cinclidotus fontinaloides* (Hedw.) P.Beauv.	Cinclidotaceae	Andika	49 68 70 E 32 40 47 N	600
2	*Cinclidotus riparius* (Host. ex Brid.) Arn.	Cinclidotaceae	Andika	49 68 70 E 32 40 47 N	600
3	*Oxyrrhynchium hians* (Hedw.) Loeske	Brachytheciaceae	Sheyvand	50 19 31 E 31 36 32 N	550
4	*Oxystegus tenuirostris* (Hook. & Tayl.) A.J.E.Sm	Pottiaceae	Andika	49 68 70 E 32 40 47 N	600
5	*Palustriella commutata* (Hedw.) Ochyra	Amblystegiaceae	Sheyvand	50 19 31 E 31 36 32 N	550
6	*Platyhypnidium riparioides* (Hedw.) Dixon	Brachytheciaceae	Sheyvand	50 19 31 E 31 36 32 N	550
7	*Syntrichia ruralis* (Hedw.) Web. & Mohr.	Pottiaceae	Sheyvand	50 19 31 E 31 36 32 N	550
8	*Tortula muralis* Hedw.	Pottiaceae	Sheyvand	50 19 31 E 31 36 32 N	550

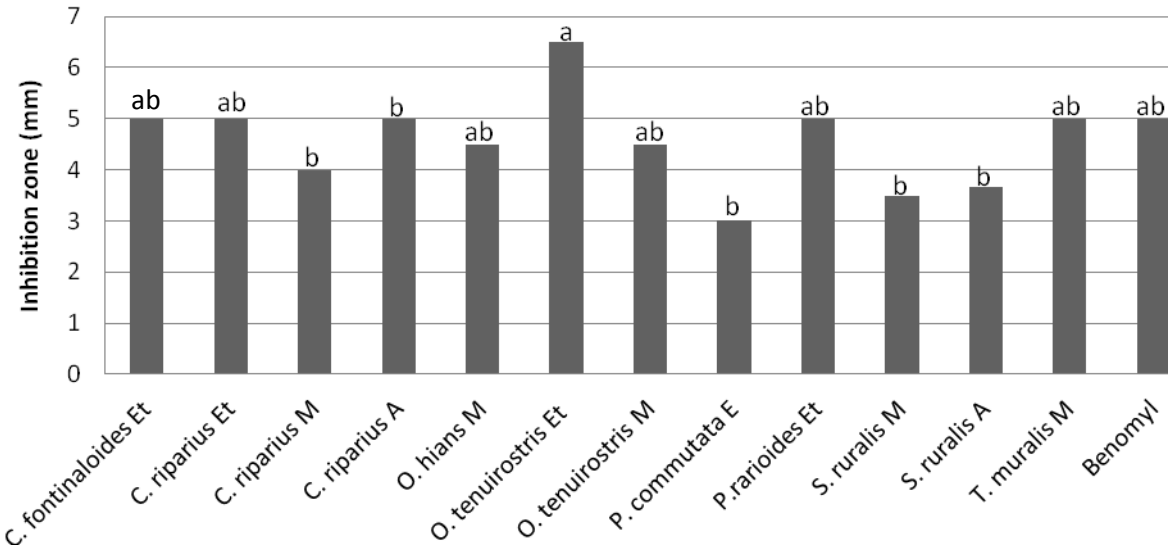

Figure 1 Comparative antifungal activity of different extracts on *Fusarium solani* (*in vitro*).
Et: Ethanol, M: Methanol, A: Aceton.

The results showed that all types of extracts have inhibitory effects on fungal growth. However, ethanolic extracts exhibited the maximum antifungal activity against fungus strain, whereas, ethanolic extracts was inhibited the most significantly, the average diameter of inhibition zone of ethanolic extracts 4.5 mm (90%) followed by methanolic extracts 4.08 mm (81%) and acetonic extracts 3.83 mm (76%) relative to Benomyl. Among the ethanolic extracts, *Oxystegus tenuirostris* extract had the highest effect on the growth fungi (6.5 mm), even better than Benomyl (5 mm), while *Cinclidotus riparius* and *Platyhypnidium riparioides* had a similar impact to Benomyl.

Among the methanolic extracts, *Tortula muralis* extract indicated a similar effect to Benomyl (5 mm), and *O. tenuirostris* and *Oxyrrhynchium hians* extracts had closed results to Benomyl (4.66 mm). Among acetonic extracts, *C. riparius* extract showed the highest effect on *F. solani* (4 mm), while other extracts had lower efficacy.

In vivo findings

Examination of root and crown of wheat plants was done based on the Wallwork scale. A 0–5 scale was used for disease assessments based on the level of stem rottenness from the crown to the first node where 0 = 0%, 1 = 1–10%, 2 = 10–25%, 3 = 25–50%, 4 = 50–75%, and 5 = > 75%. According to this, different inhibitory effects ranging 21.6 – 63.88% were observed, that ethanolic extract of *O. tenuirostris* exhibited the best effect on reducing crown and root rot (21.6%). Statistical analysis using SAS 9.3 ($p < 0.01$) showed significant variances between the effects of extracts (Tables 2 and 3). Statistical analysis of *in vitro* data also indicated that *O. tenuirostris* had a significant effect from other extracts while *C. riparius*, *O. hians*, *O. tenuirostris*, *P. riparioides* and *T. muralis* formed a group with Benomyl. The results of the analysis of *in vivo* data confirmed the results of *in vitro* analysis.

Table 2 ANOVA table for the effect of mosses extracts of *Fusarium solani* growth inhibition (*In vitro*).

Source of variance	df	Sum of Squares	Mean Square	F-value	Pr > F
Extract	12	42.064	3.505	2.62	0.0195
Error	26	34.833	1.339		
Corrected Total	38	76.897			

Table 3 Data analysis of the effect of moss extracts (ethanolic, methanolic, and acetonic) on inhibition of *Fusarium solani* growth (*in vitro* and *in vivo*).

Moss taxon	Extract	*In vitro*[1, 2]	*In vivo*[1, 2]
C. fontinaloides	Ethanolic	3.00 b	58.33 bc
	Methanolic	N. T	N. T
	Acetonic	N. T	N. T
C. riparius	Ethanolic	5.00 ab	63.88 b
	Methanolic	3.33 b	N. T
	Acetonic	4.00 b	55.55 bc
O. hians	Ethanolic	N. T	N. T
	Methanolic	4.66 ab	N. T
	Acetonic	N. T	N. T
O. tenuirostris	Ethanolic	6.50 a	21.66 d
	Methanolic	4.66 ab	N. T
	Acetonic	N. T	N. T
P. riparioides	Ethanolic	5.00ab	N. T
	Methanolic	N. T	N. T
	Acetonic	N. T	N. T
S. circinatum	Ethanolic	3.00 b	N. T
	Methanolic	N. T	N. T
	Acetonic	N. T	N. T
S. ruralis	Ethanolic	N. T	N. T
	Methanolic	2.83 b	47.22 c
	Acetonic	3.66 b	N.T
T. muralis	Ethanolic	5.00 ab	N. T
	Methanolic	N. T	N. T
	Acetonic	N. T	N. T
Benomyl	-	5.00 ab	100 a

[1] Means with the same letters in each column are not significantly different (Duncan's test, $p < 0.01$). [2] NT: Not tested.

Discussion

In this research, the different extracts of some mosses collected from Khuzestan, Iran, were tested on *Fusarium solani*, an important pathogen of crops, especially wheat, and compared to the commercial fungicide, Benomyl. The results of the present research are following the finding of Shirzadian *et al.* (2009), who investigated different extracts of 23 species of bryophytes including 21 species of mosses and two species of liverworts on

seven pathogenic fungi, namely *Alternaria alternate* (Fr.) Keissl., *F. solani*, *F. oxysporum* (Schltdl.) Snyder & Hansen, *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kuhn, *Verticillium dahlia* Kleb. and *Pythium* sp. They observed that ethanolic extract of six species of mosses, namely, *Bryum pallens Sw.*, *Drepanocladus aduncus* (Hedwig) Warnstorf, *Grimmia pulvinata, Haplocladium* sp. *Philontis marchica* (Hedwig) Brid. and, *Plagimnium rugicum* (Laurer) Kop., and two species of liverworts, namely, *D. hirsute* and *Pellia epiphylla* (L.) Corda had a wide range of antifungal effects.

Deora and Guhil (2015) also examined the antifungal activities of *Bryum cellulare* Hook in different solvents against the phytopathogenic fungi i.e. *Curvularia lunata* (Wakk.) Boed. and found that all the extracts of both the bryophytes showed varying levels of antifungal activity against the test fungi. In contrast, the ethanolic extract of *B. argenteum* was more active than other fractionated extracts.

In the present study, ethanolic, methanolic, and acetonic extracts of *C. riparius* showed inhibition effects against *F. solani*. Uyar *et al.* (2016) also indicated that ethanolic and methanolic extracts of *C. riparius*, *Calliergonella cuspidate* (Hedw.) Loesk., *Cirriphyllum crassinervium* (Taylor) Loeske & Flisch, *Leucobryum juniperoideum* (Brid.) Mull. and *Thamnobryum alopecurum* (Hedw.) Nieuwl showed effective results against *Escherichia coli* (Migul.) as applied standard antibiotic discs.

Latinovic *et al.* (2019) achieved antifungal activity of *Porella platyphylla* (L.) Pfeiff, and *C. fontinaloides* in some dosages applied to suppress mycelial growth of specific fungal plant pathogens such as *Botryosphaeria dothidea* (Moug.)Ces. & De Not. and *Calosphaeria* sp.

Colak *et al*. (2011) used four different extracts viz. (ethyl alcohol, methyl alcohol, chloroform, and acetone) of *Anomodon viticulosus* (Hedw.) Hook. & Taylor, *P. riparioides*, *Homalothecium sericeum* Schimp., *Hypnum cupressiforme* Hedw. and *Leucodon sciuroides* (Hedw.) Schw. aganist eight bacterial and fungal strains. They observed that ethanolic extracts of *P. riparioides* had inhibition effect against *Saccharomyces cerevisiae* Mey.. Veljic *et al.* (2008) indicated that methanolic extract of *P.commutata* had antifunagal potentiality against *C. albicans* human isolate, while in the present study, ethanolic extract of *P.commutata* had some inhibition effects.

In our studies, ethanolic extracts from *O. hians*, and *S. ruralis* had no antifungal activity. These results is in accordance with the findings of Karpinsky and Adamczac (2017) that investigated four ethanolic extracts from *Brachythecium albicans* (Hedw.) Schimp., *Ceratodon purpureus* (Hedw.) Brid., *O. hians*, and *S. ruralis* mosses which did not show any antibacterial activity. In the same way, Elibol *et al*. (2011) also found that ethanolic extract of *S. ruralis* was inactive against *E. coli* and *C. albicans* but it had a significant effect on *S. cerevisiae.* The possible reason behind this might be due to varying solubility of various plant metabolites in different solvents that result in no antimicrobial or antifungal effects.

In addition, some tested extracts had a more potent antifungal effect than Benomyl, which was consistent with the findings of Alam (2011), who investigated the activity of three extracts of ethanol, methanol, and chloroform *Hyophila rosea* Willi. against three fungi of *Alternaria alternate* (Fr.) Keissl, *Aspergillus flavus* Link., and *Phytophthora infestans* (Mount) de Bary showed all these extracts exhibited significant efficacy against fungi in comparison to the synthetic fungicide Bifonazol.

Moreover, the bryophyte extracts prepared in different solvents were found effective in reducing fungal growth as they possess various secondary metabolites which act as an antifungal agent. The activity of different solvent extracts was in the order of ethanolic > methanolic > acetonic as the bioactive compounds are more soluble in organic solvents (Sharma and Tripathi, 2008).

The possible reason behind this might be the varying solubility of various plant metabolites in different solvents. The possible mode of action of these plant extracts on the growth of fungi includes a few cellular changes such as granulation of cytoplasm and deformities in the cell wall structure, which ultimately affects the overall development of hyphae and subsequent mycelia (Sharma and Tripathi, 2008). Based on results obtained from the present study, all kinds of moss extracts indicated inhibitory effects on *F. solani*. In contrast, ethanolic extracts of mosses exhibited a superior impact than other treatments against *F. solani* in both tests (*in vitro* and *in vivo*). However, methanolic and acetonic extracts also showed acceptable antifungal activity against this pathogen. Therefore, it may be concluded that ethanolic extracts can be incorporated for biofungicide formulations as less hazardous natural plant products in controlling this disease, thus reducing the dependence on synthetic fungicides.

Statement of Conflicting Interests

The authors state that there is no conflict of interest in the publication of this manuscript.

Authors' Contributions

All authors have contributed equally to the writing of this manuscript.

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اثر بازدارندگی عصاره هشت گونه خزه بر رشد قارچ بیمارگر گیاهی *Fusarium solani*

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چکیده: خزه‌ها به‌دلیل دارا بودن ترکیبات شیمیایی ویژه، خواص ضدمیکروبی اثبات شده‌ای دارند. در این پژوهش، تأثیر بازدارندگی عصاره‌های مختلف برخی خزه‌های جمع‌آوری شده از استان خوزستان، بر رشد قارچ بیمارگر گیاهی *Fusarium solani*، به‌عنوان یکی از مهم‌ترین آفات محصولات کشاورزی به‌ویژه گندم، مورد بررسی و با تأثیر قارچ‌کش صنعتی بنومیل، مورد مقایسه قرار گرفت. به این‌منظور، نمونه‌های خشک شده خزه با استفاده از اتانول، متانول و استون مورد عصاره‌گیری قرار گرفتند. اثر ضدقارچی عصاره‌ها با استفاده از روش دیسک‌گذاری مورد بررسی قرار گرفت و میزان بازدارندگی رشد اندازه‌گیری شد. جهت بررسی اثر عصاره خزه‌ها بر رشد قارچ مذکور در محیط گلخانه، بذرگندم رقم چمران به عصاره خزه‌ها آغشته گردید و سپس در گلدان‌های حاوی نسبت ۱:۱۰ مخلوط خاک و خاک آلوده به قارچ بیمارگر گیاهان، کاشته شدند. پس از ۳۵ روز، میزان پوسیدگی ریشه و ساقه بوته‌های گندم طبق مقیاس والورک مورد بررسی قرار گرفت. بررسی‌های آزمایشگاهی نشان دادند که عصاره‌های اتانولی (۹۰ درصد) و عصاره‌های متانولی (۸۱ درصد) نسبت به بنومیل بهترین اثر بازدارندگی رشد بر قارچ مورد آزمایش دارا هستند درحالی‌که عصاره‌های استونی اثر کم‌تری (۷۶ درصد) داشتند. هم‌چنین مطالعات گلخانه‌ای بیانگر آن است که عصاره‌های اتانولی (۶۳/۸ درصد) نسبت به سایر عصاره‌ها به‌طور معناداری موجب کنترل میزان پوسیدگی ریشه و ساقه گندم گردید.

واژگان کلیدی: بنومیل، اثر ضدقارچی، خزه‌ها، روش انتشار دیسک