

Antifungal Activity of Aqueous Extract of Safed Musli Against Seed Mycoflora of Some Crop Species

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Abstract: In the present study, aqueous extract of safed musli tubers was used to treat the seeds of several agricultural crop species. The antifungal activity of the extract of safed musli tuber was tested against the seed mycoflora in *Sorghum Bicolor*, *Oryza sativa*, *Eleusine coracana*, *Cucumis sativus*, *Abelmoschus esculentus*, *Carthamus tinctorius*, *Lagenaria siceraria*, *Luffa acutangula* and *Momordica charantia*. The seeds treated with safed musli tuber extract showed the reduced incidence of various fungal species compared to control. The seed germination, root-shoot length and seedling vigour of all the crop species treated with extract were found significantly superior over untreated samples. In all the cases, control set showed poor germination and reduced growth of the seedlings due to the high incidence of seed-borne fungi. The confirmatory tests indicated the occurrence of glycosides, saponins, starch, flavonoids and alkaloids in safed musli extract.

Key words: Safed musli extract • antifungal activity • seed mycoflora

INTRODUCTION

For high productivity, the adequate stand of crop plants is very much essential, which largely depends on seed germinability and seedling vigour under a wide range of climatic and various physiological stress conditions. One of the major problems in the production of agricultural crops is the attack of various diseases at different stages of the growth including seed infection. The application of pesticides is not a new idea, because extensive use of pesticides produces adverse effects on crop plants and have tendency to penetrate into the tissue of the plants [1]. Such plants contain certain chemicals often protect the plants from fungal infection. Antifungal compounds derived from the plants play an important role in the plant disease control. Based on this view, in the present investigation, aqueous extract of the tubers of *Chlorophytum borivilianum*, a herb belongs to the family Liliaceae commonly called "Safed musli", which is known for its medicinal value in Indian Pharmaceuticals (anti-diabetic, anti-cancer, hepatoprotective, anti-malarial, sedative drugs) has been used to treat the seeds of some agricultural crop

species to test its efficacy against the infection of pathogenic fungi.

MATERIALS AND METHODS

Healthy, matured tubers of *Chlorophytum borivilianum* Santapau and Fernandes, were freshly harvested and washed in tap water for 3-4 times to remove the soil particles. These tubers were then macerated thoroughly with distilled water using a mortar and pestle. 100% aqueous extract (W/V) was obtained and filtered through destarched muslin cloth to remove the fibrous material. Then, this extract was used to soak the seeds of agricultural crop species. For this purpose, seed samples of agricultural crop species viz., *Sorghum bicolor*, *Eleusine coracana*, *Cucumis sativus*, *Abelmoschus esculentus*, *Oryza sativa*, *Carthamus tinctorius*, *Lagenaria siceraria*, *Luffa acutangula* and *Momordica charantia* were collected from the private seed companies at Bangalore, Karnataka, India and were subjected to soaking in the aqueous extract of safed musli. For each crop species, 400 seeds were randomly picked from the working sample and soaked in the fresh aqueous extract

(100%) of safed musli for a period of 16 h at 28 ± 2°C. The soaked seeds were then air dried under the shade and subjected to standard blotter method, following the procedures of ISTA. As per the rule the soaked seeds were equidistantly plated on three layers of wet blotters in perspex plates and incubated at 22 ± 2°C for a period of one week under alternate cycles of near ultra violet light (NUV) and darkness [2]. On the 8th day of incubation the seeds were examined carefully for the occurrence of fungi using stereobinocular and compound microscopes. Seeds of each crop species similarly incubated, but not subjected to safed musli extract treatment were served as corresponding control.

In the another set, 400 seeds of each crop species were subjected to similar treatment were sown in moistened sand as well as in between wet blotters and incubated accordingly. After incubation the seedlings were carefully examined for their essential structures. For each crop, total number of normal seedlings was counted and their root-shoot lengths were measured.

Finally, vigour index was calculated using the formula described by Abdul baki and Anderson [3]. The results were recorded and tabulated. Apart from these, the fresh tuber extract was also subjected for the analysis of its chemical constituents based on the procedures described by Evans *et al.* [4] and Harbone [5]. Different constituents present in the extract were also listed.

RESULTS AND DISCUSSION

Data provided in Table 1 represents the antifungal property of safed musli extract. In case of all the crop species, safed musli extract treatment reduced the incidence of all the associated fungi compared to their respective control. Among treated samples total fungal spectra was also reduced in *Abelmoschus esculentus* and *Luffa acutangula*. This was found to be effective even to many of the saprophytic fungal species like *Cunninghamella*, *Trichurus* and Actinomycetes. The storage molds such as *Aspergillus flavus*, *Aspergillus*

Table 1: Effect of tuber extract of safed musli on seed mycoflora of some agricultural crop species

Fungi	% occurrence of fungi in seed samples of different agricultural crop species due to safed musli extract treatment																		
	Sb		Ec		Os		Ae		La		Ls		Mc		Cs		Ct		
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	
Actinomycete sp.	--	--	--	--	--	--	42	13	28	03	--	--	--	--	--	--	--	14	03
<i>Alternaria alternata</i>	33	15	07	02	21	08	--	--	--	--	01	--	--	--	--	--	--	--	--
<i>Alternaria carthami</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	16	04
<i>Alternaria padwickii</i>	--	--	--	--	34	15	--	--	03	05	--	--	--	--	--	--	--	--	--
<i>Aspergillus flavipes</i>	03	--	--	--	08	02	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Aspergillus flavus</i>	10	04	14	03	--	--	15	06	41	07	--	--	--	--	14	03	07	--	--
<i>Aspergillus niger</i>	--	--	07	03	--	--	53	17	--	--	--	--	08	05	--	--	--	07	03
<i>Aspergillus terreus</i>	--	--	--	--	--	--	03	--	--	--	--	--	--	--	--	--	--	--	--
<i>Aspergillus versicolor</i>	--	--	--	--	--	--	20	11	10	--	06	02	--	--	05	03	--	--	--
<i>Bipolaris halodes</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Bipolaris oryzae</i>	--	--	--	--	10	02	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Bipolaris setariae</i>	--	--	33	09	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Bipolaris sorghicola</i>	15	05	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Botryodiplodia theobromae</i>	--	--	--	--	--	--	13	02	--	--	17	06	--	--	--	--	--	--	--
<i>Chaetomium globosum</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	02	--	--	--	--
<i>Cladosporium cladosporioides</i>	07	06	--	--	16	04	--	--	--	--	--	--	11	03	--	--	--	--	--
<i>Colletotrichum dematium</i>	--	--	--	--	--	--	--	--	11	00	18	04	06	02	--	--	21	06	--
<i>Cunninghamella</i> sp.	--	--	--	--	--	--	05	--	--	--	--	--	03	--	05	02	--	--	--
<i>Curvularia lunata</i>	--	--	--	--	06	01	--	--	--	--	11	03	--	--	04	02	--	--	--
<i>Fusarium oxysporum</i>	05	01	--	--	--	--	--	--	--	--	08	03	--	--	--	--	23	04	--
<i>Fusarium solani</i>	09	02	--	--	--	--	--	--	14	03	17	03	--	--	31	07	16	03	--
<i>Fusarium verticilloides</i>	--	--	--	--	--	--	23	07	09	05	02	--	09	02	08	01	--	--	--
<i>Myrothecium roridum</i>	--	--	--	--	--	--	--	--	03	00	--	--	11	04	06	02	--	--	--
<i>Nigrospora oryzae</i>	--	--	--	--	07	02	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Phoma</i> spp.	55	07	--	--	--	--	--	--	24	14	15	05	09	04	07	02	15	05	--
<i>Trichothecium roseum</i>	--	--	--	--	--	--	--	--	--	--	--	--	06	03	--	--	06	00	--
<i>Trichurus</i> sp.	--	--	--	--	--	--	--	--	--	--	08	01	--	--	18	04	08	01	--

Sb : *Sorghum bicolor*
Ec : *Eleusine coracana*

Os : *Oryza sativa*
Ae : *Abelmoschus esculentus*

La : *Luffa acutangula*
Ls : *Lagenaria siceraria*

Mc : *Momordica charantia*
Ct : *Carthamus tinctorius*

Cs : *Cucumis sativus*



Fig. 1: Enhanced growth of seedlings due to safed musli extract treatment

- A. Seedling of *Sorghum bicolor* with control (C) and treatment (T)
- B. Seedling of *Cucumis sativus* with control (C) and treatment (T)

Table 2: Effect of safed musli extract on seed germination and seedling vigour of some agricultural crop species

Crop species	Seed germination (%)		MRL ± SE (cm)		MSL ± SE (cm)		Vigor Index	
	C	T	C	T	C	T	C	T
<i>Abelmoschus esculentus</i>	74	86	12.6±0.01 ^a	13.6±0.03 ^c	9.0±0.01 ^{bc}	11.3±0.03 ^c	1598	2141
<i>Carthamus tinctorius</i>	62	71	10.6±0.03 ^d	12.6±0.02 ^d	5.0±0.02 ^e	7.2±0.02 ^e	1512	2048
<i>Cucumis sativus</i>	78	87	7.7±0.01 ^f	8.2±0.01 ^f	8.6±0.01 ^{cd}	9.3±0.02 ^{ef}	1271	1522
<i>Eleusine coracana</i>	74	82	4.7±0.03 ^e	5.2±0.01 ^h	4.6±0.05 ^{gh}	5.2±0.03 ^h	688	836
<i>Lagenaria siceraria</i>	58	69	8.3±0.02 ^e	10.1±0.01 ^e	7.5±0.01 ^e	8.6±0.03 ^e	916	1290
<i>Luffa acutangula</i>	62	71	10.6±0.01 ^d	12.6±0.03 ^d	5.0±0.03 ^e	7.2±0.02 ^e	967	1405
<i>Oryza sativa</i>	71	80	11.7±0.02 ^b	14.2±0.02 ^a	9.6±0.02 ^b	11.9±0.01 ^b	1469	2160
<i>Sorghum bicolor</i>	83	94	8.3±0.03 ^e	9.8±0.02 ^b	8.5±0.04 ^a	10.0±0.20 ^a	1394	1861

Germination percentage based on normal seedlings only

MRL: Mean Root Length, MSL: Mean Shoot Length, SE: Standard Error

According to ANOVA, data represented with the different superscript remain significantly different from each other at $p \leq 0.05$ level

Table 3: Chemical constituents in the aqueous extracts of safed musli tubers

Metabolites	Occurrence
Glycocides	+
Tannins	-
Saponins	+
Starch	+
Flavonoids	+
Alkaloides	+
Anthroquinone	-
Essential oils	+

+ = presence - = absence

flavipes, *Aspergillus versicolor* were also drastically diminished, irrespective of the crop species used. Plant pathogenic fungi which includes *Fusarium solani*, *F. oxysporum*, *F. verticilloides*, *Botryodiplodia theobromae*, *Alternaria*, *Bipolaris* and *Colletotrichum* species were also reduced by the safed musli extract treatment. Over all observations indicated the reduced incidence of almost all the fungi over control. The fungi like *B. theobromae*, *C. dematium*, *A. flavus* showed their high sensitivity to safed musli extract, whose incidence became null in many crop species. The incidence of *Phoma* sp. was reduced to 7% from 55% in *sorghum*. In paddy, the incidence of *A. padwickii* was reduced to 15% from 34%. Similarly in *Carthamus tinctorius*, the incidence of *F. oxysporum* was reduced from 23% to 4% (Table 1).

Safed musli extract, on the other hand facilitated the crop species, in which the percentage seed germination and seedling growth were enhanced to a greater extent over their respective control (Table 2). All the crop species subjected to safed musli extract treatment showed increased percentage of germination, which was ranged from 8 to 12 per cent. Correspondingly, the seedlings of safed musli extract treatment also showed enhanced growth of root and shoot. As a result of enhanced germination and seedling growth, vigor index was also upgraded by this treatment. The seedlings raised out of

safed musli extract treatment were found fresh and robust (Fig. 1).

Many angiospermic plants are known for their antimicrobial activities [6]. The usage of plant extracts for the management of microbial diseases of agricultural crop is an alternative method for chemical treatment. As the evidence, in the present findings, safed musli extract was analysed for the different constituents, which indicated the occurrence of glycocides, saponins, starch, flavonoids and alkaloids (Table 3).

The aqueous extract of safed musli tubers was found highly effective against all the pathogens. Crude preparations generally exhibit lower activity than pure substances, which is usually dose dependent. The essential oils, flavonoids, saponins, carbohydrates, proteins, alkaloides in other species have been reported to have antimicrobial properties [7]. The crude extract exhibited an antifungal property against seed mycoflora of different crop species, indicated its efficacy as a potential seed treatment. Though no much information is available on the bioactivity of the active ingredients, especially in tuber extracts, this study has proved it, as an efficient antifungal agent. This study has also confirmed the occurrence of glycocides, saponins, flavonoids and alkaloids in the fresh extracts of safed musli, which are responsible for the inhibition of fungi. Hence, this extract could be an alternative eco-friendly treating agent to overcome the fungal pathogens in agricultural crop species. Further research on the isolation and characterization of bioactive compound is necessary to come out with the promising results for chemical synthesis of the same in large scale.

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