PHYTOCHMICAL ANALYSIS AND ANTIFUNGAL POTENTIAL OF *Sorghum Halepense* (L.) Pers. FOR THE MANAGEMENT OF MYCOTOXIGENIC FUNGI

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DOI: https://doi.org/10.28941/pjwsr.v27i4.1008

ABSTRACT

Mycotoxigenic fungal species are a major cause of various infections in plants and their post-harvest produce that pose a serious threat to humans and animals. In the current study, the objective was to examine the in vitro efficacy of different concentrations of methanolic leaf extract of *Sorghum halepense* (L.) Pers. against target pathogenic mycotoxin producing fungal species (*Trichoderma viride* Pers., *Trichoderma harzianum* Rifai. and *Cladosporium cladosporioides* (Fresen.) G.A. de Vries. For this purpose, different concentrations viz. 2%, 4%, 6%, 8%, and 10% of methanolic leaf extract were prepared and tested for their antifungal potential in a completely randomized design. All the applied concentrations of *S. halepense* leaf extract inhibited the growth of all the tested fungal strains. Maximum growth inhibition (84%) was observed in 2% of the concentration of the extract against *Cladosporium cladosporoides*. On the other hand, the minimum reduction was observed in 4% of concentration of methanolic extract of *S. halepense* against *T. viride* as compared to control. The phytochemical analysis was also conducted to check which chemical entities are present in the extract that accounted for the antifungal potency of methanolic extract of *S. halepense*. Results of the phytochemical analysis revealed the occurrence of saponins, alkaloids, coumarins, flavonoids, and tannins while the plant was devoid of terpenoids, phlobatannins, and glycosides. Hence it can be concluded that the methanolic leaf extract of the tested plant effectively inhibited the growth of test mycotoxigenic fungi.

Keywords: Antifungal, Fungi, Mycotoxin, Phytochemicals

Citation: Naeem, Z.; K. Jabeen; M.K. Saeed; S. Iqbal. 2021. Phytochmical Analysis and Antifungal Potential of *Sorghum halepense* (L.) Pers. for the Management of Mycotoxigenic Fungi. Pak. J. Weed Sci. Res., 27(4): 505-512.

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INTRODUCTION

Many fungal species produce a wide array of toxic substances known as mycotoxins. These mycotoxins are mainly released by *Claviceps*, *Fusarium*, *Aspergillus*, *Alternaria*, *Penicillium*, and *Stachybotrys* species. The outcome of the existence of these toxins in pathogenic fungal strains results in the contagion of food and feedstuff during storage and nurturing (Perincherry *et al.,* 2019; Venkatesh and Keller, 2019). In addition to causing damage to crops, they are responsible for various health issues in humans and animals by the consumption of contaminated food or even by inhalation (Hojnik *et al.,* 2017). The subsistence of these mycotoxins depends on certain environmental factors like humidity, substrate composition for growth, and temperature specifically in grains, nuts, and cereals before and after reaping of crops. Sub-Saharan Africa and Asia are ideal tropical regions due to optimal growth conditions best suited to the production of these secondary metabolites (Nleya *et al.,* 2018; Balendres *et al.,* 2019).

Poor and underdeveloped countries peoples are more likely to get infected by mycotoxins resulting in a severe reduction of farming yield and trade up to 60-80% (Eskola *et al.,* 2019) which in return also poses a severe threat to food safety and their nutritional value as well (Luo *et al.,* 2018). These chemical compounds are considered to be the origin of a wide array of diseases in crops and vegetables preferably referred to as "mycotoxicosis (Liew and Redzwan, 2018). Mycotoxins zearalenone trichothecenes, aflatoxins, and fumonisins are widespread and major classes of mycotoxins agricultural significance (Dikhoba *et al.,* 2019; Celik, 2020). To limit the release of these toxins in crops; regulatory limits have been set by many countries (Haque *et al.,* 2020).

Various biological and chemical procedures have been developed and efficiently applied to manage and inhibit the production of mycotoxins (Adebiyi *et al.,* 2019). But these artificial strategies cannot be utilized on a routine basis due to their high cost, lingering venomous side effects, and low specificity in response to some mycotoxins (Mahato *et al.,* 2019). Because of these reasons, alternative ways need to be developed and implemented to detoxify fungal contaminants (Haque *et al.,* 2020). The use of botanical and herbal formulations to retard the growth of mycotoxigenic fungi has been turned out to be an eco-friendly and safer substitute in comparison to those of synthetic fungicides (Javaid *et al.,* 2020; Khan and Javaid, 2020; Khan *et al.,* 2021). It is because of the presence of phytochemicals in crude extract and essential oils of plants that we can manufacture nutraceuticals and bio fungicides, which in turn serve as bioagents for inhibition of various pathogenic fungal genera producing mycotoxins (Adebo and Meza, 2020).

Sorgham halepense L. is distributed over a third of the total world area and is considered to be a dangerous weed of the family Poaceae. The therapeutic roles reported on sorghum include anti-inflammatory, anti-carcinogenic, antibiotic, antifungal, antiviral, hepatoprotective, anti-ulcer, anti-neoplastic, cholesterol-lowering, and distastefulness slowing properties (Soetan *et al*., 2006; *Dykes et al*., 2009, Ademiluyi *et al*., 2014). Due to the presence of a large number of phytochemicals like sterols, policosanols, and sterols; it is also considered as a potent antimicrobial agent as these metabolites play a vital role in the defense mechanism of plants against pests' attacks, environmental stress, and diseases (Smith and Cherri, 2008; Salazar-Lopez *et al*., 2018) So, the present work was planned to search for eco-friendly antifungal phytochemicals from *S. halepense* against targeted mycotoxigenic fungal species.

MATERIALS AND METHODS

In vitro antifungal assay

Leaves of *S. halepense* (100 g) were surface sterilized using 10% sodium hypochlorite solution followed by sun-drying and grinding. The test mycotoxin producing fungal strains *Trichoderma viride* (FCBP-671), *Trichoderma harzianum* (FCBP-1277),

and *Cladosporium cladosporioides* (FCBP-976) were obtained from the Fungal Culture Bank of Pakistan, University of the Punjab, Lahore, Pakistan, and the cultures were maintained on 2% malt extract agar (MEA).

Antifungal activity of *S. halepense* was evaluated against the test mycotoxigenic fungi. An investigation was conducted using the protocol of (Sherazi *et al.*, 2016). For this purpose, 250 mL of methanol was used to soak 100 g test plant material. It was then subjected to filtration using an autoclaved muslin cloth to obtain the crude mass of *S. halepense.*

Five concentrations of test plant material viz. 2, 4, 6, 8, and 10% were made by adding and thoroughly mixing 3, 6, 9, 12, and 15 mL of stock solutions in 27, 24, 21, 18, 15 mL of malt extract broth medium to make total volume up to 30 mL. The *in vitro* experiment was conducted in a completely randomized design. Three replicates were made for each treatment, while plant extract was not included in the control treatment. All respective flasks were then allowed to incubate for a week at 25 °C after being endowed with 50 mg chloromycetin. Media in incubated flasks were then filtered using pre-weighed Whatman no. 1 filter papers after a week, and their dry weights were recorded after oven drying for 12 hours for each flask. The inhibition potency of each concentration towards each fungal strain was calculated using the formula mentioned below:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Phytochemical analysis

The methanol extract was tested to identify the existence of biochemical entities/phytochemicals that account for its antifungal potential (Parekh and Chanda, 2007).

Estimation of glycosides

To 2 mL of plant extract; 1 mL of concentrated sulphuric acid, two mL glacial acetic acid, and 1 drop of 1% ferric chloride were added and then shaken rapidly. The appearance of greenish-blue color established the fact that glycosides are being there in respective plant extract and this test is termed as Keller-Kiliani Test.

Estimation of alkaloids

The occurrence of creamish or orange precipitates was the confirmation of the presence of alkaloids in the tested plant extract when the mixture of 1% hydrochloric acid (HCl) and 2 mL of extract was heated and filtered. Also, six drops of Dragendroff reagent were added to the resultant filtrate which gave this color.

Estimation of terpenoids

The incidence of terpenoids had been tested by mixing 2 mL chloroform and plant extract. Later on, filtration was carried out and one drop of concentrated sulphuric acid with one mL of acetic acid was added to the solution and the appearance of blue-green confirmed the presence of terpenoids.

Estimation of saponins

In 10 mL of distilled water; 2 mL of plant extract was added and it was subjected to boiling then cooling afterward. The emergence of constant bubbles or froths gave the impression that saponins were known to exist in plant material. This test is termed as Frothing Test.

Estimation of flavonoids

In 3 mL of plant extract; 4 mL of 1% potassium hydroxide (KOH) was mixed after washing of plant material with petroleum ether followed by 80% ethanol. Flavanoids were confirmed when dark yellow color came into being.

Estimation of tannins

Twenty milliliters of distilled water was used for boiling 2 mL of plant sample in it followed by filtration. After that minimal drops of ferric chloride were added infiltrate and brownish-green or blue-black color showed the occurrence of tannins.

Estimation of phlobatanins

There were no phlobatanins in the given plant sample because on boiling 2 mL of plant extract in 1% HCl solution, red precipitates were not produced which was a clear indication of the presence of these secondary metabolites.

Estimation of coumarins

Two milliliters of residual plant extract were taken in a test tube wrapped with filter paper moistened with 0.1 N sodium hydroxide (NaOH) and subjected to heat for a few minutes in boiling water. Yellow fluorescence in filter paper was detected when observed under ultraviolet (UV) light which means that coumarins are present in plant samples.

A one-way analysis of variance (ANOVA) followed by least significant difference (LSD) using Statistix 8.1 software was used to scrutinize data statistically at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

In current studies, methanolic extract of *S. halepense* was used for controlling the *in vitro* growth of mycotoxin-producing test fungal species. Different concentrations of test plants *viz*. 2, 4, 6, 8, and 10% were made. All the applied concentrations significantly suppressed the test fungi. Results revealed that all the applied concentrations of methanolic extract of *S. halepense* inhibited the growth of up to *T. viride* 75%. The highest reduction i.e. 84% in the biomass of *C. cladosporoides* was observed at 2% conc. of *S. halepense*. The methanolic extract 4% was also found effective against *T. harzianum* that causing a 76% reduction as compared to control (Fig. 1-3). The methanolic shoot extract of *S. halepense* possessed considerable antifungal potential in minimizing the biomass of *Macrophomina phaseolina* due to the presence of antifungal constituents (Javaid *et al.,* 2012). The crude extract and fractions of *S. halepense* inhibited the growth of *Aspergillus niger, A. fumigatus, and A. flavus,* which might be due to the presence of tannic acid (Khayal *et al.,* 2019).

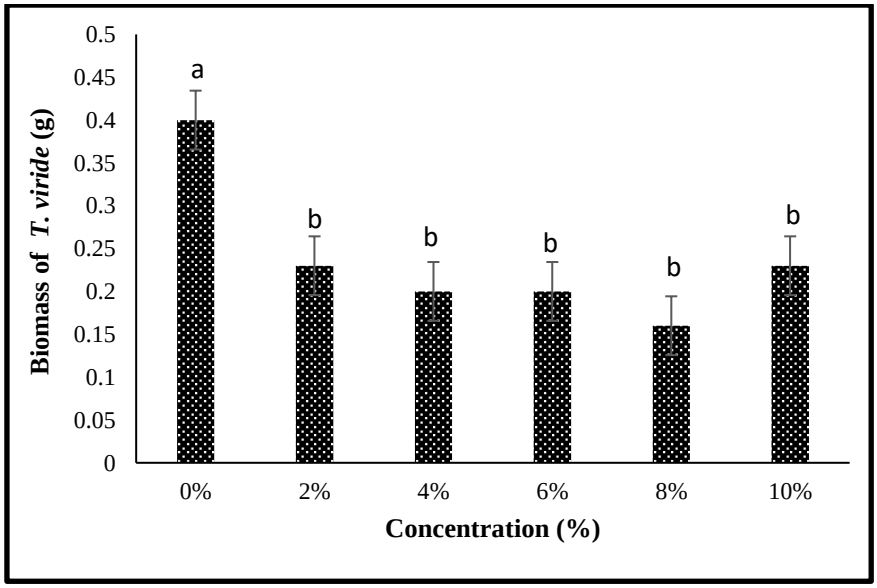

Fig. 1: Effect of various concentrations of methanolic extract of *S. halepense* on *in vitro* growth of *T. viride*. Values with different letters show significant differences as determined by LSD ($P = 0.05$) test.

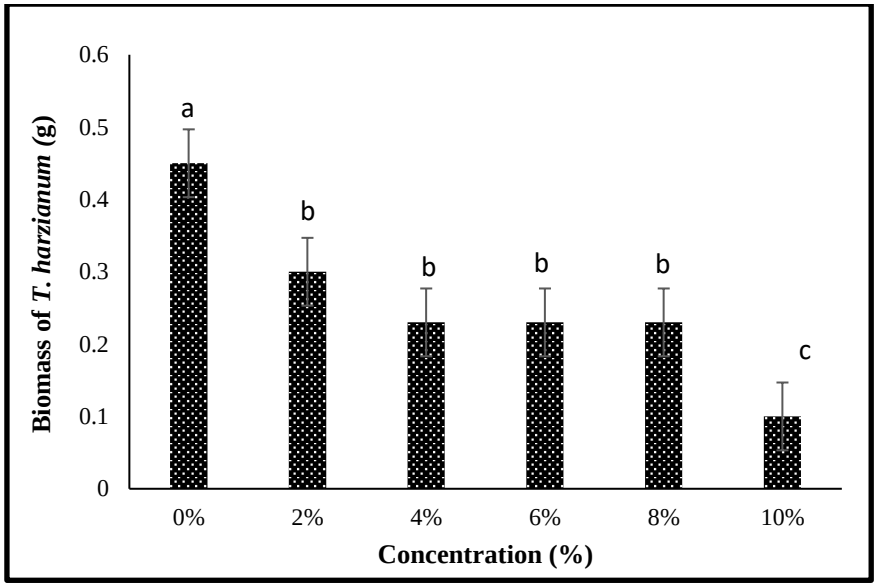

Fig. 2: Effect of various concentrations of methanolic extract of *S. halepense* on *in vitro* growth of *T. harzianum*. Values with different letters show significant differences (*P* = 0.05) as determined by LSD test.

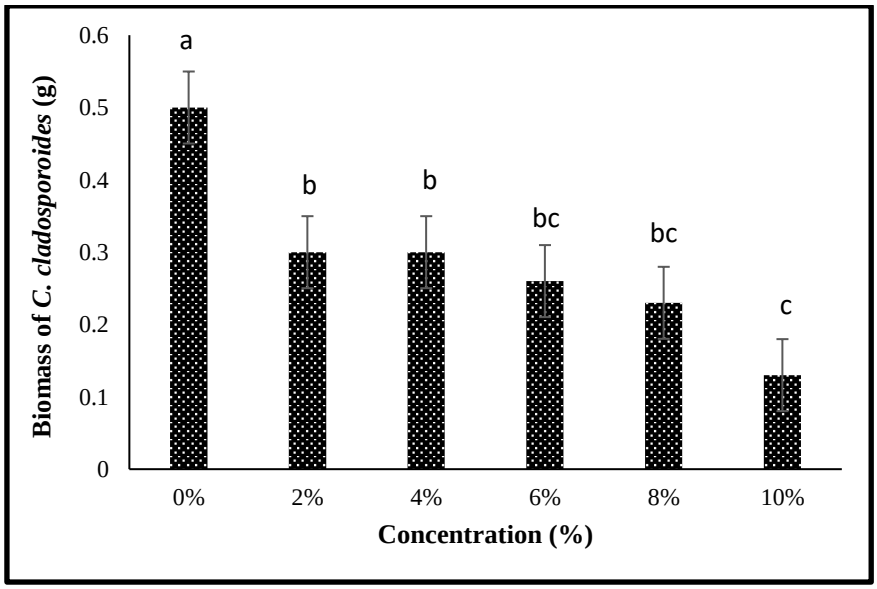

Fig. 3: Effect of various concentrations of methanolic extract *of S. halepense* on *in vitro* growth of *C. cladosporoides.* Values with different letters show significant differences (*P* = 0.05) as determined by LSD test.

The qualitative phytochemical screening of *S. halepense* was carried out which indicated the presence of saponins, alkaloids, coumarins, flavonoids, and tannins while on the other hand; terpenoids, phlobatinins, and glycosides were found to be absent in it (Table 1). The study conducted by Akande*et al.,* 2010 also revealed the presence of alkaloids, flavonoids, saponins, and tannins in *S. halepense*. The occurrence of all these chemical entities was also reported in *S. halepense* by Shah *et al.* (2019). It is due to the existence of such phytochemicals that plants got protected from disease and damage again environmental stress, drought, pollution, UV exposure, and pathogenic attack (Saxena *et al.,* 2013).

It is reported from previous studies that many people in the world have been utilizing weed plants for medicinal purposes for curing various ailments. Some varieties are edible and can be used for culinary purposes also like delicious nuts of walnuts (Islam *et al.,* 2006). All such qualities came due to the profound presence of phytochemicals in plants, for example, *Calotropis procera* possess a wide range of these chemicals like tannins, flavonoids, triterpenes, cardiac glycosides, and sterols respectively that accounts for the pharmacological and antioxidant potential of test plant (Ferdosi *et al.,* 2021).

Table 1: Phytochemical investigation of leaf methanolic extract of *S. halepense.*

Phytochemicals	**Result**
Saponins	++
Terpenoids	-
Alkaloids	++
Phylobatinnins	-
Coumarins	++
Flavanoids	++
Tannins	++
Glycosides	-

Positive sign shows that phytochemicals are present and negative sign shows the absence of phytochemicals.

Based on the work conducted, it is suggested that *S. halepense* exhibit a wide range of secondary metabolites which contribute towards its antifungal potential and it can be used medicinally as an excellent source for the isolation of potent antifungal drugs.

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