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FUNGICIDAL POTENTIAL OF FLOWER EXTRACT OF *CASSIA FISTULA* AGAINST *MACROPHOMINA PHASEOLINA* AND *SCLEROTIUM ROLFSII*

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ABSTRACT

Macrophomina phaseolina and *Sclerotium rolfsii* are the two important soil-borne phytopathogens causing diseases in hundreds of plant species. In search of alternatives to synthetic fungicides, the present study was carried out to investigate the antifungal activity of methanolic extract of flowers of *Cassia fistula* L. against these two highly destructive plant pathogens. The extract was prepared by soaking 200 g shade dried flowers of the test plant species in 1000 ml of methanol for two weeks, followed by filtration and evaporation on a rotary evaporator. Bioassays carried out with 1.562, 3.125, 6.250, 12.500, 25.000, and 50.000 mg/ml concentrations of the extract exhibited 2–36% and 31–56% reduction in biomass of *M. phaseolina*and *S. rolfsii,* respectively. The extract was partitioned with *n*-hexane followed by chloroform and the later fraction was analyzed by GC-MS that showed presence of 37 compounds. Hexacosane (16.06%) followed by heptacosane (10.94%) and benzene, 1,2,3-trimethyl- (8.12%) were recognized as the principal components of chloroform fraction. Compounds namely heptadecane (7.15%), benzene, 4-ethyl-1,2-dimethyl- (4.60%), benzene, 1,2,3,4-tetramethyl- (3.93%), benzene, 2-ethyl-1,4-dimethyl- (3.88%), aniline, N-methyl- (3.68%) and naphthalene (3.08%) were ranked as moderately abundant. Some of the identified compounds are known to possess antifungal activities against various fungal species and could be responsible for antifungal activity of the flower extract against *M. phaseolina* and *S. rolfsii* in the present study. This study also concluded that *S. rolfsii* was more susceptible to the flower extract than *M. phaseolina.*

Keywords: Antifungal; *Cassia fistula*; Flower extract; GC-MS analysis

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INTRODUCTION

Fungal pathogens are a well-known threat to crops globally (Degani *et al.,* 2020), causing up to 20% losses during production and 10% during postharvest stage (Fisher *et al.,* 2012), especially in vegetables and fruits (Zhang *et al.,* 2020). Among these, soil-borne fungal pathogens are considered a critical factor for health and productivity of plants globally (De Corato, 2020). Two soil-borne fungi namely *Macrophomina phaseolina* and *Sclerotium rolfsii* have been especially recognized as devastating pathogens because each of these two has a vast host range having the ability to cause diseases in more than 500 plant species (Khan *et al.,* 2021; Sharf *et al.,* 2021). *M. phaseolina* is an important phytopathogenic fungus that has the ability to cause pre- and post-emergence plant mortality and can survive up to 15 years as saprophyte in the soil (Kaur *et al.,* 2012). It produces microsclerotia, which serve as a primary source of inoculum and remain in the soil for prolonged period of time (Abbas *et al.,* 2019). Similarly, *Sclerotium rolfsii* is another soil-borne phytopathogenic fungus that commonly occurs in warm temperate regions of the world and affects a large number of agricultural crops. It has the ability to cause collar rot, root rot, damping-off, crown rot, stem rot, foot rot, blight and dry rot canker in different plants (Javaid and Khan, 2016; Billah *et al.,* 2017; Ali *et al.,* 2020; Bosamia *et al.,* 2020).

To control the scourge of soil-borne plant pathogens, many strategies have been tested. It is often difficult to control *S. rolfsii* through the use of conventional strategies due to its specific life cycle with production of sclerotia, which are resistant to biological and chemical degradation (Smolińska and Kowalska, 2018). Moreover, the environmental toxicity associated with chemicals and resistance development in pathogens have motivated the researchers to investigate some other possibilities to control pests and diseases of plants (Javaid and Iqbal, 2014; Ogunnupebi *et al.,* 2020). Therefore, in the recent past, extensive research has been carried out on the environmentally safe products of natural origin (Jabeen *et al.,* 2021). In this context, plant extracts are generally assumed to be the best alternative remedy for the control of fungal phytopathogens (Shuping and Eloff,

2017; Khan *et al.,* 2020; Banaras *et al.,* 2021; Javed *et al.,* 2021). The major compounds that have been found in plants for control of phytopathogens include flavonoids, terpenes, quinones, phenols, saponins, polypeptides, lectins, alkaloids and sterols (Banaras *et al.,* 2020; Khan and Javaid, 2020 a, b; Ogunnupebi *et al.,* 2020).

Cassia fistula L. is an ornamental leguminous tree native to Indian Subcontinent and adjacent Southeast Asian regions, and can be found in various countries including India, Brazil, Mexico and South Africa (Antonisamy *et al.,* 2019). It is grown as an ornamental plant in Pakistan for its beautiful yellow flowers. Extracts and compounds of this plant are known for antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, hypoglycemic and wound healing properties (Ilavarasan *et al.,* 2005; Rahman *et al.,* 2020; Ferdosi *et al.,* 2021, 2022). However, information regarding antifungal activities against the two devastating plant pathogens namely *M. phaseolina* and *S. rolfsii* are lacking. The present study was, therefore, carried out to investigate antifungal activity of flower extract of *C. fistula* against these fungal species, and identification of the possible antifungal compounds in the extract.

MATERIALS AND METHODS

Antifungal bioassays: Flowers of *C. fistula* were collected from University of the Punjab, Lahore, Pakistan during June 2020. After shade drying, 200 g flowers were soaked in 1000 ml methanol for two weeks and filtered. The solvent was evaporated on a rotary evaporator and the resultant gummy biomass was used for antifungal bioassays and GC-MS analysis (Javaid *et al.,* 2018).

For antifungal bioassays, procedure of Khan and Javaid (2020b) was adopted with some modifications. For this, 0.6 g of the extract biomass was dissolved in 50 µl dimethyl sulfoxide (DMSO) and added 2% autoclaved malt extract broth (MEB) to prepare 12 ml of the stock solution of 50.000 mg/ml concentration. This medium was repeatedly double diluted by adding MEB and the lower concentration *viz.* 25.000, 12.500, 6.250, 3.125 and 1.562 mg/ml were prepared. Antifungal bioassays against *M. phaseolina* and *S. rolfsii* were carried out by taking 1 ml of the growth medium in 10-ml volume test tubes. To each test tube, 50 µl of the fungal inocula, prepared by adding 5 ml autoclaved water to culture plates and scratching, was added and incubated at 28 ºC for one week. Experiment was carried out in a completely randomized design with three replications. Thereafter, fungal biomass was collected on a filter paper and weighed after drying at 70 ºC.

GC-MS analysis: The gas chromatograph (GC) machine model was 7890B and that of mass spectroscopy (MS) was 5977A, used for the identification of different compounds from the sample while both were branded by Agilent Technologies. The column used was DB 5 MS (30 m × 0.25 µm × 0.25 µm). Injection volume was 1 µl and carrier gas was helium. Oven ramping, initial temperature was 80 ºC that was raised 10 ºC per minute up to 300 ºC. Inlet temperature was 280 ºC. MS conditions were as: scan 50-500 m/z, the source temperature was 230 ºC and quadrupole temperature was 150 ºC. Chemical compounds were identified by comparison of their spectra with library and arranged in the ascending order of their retention times and retention indices. The relative abundance was reported by using their peak area percentages.

Statistical analysis: All the data were presented as mean ± standard error. One-way ANOVA followed by LSD test were applied for comparison of the treatments statistically ($P \leq 0.05$). The statistical analysis was performed using the software Statistix 8.1

RESULTS

Antifungal activity of methanolic extract: Lower concentrations of the extract ranging from 1.562 to 6.250 mg/ml significantly reduced biomass of *M. phaseolina* by 29–36% over control. The concentration 12.500 mg/ml and above had an insignificant effect on growth of the pathogen and reduced its biomass only by 2–12% (Fig. 1).

Growth of *S. rolfsii* was more susceptible to the applied extract than the growth of *M. phaseolina*. In this case, all the applied concentrations of methanolic extract significantly reduced biomass of the fungus by 31–56%. However, similar to that of *M. phaseolina,* the effect of lower concentrations of the extract was more pronounced than that of higher concentrations (Fig. 2).

GC-MS analysis: GC-MS chromatogram shown in Fig. 3 presents 37 peaks. Details of the compounds corresponding to these peaks are given in Table 1. The most abundant compound was hexacosane (16.06%) followed by heptacosane (10.94) and benzene, 1,2,3-trimethyl- (8.12%). Moderately abundant compounds included heptadecane (7.15%), benzene, 4-ethyl-1,2-dimethyl- (4.60%), benzene, 1,2,3,4-tetramethyl- (3.93%), benzene, 2-ethyl-1,4-dimethyl- (3.88%), aniline, N-methyl- (3.68%) and naphthalene (3.08%). Less abundant compounds were octane, 2,6-dimethyl- (1.16%), heptadecyl trifluoroacetate (1.41%), nonadecane (1.44%), 3,4-octadiene, 7-methyl- (1.46%), benzene, 1-ethyl-3,5-dimethyl- (1.51%), benzene, 1-ethyl-2-methyl- (1.59%), mesitylene (1.71%), benzene, 1,2,4,5-tetramethyl- (2.08%), *o*-cymene (1.88%), heptacosane, 1-chloro- (2.14%), tetradecane (2.22%), benzene, 1-methyl-3-propyl- (2.40%), hexadecanoic acid, 15-methyl-, methyl ester (2.48%), tetracosane (2.56%), and acetic acid, 2-ethylhexyl ester (2.98%). The minor compounds with less than 1% peak areas were benzene, 1,3-diethyl-

5-methyl- (0.50%), benzene, propyl- (0.57%), nonane, 4-methyl- (0.59%), benzene, 2-ethyl-1,3-dimethyl-(0.61%),benzene, 1-ethyl-2,3-dimethyl-(0.69%), benzene, 1,3-diethyl-5-methyl- (0.71%), benzene, 1-methyl-4-(2-methylpropyl)- (0.73%), trifluoroacetoxy hexadecane (0.73%), pentadecane (0.79%), tetradecane (0.83%), cyclohexane, butyl-(0.85%), 1-phenyl-1-butene (0.86%) and cyclohexane, propyl- (0.89%).

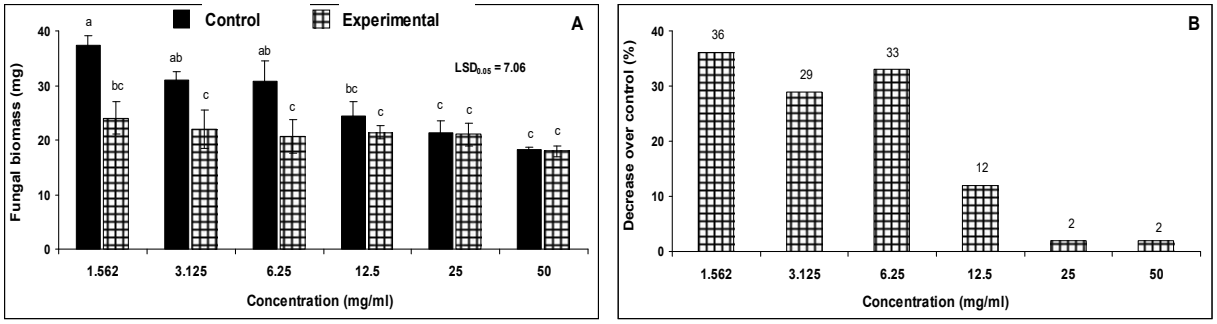

Fig. 1: Effect of different concentrations of methanolic flower extract of *Cassia fistula* on growth of *Macrophomina phaseolina*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

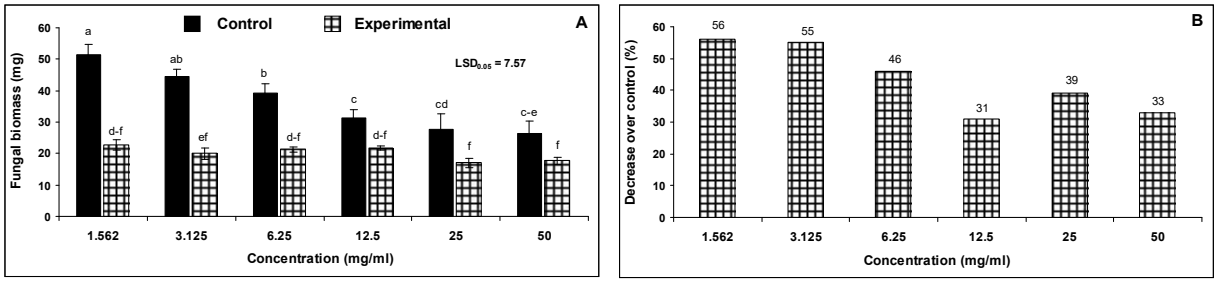

Fig. 2: Effect of different concentrations of methanolic flower extract of *Cassia fistula* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

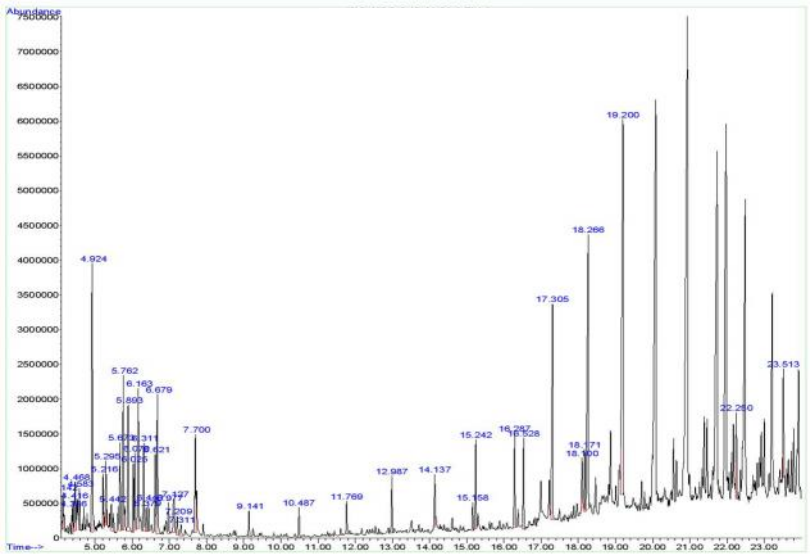

Fig. 3: GC-MS chromatogram of chloroform fraction of methanolic flower extract of *Cassia fistula.*

Table 1: Compounds identified in chloroform fraction of methanolic flower extract of *Cassia fistula* through GC-MS analysis.

Sr.	Names of compounds	Molecular	Molecular	Retention	Peak area

No.		formula	weight	time (min)	(%)
1	Cyclohexane, propyl-	C_9H_{18}	126.24	4.142	0.89
2	Benzene, propyl-	C_9H_{12}	120.19	4.386	0.57
3	Nonane, 4-methyl-	$C_{10}H_{22}$	142.28	4.416	0.59
4	Benzene, 1-ethyl-2-methyl-	C_9H_{12}	120.19	4.468	1.59
5	Benzene, 1,2,3-trimethyl-	C_9H_{12}	120.19	4.924	8.12
6	Octane, 2,6-dimethyl-	$C_{10}H_{22}$	142.28	5.216	1.16
7	Mesitylene	C_9H_{12}	120.19	5.295	1.71
8	Cyclohexane, butyl-	$C_{10}H_{20}$	140.27	5.442	0.85
9	Benzene, 1-methyl-3-propyl-	$C_{10}H_{14}$	134.22	5.673	2.40
10	Benzene, 4-ethyl-1,2-dimethyl-	$C_{10}H_{14}$	134.22	5.762	4.60
11	Aniline, N-methyl-	C_7H_9N	107.15	5.893	3.68
12	*o*-Cymene	$C_{10}H_{14}$	134.22	6.070	1.88
13	Benzene, 2-ethyl-1,4-dimethyl-	$C_{10}H_{14}$	134.22	6.163	3.88
14	Tetradecane	$C_{14}H_{30}$	198.39	6.311	2.22
15	Benzene, 1-methyl-4-(2-methylpropyl)-	$C_{11}H_{16}$	148.24	6.379	0.73
16	Benzene, 1-ethyl-2,3-dimethyl-	$C_{10}H_{14}$	134.22	6.440	0.69
17	Benzene, 1,2,4,5-tetramethyl-	$C_{10}H_{14}$	134.22	6.621	2.08
18	Benzene, 1,2,3,4-tetramethyl-	$C_{10}H_{14}$	134.22	6.679	3.93
19	1-Phenyl-1-butene	$C_{10}H_{12}$	132.22	6.977	0.86
20	Benzene, 1-ethyl-3,5-dimethyl-	$C_{10}H_{14}$	134.22	7.127	1.51
21	Benzene, 2-ethyl-1,3-dimethyl-	$C_{10}H_{14}$	134.22	7.209	0.61
22	Benzene, 1,3-diethyl-5-methyl-	$C_{11}H_{16}$	148.24	7.311	0.50
23	Naphthalene	$C_{10}H_8$	128.17	7.700	3.08
24	Hexadecane	$C_{16}H_{34}$	226.44	9.141	0.71
25	Pentadecane	$C_{15}H_{32}$	212.41	10.487	0.79
26	Tetradecane	$C_{14}H_{30}$	198.39	11.769	0.83
27	Nonadecane	$C_{19}H_{40}$	268.52	12.987	1.44
28	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338.44	15.158	0.73
29	Tetracosane	$C_{24}H_{50}$	338.65	16.287	2.56
30	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.48	16.528	2.48
31	Heptadecane	$C_{17}H_{36}$	240.46	17.305	7.15
32	Heptadecyl trifluoroacetate	$C_{19}H_{35}F_3O_2$	352.47	18.100	1.41
33	3,4-Octadiene, 7-methyl-	C_9H_{16}	124.22	18.171	1.46
34	Heptacosane	$C_{27}H_{56}$	380.73	18.266	10.94
35	Hexacosane	$C_{26}H_{54}$	366.70	19.200	16.06
36	Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	415.17	22.250	2.14
37	Acetic acid, 2-ethylhexyl ester	$C_{10}H_{20}O_2$	172.26	23.513	2.98

DISCUSSION

In the present study, flower extract of *C. fistula* significantly retarded the growth of both the fungal pathogens. Most of the previous antifungal studies were carried out using extracts of leaves, stem-bark, fruits and seeds of *C. fistula* against various *Candida* and *Aspergillus* species (Bhalodia and Shukla, 2011; Bhalodia *et al.,* 2012; Sony *et al.,* 2018; Nascimento *et al.,* 2020). However, a few studies were also carried out using flower extracts of this plant which showed antifungal activity against other fungi that support the results of the present study (Bhalodia *et al.,* 2011; Sharma *et al.,* 2021). Flowers of *C. fistula* possess alkaloids, flavonoides, phenols and tenins, which could be responsible for antifungal activity (Rahman *et al.,* 2020). A crystalline compound 4-hydroxy benzoic acid hydrate present in *C. fistula* flower has been reported as an antifungal agent against *Trichophyton mentagrophytes* and *Epidermophyton floccosum* (Duraipandiyan and Ignacimuthu, 2007).

Although growth of both the targeted fungal species was suppressed by the application of flower extract of *C. fistula,* however, growth of *S. rolfsii* was more susceptible to the applied extract as compared to growth of *M. phaseolina*. Similar differential effects of the plant extracts have also been reported in other similar interactions. Salhi *et al.* (2017) evaluated antifungal activity of four Algerian medicinal plants against two species of *Fusarium* namely *F. graminearum* and *F. sporotrichioides* and observed a marked variability between the sensitivity of the two fungal species towards various plant extracts. Likewise, Liu *et al.* (2019)

investigated the effect of crude extracts of *Smilacina japonica* against *Candida albicans, Cryptococcus neoformans, Cryptococcus tropicalis* and *Cryptococcus glabrata.* Marked variation in MICs of the extract (208 to 1665 µg/ml) against different fungal species demonstrated highly variability in sensitivity of the test fungi towards applied extract.

In the present study, lower concentrations of the flower extract were more effective in controlling growth of the two fungal species as compared to higher concentrations. This irregular antifungal activity of the extract might be due to the presence of higher concentrations of DMSO in the higher concentrations of flower extract. DMSO was used to dissolve the extract. However, there are reports that its higher concentrations also suppressed growth of different fungal species to variable extents (Akhtar *et al.,* 2020; Khan and Javaid, 2020a). In the present study, a set of control treatments was used keeping the same concentrations of DMSO as that in various concentrations of the extract treatments. Suppression of *M. phaseolina* and *S. rolfsii* growth by 36% and 56% due to the lowest extract concentration (1.562 mg/ml) clearly indicated the significant antifungal potential of the extract. However, at 50.000 mg/ml concentration of the extract, higher concentration of DMSO markedly suppressed fungal growth in the corresponding control treatment and thus no significant difference was observed between control and experimental treatments.

Chloroform fraction of methanolic flower extract was analyzed by GC-MS to identify various possible antifungal compounds. This fraction was selected on the basis of results of various previous studies where chloroform fraction of methanolic extracts of *Chenopodium quinoa, Datura metel* and *Sonchus oleraceous* exhibited markedly higher antifungal potential than the non-polar *n*-hexane fraction as well as more polar ethyl acetate and *n*-butanol fractions against *M. phaseolina* and *S. rolfsii* (Jabeen *et al.,* 2014; Banaras *et al.,* 2020; Khan and Javaid, 2020a). GC-MS analysis of the chloroform fraction showed 37 organic compounds. Among these, a number of compounds are known for their antifungal activities against different fungal species. As for example, hexadecanoic acid, 15-methyl-, methyl ester belongs to fatty acid methyl esters. Members of this group of compounds generally possess antifungal properties (Belakhdar *et al.,* 2015; Ali *et al.,* 2017). Other compounds namely trifluoroacetoxy hexadecane and tetradecane are also known for their antifungal properties (Ibrahim *et al.,* 2017).

Conclusion: This study concludes that methanolic extract of *C. fistula* possess selective antifungal activity. It showed more pronounced activity against *S. rolfsii* than against *M. phaseolina.* Compounds such as hexadecanoic acid, 15-methyl-, methyl ester and trifluoroacetoxy hexadecane might be responsible for antifungal activity of flower extract.

Author's contributions: MFHF supervised the work. HA carried out research work. IHK contributed in paper writing. AJ carried out statistical analysis, draw graphs, arranged GC-MS data in the form of a table and also finalized the manuscript.

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