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Research Note

Quantitative analysis of secondary metabolites produced by *Chaetomium globosum* Krunze ex Fr.

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ABSTRACT: ***Chaetomium globosum*** **Krunze ex Fr. (Cg) has been identified as a potential antagonist of** ***Bipolaris sorokiniana*** **and** ***Ascochyta rabiei*****. This biocontrol agent has been found to be producing antifungal metabolites. Six different isolates of** ***Cg*** **were characterized for the production of antifungal metabolites. The quantitative estimation of crude extracts showed maximum production by isolate Cg3 (47.2μg/mg mycelium) followed by Cg2 (44.2μg/mg). Isolate Cg1 produced minimum quantity of metabolites (7.44μg/mg). Crude extracts of secondary metabolites from all the isolates of** ***C. globosum*** **when resolved by thin layer chromatography showed that the isolate of Cg1 produced only two compounds of R_f value 0.17 and 0.48, while isolate Cg2 produced thirteen compounds, followed by 11 compounds produced by isolate Cg3. The metabolite from spore germination fluid of isolate Cg2, when resolved on TLC plate along with crude extracts from culture filtrate of Cg2, showed production of about same number of compounds. One of the purified compounds from isolate Cg2 when used for bioassay against** ***Bipolaris sorokiniana***, ***Rhizoctonia solani***, ***Fusarium udum*** **and** ***Macrophomina phaseolina*** **pathogens proved effective in inhibiting the growth up to 70 percent.**

KEY WORDS: Bioefficacy, *Chaetomium globosum*, secondary metabolites, soil borne pathogens

Recent advances in the development of biopesticides offer opportunities for the worldwide exploitation of biocontrol agents (BCA) as replacement for more hazardous and environmentally unacceptable chemical pesticides and for inclusion in integrated pest management programmes. Some biocontrol agents secrete a wide array of compounds with biological activity against other organisms, mostly products of secondary metabolism. These metabolites serve different functions, depending upon the ecological niche of the organisms. Some metabolites may be antibiotics to protect the BCA against antagonistic microorganism or may prevent growth of saprophytic microbes on the host or suppress the growth of plant pathogens. Metabolite production by fungi exhibiting biocontrol activity has been most commonly reported for isolates of *Gliocladium* spp., *Talaromyces flavus*, *Trichoderma* spp., *Chaetomium* spp., *Minimedusa polyspora*,

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Verticillium biguttatum and also by fungal entomopathogens such as *Metarhizium ansiopliae* and *Beauveria bassiana*.

Chaetomium globosum has been identified as potential biocontrol agent against *Bipolaris sorokiniana* causing spot blotch of wheat (Mandal *et al.*, 1999; Biswas *et al.*, 2000) and *Ascochyta rabiei* causing blight of chickpea (Rajkumar *et al.*, 2005). Recent studies conducted in our laboratory have indicated that some isolates of *C. globosum* are mycoparasitic while others cause antibiosis (Aggarwal *et al.*, 2004). Keeping this in view, the present study was undertaken to analyze the secondary metabolite production by *C. globosum* and their role in antagonizing soil borne pathogens.

Chaetomium globosum isolates designated as Cg1, Cg2, Cg3, Cg4, Cg5 and Cg6 obtained from various sources were used. An isolate each of *Bipolaris sorokiniana, Rhizoctonia solani, Fusarium udum* and *Macrophomina phaseolina* were also taken up for *in vitro* bioassay studies. All the microorganisms were maintained on potato dextrose agar (PDA) slants and stored at 4°C.

Quantitative analysis of antifungal metabolites

One hundred ml conical flasks with 30ml Potato dextrose broth (PDB) were incubated with *C.globosom* isolates individually and incubated for 21 days at 25°C. The fresh mycelium from liquid media was taken and weighed. The culture filtrate was used for extraction of antifungal metabolites by solvent extraction procedure using ethyl acetate as solvent. After extraction, antifungal metabolites were weighed and production of antifungal metabolites per mg fresh weight of mycelium was calculated.

Crude extracts of each isolate at 1000ppm concentration was added in flasks containing PDA just before pouring in Petri-plates at 40°C. Petri-plates were inoculated at the center with agar plugs of 4-day-old culture of *B. sorokiniana*. Plates with plain PDA were kept as check. Three replications were kept for each treatment. The inoculated plates were incubated at 26°C and colony growth was measured after one week.

Concentrated crude extract from each isolate was spotted on silica gel plates for TLC. The plates were developed in previously standardized solvent system-benzene: ethyl acetate: glacial acetic acid (10:1:1) (Aggarwal, 2000). Banding pattern was observed under transilluminator at 254 nm UV. The R_f values of all the bands appeared on the TLC plate were calculated.

Metabolite profile of spore germinating fluid (SGF) of Cg2 isolate of *Chaetomium globosum*

One mg ascospores of Cg2 were mixed with 100ml-distilled water. The ascospores suspension was incubated at 25°C for 48 hours. After incubation the ascospores were mounted on the slide and observed for the germination. The suspension was centrifuged at 10,000 rpm for 30 minutes and clear supernatant was decanted and stored for isolation of antifungal metabolites.

Cell free SGF was taken in 250ml separating funnel, 125ml of ethyl acetate was added and shaken thoroughly by gently releasing the air. The separating funnel was kept at rest on stand for 5 minutes and solvent portion was taken out and filtered through sterilized blotter sheet. This procedure was repeated three times. The solvent extracted portion was vaccum dried in rotatory evaporator and the compound obtained was dissolved in methanol and stored in small vials for further use

Thin layer chromatography was done using crude antifungal metabolites of culture filtrate of Cg2 and SGF of Cg2. The bands were observed under UV-transilluminator. The R_f value of each band was calculated.

Efforts were made to purify individual compounds through column chromatography using activated silica gel and hexane as solvent. Each fraction after drying was run on TLC plate in different solvent systems to see if the compound was pure. Column chromatomatography and subsequent thin layer chromatography was repeated till the compound obtained was pure.

The inhibitory effect of purified compound

of *C.globosum* on mycelial growth of *Bipolaris sorokiniana*, *Rhizoctonia solani*, *Fusarium udum*, *Macrophomina phaseolina* and *Pythium aphanidermatum* was studied by food poisoning technique. The bioassay was done taking three concentrations viz: 1000ppm, 500ppm, and 100ppm and three replicates were kept for each treatment.

The crude extracts obtained by all the isolates showed quantitative differences and differences in colour. It was found that maximum antifungal metabolites (47.0μg/mg of mycelium) were produced by Cg3 isolate followed by Cg2, producing 44.2μg/mg of mycelium. Isolate Cg4 produced 20.2μg metabolites, which was at par with Cg5. *C.globosum* isolate Cg1 produced minimum antifungal metabolites (7.44μg/mg of mycelium) among all the isolates (Table 1).

Thin layer chromatography showed that there are differences in the production of compounds by different isolates. Only two bands of R_f value 0.17 and 0.48 were found in isolate Cg1. Thirteen compounds were produced by Cg2 and eleven by Cg3. The band of R_f value, 0.19 and 0.82 were found extra in Cg2, which was absent in all other isolates. Isolate Cg4 produced seven bands and Cg5 produced only two bands, seven bands were resolved from Cg6.

The inhibitory effect of crude extracts of antifungal metabolites from *C. globosum* isolates on mycelial growth of *B. sorokiniana* showed that Cg2 isolate most significantly reduced the radial growth of the test pathogen causing 87.7percent reduction in growth, while Cg1 showed 65.4percent reduction over check (Table 2). The next best isolate was Cg6 showing 83.5percent reduction followed by Cg4 (83.1%) and Cg5 (81.1%).

Total 14 bands were observed in antifungal metabolites obtained from mycelium of Cg2, while in SGF of Cg2, 12 bands were observed. Bands of R_f value 0.8 and 0.9 were missing in SGF.

Through column chromatography eight compounds were purified. Out of these, one of the compounds Cg2 (A6) was purified in sufficient quantities for further *in vitro* bioassay against *B. sorokiniana*, *R. solani*, *F. udum*, *M. phaseolina* and *P. aphanidermatum*. *In vitro* bioassay with this compound showed maximum inhibitory effect on mycelial growth of *B.sorokiniana* followed by *F.udum* and *R.solani* at 1000ppm. As the concentration of compound decreased, the inhibitory effect also decreased. At 1000ppm concentration minimum colony diameter was observed in *B. sorokiniana* (4.06cm), followed by *F.udum* and (5.3cm), *M. phaseolina* (5.7cm) and *R.solani* (6.1cm) (Table 3).

Table 1. Total metabolites produced by *Chaetomium globosum* isolates

Isolate of *C. globosum*	Fresh mycelial weight in liquid media (mg)	Weight of antifungal metabolites (ug)/mg of mycelium
Cg1	46.4	7.44
Cg2	38.5	44.20
Cg3	30.6	47.00
Cg4	38.2	20.20
Cg5	28.0	24.00
Cg6	38.0	35.50
CD (P=0.05)	-	3.35

*Average of three replications

Table 2. Effect of crude extracts of antifungal metabolites of *C. globosum* isolates on growth of *Bipolaris sorokiniana*

Chaetomium globosum Isolate	Growth of *Bipolaris sorokiniana*	
	Colony Diameter (mm)	Reduction in growth (%)
Cg1	30.00c	65.38
Cg2	10.67a	87.68
Cg3	20.00b	76.92
Cg4	14.67b	83.07
Cg5	16.33	81.15
Cg6	14.33a	83.46
Check	86.67	-
CD (P=0.05)		5.56

Table 3. Effect of purified compound from *C. globosum* on growth of some soil borne pathogenic fungi

Concentration ppm	Colony Growth							
	Bipolaris sorokiniana		*Rhizoctonia solani*		*Fusarium udum*		*Macrophomina phaseolina*	
	Diam (cm)	% inhibition	Diam (cm)	% inhibition	Diam (cm)	% inhibition	Diam (cm)	% inhibition
1000	2.40	70.58	3.26	58.20	2.50	60.06	4.26	45.10
500	2.86	64.95	5.80	25.64	3.10	50.47	5.13	31.70
100	4.06	50.24	6.10	21.79	5.30	15.33	5.70	26.54
Control	8.16	-	7.80	-	6.26	-	7.6	-
CD (P=0.05)	0.83		1.10		0.60		0.81	

Understanding the mechanism (s) of action involved in biocontrol process is of primary importance for establishing the effectiveness of biocontrol agent and will provide much insight into where and when the interaction occurs and how the pathogen will be affected (Larkin *et al*, 1996), and also have the potential to interfere in the life process of plant pathogens. Our present results provide substantial evidence for antibiosis as the mechanism of antagonism by *C.globosum* against *B. sorokiniana* and other soil borne pathogens, as the potential isolate produced a large number of compounds. The isolates differed in their antagonistic activity and also differences in the production of secondary metabolites were reported. The potential isolate Cg2 produced thirteen compounds. One of the compounds purified was highly inhibitory to mycelial growth of *B.sokiniana*, *F.udum*, and *R.solani* at 1000ppm concentration. The spore germinating fluid of the potential isolate (Cg2) also produced same number of compounds as the culture grown in the laboratory, indicating

the role of antifungal compounds released at the site of application for the control of disease. Our earlier findings have also suggested antibiosis as the mechanism of antagonism by *C.globosum* (Aggarwal *et al.*, 2004). The role of antibiosis in the antagonists' activity of *C. globosum* against *Venturia inaegualis*, *Fusarium nivale*, *Pythium ultimum* and *Helminthosporium* sp. has been reported by various workers (Di Pietro *et al.*, 1992; Walther and Gindrat, 1988: Cullen and Andrews, 1984: Hubbard *et al.*, 1982 and Tveit and Wood, 1955).

The positive relation between the production of antifungal metabolite by *C.globosum* isolates in liquid culture or soil and efficacy in suppressing *Pythium* damping off has been reported by Di Pietro *et al.* (1992). The antagonism of *C. globosum* to *Ascochyta rabiei* has also been reported (Rajkumar *et al.*, 2005). Antifungal metabolite production by fungi exhibiting biocontrol activity has been most commonly reported for isolates of *Gliocladium*, *Talaromyces flavus* and *Trichoderma*. In our findings also, a potential isolate, Cg2 has been found to produce antifungal compounds and further work on characterization of the compounds and their mass production are the potential areas of the research.

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