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Performance evaluation of chaetoglobosin against fruit rot disease on chilli

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Abstract
Chaetoglobosin is a biomolecule produced by the fungus *Chaetomium globosum.* In the present study Chaetomium isolate Ch-1 was isolated from *Solanum tuberosum* and used for the extraction of secondary metabolite named as Chaetoglobosin. The extracted metabolite was injected in HPLC for confirmation and showed the retention time between between 7.51 and 7.53 min which is matching with the retention time of chaetoglobosin standard obtained from Sigma Aldrich. Co.LLC, USA. The efficacy of chaetoglobosin was tested against *Colletotrichum capsici* causing fruit rot disease on chilli under *in vitro* and glass house conditions. Chaetoglobosin at 2000 ppm concentration recorded 67.14 per cent inhibition of mycelial growth whereas at 1500 ppm showed 52.87 per cent mycelial inhibition over control. Among the different concentrations, chaetoglobosin at 2000 ppm showed 53.31 per cent inhibition of total biomass production against *C. capsici*. In the *in vivo*, chaetglobosin applied both individually and in combination with azoxystrobin and tebuconazole fungicides. The individual application of chaetoglobosin at 0.2 per cent concentration showed 56.78 per cent disease reduction over control. The combination of azoxystrobin (Willowood) with chaetoglobosin exhibited 81.82 per cent reduction of (10.69 PDI) fruit rot incidence. Tebuconazole and chaetoglobosin combination showed 76.73 per cent disease reduction (13.24 PDI) over control.

Keywords: Chaetoglobosin, chilli, fruit rot, biomass production

Introduction
Screening and utilization of *Chaetomium* species as potential biological control agents commenced in Thailand in 1989 (Soytong, 1992) [22]. *Chaetomium* species are normally found in soil and organic compost and it is one of the largest genera of saprobic ascomycetes with more than 300 species distributed worldwide (Arx *et al.,* 1986; Soytong and Quimio, 1989) [1, 21]. *Chaetomium* species are potential degraders of cellulosic residues and other organic materials (Domsch *et al.,* 1972) [8]. In addition, the antagonistic abilities of *Chaetomium* species against several phytopathogens have been well established. *Chaetomium* spp have also been reported to be potentially antagonistic to seed borne pathogens (Cullen *et al.,* 1984, Soytong and Quimio, 1989; Park *et al.,* 2005) [7, 21, 13]. Competition (for substrate and nutrients), mycoparasitism, antibiosis or various combinations of these mechanisms have been suggested as modes of action of *Chaetomium* species (Zhang and Yang, 2007, Cheng *et al*., 2017) [29, 6].

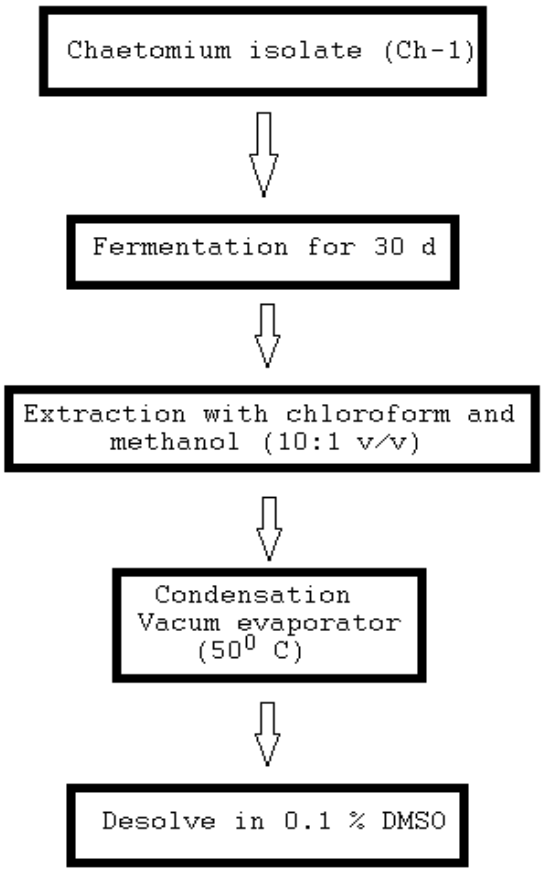
C. globosum and *C. cupreum* have been extensively studied and successfully used to control root rot disease of citrus, black pepper, strawberry and damping off disease of sugar beet (Tomilova and Shternshis, 2006) [27]. *C. globosum* has also been found to be an effective antagonist against *Colletotrichum* sp on soybean (Manandhar *et al.,* 1986). Chaetoglobosin is a secondary metabolite isolated from the fungus *Chaetomium globosum.* Sibounnavong *et al.,* (2011) [19] reported that Chaetoglobosin-C had showed greater antifungal activity against *F. oxysporum* f. sp. *lycopersici*, with an effective dose (ED50) of 5.98 µg / ml (Phonkerd *et al.,* 2008) [14]. Di Pietro *et al.,* (1992) [9] reported that *C. globosum* had produced chetomin, which effectively inhibited *Pythium ultimum*, the damping-off pathogen in sugar beet. *C. globosum* strain KMITL 0802 has been shown to produce chaetoglobosin - C (Kanokmedhakul *et al.,* 2002) [11]. Park *et al.,* (2005) [13] isolated chaetoviridin A from *C. globosum* strain F0142 and successfully contained rice blast, wheat leaf rust and tomato late blight pathogens. Soytong (1992) [10] and Soytong *et al.,* (2001) [23] showed that a specific isolate of *Chaetomium cupreum* had produced secondary metabolites, which were found to significantly suppress tomato wilt caused by *F. oxysporum* f.sp. *lycopersici* in Thailand.

The same isolate also produced rotiorinols A to C and rotiorin, which exhibited antifungal activity against *Candida albicans* (Kanokmedhakul *et al.,* 2006) [10]. *Chaetomium cochlioides* - strains VTh01 and CTh05 produced sufficient quantities of chaetoglobosin and exhibited antimicrobial activities against a *Phytophothora* sp, *Colletotrichum gloeosporioides*, and *F. oxysporum* f. sp. *lycopersici* (Pornsuria *et al.,* 2008, Song *et al.,* 2016) [20]. With this background the present investigation was carried out to estimate the efficacy of chaetoglobosin biomolecule against *Colletotrichum capsici* causing fruit rot disease in chilli.

Materials and methods

Extraction of the chaetoglobosin

The Schematic procedure followed is explained in Fig.1. Briefly, after fermentation, the culture medium and the mycelial mat were separated by filtration. The filtrate was extracted with organic solvents *viz*., chloroform and methanol (10:1 v/v). The extracted substance was condensed with the help of vacuum evaporator at 55° C until dry. The dry form of metabolite was collected by dissolving in 0.1 per cent dimethyl sufoxide (DMSO) and used for further study.



Scheme on extraction of chaetoglobosin from endophytic *Chaetomium* isolate Ch-1

Detection of chaetoglobosin through HPLC

The extracted chaetoglobosin was confirmed by High Performance Liquid chromatography (HPLC). The instrumental Parameters for HPLC as follows.

Instrumental method details	HPLC method
Name of equipment	Agilent 1200 HPLC System and AB Sciex API 4000
Column	Atlantis - dC18 (100 mm x 2.0 mm, 5 µm)
Guard Column	C18 (10 mm x 2.1 mm, 5 µm)
Injection Volume	10 µL
Autosampler Temp.	25 °C
Column Temp.	35 °C
Mobile Phase A	5 mM Ammonium Acetate in 80% water/20% MeOH
Mobile Phase B	5 mM Ammonium Acetate in 10% water/90% MeOH
Flow Rate	0.4 ml/minute
Wave length (λ_{max})	224 nm

Chaetoglobosin standard obtained from Sigma Aldrich. Co. LLC, USA. was made to 1000 ppm and diluted serially to acquire 0.1, 0.3 and 0.5 ppm. These dilutions were injected in HPLC at the rate of 10 µl and observed for the retention time and peak area. The dry form of extracted sample was collected after condensation and dissolved in methanol at the rate of 1mg / ml to attain 1000ppm concentration. Further, it was serially diluted to 0.1, 0.3 and 0.5 ppm. All these dilutions were injected in HPLC at the rate of 10 µL and noticed for the retention time and peak area. The similarity between chaetoglobosin standard and extracted sample was analyzed by comparing the data.

Determination of antifungal activity of chaetoglobosin

The antifungal activity of chaetoglobosin produced by the endophytic *Chaetomium* isolate Ch-1 was evaluated under *in vitro* by poisoned food technique (Schmitz, 1930) [17]. An eight mm diameter mycelial disc of seven d old pathogen culture was aseptically placed at the centre of Petri plates containing the PDA medium amended with the extracted chaetoglobosin at 500, 1000, 1500 and 2000 ppm. Growth medium inoculated without chaetoglobosin served as control.

The plates were incubated at 28 ± 2° C until the control plate was fully covered by the test fungus. Each treatment was replicated four times. The per cent growth inhibition of the test pathogen was calculated by using the following formula:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent inhibition
C = Growth in control
T = Growth in treatment

Efficacy of Azoxystrobin and Chaetoglobosin on Biomass Production of *C. capsici*

One hundred ml of potato dextrose broth was distributed in 250 ml Erlenmeyer flasks, autoclaved at 1.4 kg / cm^2 for 20 min and cooled at room temperature. The test fungicides were suspended aseptically in sterile distilled water and added to the broth to get the required final concentration. The flasks were inoculated separately with a 15 d old nine mm disc of the respective isolate of *C. capsici*. The flasks were incubated at room temperature (28 ± 2 ° C) for 20 d. Three replications were maintained. The mycelial mat was filtered through Whatman No. 1 filter paper, dried in the hot air oven at 60ºC for 24 h till a constant weight was obtained. The mycelial dry weight was calculated as per procedures suggested by Awadhiya, 1991 [2].

Efficacy of Chaetoglobosin in Comparison to Azoxystrobin and tebuconazole Against Fruit rot disease on Chilli.

Preparation of fungicide concentrations

The chaetoglobosin extracted from the endophytic *Chaetomium* and a new formulation of azoxystrobin biomolecule obtained from Willowood Agrochemicals Pvt. Ltd, New Delhi were used in the present study. These two biomolecules were compared with commercially available fungicides such as tebuconazole against fruit rot disease of chilli. All the fungicides at 0.2 per cent concentration (2 g / l) were sprayed as per the treatments given below:

T1 Azoxystrobin
T2 Chaetoglobosin
T3 Tebuconazole
T4 Azoxystrobin + Tebuconazole
T5 Azoxystrobin + Chaetoglobosin
T6 Tebuconazole + Chaetoglobosin
T7 Control

Results

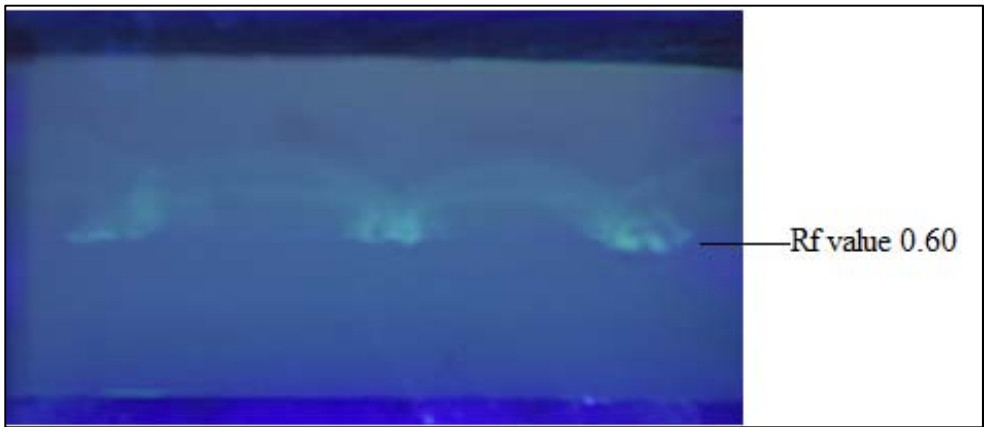
The *Chaetomium* isolate Ch-1 was selected based on its performance against the pathogens tested. The isolate Ch-1 was inoculated in 250 ml potato dextrose broth (PDB) and incubated at 28° C for30 d. After fermentation, the broth was filtered and extracted with the solvents such as choloform and methanol (10:1 v/v). The extracted substance was condensed with the help of vacuum evaporator at 55° C until dry. Finally it was suspended in 2 ml methanol and subjected to TLC and HPLC for confirmation.

The antifungal metabolite chaetoglobosin produced by the endophytic *Chaetomium* isolate Ch-1was verified by TLC. The metabolite chaetoglobosin exhibited the band with them R_f value of 0.60 in TLC plate. The extracted chaetoglobosin was further confirmed by HPLC. The chaetoglobosin pure standard was purchased from Sigma Aldrich. Co. LLC, USA. The standard was injected in 0.1, 0.3 and 0.5 ppm concentrations. The metabolite extracted from the isolate Ch-1 was also injected in the same concentrations. The retention time for chaetoglobosin standard was found between 7.50 and 7.52 min and the extracted metabolite showed the same range of retention time between 7.51 and 7.53 min. Chaetoglobosin at 2000 ppm concentration recorded 67.14 per cent inhibition of mycelial growth, whereas at 1500 ppm, 52.87 per cent mycelial inhibition of *C.capsici* over control was observed. Among the four different concentrations tested, chaetoglobosin at 2000ppm concentration showed the highest inhibition of biomass production of *C. capsici* (53.31 per cent) followed by 1500 ppm exhibited 35.69 per cent reduction of biomass production.

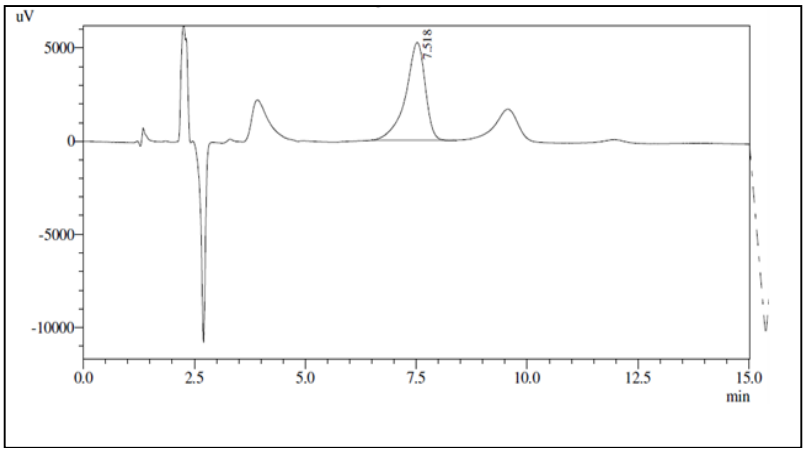
In the *in vivo* study, the individual application of chaetoglobosin, azoxystrobin (Willowood), and tebuconazole at 0.2 per cent concentration showed 56.78, 79.01, and 75.09 per cent disease reduction, respectively over control. The combination of azoxystrobin (Willowood) with tebuconazole showed the maximum disease reduction of 89.94 per cent (6.39 PDI) over control. This treatment was followed by the combinations of azoxystrobin (Willowood) with chaetoglobosin which exhibited 81.82 per cent reduction of (10.69 PDI) fruit rot incidence. Tebuconazole and chaetoglobosin combination showed 76.73 per cent disease reduction (13.24 PDI) over control.

Detection of chaetoglobosin (TLC & HPLC)

Detection of Chaetoglobosin through TLC



Chaetoglobosin Standard 0.5 ppm (HPLC)



Chaetoglobosin sample 0.5 ppm (HPLC)

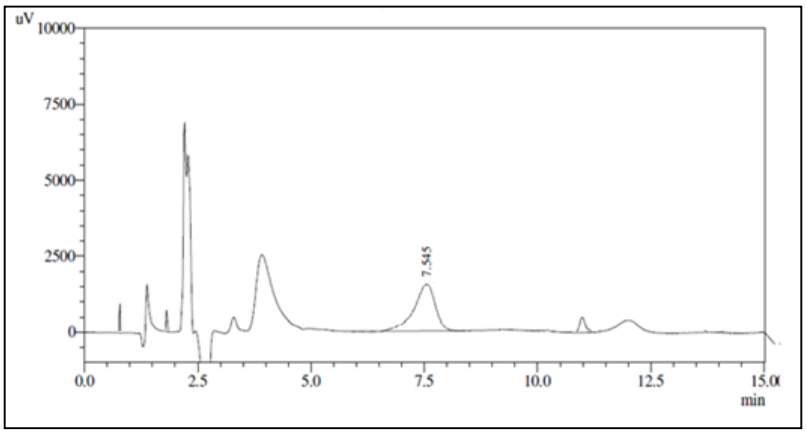


Table 1: *In vitro* evaluation of chaetoglobosin against *C. capsici*

Chaetoglobosin concentration (ppm)	*C. capsici*	
	Mycelial growth (mm)	Per cent inhibition over control
500	63.59[d] (52.88)	28.55
1000	59.28[c] (50.34)	33.39
1500	41.94[b] (40.36)	52.87
2000	29.57[a] (32.94)	67.14
Control	89.00[e] (70.63)	-

Mean of four replications
Values in parentheses are arcsine-transformed
In a column, means followed by same letter are not significantly different at the 5 per cent level by DMRT

Table 2: Efficacy of chaetoglobosin and azoxystrobin on biomass production by *C. capsici*

Biomolecules (ppm)	*C. capsici*	
	Mycelial dry weight (mg)	Percent inhibition over control
500	514.33[g]	10.60
1000	431.48[f]	25.00
1500	369.94[e]	35.69
2000	268.60[d]	53.31

Mean of three replications
In a column, means followed by same letter are not significantly different at 5 per cent level by DMRT

Table 3: Efficacy of Chaetoglobosin against fruit rot incidence on Chilli

Treatment	PDI				Per cent decrease over control
	Before spray	After I spray	After II spray	After III Spray	
Azoxystrobin 0.2%	30.76 [a] (33.68)	20.27 [c] (26.75)	16.34 [c] (23.84)	13.83[c] (21.83)	79.01
Chaetoglobosin 0.2 %	36.01 [f] (36.87)	31.78 [f] (34.31)	27.46 [f] (31.60)	23.51 [f] (29.00)	56.78
Tebuconazole 0.2%	35.42 [e] (36.52)	26.33 [e] (30.87)	19.83 [e] (26.44)	15.17 [e] (22.92)	75.09
Azoxystrobin 0.2% + Tebuconazol 0.2 %	34.95 [d] (36.24)	17.48 [a] (24.71)	9.54 [a] (17.99)	6.39 [a] (14.64)	89.94
Azoxystrobin 0.2% + Chaetoglobosin 0.2 %	33.15 [c] (35.15)	18.68 [b] (25.60)	13.55 [b] (21.59)	10.69 [b] (19.08)	81.82
Tebuconazol 0.2% + Chaetoglobosin 0.2 %	35.83 [f] (36.76)	23.84 [d] (29.22)	18.78 [d] (25.68)	13.24[d] (21.33)	76.73
Control	32.84 [b] (34.96)	46.75 [g] (43.13)	56.91 [g] (48.97)	63.54 [g] (52.85)	0.00

Mean of three replications
Values in parentheses are arcsine-transformed
In a column, means followed by same letter are not significantly different at the 5 per cent level by DMRT

Discussion

In the present study, the metabolite chaetoglobosin was extracted from the endophytic *Chaetomium* isolate Ch-1 as per the procedure derived by Yang *et al.,* (2012) [28] for testing its efficacy against *C. capsici.* Production of secondary metabolite by *Chaetomium* spp has been reported by several scientists. Cytochalasan-based alkaloid named chaetoglobosin U, along with four known analogues, chaetoglobosin C, F, and E and penochalasin A, have been characterized from the culture extract of *Chaetomium globosum* IFB-E019 (Brakhage *et al.,* 2008) [3]. Hui *et al.,* (2011) have extracted four different secondary metabolite namely, Chaetoglocin A, B, C and D from *Chaetomium*s pp. Ting *et al.,* (2010) [26] extracted Chaetoglobosin A and C from *Penicillium discolour* and confirmed by TLC and HPLC by comparing extracted compound with authentic standards. Burlot *et al.,* (2003) [4] extracted chaetoglobosin and confirmed through TLC which showed the Rf value of 0.55. The TLC result of the present experiment exhibited the Rf value of 0.60 which exemplified 91.67 per cent similarity with the earlier report. Susheel *et al.,* (2013) [25] extracted chaetoglobosin from *Chaetomium globosum* and confirmed through LCMS which showed the retention time of 33.33 min. Yang *et al.,* (2012) [28] extracted chaetoglobosin from eight different *Chaetomium* isolates and confirmed through HPLC. Chaetoglobosin extracted from all the eight isolates showed the retention time between 10.90 to 11.10 min. In the current study, the chaetoglobosin standard obtained from Sigma Aldrich. Co.LLC, USA and injected in HPLC at various concentrations *viz.,* 0.1, 0.3 and 0.5 ppm showed the retention time of 7.50 min. The extracted metabolite injected at the same concentrations exhibited retention time ranging between 7.51 and 7.53 min. By comparing the HPLC results of chaetoglobosin standard, the extracted metabolite was confirmed as chaetoglobosin.

The extracted chaetoglobosin was evaluated at four different concentrations against *C. capsici*. Among which, chaetoglobosin at 2000 ppm showed 67.14 inhibition of *C. capsici* under *in vitro*. Results of this experiment clearly revealed that the antifungal potential of chaetoglobosin increased with a concomitant increase in the concentration used. The secondary metabolite chaetoglobosin also showed significant reduction of mycelial dry weight over control. But the rate of inhibition was not up to the level of azoxystrobin. Soytong, (2007) [24] reported that the bioactive compound extracted from *Chaetomium cochliodes* and *C. cupreum* inhibited plant pathogenic fungi, *Phytophthora palmivora* (root rot of Pomelo) and *Fusarium oxysporum* f. sp. *lycopersici* causing wilt disease in tomato (Qin *et al.,* 2009 and Shuang, *et al.,* 2017) [16, 18]. Charoenpoen *et al.,* (2010) reported that crude extract of *Chaetomium lucknowense* CLT significantly inhibited the mycelial growth and conidial production of *F.oxysporum* f. sp. *lycopersici.* In the bioefficacy experiment, the efficacy of azoxystrobin, chaetoglobosin and tebuconazole are tested individually and in combinations. It revealed that the individual effect of each compound was comparatively lesser than the combined applications. Among the different treatments, azoxystrobin with tebuconazole combination showed the maximum disease reduction against fruit rot incidence on chilli. Azoxystrobin combined with chaetoglobosin also showed considerable rate of inhibition when compared to control. Through this study it is evident that the metabolite chaetoglobosin can be used as an antifungal compound against plant pathogenic fungi. But the mode of action of chaetoglobosin against the pathogenic fungi is not identified clearly sofar. It needs further elaborate study.

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