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Deciphering anti-Microbial compounds Produced by *Chaetomium globosum* (TNAU *Cg*- 6) against Early Blight (*Alternaria solani)* in Potato- an Eco-friendly Approach

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ABSTRACT: The escalating demand for sustainable and eco-friendly strategies to combat plant pathogenic fungi has fueled the exploration of biocontrol agents. Among the biocontrol agents, *Chaetomium globosum* has shown promising antagonistic potential against various soil-borne plant pathogens. They are considered to be a rich source of novel and bioactive secondary metabolites of great importance. In this study, we investigated the antagonistic interactions between *Chaetomium globosum* and *Alternaria solani*, a notorious fungal pathogen causing early blight disease in potato crops, using dual culture technique. The result revealed that TNAU Cg-6 has recorded the maximum inhibition of 75.22% compared to control. The secondary metabolites are identified using GC-MS technique and 7 beneficial compounds are identified with anti-microbial properties. Harnessing the anti-microbial potential of *Chaetomium globosum* (TNAU Cg-6) could pave the way for innovative, biologically-based disease management solutions in agriculture, promoting sustainable crop production and safeguarding the environment. Some of the challenges include identifying and characterizing the specific antimicrobial compounds, antimicrobial activity may arise from the synergistic effects of multiple compounds, determining the optimal concentration of the antimicrobial compounds for effective disease control without causing harm to the environment, the crop, or beneficial organisms is essential, ensuring that the antimicrobial compounds target only the pathogen (*Alternaria solani*) while sparing beneficial microorganisms and non-target organisms is crucial. Before commercial application, any novel antimicrobial compound must go through rigorous testing to meet regulatory standards for safety, efficacy, and environmental impact, developing and producing antimicrobial compounds on a scale that is economically viable for farmers can be challenging. Some of the contributions made by the researchers include, compound identification, mechanism of action, conducting field trials to evaluate the efficacy of the antimicrobial compounds under real-world conditions, etc. Further investigations are warranted to optimize the formulation and application methods for large-scale implementation of this eco-friendly approach in potato cultivation.

Keywords: Potato, *Alternaria solani*, *Chaetomium globosum* (TNAU Cg-6), *in vitro* screening, GC-MS and Secondary metabolites.

INTRODUCTION

Potato is the world's largest vegetable crop with an average production of 325 million tons/year whereas India produces an average of 123.06 million metric tons annually, with an area and productivity of 4.62 million hectares and 26.61MT/hectares, respectively (Anonymous, 2015). It is a staple food in most of countries across the world. During the crop growth, biotic and abiotic stresses are the major production constrains (Wang *et al*., 2016). Some of the emerging diseases include powdery scab, wart, tuber necrotic viruses and phytoplasma. In India, the major diseases infecting potato are early blight, late blight and potato scab. Early blight of potato caused by *Alternaria solani* was identified by Sorauer. The average annual losses due to blight have been estimated to be 15% of total production in the country. With this background of study, the present study has been focused to combat the early blight of potato using *Chaetomium globosum.*

The management for early blight of potato includes clearing of the infected debris from the field to reduce inoculums for the next year, planting resistant cultivars, spraying of fungicide like azoxystrobin, pyraclostrobin etc. As the residual effects of fungicides are more, we opt for an environmentally friendly biocontrol agent (Kumar *et al*., 2020a). One such effective biocontrol agent for potato against early blight is *Chaetomium globosum.* In that, *Cg*6 is an elite strain and already tested against several diseases of horticultural crops. *Chaetomium globosum* is a hyperparasitic fungus frequently isolated in different ecosystem of plant and soil. The *C. globosum* reported as potential biocontrol agent and also produces several antibiotic namely chaetoglobosins A and C (Kumari *et al*., 2022). It is also helpful in growth promotion of plants.

MATERIALS AND METHODS

Collection of Pathogen and biocontrol agent. The strain of the phyto-pathogen, *Alternaria solani* were collected from ITCC (Indian Type Culture Collection), Division of Plant Pathology, ICAR-IARI, New Delhi. The cultures were maintained at 25±l °C by periodic sub-culturing on Potato Dextrose Agar (PDA). *C. globosum* TNAU *Cg*6 were collected from the Department of Plant Pathology, TNAU, Coimbatore. It is used for screening as biocontrol agent in the study. The strains were maintained on PDA slants at 4°C after growing for seven days at 25±1 °C for further study.

Radial growth of *C. globosum* strain. On PDA medium, the colony growth of *C. globosum* was observed. For this, a sterilized Petri plate containing PDA medium was aseptically filled with mycelia of a 4 day old *C. globosum* culture with a diameter (disc) of 5 mm. After 6 days of incubation at 25 1 °C, the radial growth (cm) of the plates was determined (Rashmi, 2015). There were kept three replications. *C. globosum* strains were raised in Petri plates filled with PDA medium and kept at a temperature of 25°C (Biswas *et al*., 2012). 20-day samples of 5 mm discs were homogenized in 10 mL of sterile, distilled water after being randomly cut at four locations. Haemocytometer was used to count the ascospores, which were then expressed as spores/petridish.

***In-vitro* antagonistic assay.** Antagonistic potential of *C. globosum* strain against phyto-pathogens were observed by Dual Culture Technique (Dennis and Webster 1971) on PDA medium. One end of Petri dish (9 cm) containing PDA was inoculated with 5mm mycelial disc of five days old culture of *C. globosum* and the opposite side with 5mm mycelia disc of *Alternaria solani*. All the treatments were replicated three times. The plates were sealed with parafilm and kept at 25±1 °C for 7 days in room temperature.

Per cent growth inhibition (I %) = (C-T)/C× 100

Where C is the radial growth in control and T is the radial growth in treatment.

Mass production of *C. globosum.* PDB was added to 1000 mL culture flasks containing *Chaetomium globosum* (TNAU *Cg*-6), which were then inoculated at 25 °C. Under laminar air flow, 3–4 (5mm) discs of a *C. globosum* culture that was actively developing were put into each flask. 1 L of culture was produced in total. The fungal biomass was extracted aseptically following a 21-day incubation period (Jiao *et al*., 2004). PDB also received an injection of *Alternaria solani* and TNAU *Cg*-6. The biomass was extracted and put through additional processing after a few days.

Gas Chromatograph-Mass Spectrometry (GC-MS) Analysis. A triple axis mass spectrometer (Thermo Fisher, Mo, USA) and a 30 m × 0.25 mm HP-5MS column (30 m × 0.25 mm, Thermo Co., USA) were used for the analysis. With split control enabled, the injection volume was 1 mL. Helium 1 mL/min was chosen as the carrier gas flow (Zhao *et al*., 2017). Here is a description of the GCMS condition. For the first 2 minutes, the oven's temperature was maintained at 60 °C. The temperature was then raised with a gradient of 3 °C/min until it reached 120 °C and was held there for 2 min before being elevated again with a gradient of 5 °C/min up to 220 °C (Kaur *et al*., 2020). With an increase of 4 °C/min, the temperature was finally raised to 280 °C (Aggarwal *et al*., 2007). Using the NIST (National Institute of Standards and Technologies) Mass Spectra Library as a guide for identifying the volatile components and their mass fragmentation pattern, compounds were identified by comparing their mass spectral data (Zhang *et al*., 2012).

Statistical analysis. All the data were subjected to statistical analysis with software, Microsoft excel for windows 2007 add-in with XLSTAT version 2010.5.05 (XLSTAT, 2010).

RESULT AND DISCUSSION

Cultural and morphological characteristics of *A. solani.* The pathogen was collected from ITCC (Indian Type Culture Collection), Division of Plant Pathology, ICAR-IARI, New Delhi. The pathogen was maintained on PDA medium. The Colony morphology of *A. solani* varies widely, but is generally effuse, grayish brown to black, with cotton-, felt- or velvet-like texture. Similarly, Orange to dark red pigments, cottony irregular with concentric zonation and agar green white margin are produced (Plate 1). Cells of *A. solani* are multinucleate, but different organs vary in the number of nuclei. Nuclear division in hyphal cells is followed by multiple septations, which results in the division of elongated tip cells into several multinucleate cells (Marak *et al.*, 2014).

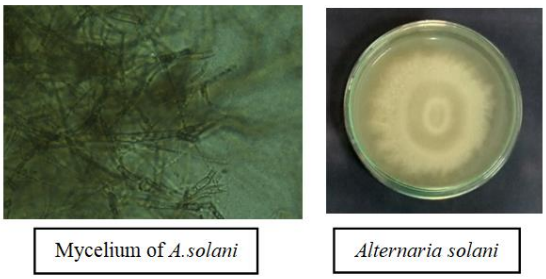


Plate 1. Morphological and cultural characteristics of *Alternaria solani.*

Conidiophores are dark or olivaceous brown, thick-walled, straight to flexuous, septate; arise singly or in small groups, up to 110 µm in length and 6–10 µm in

diameter (Aggarwal, 2015). Conidia are usually pale to olivaceous-brown, produced singly or seldom in short chains, straight or slightly flexuous, obclavate to elongate, double walled with 0–8 longitudinal or oblique and 6–19 transverse septa, 75–350 µm in length and 20–30 µm in diameter in the broadest part. Beaks are about one-half to double the length of the conidium, filiform, straight of flexuous, septate, hyaline to pale brown and 5–9 µm in diameter (Wang *et al*., 2016).

Cultural and morphological characteristics of TNAU *Cg*-6. The biocontrol agent *C. globosum* (TNAU *Cg*6) was collected from the Department of Plant Pathology, TNAU, Coimbatore. The colony diameter is 9cm with rapid colony growth and the colour of the colony varied from white to olive green. The margins are smooth with raised fluffy growth and without zonation. They produce perithecia and asci with ascospores. When observed under microscope, the sporulations were 8 to 10 ascospores per microscopic view at 40x. Dark green globose ascomata with terminally coiled ascomatal hairs are observed. The ascomata size is 62.05 × 44.86µm, the ascospores are slightly elliptical with 8.6×7.7 µm at 40x. It is maintained in PDA medium (Plate 2).

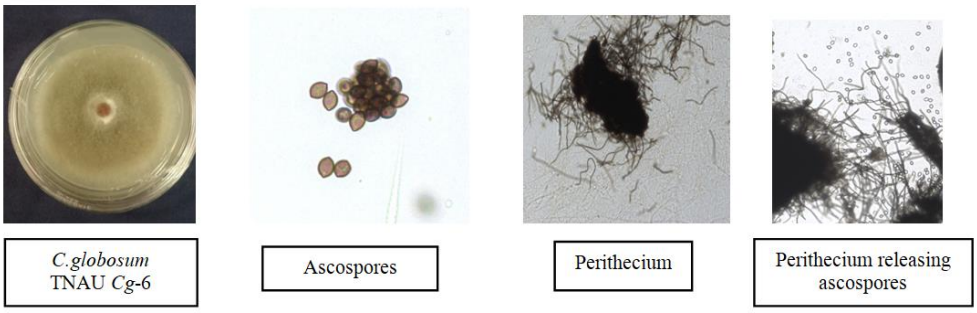


Plate 2. Morphological and cultural characteristics of *Chaetomium globosum* TNAU *Cg*-6

Aggarwal (2015) noted that the perithecia of *C. globosum* are ostiole-shaped, subglobose, varied in shape, opaque when mature, and black (200-320 × 200-280 m). Asci have eight spores and are atypically club-shaped. The ascospores of *Chaetomium* sp. varied in size from 9 to 13 × 6 to 9.5 m and contained numerous sizable refractive globules that were olive-brown, with apiculate or rounded ends. Similar to the above, Uma maheswari *et al.* (2015) found that ascospores are olive green in colour, lemon shaped (8-12 × 6-9 m), and produce club-shaped asci and black-colored ovoid globose perithecia (180-370 × 180-300 m). The initial descriptions of these traits provided by Ames (1961); Soytong *et al*. (2001) all matched. Similar results of variation in the cultural characteristics were reported in the present study.

***In vitro* screening of TNAU *Cg*-6 against pathogen *Alternaria solani*.** TNAU Cg-6 was tested against *Alternaria solani* under *in vitro* condition. The mycelial growth of the pathogen was analyzed at 13th day after inoculation. TNAU *Cg*-6 recorded maximum inhibition against pathogen (2.23cm) with the percent inhibition of 75.22. This effective isolate were used for further study (Table 1, Plate 3). Similarly, all eight *Chaetomium* isolates were found to inhibit the growth of *P. infestans* in vitro, however, the isolate Cg-6 showed higher inhibition to *P. infestans* followed by *Cg*-5 and *Cg*-2. The percent inhibition of *P. infestans* in vitro was significantly higher in Cg-6 (72.3%) when compared to other isolates Cg-1 (64.5%), Cg-3 (62.2%), Cg-4 (61.2%) and Cg-5 (60%) (Shanthiyaa *et al*., 2013).

Table 1: *In vitro* screening of TNAU *Cg*-6 against pathogen *Alternaria solani*.

Sr. No.	Isolate name	Radial growth of mycelium (cm)*	% inhibition	SE(d)	CV
1.	*A. solani*	2.23	75.22	0.11	6.08
2.	Control	9.00	-	-	-

*values are mean of three replications. Means in a column are not significantly different according to DMRT at P≤ 0.05

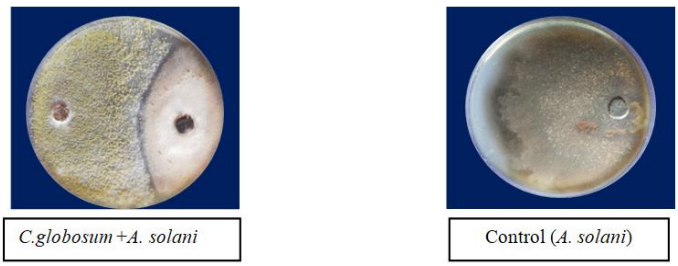


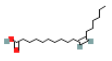
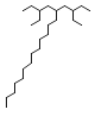
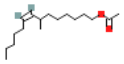
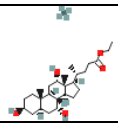
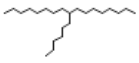
Plate 3. *In vitro* antagonistic activity of *Chaetomium globosum* (TNAU *Cg*-6) against *Alternaria solani* causing early blight in potato.

The experiment's findings demonstrated that each bioagent employed as spore suspension lessened the severity of the tomato leaf spot disease brought on by *A.alternata*. The effectiveness of *C.globosum* appears to have outweighed that of *A. niger* and *Penicillium* sp. *A.alternata* + *C.globosum* treatment reduced illness severity by 21.6% compared to control treatment (*A.alternata* alone), which reduced disease severity by

49%. The remaining treatments reduced disease severity by 23.3 to 25% (Fayyadh & Yousif 2019). Additionally, *C.globosum* was found to stimulate the production of pathogenesis-related proteins (PRP) in plants (Ahammed *et al.*, 2008).

Identification of secondary metabolites from TNAU *Cg*-6 by GC-MS. Bioactive substances with a range of retention durations (RTs) were found by GC-MS analysis. In the potential isolates, 40 substances were found. The putative antibiotic substances such as caprolactam (6.52 RT), n-hexadecanoic acid (18.78 RT), 9-octadecenoic acid (21.61 RT), abietic acid (25.83 RT), and hexadecanoic acid (28.06 RT) were discovered in the TNAU Cg-6 isolate (Rajendran *et al.*, 2023). When it comes to the interaction of TNAU *Cg*-6 and *Alternaria solani*, anti-microbial substances like 1-Heptatriacotanol, cis-Vaccenic acid, 17-Pentatriacontene, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Ethyl iso-allocholate, and Heptadecane, 9-hexyl- were found (Table 2, Fig. 1).

Table 2: Identification of secondary metabolites from TNAU *Cg*-6 by GC-MS.

Sr. No.	RT	Compound name	Molecular formula	Molecular weight (g/mol)	Molecular structure	Biological activity	Area (%)
1.	22.791	1-Heptatriacotanol	$C_{37}H_{76}O$	537		Anti hypercholesterolemic effect	0.511
2.	23.802	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5		Anti bacterial	1.273
3.	28.199	17-Pentatriacontene	$C_{35}H_{70}$	490.9		Anti bacterial, Anti inflammatory, Anti cancer	1.213
4.	28.314	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7		Antimicrobial, Antifungal, anticancer, anti arthritic	1.401
5.	28.399	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.4		Anticancer, Anti inflammatory	0.586
6.	28.424	Ethyl iso-allocholate	$C_{27}H_{48}O_5$	452.7		Anti tumour	0.684
7.	28.889	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324.6		Antifungal	0.947

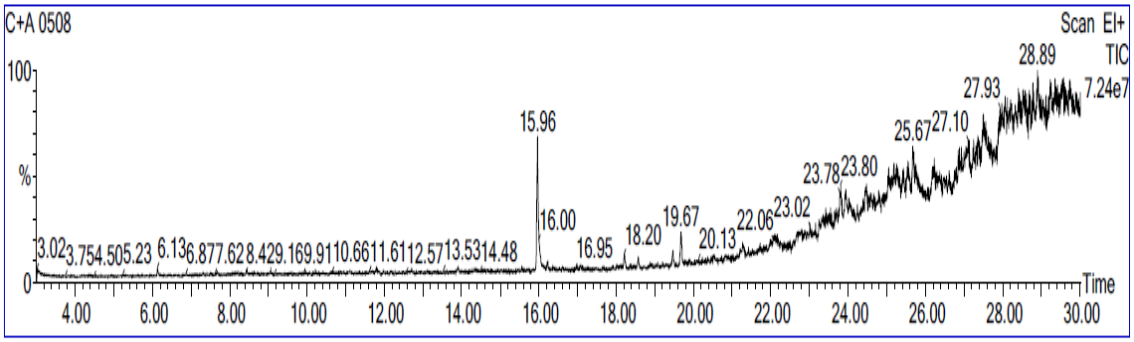


Fig. 1. Identification of secondary metabolites during di-trophic interaction by GC-MS.

(TNAU *Cg*-6+*Alternaria solani*). *Chaetomium globosum* Kunze (*Chg1–Chg9*), screened to analyse the secondary metabolites using Gas Chromatography-Mass Spectrometry (GC-MS), *Chg2* revealed the presence of major compounds, *viz*., benzothiazole, 2-(2-hydroxyethylthio) (7.51%); 9,12,15- octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester (3.13%); and hexadecanoic acid, 1- (hydroxymethyl)-1,2-ethanediyl ester (2.69%).

The presence of hydrocarbons, phenols, terpenoids, and sulphur compounds, accounting for 73.6% of the total concentrate, was discovered by Kumar *et al.* (2021b) in

the crude extract of *C. globosum* isolates. In the tomato crop, during the interaction with TNAU *Cg*-6, the secondary metabolites were observed *viz*., 4-methyl-1,5-dimethyl-4-hexenyl-benzene (9.3%), tetradecane (6.8%), dodecane (6.3%), hexadecane (6.1%), -bisabolene (3.5%), and dimethyl-propyl-disulphide (1.34%) were the most significant anti-microbial chemicals (Rajendran *et al*., 2022).

CONCLUSIONS

The biological management of foliar pathogens has drawn more attention recently as a potential candidate for substituting chemical control. Overall, our findings indicate that TNAU Cg-6 have the potential to serve as effective biocontrol agents against *Alternaria solani.* This study contributes to the exploration of eco-friendly and sustainable strategies to manage early blight disease in solanaceous crops. Further research on field trials is warranted to assess the practical applicability and long-term efficacy of dual-plate *Chaetomium* as a biocontrol agent for *Alternaria solani* in agricultural settings.

FUTURE SCOPE

The future scope is highly promising. It aligns well with the global push towards sustainable and eco-friendly agricultural practices while addressing the challenges associated with conventional chemical-based disease management. Effective metabolites which are identified can be further used for promoting the growth of the crop and can be designed as a biomolecule. However, further research, field trials, and commercialization efforts are necessary to fully realize the potential benefits of this approach in real-world farming scenarios.

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Conflict of Interest. None.

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