



RESEARCH ARTICLE

ANTAGONISM OF INDIGENOUS FUNGAL ISOLATES AGAINST *BOTRYTIS CINERIA* THE CAUSAL OF GRAY MOLD DISEASE OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

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ABSTRACT

The efficacy of indigenous fungal isolates associated with tomato plant against the Gray mold pathogen *Botrytis cineria* was evaluated *in vitro*. The investigation has dual purpose, firstly identification of indigenous fungal isolates from *Solanum lycopersicum* L.; secondly, to evaluate their antagonistic property against *Botrytis cineria* using the dual culture method. A total of 96 fungal species were isolated from the rhizospheric soil of *S. lycopersicum* of which, 81 species belonged to Ascomycota and 12 species belonged to Zygomycota. Among the fungal isolates, 14 fungal species, namely *Aspergillus flavus*, *A. fumigatus*, *Chaetomium elatum*, *C. globosum*, *Gliocladium roseum*, *G. viride*, *Myrothecium verrucaria*, *Trichoderma hamatum*, *T. harzianum*, *T. polysporum*, *T. pseudokoningii* and *T. viride* were selected for antagonistic effect against *B. cineria*. Of 14 selected fungal species, *C. globosum* have the highest percentage of inhibition of 80% and *G. viride* have the least percentage of inhibition of 29.33% against *B. cineria*. Among the test organisms, a zone of inhibition was produced only by *C. elatum* (0.2cm), *G. viride* (0.3cm), *M. verrucaria* (0.4cm), *T. hamatum* (0.3cm) and *T. harzianum* (0.2cm) while an intermingled zone was produced only by *A. fumigatus* (1.8cm) and *Penicillium canescens* (0.5cm). Out of 14 test organisms, *C. globosum*, *Penicillium chrysogenum*, *A. flavus* and *A. fumigatus* and *Trichoderma* species may be recommended as good sources of biocontrol agents against *B. cineria* the causal organism of tomato gray mold disease.

Key words: *Solanum lycopersicum* L., fungal isolates, *Botrytis cineria*, antagonistic effect.

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INTRODUCTION

Microorganisms play important roles in the growth and ecological fitness of their plant host (Martin *et al.*, 2001). The role of soil fungi in nutrient cycling, and plant health and development is extremely complex and is fundamental to the soil ecosystem (Thorn, 1997 and Bridge and Spooner, 2001). Some fungi antagonize plant pathogens, decompose plant residues, provide nutrients to plants, and stimulate plant growth, while others cause a range of plant diseases (Thorn, 1997). Fungal plant diseases are one of the major concerns to agricultural production. More than 10,000 species of fungi can cause disease in plants (Agrios, 2005). Tomato (*Solanum lycopersicum* L.) which is a member of Solanaceae (nightshade) family (Nonnecke, 1989) is one of the world's most widely cultivated, popular and economically important 'vegetable crops'. It is the second most important vegetable crop next to potato (Dorais *et al.*, 2008). Tomato plants are subjected to attack by several soil borne fungal pathogens such

as early blight, *Fusarium* wilt and bacterial wilt which cause serious diseases under greenhouse as well as field conditions (Agrios, 1997). Gray mold, caused by the fungus *Botrytis cineria*, is a common disease of Solanaceous crops that can be particularly damaging in greenhouse environments. It is a major cause of post-harvest rot at harvest and in storage (Coley Smith *et al.*, 1980). The most common method to check the diseases is by using fungicides. However, due to the non-biodegradable nature and the pollution caused by the use of such chemicals and the development of resistance by pathogens to fungicides biological control using potential microorganisms having strong fungal activity offers an important alternative to synthetic chemicals for disease management. Although chemical measures improve the crop health but it may establish imbalances in the microbiological community unfavourable for the activity of beneficial organisms. Owing to the concerns about the safety and environmental aspects of chemicals, biological control avoids the problem of pesticide resistance and also offers the chance to improve crop production within the existing resources (Khan *et al.*, 2014). The present investigation focuses on

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screening and identification of fungi isolated from rhizospheric soil of *Solanum lycopersicum* L. and to assess antagonistic effects of certain selected fungi against *Botrytis cineria* the causal organism of gray mold disease in tomato.

MATERIALS AND METHODS

Isolation and Identification of Rhizospheric Fungi

Study site

The present study was carried out in Mawryngkneng village, East Khasi Hills, Meghalaya, India which is located between 25°32.907'N and 92°02.495'E.

Collection of soil samples

Soil samples were collected aseptically from the rhizospheric soil at monthly intervals for a period of two years in the year 2015-2016. For rhizospheric soil sampling, three tomato plants were uprooted and complete root system with soil adhering to it was removed with the help of a sterilized digger and collected in sterilized polythene bags. The soil samples collected were stored at 4°C for further analysis.

Isolation and enumeration of fungi

Serial dilution plate method (Johnson and Curl, 1972) was followed for the isolation of rhizospheric and non rhizospheric fungi using Rose Bengal Agar medium (Martin, 1950). The plates were incubated at 25°C for 5 days. Colony forming unit (CFU) of fungi was estimated by counting the number of fungal colonies. The CFU per gram soil was calculated on the dry weight basis.

Identification of fungi

The fungal species were identified on the basis of their morphology and reproductive structures by consulting monographs by Subramaniam (1971), Barnett and Hunter (1972), Ellis (1972) and Domsch *et al.* (1980).

Isolation of fungal pathogen

Method of sample collection

Tomato leaves showing typical symptoms of gray mold disease were collected from the farmer's field. These infected leaves sample were kept in a sterilised polythene bag and taken to the laboratory for further investigation.

Isolation of *Botrytis cineria*

B. cineria was isolated from the tomato leaves with typical symptoms. Young and healthy tomato fruits were picked from tomato plants grown in the field. The fruits were surface sterilize in 96% ethanol for 10 minutes and placed on a sterile slicing board for about 1 minute to allow the ethanol to evaporate. Using the sterile knife, the tomato fruits were cut into 5mm thick slices. The infected tomato leaves were cut into small pieces (10-15mm). The infected discs were then covered by the tomato fruit slices in 94mm diameter sterile petri dishes and incubated at room temperature (25°C) for 5 days.



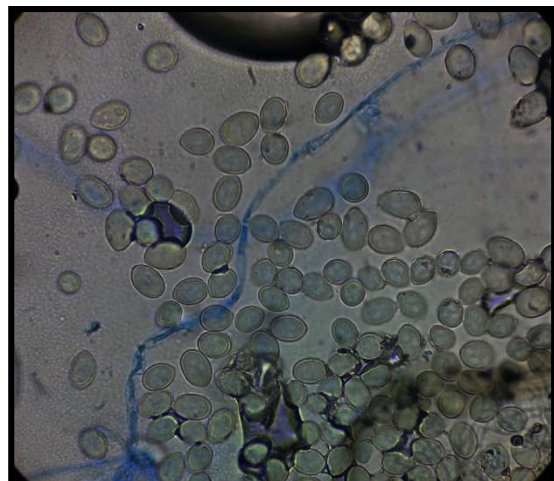
Plate 1. *Botrytis cineria* culture on tomato fruit slices

Identification of *B. cineria*

The colony was morphologically and reproductively identified by staining with lacto phenol cotton blue and observing under microscope. Conidiophores arising irregularly, often in patches without a basal swelling, 750µm to over 2mm long, brown below, smooth walled, 16-30µm wide, with an apical head of alternate branches.



(a)



(b)

Plate 2. (A) Pure culture of *B. cineria* isolate and (B) Morphological structure of *B. cineria* isolate grown at 25°C for 7 days- microscopic view (40x magnification)

Study of Antagonistic Effects

The antagonistic activity of selected fungal isolates was studied following the method of Skidmore and Dickinson (1976) by the presence or absence of inhibition zone observed in dual cultures. The fungal isolates were categorized as effective based on their ability to overgrow and inhibit the growth of the pathogens by giving them a score as per modified Bell's scale (Bell *et al.*, 1982). Fourteen fungal isolates namely *Aspergillus flavus*, *A. fumigatus*, *Chaetomium elatum*, *C. globosum*, *Gliocladium roseum*, *G. viride*, *Myrothecium verrucaria*, *Trichoderma hamatum*, *T. richoderma harzianum*, *T. polysporum*, *T. pseudokoningii* and *T. viride* were tested for antagonism against *B. cineria*, by dual culture techniques. In this method the mycelial discs of 5mm diameter were cut from the margins of actively growing region of 5 day old cultures of the fungal isolate and the pathogen and were placed on the opposite end of the sterile Czapek Dox (CDA) plates. For each treatment three replicates were maintained with one control set in which only the pathogen was inoculated. In dual cultures the fungal isolates were categorized as effective based on their ability to overgrow and inhibit the growth of the pathogens by the presence or absence of the inhibition zone, which was measured as percentage of inhibition of radial growth of *Botrytis cineria*.

Percentage of inhibition was calculated using the formula:

$$\frac{R1 - R2}{R1}$$

Where, R1 = radius of the radial growth of the pathogen towards the opposite side in control plate:

R2 = radius of the radial growth of the pathogen towards the opponent antagonist in test plate.

RESULTS AND DISCUSSIONS

Isolation and Identification of Fungi

A total of 96 fungal species were isolated from rhizospheric soil of *S. lycopersicum* L. for a period of two years in the year 2015 and 2016. Amongst the fungal species isolated 81 species belonged to the Ascomycota, 12 species belonged to Zygomycota and 3 sterile mycelia. Ascomycota was represented by 29 genera and 81 species and Zygomycota by 4 genera and 12 species. Qualitatively, there was not much difference in the composition of the fungal species isolated from rhizosphere soil for both the years. *Ascomycota* is the largest and widespread phylum of fungi and is abundant in soil and composts (Abed *et al.*, 2013; De Gannes *et al.*, 2013; Kazeeroni and Al-Sadi, 2016). They are considered important decomposers and causal agents of several soil-borne diseases.

Species such as *Absidia corymbifera*, *A. glauca*, *Acremonium butyri*, *A. cerealis*, *A. furcatum*, *Aspergillus flavus*, *Chrysosporium merdorum*, *Eupenicillium lapidosum*, *Gliocladium virens*, *Gonytrichum macrocladum*, *Oidiodendron griseum*, *Penicillium corylophyllum*, *P. daleae*, *P. expansum*, *P. fellutanum*, *P. italicum*, *P. nigricans*, *P. sacculum*, *P. spinulosum*, *P. steckii*, *P. variabile*, *P. verrucosum*, *Phoma destructiva*, *Sesquicillium buxi*, *Sporothrix schenckii*, *Verticillium species*, *Mortierella verticillata*, *Rhizopus oryzae* and *R. stolonifer* were isolated only from the first year of sampling i.e., 2015. Whereas, *Absidia cylindrospora*, *A. spinosa*, *Acremonium murorum*, *Arthrinium*

phaerospermum, *Aspergillus fumigatus*, *Chaetomium elatum*, *Cladosporium cladosporioides*, *C. herbarum*, *C. macrocarpum*, *C. sphaerospermum*, *Cylindrocladium parasiticum*, *C. scoparium*, *Daratomyces sinema*, *Gliocladium viride*, *G. species*, *Gongronella butleri*, *Iyengarina elegans*, *Mortierella alpina*, *Mucor circinelloides*, *Nannizzia grubyia*, *Paecilomyces forinoseus*, *P. marquandii*, *Penicillium crustosum*, *P. digitatum*, *P. restrictum*, *P. stoloniferum*, *Sclerotinia sclerotiorum*, *Scopulariopsis brevicaulis*, *Sesquicillium candelabrum*, *Stachybotrys echinata*, *Trichocladium asperum*, *Trichoderma hamatum*, *Trichodermaharzianum*, *T. polysporum*, *T. pseudokoningii* and *Verticillium chlamydo sporium* were isolated only from the second year of sampling i.e., 2016. *Myrothecium verrucaria* was found to be the dominant species in both the years. *Gliocladium* and *Chaetomium* species were common occurrence in all the sampling periods. Out of the total 83 fungal species isolated, 14 fungal species were selected to assess the antagonism against the plant pathogen, *Botrytis cineria*.

Antagonism in dual culture

The selected fungal species tested in this study exhibited antagonistic activities against the pathogen *B. cineria*. Radial growth of the pathogen was considerably hindered and there was a significant difference in percentage inhibition of radial growth of pathogen by all the test antagonists. Among the test organisms, *Chaetomium globosum* was found to be the most antagonistic and inhibited the radial growth of the pathogen while *Gliocladium viride* was found to be the least antagonistic. Among the test organisms, zone of inhibition were produced only by *Chaetomium elatum*, *Gliocladium viride*, *Myrothecium verrucaria*, *Trichoderma hamatum* and *T. harzianum*. The intermingle zone between *Aspergillus fumigatus* and *Penicillium canescens* was found to be significantly different. *Chaetomium globosum* inhibits the maximum growth of inhibition of *B. cineria* as compared to the other 13 test antagonists i.e. *Aspergillus flavus*, *A. fumigatus*, *Chaetomium elatum*, *Gliocladium roseum*, *G. viride*, *Myrothecium verrucaria*, *Penicillium canescens*, *Penicillium chrysogenum*, *Trichoderma hamatum*, *Trichoderma harzianum*, *T. polysporum* *T. pseudokoningii* and *T. viride*. The percentage (%) of maximum growth inhibition of *Botrytis cineria* in presence of *Chaetomium globosum* was 80% with no inhibition or intermingle zone between the pathogen and the antagonist. This result was in accordance with the study from Phong *et al.* (2013) who reported that *C. globosum* significantly inhibited the colony growth of *Pestalotia* spp. causing leaf spot of tea when compared to the control plate and had tendency to give higher inhibition percentage of colony growth as compared to *Ch. lucknowense* and *Ch. cupreum*. Sibounnavong (2012) reported that *Chaetomium* species had the abilities to inhibit the pathogen colony growth of *Fusarium oxysporum* f.sp. *lycopersici* NKSC02 causing wilt disease of Tomato in bi-culture plates. Tathan, S. *et al.* (2012) also reported that *Ch. globosum* and *Ch. cupreum* had the abilities to inhibit the pathogen colony growth of *Drechslera oryzae* causing leaf spot of rice in bi-culture plates. The percentage (%) of maximum growth inhibition of *B. cineria* in presence of *Chaetomium elatum* was 44% and the inhibition zone between the pathogen and the antagonist was found to be 0.2cm. This indicates that there is growth inhibition of *B. cineria* in presence of *Ch. elatum*. Soyong (2015) also reported the ability of *Ch. elatum* *ChE01*

to antagonize *Fusarium oxysporum f.sp. lycopersici*. The antagonism test demonstrated the antagonistic activity of *Ch. elatum* ChE01 to inhibit the conidial production of *Fusarium oxysporum f.sp. lycopersici*. The percentage (%) of maximum growth inhibition of *B. cineria* in presence of *Aspergillus flavus* and *A. fumigatus* was 79.66% and 76.92% respectively. *A. fumigatus* showed intermingle zone of 1.8cm. There is growth inhibition of *B. cineria* in presence of *A. fumigatus*. *Aspergillus* species inhibit the growth of the pathogen in a dual culture due to the production of secondary metabolites such as aflatoxin. Adebola and Amadi (2010) reported similar findings where three *Aspergillus* species i.e., *A. fumigatus*, *A. repens* and *A. niger* were used as biological control agents against *Phytophthora palmivora*, the causal agent of black pod disease of cocoa. Among the *Trichoderma* species, *T. viride* inhibits the maximum growth inhibition of *B. cineria* with the percentage growth inhibition of 74.94% followed by *Trichoderma pseudokoningii* (74.44%), *T. polysporum* (67.44%), *T. harzianum* (67.11%) and *T. hamatum* (63.77%). This result is congruent with work of Seema and Devaki (2012) who have reported the inhibitory effect of *T. viride* against growth reduction of *R. solani* under *in vitro* conditions when compared to *T. harzianum*. Several workers have reported that *Trichoderma* spp. produces large variety of volatile secondary metabolites such as ethylene, hydrogen cyanide, aldehydes and ketones which play an important role in controlling the plant pathogens (Vey *et al.*, 2001).

Table 1. List of fungal species isolated from rhizospheric soil of *Solanum lycopersicum* L

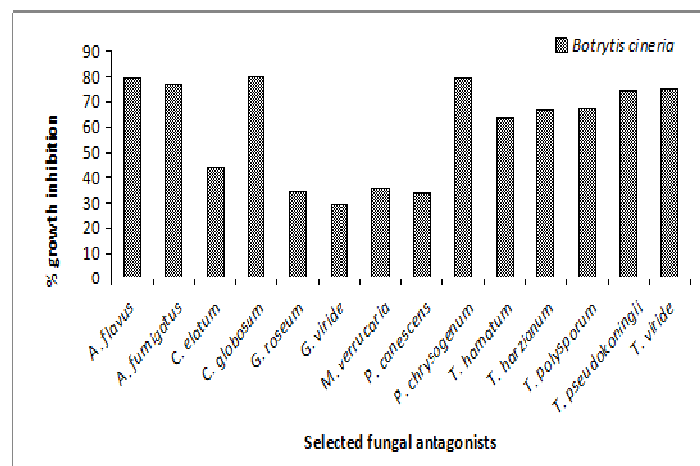
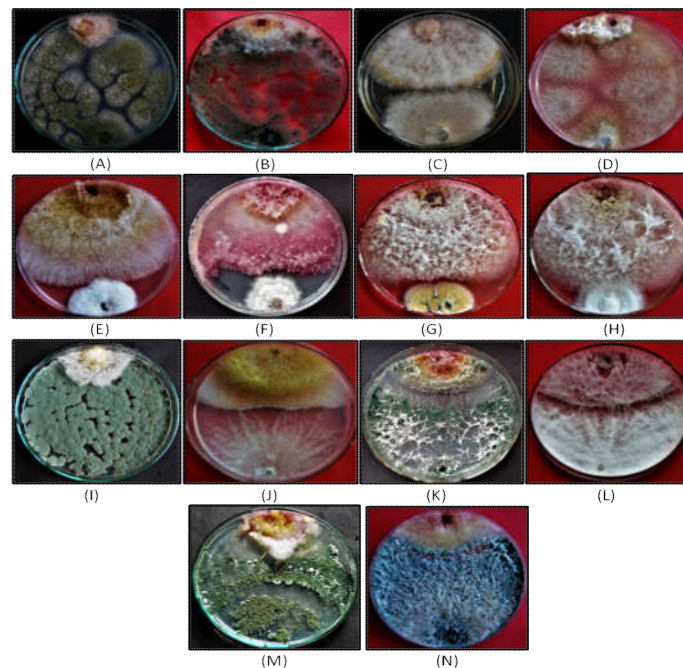
Sl. No.	Isolated fungal species	Rhizospheric soil	
		2015	2016
ZYGOMYCOTA (4 genera, 12 species)			
1	<i>Absidia corymbifera</i>	+	-
2	<i>A.cylindrospora</i>	-	+
3	<i>A. glauca</i>	+	-
4	<i>A. spinosa</i>	-	+
5	<i>Mortierella alpina</i>	-	+
6	<i>M. minutissima</i>	+	+
7	<i>M.parvispora</i>	+	+
8	<i>M.verticillata</i>	+	-
9	<i>Mucor racemosus</i>	+	+
10	<i>M. circinelloides</i>	-	+
11	<i>Rhizopus oryzae</i>	+	-
12	<i>R. stolonifer</i>	+	-
ASCOMYCOTA (29 genera, 81 species)			
1	<i>Acremonium butyri</i>	+	-
2	<i>A. cerealis</i>	+	-
3	<i>A. furcatum</i>	+	-
4	<i>A. kiliense</i>	+	+
5	<i>A. murorum</i>	-	+
6	<i>Arthrinium sphaerospermum</i>	-	+
7	<i>Aspergillus fumigatus</i>	-	+
8	<i>A. flavus</i>	+	-
9	<i>Chaetomium elatum</i>	-	+
10	<i>C. globosum</i>	+	+
11	<i>Chrysosporium merdorum</i>	+	-
12	<i>Cladosporium cladosporioides</i>	-	+
13	<i>C. herbarum</i>	-	+
14	<i>C. macrocarpum</i>	-	+
15	<i>C. sphaerospermum</i>	-	+
16	<i>Cylindrocladium parasiticum</i>	-	+
17	<i>C. scoparium</i>	-	+
18	<i>Daratomyces sinema</i>	-	+
19	<i>E. javanicum</i>	+	+
20	<i>E. lapidosum</i>	+	-
21	<i>Fusarium oxysporum</i>	+	+
22	<i>Gliocladium catenulatum</i>	+	+
23	<i>G. roseum</i>	+	+
24	<i>G. viride</i>	-	+
25	<i>G. virens</i>	+	-
26	<i>G. Species</i>	-	+

27	<i>Gongronella butleri</i>	-	+
28	<i>Gonytrichum macrocladum</i>	+	-
29	<i>Humicola fuscoatra</i>	+	+
30	<i>H. grisea</i>	+	+
31	<i>Iyengarina elegans</i>	-	+
32	<i>Myrothecium verrucaria</i>	+	+
33	<i>Nannizzia grubyia</i>	-	+
34	<i>Nectria ventricosa</i>	+	+
35	<i>Oidiodendron griseum</i>	+	-
36	<i>Paecilomyces carneus</i>	+	+
37	<i>P. forinoseus</i>	-	+
38	<i>P. lilacinus</i>	+	+
39	<i>P. marquandii</i>	-	+
40	<i>Penicillium canescens</i>	+	+
41	<i>P. chrysogenum</i>	+	+
42	<i>P. citrinum</i>	+	+
43	<i>P. corylophyllum</i>	+	-
44	<i>P. crustosum</i>	-	+
45	<i>P. daleae</i>	+	-
46	<i>P.digitatum</i>	-	+
47	<i>P. expansum</i>	+	-
48	<i>P. fellutanum</i>	+	-
49	<i>P. italicum</i>	+	-
50	<i>P. janthinellum</i>	+	+
51	<i>P. jensenii</i>	+	+
52	<i>P. lanosum</i>	+	+
53	<i>P.nigricans</i>	+	-
54	<i>P. paradoxum</i>	+	+
55	<i>P. restrictum</i>	-	+
56	<i>P. rubrum</i>	+	+
57	<i>P. sacculum</i>	+	-
58	<i>P. simplicissimum</i>	+	+
59	<i>P. spinulosum</i>	+	-
60	<i>P. steckii</i>	+	-
61	<i>P. stoloniferum</i>	-	+
62	<i>P. variabile</i>	+	-
63	<i>P. verrucosum</i>	+	-
64	<i>Phoma eupyrena</i>	+	+
65	<i>P. destructiva</i>	+	-
66	<i>Sclerotinia slerotiorum</i>	-	+
67	<i>Scopulariopsis brevicaulis</i>	-	+
68	<i>S. brumptii</i>	+	+
69	<i>Sesquicillium buxi</i>	+	-
70	<i>S. candelabrum</i>	-	+
71	<i>Sporothrix schenckii</i>	+	-
72	<i>Stachybotrys echinata</i>	-	+
73	<i>Trichocladium asperum</i>	-	+
74	<i>Trichoderma hamatum</i>	-	+
75	<i>T. harzianum</i>	-	+
76	<i>T. polysporum</i>	-	+
77	<i>T. pseudokoningii</i>	-	+
78	<i>T. viride</i>	+	+
79	<i>Verticillium albo-atrum</i>	+	+
80	<i>V. chlymydosporium</i>	-	+
81	<i>Verticillium sp.</i>	+	-
STERILE MYCELIA			
1	Brown sterile mycelium	-	+
2	White sterile mycelia	+	+
3	Yellow sterile mycelia	+	-

The antifungal activity of *Trichoderma* involves production of antibiotics, including compounds affecting the integrity of fungal membranes, competition for key nutrients, and production of fungal cell wall-degrading enzymes (Hjeljord and Tronsmo, 1998). *T. viride* recorded the fastest growth rate in all the dual cultures, followed by *T.hamatum*. The rapid growth gives them an important advantage in the competition for space and nutrients with plant pathogenic fungi even before it deploys its arsenal of mycotoxin (Cook, R.J. and Baker, K.F. ,1989). The blistering pace of the colonial growth severely retards the growth of the pathogens. The percentage (%) of maximum growth inhibition of *B. cineria* in presence of *Myrothecium verrucaria* was 36% and the inhibition zone between the pathogen and the antagonist was 0.4cm. Thus there is growth of inhibition of *B. cineria* in presence of *M. verrucaria*.

Table 2. Antagonistic activity (% inhibition) of rhizospheric fungal isolates of *Solanum lycopersicum* L. against *Botrytis cineria* using dual culture technique

Dual culture	Radial growth in cm		% of inhibition	Inhibition zone in cm	Intermingled zone in cm
	R ₁	R ₂			
<i>B. cineria</i> and <i>A. flavus</i>	9.0	1.83	79.66	-	-
<i>B. cineria</i> and <i>A. fumigatus</i>	7.8	1.80	76.92	-	1.8
<i>B. cineria</i> and <i>C. elatum</i>	8.5	4.76	44.00	0.2	-
<i>B. cineria</i> and <i>C. globosum</i>	9.0	1.80	80.00	-	-
<i>B. cineria</i> and <i>G. roseum</i>	9.0	5.90	34.44	-	-
<i>B. cineria</i> and <i>G. viride</i>	9.0	6.36	29.33	0.3	-
<i>B. cineria</i> and <i>M. verrucaria</i>	9.0	5.76	36.00	0.4	-
<i>B. cineria</i> and <i>P. canescens</i>	9.0	5.96	33.77	-	0.5
<i>B. cineria</i> and <i>P. chrysogenum</i>	9.0	1.86	79.33	-	-
<i>B. cineria</i> and <i>T. hamatum</i>	9.0	3.26	63.77	0.3	-
<i>B. cineria</i> and <i>T. harzianum</i>	9.0	2.96	67.11	0.2	-
<i>B. cineria</i> and <i>T. polysporum</i>	9.0	2.93	67.44	-	-
<i>B. cineria</i> and <i>T. pseudokoningii</i>	9.0	2.30	74.44	-	-
<i>B. cineria</i> and <i>T. viride</i>	8.5	2.13	74.94	-	-

**Plate 3. Graphical representation of the antagonistic activity of rhizospheric fungal isolates showing the % of growth inhibition of the fungal pathogen *Botrytis cineria*****Plate 4. Dual culture method showing colony interaction of the pathogen *Botrytis cineria* and antagonist (A) *Aspergillus flavus*, (B) *A. fumigatus*, (C) *Chaetomium elatum*, (D) *C. globosum*, (E) *Gliocladium roseum*, (F) *G. viride*, (G) *Myrothecium verrucaria*, (H) *Penicillium canescens*, (I) *P. chrysogenum*, (J) *Trichoderma hamatum*, (K) *T. harzianum*, (L) *T. polysporum*, (M) *T. pseudokoningii* and (N) *T. viride***

Similar findings were reported by Tuset *et al.* (1994) where *M. roridum* and *M. verrucaria* produce antibiotics and toxins highly active against *Phytophthora* spp. *in vitro* and *in vivo*. Culture filtrates of *M. roridum* and *M. verrucaria* obtained on

a liquid basic salts medium with low glucose content significantly inhibited mycelial growth of *P. palmivora* and *P. katsurae* isolates *in vitro*. The percentage (%) of maximum growth inhibition of *B. cineria* in presence of *Gliocladium*

roseum and *G. viride* was 34.44% and 29.33% respectively. Sutton *et al.* (1997) reported that the *Gliocladium* species are effective and versatile antagonists. These fungi have the advantage of abundant production and the long term viability of the inoculums attributes of key importance for commercial attributes of key importance for commercial use. The percentage (%) of maximum growth inhibition of *B. cineria* in presence of *Penicillium chrysogenum* and *P. canescens* was 79.33% and 36% respectively. There is inhibition growth of *B. cineria* in presence of *P. chrysogenum* and *P. canescens*. Druvefors *et al.* (2002) reported that *Penicillium* species secretes some secondary metabolites such as Penicillin and enzyme like β -1-3-glucanase, a cell wall lytic enzyme which inhibit the growth of the pathogen.

The varying zones of inhibition as well as intermingled zones were observed with various fungi. Maximum inhibition zone of 0.4cm was observed in the dual culture of *Myrothecium verrucaria* and *B. cineria* and maximum intermingled zone of 0.8cm was observed in case of *P. canescens*. There were instances where the antagonistic fungi over grew the other colonies as was observed in the case of *Trichoderma spp.*, *Aspergillus spp.* and *Penicillium spp.* *Penicillium* and *Trichoderma* species are known to produce a variety of beneficial compound to suppress the pathogens (Hyakumachi, 1994; Narisawa *et al.*, 2004; Dubey *et al.*, 2007) and stimulate plant growth by the production of phytohormones (Hasan 2002) and/or degradation of the complex substrate (Altmore *et al.*, 1999). An environmentally friendly and sustainable alternative to protect plants against soil borne pathogens is the biological control using antagonistic microorganisms as bioagents (El-Kot and Belal 2006). Using of fungicides for long time will result in development of resistant strains (Rosslensbroich and Stuebler, 2000). Consequently, biological control, including the use of microorganisms or their antibiotics, offers an attractive alternative or supplement to pesticides for the management of plant diseases without the negative impact of chemical control (Wang *et al.*, 1999). Eziashiet *al.* (2007) reported that biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods.

Conclusion

The study highlights the efficiency of rhizospheric fungal isolates as biocontrol agents against phytopathogen *Botrytis cineria*, the causal agent of Gray mold disease of tomato. Among the isolates, *Chaetomium globosum* has the highest percentage of inhibition and effectively controlled the pathogen *B. cineria* under *in vitro* conditions followed by *Aspergillus flavus*, *Penicillium chrysogenum*, *Aspergillus fumigatus*, *Trichoderma viride*, *T. pseudokoningii*, *T. polysporum*, *T. harzianum* and *T. hamatum*. *Chaetomium elatum* showed low percentage of inhibition against *B. cineria* followed by *Myrothecium verrucaria*, *Gliocladium roseum* and *Penicillium canescens*. *Gliocladium viride* showed the least percentage of inhibition against *B. cineria* among all the selected fungal isolates. Thus it can be concluded that out the 14 test antagonists, *C. globosum*, *A. flavus*, *P. chrysogenum*, *Aspergillus fumigatus*, *T. viride*, *T. pseudokoningii*, *T. polysporum*, *T. harzianum* and *T. hamatum* may be recommended as good biocontrol agents of *Botrytis cineria* the pathogen of tomato as all the 9 fungal isolates showed good inhibition growth of the pathogen.

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REFERENCES

- Abed R. M. M., Al-Sadi A. M., Al-Shehi M., Al-Hinai S., Robinson M. D. 2013. Diversity of free living and lichenized fungal communities in biological soil crusts of the Sultanate of Oman and their role in improving soil properties. *Soil Biology Biochemistry*, 57 695–705. 10.1016/j.soilbio.2012.07.023.
- Adebola, M.O. and Amadi, J.E. 2010. Screening three *Aspergillus* species for antagonistic activities against the cocoa black pod organism (*Phytophthora palmivora*). *Agriculture and Biology Journal of North America*, 1(3):362-365.
- Agrios GN 1997. *Plant pathology*, 4th edn. Academic Press, San Diego.
- Agrios, G.N. 2005. *Plant Pathology*. Fifth Edition. Elsevier Academic Press, London, UK, pp. 922.
- Altmore C, Norvell WA, Bjorkman T, Harman GE 1999. Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22 // *Applied and Environmental Microbiology*.vol. 65, p. 2926-2933.
- Barnett, K.L. and Hunter, B.B. 1972. *In: Illustrated genera of imperfect fungi*. Burgess Publishing Company, Minneapolis.
- Bell, D.K., Wells, H.D. and Markham, C.R. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal pathogens. *Phytopathology*, 72: 379-382.
- Bridge, P. and Spooner, B. 2001. Soil fungi: diversity and detection. *Plant Soil*, 232:147–154.
- Coley-Smith JR, Verrhoeff K, Jarvis WR 1980. *The biology of Botrytis*, Academic Press, London, UK, 318 pp.
- Cook, R.J.; Baker, K.F. *The nature and practice of biological control of plant pathogens*. APS Press, St. Paul, 1989. 539p.
- De Gannes V., Eudoxie G., Hickey W. J. 2013. Insights into fungal communities in composts revealed by 454-pyrosequencing: implications for human health and safety. *Frontiers in Microbiology*, 4:164 10.3389/fmicb.2013.00164.
- Domsch, K.H., Gams, W. and Anderson, T.H. 1980. *In: Compendium of soil fungi*. Academic Press, London.
- Dorais, M., Ehret, D.L. and Papadopoulos, A.P. 2008. Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. *Phytochemistry Reviews*, 7:231–250.
- Druvefors, U., Jonsson, N., Boysen, M.E. and Schnürer, J. 2002. Efficacy of the biocontrol yeast *Pichia anomala* during long-term storage of moist feed grain under different oxygen and carbon dioxide regimens. *FEMS Yeast Research*, 2: 389- 394.
- Dubey, S.C. and Singh, M. S. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris*, for integrated management of chickpea wilt. *Biological Control*, 40: 118-127.
- El-Kot, G.A.N. and Belal, E.B.A. 2006. Biocontrol of *Fusarium* damping-off of pea by certain bacterial

- antagonists. *Journal of Agricultural Research Tanta University*, 32: 225-242.
- Ellis, M.B. 1972. *Dematiaceous Hypomycetes*. CAB International, UK.
- Eziashi EI, Omamor IB and Odigie EE 2007. Antagonism of *Trichoderma viride* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. *African Journal of Biotechnology*, 6 388-392.
- Hasan H.A.H. 2002. Gibberellin and auxin-indole production by plant root-fungi and their biosynthesis under salinity-calcium interaction // *Rostlina vyroba*. vol. 48, iss. 3, p. 101-106.
- Hjeljord, L and Tronsmo, A. 1998. *Trichoderma and Gliocladium: enzymes, biological control and commercial applications*, London: Taylor and Francis, Ltd. *Trichoderma and Gliocladium* in biological control: an overview, Vol(2): 131-151.
- Hyakumachi M. 1994. Plant-growth-promoting fungi from turfgrass rhizosphere with potential for disease suppression // *Soil Microorganisms*. vol. 44, p. 53-68.
- Johnson, L.F. and Curl, A.E. 1972. *In: Method for the research on ecology of soil borne plant pathogens*. Minneapolis Burgess Publishing Company, pp. 247.
- Kazeeroni E. A., Al-Sadi A. M. 2016. 454-pyrosequencing reveals variable fungal diversity across farming systems. *Frontiers in Plant Science* 7:314 10.3389/fpls.2016.00314.
- Khan F, Mazid M, Khan TA, Patel HK and Roychowdhury R. 2014. Plant derived pesticides in control of lepidopteran insects: dictum and directions. *Research Journal of Biology*, 2: 1 - 10.
- Martin, F.M., Peretto, S. and Bonfante, P. 2001. Mycorrhizal fungi. *In: The rhizosphere – a fungal community at the interphase between soil and roots* (eds. R. Pinton, Z. Varanini, P. Nannipieri). New York, Marcel Dekker, pp.263-296.
- Martin, J.P. 1950. Use of acid rose bengal and streptomycin in plate method for estimating soil fungi. *Soil Science*, 69: 215 - 232.
- Narisawa R, Quimio TH 2004. Soil mycoflora of black pepper rhizosphere in the Philippines and their in vitro antagonism against *Phytophthora capsici* // *Indonesian Journal of Agricultural Sciences*. vol. 5, iss. 1, p. 1-10
- Nonnecke, I.B.L. 1989. *In: Vegetable Production*. Avi Book Publishers. New York, USA, pp. 200-229.
- Nonnecke, I.B.L. 1989. *In: Vegetable Production*. Avi Book Publishers. New York, USA, pp. 200-229.
- Phong N. H., Pongnak, W. and Soyong, K. 2013. Biological control of plant pathogenic fungi from tea using *Chaetomium* spp. King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand, pp. 416-427.
- Rosslbroich, H.J., Stuebler, D., 2000. Botrytis cinerea-history of chemical control and novel fungicides for its management. *Crop Protection*, 19, 557-561.
- Seema M, Devaki N.S. 2012. *In-vitro* evaluation of biological control agents against *Rhizoctonia solani*. *Journal of Agricultural Technology*, 8(1):233-240.
- Sibounnavong, P. 2012. Biological activities of antagonistic fungi to control fusarium wilt of tomato. (Ph.D thesis) pp. 86.
- Skidmore, A.M. and Dickinson, C.M. 1976. *Transaction of the British Mycological Society*, 66: 57-64.
- Soyong, K. 2015. Testing bioformulation of *Chaetomium elatum* ChE01 to control *Fusarium* wilt of tomato. *Journal of Agricultural Technology*, 11: 975-996.
- Subramaniam, C.V. 1971. *In: Hyphomycetes; an account of Indian species, except Cercospora*. ICAR, New Delhi.
- Sutton JC, Li D-W, Peng G, Yu H, Zhang P 1997. *Gliocladium roseum*: a versatile adversary of *Botrytis cinerea* in crops. *Plant Disease* 81, 316-328.
- Tathan, S., Sibounnavong, P., Sibounnavong, P.S., Soyong, K. and To-anun, C. 2012. Biological metabolites from *Chaetomium* spp to inhibit *Drechslera oryzae* causing leaf spot of rice. *Journal of Agricultural Technology* 8(5): 1691-1701.
- Thorn, G. 1997. The fungi in soil. *In: Modern Soil Microbiology*. Van Elsas, J.D., J.T. Trevors and E.M.H. Wellington (Eds.). New York, Marcel Dekker, pp. 63-127.
- Tuset, J, J., Hinarejos, C. and Mira, J. L. 1994. *Myrothecium roridum* and *M. verrucaria* as potential antagonists of *Phytophthora* species of coconut. *Investigación Agraria, Producción y Protección Vegetales*, pp.385-393.
- Vey, A., Hoagland, R.E. and Butt, T.M. 2001. Toxic metabolites of fungal biocontrol agents, pp 311-345. *In: Butt T.M. and Jackson C. (Eds). Fungi as Control Agents: Progress, Problems and Potential*. CAB International, Bristol.
- Wang S.L., Yieh T.C., Shih I.L., 1999. Production of antifungal compounds by *Pseudomonas aeruginosa* K-187 using shrimp and crab shell powder as a carbon source. *Enzyme Microbiology Technology*, 25: 142-148.
