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RESEARCH ARTICLE

Exploration of Antagonistic Fungi From Rhizospheric Soil Against Phytopathogens of *Solanum melongena* and *Citrus sinensis*

K Sabina[1]*, S Gayathridevi[1], S Pavithraa[2]

1 Assistant Professor, Department of Microbiology, Prince Shri Venkateshwara Arts and Science College, Gowrivakkam, Chennai, 600073, Tamil Nadu, India
2 Director and trainer, TPSJ Academy, Chennai, Tamil Nadu, India

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* **Corresponding author**.

sabinanair2008@gmail.com

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Abstract

Objectives: To explore the antagonistic activity of rhizospheric fungi against the phytopathogens of *Solanum melongena* (Brinjal) and *Citrus sinensis* (Orange), as these diseases cause major economic loss in the respective plants. **Methods:** Phytopathogens were isolated from infected leaves and fruits. Fungi were isolated from the rhizosphere of respective plants. Fungal isolates were screened for antagonism against phytopathogens and positive isolates were identified. Assays were conducted for volatile and non-volatile compounds from the antagonistic fungi and antagonistic metabolites were extracted. Antagonistic extracts were characterized and confirmed the antagonistic activity of the extract against the phytopathogens. **Findings:** Phytopathogens were identified as *Curvularia sp.* and *Penicillium sp.* Among the 12 rhizospheric fungal isolates, *A. niger, A. fumigatus* and *A. flavus* exhibited good antagonistic activity against the phytopathogens. The non-volatile metabolites were mostly responsible for antagonism when compared to the volatiles. The non-volatile compounds from *A. niger* showed highest activity against *Curvularia* (95% inhibition) followed by *A. flavus* (79%) and *A. fumigatus* (76%). This study demonstrates the potential applications of antagonistic fungi as effective biocontrol agents against various phytopathogens. **Novelty:** As high as 95% inhibition of phytopathogens was observed in the study which suggests the effectiveness of non-volatile metabolites from antagonistic fungi as potent biocontrol agents to prevent the crop-loss.

Keywords: Antagonism; Antagonistic Metabolites; Phytopathogens; Rhizospheric Fungi; Volatile Assays

1 Introduction

Plant pathogens pose a major threat to agroecosystem, responsible for about 10–50% of crop loss depending on the crop and country. With global population explosion, it is necessary to manage agricultural systems in an ecofriendly and sustainable manner. This can only meet the rising need for food for the growing population. In the recent

years, scientists have begun to understand the consequences of the widespread and repeated use of chemical fungicides that threaten our environment while also cause many health issues. For several years, a great deal of money and manpower is invested to protect the crop plants from fungal pathogens for ensuring good quantitative and qualitative yield, but still, it remains as a burning problem. The potential issues as toxicity, environmental pollution and side effects on human health hinder the applicability of chemical agents for fighting the phytopathogens [1].

The concept of "sustainable agriculture" is feasible only when coupled with natural microbicidal products that can control the infections in agricultural ecosystems [2]. Biological control is the most feasible method for protecting plants from such phytopathogens. Rhizosphere is colonised by a diversity of complex microorganisms including different species of bacteria and fungi, which can play a central role for plant health and growth [3]. Antagonistic soil microorganisms are of great practical importance since they produce antibiotics which affect the normal growth processes of another species of organism, which can be extracted and used directly as biocontrol agent. Mechanism of antagonism include antibiosis by production of enzymes, toxins or antibiotics or direct parasitism either as biotrophic or necrotrophic fungi. It may also involve competition for nutrients or indirect mechanism as induced resistance [4]. Fungi are potentially better biocontrol agents suggesting one of the alternatives for chemical fungicides. Most of the fungi used as biocontrol agents include antagonists of phytopathogens. Mycoparasitism of plant pathogens is an important biocontrol trait which can be exploited in developing effective biocontrol agents.

At present, different species of antagonistic fungi such as those belonging to genera of *Trichoderma, Fusarium, Alternaria* etc. are approved to be used as BCA (Biocontrol Agents). But there are certain obstacles in the use of such biocontrol agents. Commercialization of biological control agents for plant diseases is following a slow pace mainly due to their variable performances under different environmental conditions in the field, their host specificity and low efficacy to manage the disease [5]. The performance of a biocontrol agent depends on the survival rate in the soil, its compatibility with the crop plant, interaction with other soil microbial species and the environmental factors [1]. These limitations can be overcome if we can identify the biologically active compound from the antagonistic organism and extract it in the pure form. Hence, it is important to identify novel fungal strains which can effectively control specific plant diseases affecting economically important plant species and to identify the active compound from the strain and extract and purify it for further use. The present study is aimed to isolate novel antagonistic fungi against plant diseases and extract their active metabolite in pure form.

Brinjal or eggplant (*Solanum melongena*) and orange plant (*Citrus sinensis*) are rich in nutrients. They are good source of minerals and vitamins. They possess several medicinal properties. Among the diseases affecting these plants, leaf spots of brinjal and the fruit rots of the orange plant have significant impact on the crop yield of respective plants. Though these are two common infections, there are no much studies conducted to identify effective biocontrol agents against these diseases of such economically important plants. Aktar et al. (2015) [6] have studied the antagonistic potential of rhizosphere fungi against leaf spot and fruit rot pathogens of brinjal. They have identified *Trichoderma harzianum* as the most effective to control the growth of pathogens and they have studied the effects of volatile and non-volatile metabolites. But they have not identified the active ingredient responsible for the antagonistic activity. The current study is aimed to isolate antagonistic fungi from rhizospheric soil and study the antagonistic potential of these isolates against the phytopathogenic fungi responsible for leaf spot disease of brinjal plant and fruit rot disease of orange plant. This study can fill the research gap existing in the field by finding out effective fungal biocontrol agents against most common plant diseases.

2 Methodology

Infected plant parts and rhizospheric soil were collected in sterile zip lock covers from the agricultural fields in and around Chennai, Tamil Nadu. The collected samples consisted of fungal infected leaves from brinjal plant, fungal infected orange fruits directly from the plant and rhizospheric soil near the respective plants. All the culture media used were obtained from Hi-Media and chemicals were obtained from Sigma-Aldrich. Mercuric chloride used was of analytical grade with 98% purity.

2.1 Isolation of rhizospheric fungi and phytopathogens

The soil fungi were isolated and enumerated by serial dilution and plating method on Sabouraud dextrose agar with chloramphenicol. Collected infected leaves of brinjal plant and infected fruit from orange plant exhibiting clear symptoms were cut into small pieces. After washing the tissues thoroughly in sterile water, the infected tissues were cut into small pieces (2–5 mm squares) and using sterile forceps, they were transferred to sterile petridishes containing 0.1% mercuric chloride solution for surface sterilization of plant tissues. The plant parts were transferred to SDA plates and incubated for 5-7 days for the complete growth of fungi. The growth of fungi from the infected tissues were purified using the hyphal tips technique on SDA medium.

2.2 Screening for Antagonism

Screening for antagonistic effect was done on SDA plates with chloramphenicol by dual culture method. Agar disc (5 mm in diameter) of 7 days old culture of phytopathogenic fungal isolate was placed 1 cm away from the periphery of petri dish. An equal sized agar disc of 7 days old culture of isolated rhizospheric soil fungus was placed 1 cm away from the edge of the same petri plate on the opposite side of phytopathogen. Same procedure was followed for all the isolates against each phytopathogen. Plate with each pathogenic fungus alone, similarly placed in the medium served as control. The plates were incubated at 30°C for 5-7 days. The growth radius of the phytopathogenic fungi in the direction of the antagonistic colonies (R_2) and the growth radius of phytopathogenic fungal colonies in the respective control plates (R_1) were measured. This R_1 and R_2 reading were used to calculate percentage inhibition of radial growth (PIRG).

$$\text{PIRG} = \frac{R_1 - R_2}{R_1} \times 100 \quad (1)$$

2.3 Identification of fungi

Isolated phytopathogenic fungi and the fungi observed to be positive after antagonistic screening were identified based on fungal morphology. Morphological studies were done macroscopically by observing colony features (colour and texture) and microscopically by lactophenol cotton blue (LPCB) staining and observed under light microscope to study the structural features.

2.4 Assay for volatile compounds from the antagonistic fungi

SDA plates were centrally inoculated with 5 mm diameter discs of antagonistic fungi - *A. niger*, *A. flavus*, *A. fumigatus* and the phytopathogenic fungi *Curvularia* and *Penicillium* in separate plates and covered with parafilm to avoid the volatilization of compounds. The plates were incubated for 3 days at room temperature. After incubation, the lids were removed aseptically and the plate containing pathogenic fungus (*Curvularia* and *Penicillium*) was placed over the plate containing the antagonistic fungus (*A. niger, A. flavus, A. fumigatus*). The plates were enclosed by three layers of parafilm to prevent the loss of volatile substances and incubated for 5 days at room temperature. The same procedure was followed for each antagonistic isolate with each phytopathogen. The average diameter of the fungal growth was measured and compared with that on control plates. SDA plates of pathogen without antagonist served as control. Each assay was performed in triplicate. The percentage inhibition was obtained using the following formula:

$$\text{Inhibition } (\%) = (\text{D1} - \text{D2})/\text{D1} \times 100 \quad (2)$$

Where, D1 represents the diameter of radial growth of pathogens in control and D2 represents the diameter of radial growth of pathogens in antagonistic tests.

2.5 Assay for non-volatile compounds

Dual culture method as stated in screening, was done with each antagonistic fungus and phytopathogen. Pathogenic fungi without antagonist served as control. The growth radius of the phytopathogenic fungi *Curvularia* and *Penicillium* in the direction of the antagonistic colonies (R_2) and the growth radius of phytopathogenic fungal colonies in the respective control plates (R_1) were measured. This R_1 and R_2 values were used to calculate percentage inhibition of radial growth (PIRG) using the formula.

$$\text{PIRG} = \frac{R_1 - R_2}{R_1} \times 100 \quad (3)$$

After the assay, different grades (1 to 5) were given to the interaction between each pair of pathogen and antagonist based on the observation of their growth characteristics on SDA plate. The standard grade chart [6] is given below the Table 1.

2.6 Extraction of antagonistic substances

Active antagonistic cultures were grown separately in 100 ml of Sabouraud dextrose broth in 500 ml Erlenmeyer flasks. After 5 days, the contents of each flask were filtered under vacuum through four layers of Whatmann No. 1 paper to remove the mycelium. The concentrated culture filtrates were then extracted with chloroform. Two successive extractions, each with 50 ml chloroform, were carried out by shaking the chloroform with aqueous fraction in a separating funnel. The heavier chloroform fraction was allowed to separate out and run off from the aqueous layer. The chloroform was allowed to evaporate and the extracts were collected.

Table 1. Assay for non-volatile compounds

Pathogen	Antagonist	Radial growth of pathogen (in cm)		PIRG (%)	Grade in colony interaction
		D1	D2		
Curvularia	*A.niger*	4.2	0.2	95	4
	A.flavus	4.2	0.9	79	2
	A.fumigatus	4.2	1.0	76	2
Penicillium	*A.niger*	3.4	0.2	67	2
	A.flavus	3.4	0.5	66	2
	A.fumigatus	3.4	0.8	88	4

Standard Grade Chart (Grades from 1 to 5)
Grade 1 – Mutual intermingling without any macroscopic sights of interaction.
Grade 2 – Mutual intermingling growth where the growth of the fungus is ceased and being over grown by the opposed fungus.
Grade 3 – Intermingling growth where the fungus under observation is growing into the opposed fungus either above or below.
Grade 4 – Sight inhibition of both the interacting fungi with narrow demarcation line (12 mm).
Grade 5 – Mutual inhibition of growth at a distance of > 2 mm

2.7 Characterization of the antagonistic substances

The Characterization was done by the TLC method. The mobile phase used was a mixture of ethyl acetate and Hexane (1:1). The extracts were allowed to run on the stationary phase by mixing with the solvent mixture used for mobile phase. 2% vanillin – sulphuric acid and ninhydrin were used as the spraying agent. Rfvalue was calculated by using the formula,

$$\mathrm{Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance traveled by solvent front}} \tag{4}$$

2.8 Antagonistic activity of the extracts

The dried chloroform extracts from culture filtrates of antagonistic fungal isolates were tested for antibiotic activity by well diffusion method. 50 μl chloroform suspension was placed in the centre well of the SDA plate, a 12 mm disc cut from the margin of the 3-day old culture of pathogenic fungus was placed covering the well. SDA plates with 50 μl of chloroform in the central well and fungal disc of pathogenic isolate placed over the well served as the control. Test and control plates were incubated at room temperature for 3 to 5 days. The radial growth of phytopathogens were measured and compared with the control plates. PIRG value was calculated.

3 Results and Discussion

3.1 Isolation of fungi

By soil dilution and plating, growth of 12 fungal colonies were isolated from the rhizospheric soil sample and named as Isolate 1 to Isolate 12. By direct culturing of phytopathogens from infected plant tissues, growth of 2 fungal colonies were isolated and the isolates were named as Isolate A from leaf spot of brinjal plant and Isolate B from fruit rot of orange plant.

3.2 Screening for antagonism

Among the 12 rhizospheric fungal isolates, 3 isolates exhibited antagonistic activity against both the phytopathogenic fungal isolates, while both pathogenic fungi were found to grow well near the other 9 rhizospheric fungi. Hence, isolates 1, 2 and 3 were selected as antagonistic fungi against phytopathogens, for further studies. Isolates 1, 2 and 3 exhibited good antagonistic activity against the phytopathogenic isolates from orange plant (Isolate A) and brinjal plant (Isolate B), measured as PIRG values. The antagonistic activity is expected to be due to the inhibitory property of their metabolites against the pathogenic fungi which may include volatile and non-volatile metabolites.

3.3 Identification of isolated fungi

All the antagonistic fungi and phytopathogenic fungi were identified based on the macroscopic appearance on SDA and microscopic properties as described in Table 1. All the antagonistic fungal isolates were identified to be belonging to the same genus *Aspergillus*. Isolates 1, 2 and 3 were identified as *A. niger, A. flavus* and *A. fumigatus* respectively. The phytopathogen

isolated from leaf spot disease of brinjal plant (Isolate A) was identified as *Curvularia sp.* and that from fruit rot of orange plant (Isolate B) as *Penicillium sp.*

Table 2. Identification of fungal isolates

Isolates	Macroscopic appearance	Microscopic appearance	Identification
Isolate 1	Wooly at first, then turning black with white to yellow reverse	Long and smooth structure, phialides are biserate forming radiate head	*A. niger*
Isolate 2	Yellow to green velvetty, and goldish to red brown reverse	Variable length, rough and spiny, phialides are uniserate and biserate, point out in all direction and cover 2/3 vesicles.	*A. flavus*
Isolate 3	Dark greenish to grey velvetty with white to tan reverse	Short and smooth, phialides are uniserate appearing on upper 1/3 of the vesicle parallel to axis of conidiophores	*A. fumigatus*
Isolate A	Brownish black with a pinkish grey wooly surface and dark reverse	Dark, septate hyphae, knobby conidiophores at points of conidium formation, large and curved conidium containing 4 cells	*Curvularia sp.*
Isolate B	Bluish green with a white border colonies and reverse of red or brown	Septate hyphae with branched or unbranched conidiophores further branching into metulae that contain phialides and conidia forming brush like appearance	*Penicillium sp.*

3.4 Assay for volatile compounds from antagonistic fungi

The rhizospheric fungal isolates were found to have a low level of antagonistic activity against the phytopathogenic fungi when the effect of volatile compounds in antagonism were assayed. Volatile substances emanating from the soil fungi inhibited radial growth of the test pathogens to varied degree ranging from 3- 18%. The maximum antagonistic activity was exhibited by A. fumigatus against both *Penicillium* (PIRG -18%) and *Curvularia* (PIRG-14%). Table 2 shows the results of antagonistic assay for volatile metabolites and Figure 1 demonstrates the antagonism between *A. fumigatus* and *Penicillium*. The first plate shows the growth of *A. fumigatus* after the assay and the second plate shows 18% growth inhibition of phytopathogen after the assay compared to the control growth in the third plate.

Table 3. Assay for volatile compounds from antagonistic fungi

Pathogen	Antagonist	Radial growth of pathogen (in cm)		PIRG (%)
		D1	D2	
Curvularia	*A.niger*	3.4	3.2	6
	A.flavus	3.4	3.3	3
	A.fumigatus	3.4	2.9	14
Penicillium	*A.niger*	3.9	3.7	5
	A.flavus	3.9	3.4	13
	A.fumigatus	3.9	3.2	18

Though there is inhibition of pathogen growth, it is not much prominent. It is clear from the results that, the volatile metabolites produced by the isolated *Aspergillus sp.* play only a minor role in their antagonistic activity. The results obtained were in agreement with previous findings[6]. Hence, the major role in antagonistic activity might be due to the non-volatile metabolites which diffuse through the medium and directly inhibit the pathogen growth.

3.5 Assay for non-volatile compounds

The antagonistic effects owing to non-volatile metabolites of the antagonistic fungi against the test pathogens ranged from 65 to 95%. It is evident from the data presented in the table 3 that the growth inhibition due to non-volatile metabolites of the antagonistic fungi was much higher when compared to the effect of volatile substances. The highest inhibition was exhibited by *Aspergillus niger* against *Curvularia* (PIRG-95%). This interaction was designated as Grade 4, as there was clear inhibition

Fig 1. Plate assay for antagonistic activity of volatile compounds- *A.fumigatus* against *Penicillium*

with a narrow demarcation. The antagonistic action of *A. fumigatus* on *Penicillium* also belonged to Grade 4 inhibition with a PIRG of 88%. The inhibition of the radial growth of the test fungi due to non-volatile metabolites, may be attributed to the production of antagonistic substances in the culture filtrates and impoverishment of nutrients. The plates showing antagonistic activity by non-volatile metabolites are shown in the figures. Figure 2 demonstrates the antagonistic activity of three species of *Aspergillus* on *Curvularia* and Figure 3 illustrates the antagonistic activity of three species of *Aspergillus* on *Penicillium* by dual culture method. Control plate showing the growth of pathogen alone is also shown in Figures 2 and 3. Since the assay was done by dual culture method on the same plate, the role of non-volatile diffusible metabolites in inhibiting the growth of tested pathogen is evident.

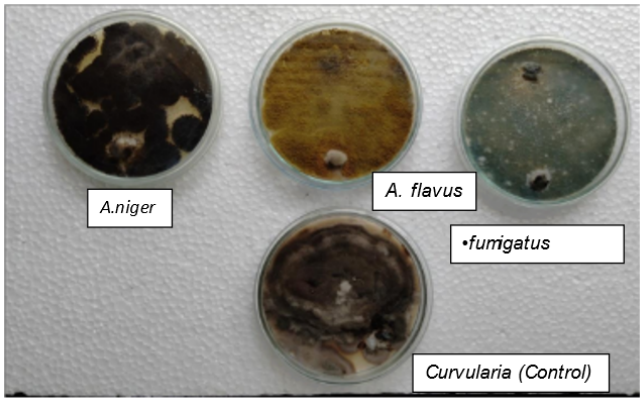

Fig 2. Growth inhibition of *Curvularia* by antagonistic fungi

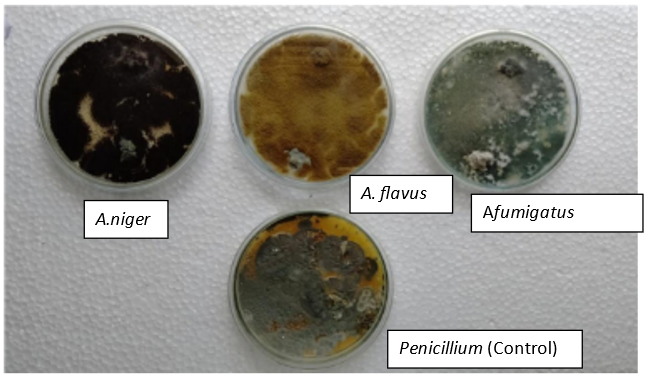

Fig 3. Growth inhibition of *Penicillium* by antagonistic fungi

Many non-volatile antagonistic metabolites have been extracted and characterized from different fungi. It has been reported that *A. flavus* produce Aspergillic acid (pyrazine derivative) which is a potent anti-fungal metabolite. It is non-volatile in nature

but volatile in steam. *A. fumigatus* produces Gliotoxin ($C_{13}H_{14}N_2O_4S_2$), a potent antifungal metabolite that inhibits the growth of several fungi. Gliotoxin is a diketopiperazine with 2 amide groups. *A. niger* synthesises compounds like Tensidols-A and B and it also exhibits antagonistic activity against some yeast and fungi. This may be due to their secondary metabolites like Aspergillic acid, Tensidols, Gliotoxin, Fumigacin and Spinulosin. Tensidols – A ($C_{13}H_{11}NO_3$) & B ($C_{18}H_{17}NO_6$) from A. niger, Aspergillic acid ($C_{12}H_2ON_2O_2$) from *A. flavus* and Gliotoxin ($C_{13}H_{14}N_2O_4S_2$) from *A. fumigatus* are potent antifungal metabolites.

3.6 Extraction and characterization of antagonistic metabolites

The extraction of antagonistic metabolites from culture filtrate was done by solvent extraction using chloroform. The appearance of the extract from *A. niger* was found to be fine white to pale brown colored substance. The extract appeared as pale yellow crystalline and resinous substance for *A. flavus* and as white, crystalline substance for *A. fumigatus***.**

On TLC plate, there were spots which positively reacted to 366 nm UV (Rf values of 0.45 for *A. niger* and *A. flavus* and 0.26 for *A. fumigatus*). Fluorescent blue compounds, when exposed to UV are considered to be a group of organic compounds that possess double bonds (Diene / Polyene or conjugation) [7]. Compounds which produced fluorescent blue spots with 2% vanillin-sulphuric acid reagent showed the presence of alcohol and carbonyl functional groups (ketones, aldehydes) and no spots appeared with ninhydrin reagent for the extracts of *A. niger* and *A. flavus*. Purple spot appeared with ninhydrin reagent for the extract of *A. fumigatus* as gliotoxin contains 2 amide groups. Hence the presence of active antagonistic metabolites was detected by TLC of the extracts from fungal culture filtrates.

3.7 Antagonistic activity of the extract

When the activity of the antagonistic fungal extracts was tested against *Curvularia* and *Penicillium*, there was considerably higher or similar antagonistic activity observed when compared to the activity by the whole fungal culture. Extracts from *A. niger* exhibited the maximum antagonistic activity against the phytopathogen *Curvularia* with a PIRG value of 96%, followed by *A. flavus* and *A. fumigatus* with PIRG values of 81% and 75% respectively. The extract of *A. fumigatus* was very effective against *Penicillium* than the extracts of *A. niger* and *A. flavus*. The PIRG values were 90%, 70% and 72% for *A. fumigatus*, *A. niger* and *A. flavus* respectively against *Penicillium.*Figures 4 and 5 illustrate the antagonistic activities of *A. niger* extract against *Curvularia* and *A. fumigatus* extract against *Penicillium* respectively, where clear inhibition of pathogen growth is visible in the plates.

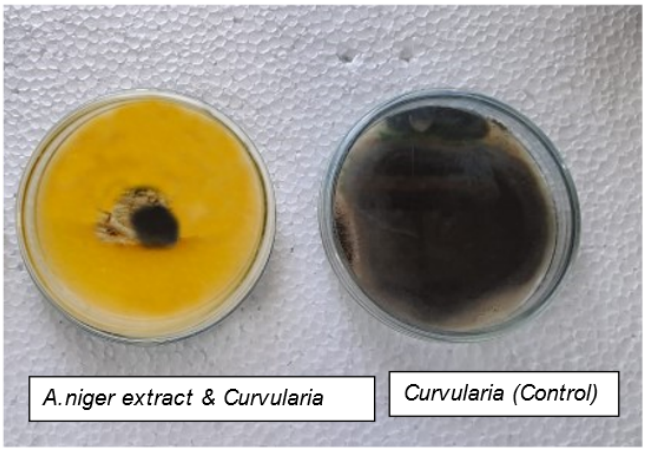

Fig 4. Antagonistic activity of the fungal extracts: *A.niger* extract on *Curvularia*

Biocontrol products based on microbial antagonists are safer alternative tools for agrochemical products. Keeping this in mind, several studies are conducted in the last few decades on the selection, characterization and commercial development of biocontrol agents. Many studies have isolated fungal biocontrol agents from soil and several species like *Trichoderma, Fusarium, Alternaria, Aspergillus* etc. have been reported to possess antagonistic action against many plant pathogens. All the researchers followed the similar methods for initial isolation of antagonists- the dual culture method, where the pathogens were cocultured with potential isolates to assess its ability to inhibit its growth. Alqarawi et al. (2021) [8] isolated and characterized potential fungal antagonists of the phytopathogenic fungus *Fusarium oxysporum* f. sp. radicis-lycopersici from date palm rhizosphere

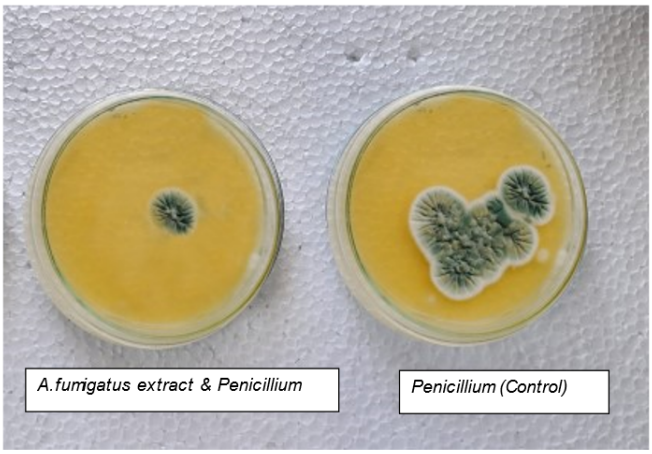

Fig 5. Antagonistic activity of the fungal extracts: *A.fumigatus* extract on *Penicillium*

soil. The potential fungal antagonists were identified as belonging to the genera *Aspergillus, Penicillium, Trichoderma, and Fusarium*. Kowsari et al. (2021)[9] have isolated *Aspergillus, Penicillium, Trichoderma*, and *Chaetomium* from rhizospheric and non-rhizospheric soil against phytopathogen of Fusarium wilt disease in tomato plants. *Trichoderma* was found to have highest antagonistic activity against the target pathogen. The findings from all these studies like the current study have implications for the development of sustainable and environmentally friendly strategies for the management of plant diseases caused by soilborne pathogens. Thambugala et al. have reviewed the mechanism of antagonistic fungi, enlisted different antagonistic fungi against many plant diseases and analyzed the limitations in commercializing fungal biocontrol agents[5]. They have reported that the use of more than one antagonistic fungus in consortium can be considered as a useful strategy in achieving an enhanced performance against plant diseases. But the practical applications of all these biocontrol agents have been hindered mainly by a few common problems involving variability in their performance, their survival rate in the field and the dependance on other factors including environmental conditions. Hence the authors in the current study suggest a method where the active compound(s) extracted from the antagonistic fungi is applied for plant protection instead of using the fungi directly.

In the present study, three species of *Aspergillus viz. A. niger, A. flavus and A. fumigatus* were found to have good antagonistic activity against the pathogenic isolates from brinjal plant and orange fruit. Further the non-volatile metabolites extracted from the isolates in crude forms were also found to possess higher inhibitory effects on the target pathogen. Similar evaluation of antagonistic activity of fungal isolates against plant diseases was done in previous studies[5,10] and identified many bacterial and fungal antagonists. But in most of these studies, they have neither extracted the metabolites nor assessed their effectiveness. The current study has taken an extra step in assessing the effectiveness of fungal metabolites. Partial characterization of these metabolites was also done by TLC. The plant growth promoting ability of *Aspergillus spp.* on brinjal plant was already reported and it was attributed to the production of organic acids by the fungus[11]. Potential of endophytic fungus *Aspergillus terreus* as a plant growth promoter was found to be due to the production of hydrogen cyanide, indole acetic acid (IAA), phenols and flavonoids[12]. This property can add value to the application potential of *Aspergillus spp.* as a biocontrol agent and also as a biofertilizer for sustainable agriculture.

In contrast to these beneficial aspects of Aspergillus spp., certain species like *A.niger* and *A.flavus* are reported as plant pathogens causing many plant diseases, for example, black rot in onions and black mould disease in date fruits caused by *A.niger* and fruit rot of peaches caused by *A.flavus.*[13,14]. Moreover *A.flavus* is well known for production of aflatoxins[15]. Hence, it is not safe to apply these BCAs directly to the soil. But the method used in the present study, ie. the extraction of the antagonistic metabolites from the fungal isolates and use of it for further inhibition, can eliminate this risk. In addition to this, the other limitations of using the BCA directly in the soil such as low survival rate in the field, interaction with other micro-organisms and environmental factors can also be overcome by this method where fungal metabolites are used for plant protection.

4 Conclusion

Management of plant infections by biological control is one of the most promising approaches for sustainable agriculture. Hence, research on the selection, characterization and commercial development of BCAs have been progressively increasing

over the last few decades. The current study is an *in-vitro* screening and evaluation of antagonistic fungi from rhizospheric soil against potential phytopathogens responsible for leaf spot of brinjal and fruit rot of orange. By antagonistic screening, three species of *Aspergillus* identified as *A. niger, A. flavus* and *A. fumigatus* were found to have good antagonistic activity against the pathogenic isolates. Assays were performed to determine the role of volatile and non-volatile compounds in antagonistic action and it was observed that the non-volatile metabolites revealed higher antagonistic activity (65-95%) compared to volatile metabolites. Antagonistic substances were extracted from the culture filtrates and characterized. When these extracts were used in crude form, there was higher inhibition of the growth of tested phytopathogenic fungi. This study suggests the effectiveness of using antagonistic fungi and their non-volatile metabolites as potent biocontrol agents to prevent the crop loss of economically important vegetable and fruit plants. This study highlights exploration of antagonistic fungal strains against not much studied, but important plant diseases. A more practical way of managing the plant diseases by using the antagonistic metabolites instead of using the fungus directly is suggested in the study. The current study has been conducted *in-vitro*, hence further field studies are required to assess the efficacy of fungal metabolites for practical applications. This vital area of research demands more focus for developing novel biocontrol agents and technologies. Further, it is important to explore the interaction between the biocontrol agent, pathogen and the plant in order to establish a better biocontrol technology.

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