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**A Thoyajakshi Bai**

Department of plant pathology,  
College of Horticulture, Dr.  
Y.S.R. Horticultural university,  
Anantharaju Peta, Y.S.R  
Kadapa, Andhra Pradesh, India

**Ch. Ruth**

Department of plant pathology,  
College of Horticulture, Dr.  
Y.S.R. Horticultural university,  
Anantharaju Peta, Y.S.R  
Kadapa, Andhra Pradesh, India

**K Gopal**

Department of plant pathology,  
College of Horticulture, Dr.  
Y.S.R. Horticultural university,  
Anantharaju Peta, Y.S.R  
Kadapa, Andhra Pradesh, India

**M Surya Prakash Reddy**

Department of Plant Pathology,  
JNKVV, College of Agriculture,  
Jabalpur, Madhya Pradesh,  
India

**Correspondence****A Thoyajakshi Bai**

Department of plant pathology,  
College of Horticulture, Dr.  
Y.S.R. Horticultural university,  
Anantharaju Peta, Y.S.R  
Kadapa, Andhra Pradesh, India

## Effect of volatile and non-volatile inhibitory compounds of rhizosphere fungal antagonistic microbes on the growth and development of chilli wilt pathogen *Fusarium oxysporum*

**A Thoyajakshi Bai, CH Ruth, K Gopal and M Surya Prakash Reddy**

### Abstract

Rhizospheric fungal antagonists can act as potential biocontrol agents towards fungal pathogens. They show antagonistic activity against phytopathogen by production of volatile and non volatile metabolites. *In vitro* assessments revealed their antibiosis and mycoparasitic ability to affect growth of the pathogen. This study explored the potential of rhizospheric fungal antagonists in controlling the wilt pathogen *Fusarium oxysporum* due to production of toxic volatile and non volatile metabolites. A total of twenty rhizospheric microbes were isolated from healthy chilli plants out of which eight fungal antagonists were tested for their ability to inhibit the growth of the pathogen *Fusarium oxysporum* by production of volatile and non volatile inhibitory metabolites. Among them four promising antagonists were tested RFA 2 showed the highest mycelial inhibition of the pathogen (24.35% and 74.5%) followed by RFA 4(17.29% and 60.47%) and RFA 1 (5.17% and 31.76%), whereas RFA 3 showed less inhibitory effect (2.35% and 22%) by production of volatile and non volatile inhibitory compounds respectively after seven days of inoculation.(Table-1). All the tested rhizospheric fungal antagonists showed positive results by significantly reducing the growth of pathogen.

**Keywords:** Volatile, non volatile inhibitory compounds, biocontrol, *Fusarium*, chilli

### 1. Introduction

*Fusarium oxysporum*, is one of the major disease in chilli, which leads to severe crop loss. *Fusarium* wilt became most destructive disease in chilli all over the India. The management of wilt disease is essential criteria to reduce crop loss but increasing use of pesticides in the process of controlling the disease incidence leads to several ecological problems, such as pest resistance, resurgence effect which ultimately results in decrease in population of beneficial microbes those are residing in the rhizosphere portion of soil. So there is a world wide need to adopt the practice of sustainable agriculture, using biocontrol strategies that are environment-friendly. One of the key elements of such sustainable agriculture is the application of biological controlling strategies, for plant protection. The use of beneficial rhizosphere microbes residing soil near to the roots of the healthy plant like *Trichoderma*, *Bacillus*, *Pseudomonas* etc.) for the control of fusarium wilt disease.

Antimicrobial compounds are produced by the rhizosphere fungal antagonists most usually in the form of volatile or nonvolatile metabolites which are toxic and can directly affect of mycelial growth and conidiation of the pathogens. The inhibitory effect of the antagonists by the production of nonvolatile compounds can be detected using simple plate assay (Chaurasia *et al.*, 2005) [2].

Rajeshwari and Kannabiran (2011) found that volatile and non-volatile metabolites of *Trichoderma* sp. more effective in reducing conidial germination rate and mycelia growth *Fusarium oxysporum* *in vitro*.

Sree Deepthi *et al.* (2016) [8] observed the antimycotic effect of *Trichoderma* species by producing volatile and non volatile inhibitory compounds on *Fusarium oxysporum* f. sp. *capsici*.

### 2. Materials and Methods

Rhizosphere antagonists were isolated from healthy rhizosphere soil samples collected from Kurnool, Kadapa and Guntur districts of A.P.

A total of 20 rhizosphere microbes were isolated. Among which eight fungi, ten bacteria and two fluorescent Pseudomonads were found to exhibit antagonism against chilli wilt pathogen. On further *in vitro* evaluation, nine isolates including four fungi, four bacteria and one Pseudomonas sp. were found to be more efficient antagonists through dual culture technique.

By observing the performance of respective fungal antagonists *in vitro*, the most promising rhizosphere fungal antagonists were tested for production of volatile and non-volatile inhibitory metabolites.

### 2.1 Antibiosis test for the production of diffusible, non volatile inhibitory metabolites

Antibiosis test for production of diffusible, non volatile inhibitory metabolite was carried out using cellophane paper method described by Dennis and Webster (1971) [3]. A sterilized cellophane paper of 90 mm diameter was taken and then each sterilized disc was aseptically placed over the PDA inoculated plates. Ten mm discs were taken from the growth of rhizosphere antagonistic fungi and was placed at the centre of the cellophane paper and incubated for 72 hrs. After this, the cellophane paper along with adhering antagonists was removed carefully and 10 mm disc of *Fusarium* wilt pathogen was immediately placed on the medium at the central position previously occupied by antagonist. The growth of the pathogen was calculated from 48 hrs. up to seven days and the growth was compared with that in control. Three replications were maintained and the per cent inhibition of the pathogen was calculated.

### 2.2 Evaluation of volatile metabolites

The effect of volatile metabolites released by antagonists on the mycelial growth of pathogen was evaluated by inverted plate technique as described by Dennis and Webster (1971) [3]. The 5 mm mycelial discs of fungal antagonists obtained from the margin of young cultures were placed centrally on the PDA petridish and incubated in 25±1° c for 72 hrs. In the control plates, sterile PDA media discs 5mm in diameter were placed on the plates as mentioned above. At the end of the incubation period, the top of each petridish was replaced with the bottom of the petridish inoculated with pathogen and sealed together with adhesive tape. Sealing petridishes avoided the escape of volatile compounds of the antagonist and ensured their inhibitory effect on the pathogen. A completely randomized design was followed with three replicates in the experiment. Radial growth of the pathogens was recorded on seventh day of incubation and percent inhibition of the pathogen was recorded.

## 3. Results and Discussion

### 3.1 Volatile and non-volatile inhibitory compounds produced by rhizosphere fungal antagonists

Evaluation for the production of volatile and non-volatile components was conducted *in vitro* for the efficient

rhizosphere fungi RFA 1, RFA 2, RFA 3 and RFA 4 and the results revealed that RFA 2 (24.35% and 74.5%) followed by RFA 4 (17.29% and 60.47%), RFA 1(5.17% and 31.76%), and RFA 3(2.35% and 22.0%) have significantly inhibited the mycelial growth of pathogen by the production of volatile and non-volatile metabolites respectively after seven days of inoculation (Table 1). All the tested fungal antagonists were positive to both volatile and non-volatile metabolites production.

Similarly, Rajeshwari and Kannabiran (2011) observed the reduction of mycelial growth as well as conidial germination rate of *Fusarium oxysporum* due to production of volatile organic metabolites by *Trichoderma harzianum*.

The similar line of work done by Hindumathi *et al.* (2017), Stoppacher *et al.* (2010) [9] and Pan *et al.* (2013) [7], by (Dennis and Webster, 1971b; Fiddman and Rossall (1993) [3,4], Mohammad Akrami *et al.* (2011) [5] in their results suggested that from the isolates of *Trichoderma* used in their study, *T. harzianum* T149 and *T. asperellum* T90 strains were more capable of influencing the growth of all tested pathogens in dual culture and through production of volatile and non-volatile inhibitors under controlled condition.

Amin *et al.* (2010) [1] reported volatile activity of six isolates of *Trichoderma* spp. against seven different fungal plant pathogens. Out of which *T. viride* (Tv-1) was found to be more effective in reducing the mycelial growth of *F. oxysporum* (41.8%). Similarly Nagamani *et al.* (2017) [6] in their studies noticed that the radial growth of test pathogens significantly reduced by the production of toxic volatile metabolites.

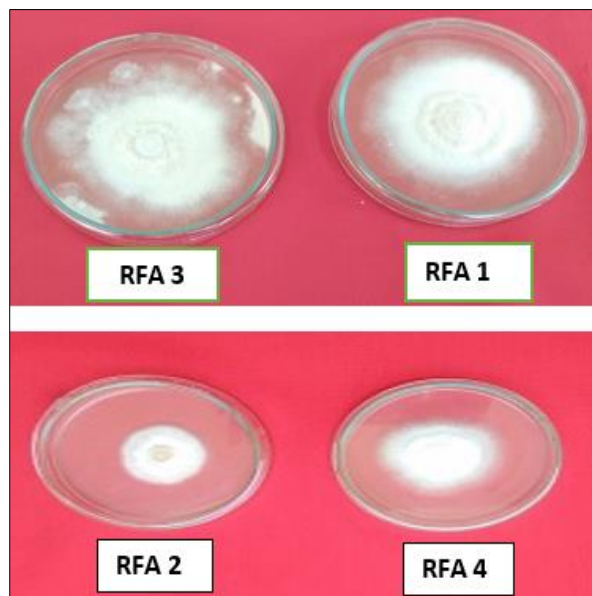


Fig 1: Effect of volatile inhibitory compounds on mycelial growth of *Fusarium* wilt pathogen

Table 1: Effect of Volatile and non volatile inhibitory compounds on radial growth of mycelium

S. No	Rhizosphere antagonistic fungi	Volatile inhibitory compounds		Nonvolatile inhibitory compounds	
		Radial growth (mm)	PIOC (%)	Radial growth (mm)	PIOC (%)
1	RFA 1	80.66	5.17 (13.05) **	58.00	31.76 (34.27) **
2	RFA 2	64.3	24.35 (29.53)	21.66	74.5 (59.67)
3	RFA 3	83.00	2.35 (8.72)	66.33	22.0 (27.97)
4	RFA 4	70.3	17.29 (24.50)	33.66	60.47 (51.00)
5	Control	85	0.00 (0)	85	0.00 (0)
	CD at 5%	0.350		0.273	
	Sem ±	0.110		0.086	

\*Mean of three replications

\*\* Figures in parentheses are angular transformed values

Yuan *et al.* (2012) <sup>[10]</sup> conducted detailed study on VOCs of bacterial bioagents against *Fusarium oxysporum* and noted that the volatile compounds produced by the bacteria significantly reduced the Mycelial growth 30-40 % compared to control and also inhibited spore germination of *F. oxysporum*.

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