



## Antagonistic activity and culture filtrate of fungi against *Fusarium oxysporum* causing wilt disease in banana

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### Abstract

Totally 24 species belonged to 20 genera were isolated and identified from banana field soil. A preliminary screening of all the species isolated from soils were made for antifungal (antagonistic) activity against *Fusarium oxysporum*, a known soil borne fungal pathogen. Among the fungi promising activity of *Trichoderma viride* inhibited the pathogenic fungi for the maximum inhibition in dual culture and food poisoning technique were performed. The maximum percentage of inhibition against *Aspergillus niger*, followed by *A. awamori*, *A. flavus*, *A. fumigatus*, *A. terreus*, *Curvularia lunata* *A. niger*, *Memnoniella* sp., *Penicillium citrinum*, *Trichoderma viride*, and *T. harzianum* against *F. oxysporum*. were observed whereas, culture filtrates of fungi like *Aspergillus awamori*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A. niger*, *Curvularia lunata*, *Memnoniella* sp., *Penicillium citrinum*, *Trichoderma viride* and *T. harzianum* showed the inhibitory effect of different concentration 5, 10, 15 and 20% were determined against *F. oxysporum*. However, the maximum inhibitory effect was observed at 20 percent concentration of the cell free filtrate of *Trichoderma viride* against *F. oxysporum*. The investigation was carried out and of results were discussed in detail.

**Keywords:** dual culture, culture filtrate, fungi *f. oxysporum*

### Introduction

Biological control of plant diseases including fungal pathogens has been considered as environmentally safe and viable alternative measure to chemical control. Nonpathogenic species of the fungal genus *Fusarium* were numerous reported to successfully control *Fusarium* wilt of various crops worldwide (Nel *et al.*, 2006) [10]. In Thailand, non-pathogenic *F. oxysporum* (F221-B), originally isolated from root of lettuce grown in hydroponics, has shown its ability as plant growth promoting fungus (PGPF) on various crops such as Butter head, Cos, Green oak, Red oak lettuces, kale and mung bean and as biocontrol agent (BCA) against plant pathogenic fungi, namely *Curvularia* spp., *Colletotrichum* spp. and *F. oxysporum* f. sp. Lactucae. Non-pathogenic F221-B showed remarkable consistency in retaining its morphology characteristics and its efficacy as BCA and PGPF during 24 months storage. In addition, F221-B was proved to be able to colonize on root surface (epiphyte) as well as inside the root of lettuce (endophyte) without producing any disease symptoms. *Fusarium* root rot and wilt disease was regarded as one of the most seriously disease of lettuce grown in soil and hydroponic cultivation (Cabral *et al.*, 2014) [5]. The disease was caused by *Fusarium oxysporum* f.sp. lactucae. Effective control methods against this disease are limited whereas chemical control has adverse effect to the consumer and environment. On this regard, non-pathogenic F221-B would be one of the strong candidates as biological control agent to solve this problem. Based on the above-mentioned good characteristics of F221-B as PGPR and BCA from which the feature of antibiosis was also observed in confrontation experiment between F221-B and test fungal pathogens at the early stage of incubation period, it is interesting to further research on the antifungal ability of

secondary metabolites in culture filtrate of non-pathogenic F221-B. Therefore, the research was conducted to determine the potential of culture filtrate (CF) of non-pathogenic *Fusarium oxysporum* (F221-B) against 10 plant pathogenic fungi namely, *Curvularia* sp. (C11, C12), *F. semitectum* (F113), *F. oxysporum* f.sp. lactucae (F221-R, F422-G), *Rhizoctonia* sp. (R11, R12, R13) and *R. solani* (R11, R12) *in vitro* and *Fusarium* root rot disease in hydroponics.

*Fusarium* wilt also known as panama wilt caused by *Fusarium oxysporum* f. sp. cubense is a major constraint to banana production worldwide. It is active saprophyte in organic matter, soil and several forms are pathogenic to plants (Smith *et al.*, 1988). This pathogen survives and spread through infested soil and infected plants. It can infect fruits and seeds but dispersal through seed is very rare. Characteristic symptoms of *F. oxysporum* f. sp. cubense infection are discoloration or browning of foliar plant tissues. However, symptoms are more obvious on old plant during flowering stage and fruit maturity stages. The fungus causes root rot, vascular wilt and damping off in a number of plant species. *F. oxysporum* f. sp. cubense cause vascular wilt syndrome in banana during 1960s it demolished whole banana industry (Leslie *et al.*, 2006) [9]. This fungus has many famous plant pathogenic strains and *F. oxysporum* the cause of panama wilt have four different races. Control of soil borne phytopathogens through biological control agents is effective way (Rakh *et al.*, 2011) [12]. Only one application of a biological control agent as seed treatment reduces the seed decay and damping off of seedlings while controlling such, diseases mainly depend on fungicides treatments (Ahmad *et al.*, 1996) [1]. Application of fungicides hazardous for environment and human health, therefore, environment friendly approach against pathogens required for safety. Many studies have proved the potential

of *Trichoderma* sp. as biological agent antagonistic behavior to several plant pathogens. Among different bio control agents *Trichoderma* sp. are common free-living fungi present in roots and soil. *Trichoderma* species is very interactive to root, soil and plant foliar environments. Due to release of different compounds it triggers the plant immune system and resistance in the form of local or systemic.

*Trichoderma* is well recognized as biological weapon against different plant pathogens and used as growth regulator for roots, yield increase, flexibility towards stress and nutrients uptake from soil. *Trichoderma* spores are more tolerant against unfavorable environmental conditions when used in the form of product formulation and field applications. Presence of mycelia mass plays a key role in antagonistic metabolites (Yedidia *et al.*, 2000) [19]. The efficacy of *T. harzianum* against *F. oxysporum* f. sp. cubense and to detect and quantify the reduction in pathogen population.

## Materials and Methods

### Dual culture method (Skidmore and Dickinson, 1976)

Potential antagonist's culture blocks of 5mm diameter cut from the margin of 8 days old cultures of both test pathogen and antagonists were placed opposite to each other on PDA in 90mm glass petriplates. The distance between inoculum block was 50mm. The inoculated plates were incubated at room temperature (28±2°C). The colony growth and radial growth of the pathogen was measured at both sides i.e. towards and opposing each other from their central loci. Intermingled or inhibition zone was also measured at the same period. The assessment of interaction was made following the model of Skidmore and Dickinson, 1976. The parameters used for the assessment of colony interaction were the width of inhibition zone of growth intermingled zone and percent inhibition of radial growth, I. e.  $100 \times (r_1 - r_2) / r_1$ , where,  $r_1$  denotes the radial growth of the paddy pathogen towards the opposite side and  $r_2$  denotes the radius of the pathogen towards the antagonistic fungus.

### Culture filtrate method (Rabindran and Vidyasekaran, 1996)

To assess the effect of culture filtrate, the antagonist fungi were grown on PDA medium. Three mycelia agar discs (each of 5mm diameter) of an individual antagonist, were cut from the actively growing margins of 5 day old culture were inoculated into a 250ml conical flask containing 100ml Potato Dextrose broth medium. After 10 days of incubation at (30 ± 2°C) the culture of an antagonist was filtered through a filter paper and then centrifuged at 3000rpm for 20 minutes and finally filter through a micro pore filter paper under vacuum pressure to obtain the cell free culture filtrate. The Culture filtrate of an antagonist containing its non – volatile metabolites was tested in four concentrations (5, 10, 15 and 20%) against rice pathogen. The culture filtrate of a particular concentration was obtained by supplemented it with required amount of sterilized PDA medium. The each Petri plate was inoculated centrally with a 5mm. mycelia agar disc cut from the margin of actively growing culture of a *Fusarium oxysporum* pathogen. All the plates were incubated at room temperature (28 ± 2°C). The radial growth of the colonies was measured after five days

of incubation. The percent growth inhibition of a rice pathogen was calculated by using the formula.

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

Where,

I-Percent inhibition of the pathogen

C-Radial growth of the pathogen in control

T-Radial growth in of the pathogen in treatment

## Result and Discussion

In dual culture technique results revealed that *Trichoderma* spp. exhibited the maximum resistance of pathogen mycelial growth. During inhibition zone in dual culture distance between hyphae of both fungi suggests that some secretion which are diffusible non-volatile inhibitory material produced by the *Trichoderma* isolate. *Trichoderma* species interacts and produces small quantities of extracellular exochitinases (Brunner *et al.*, 2003) [4]. *T. harzianum* inhibits the growth of *F. oxysporum* (61.4%) in the dual culture technique described by (Muthu Kumar *et al.*, 2008). The maximum inhibition of *F. oxysporum* was recorded in 20% culture filtrate of *T. harzianum* 75.5% showed in. *T. harzianum* significantly (P <0.05) reduce the growth of *F. oxysporum*, on PDA after incubation for 72 h at 30°C reported (Ullah *et al.*, 2011) [17]. Researchers reported that *Trichoderma* release different antibiotics which are trichodermin, trichodermol, harzianum A and harzianolide (Howell, 1998 and Woo *et al.*, 2006) [7, 18]. *Trichoderma* against *Fusarium* wilt showed that the *T. harzianum* significantly (P ≤ 0.05) reduced the *Fusarium* wilt disease severity (Srivastava *et al.*, 2010) [15].

The present investigation reported that the effect of antagonistic fungi on the growth of inhibition of *Fusarium oxysporum* by beneficial fungi like *Aspergillus awamori*, *A. flavus*, *A. fumigatus*, *A. terreus*, *Curvularia lunata*, *Memnoniella* sp., *Penicillium citrinum*, *Trichoderma harzianum* and *T. viride* were maneuvering results noticed. The maximum zone of growth inhibition from *Aspergillus flavus* to *F. oxysporum*. According to the either fungal interactions of beneficial fungi against the *F. oxysporum* by causing banana field disease can be reduced respectively and minimum zone of inhibition was observed in *Aspergillus flavus* against *F. oxysporum*. However, the antagonistic potential of fungi has shown an extraordinary performance against pathogenic fungi *F. oxysporum* because fungal secondary metabolites which contain rich amount of bioactive compounds (Table-1).

Three *Trichoderma* isolates viz., *T. reesei* (CSR-T-3), *T. koningiopsis* (CSR-T-2), and *T. asperellum* (CSR-T-4) were assessed for their *in vitro* efficacy by a dual co-culture technique on PDA medium. All of the three isolates utilized the nutrient source and showed a significant increase in the inhibition percentage between 48 and 72 h after inoculation by inhibiting the mycelia growth of Foc TR4 (CSR-F-1). The maximum reduction of the mycelia growth of the pathogen was elicited by the isolate CSR-T-3 that showed the highest inhibition percentage of 85.19% at 120 h after inoculation, whereas CSR-T-1 Inhibitory effect of CSR-T-3 (*Trichoderma reesei*) against pathogenic isolate CSR-F-1 of Foc TR4. Percentage inhibition of Foc TR4 was found to be highest in CSR-T-3 (*Trichoderma reesei*) over a period of time in comparison with *T. asperellum* and *T. koningiopsis*.

Showed the least inhibition (50.00%). The inhibition percentage of CSR-T-2 (*T. koningiopsis*) was 62.65%. Based on these results, it is evident that CSR-T-3 (*T. reesei*) is the most effective *Trichoderma* isolate with a tremendous antagonistic ability against Foc TR4. (Damodaran *et al.*, 2020) [6].

The inhibitory effect of F221-B culture filtrate was in accordance with those of Benhamou *et al.*, (2002) [3, 19] and Khurshid *et al.*, (2014) [8] who reported that various non-pathogenic *Fusarium* isolates have shown their ability to suppress plant pathogens. Tayung *et al.*, (2010) [16] reported that the metabolite produced by endophytic *Fusarium* (MTCC-9622) showed significant antifungal activity against 3 postharvest pathogens including *F. oxysporum*.

The current study proved that the antagonistic efficacy of fungal culture filtrate of different concentrations of 5, 10, 15 and 20% against *F. oxysporum*. About 20% concentration of culture filtrate shown no growth of *F. oxysporum*, but 5% of culture filtrate expressed moderate growth on *F. oxysporum*. Among the culture filtrate of antagonistic fungi, the *Trichoderma viride* has been observed an excellent property in the suppression of test pathogenic fungus (Table-2).

Even though proliferation of a fungus *in vitro* is not necessarily related to pathogenicity of its culture filtrate there are various reports indicating such a relation for several plant-pathogen interactions (Alberts *et al.*, 1990) [2].

Found a positive relation between *Fusarium moniliforme in vitro* growth and production of fusarin C toxin. Reported maximum *Fusarium subglutinans* growth at 21 days of incubation, coinciding with the highest phytotoxicity of the culture filtrate over pineapple plants. Seem to correspond to the second group where fungal culture mass maximised at 24 days coinciding with the highest capacity of culture filtrate to promote leaf lesions. Additionally, autolysis seems to have taken place after 24 days of medium inoculation with the fungus.

The culture filtrate of the three antagonistic fungi that are used for the biological control studies, *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma asperellum* were also evaluated for the effect on the seed germination. Amongst the three species, *T. asperellum* and *T. harzianum* culture filtrate were found to have stimulatory effect on seed germination and was found to cause increase in the seed germination percentage. Culture filtrate of *T. viride* cause inhibition in seed germination of *B. rapa* and *R. sativus*. Culture filtrate of all *Trichoderma* sp. were found to increase the seed germination percentage of *Solanum lycopersicum* and *Trigonella melongena*, whereas a negative effect was found on the germination percentage of *B. rapa* and *R. sativus* seeds by culture filtrate of *T. viride*. (Parveen *et al.*, 2019) [20].

**Table 1:** Colony interactions between *Fusarium oxysporum* and some soil fungi in dual culture experiments

Growth response of the antagonistic and test fungus	Antagonistic fungi Tested (Growth rate in mm)									
	Aa	Af	Af	At	An	Cu	Me	Pc	Tv	Th
Colony growth of pathogen towards antagonist (r1)	19.0	52.0	20.0	20.0	30.0	17.0	16.0	18.0	55.0	18.0
Colony growth of pathogen away from the antagonist	21.0	55.0	24.5	24.0	37.0	18.5	19.0	22.0	58.0	20.0
% growth inhibition of pathogen in the zone of interaction	65.0	73.0	70.0	63.0	75.0	70.0	62.0	65.0	75.0	63.0
Colony growth of antagonist in control (ie) growth of the plate in absence of the pathogen. (r)	64.0	75.1	69.0	56.9	73.8	53.8	70.0	72.3	71.4	61.0
Colony growth of antagonist towards the pathogen (r1)	67.0	27.0	63.0	49.0	67.0	51.0	43.0	65.0	62.6	48.0
Colony growth of antagonist away from the pathogen	58.0	50.0	21.0	41.0	56.0	45.0	25.0	51.0	45.0	38.3
% of grow the inhibition in the zone interaction.	7.0	55.4	8.0	8.2	20.5	10.2	13.6	15.9	26.3	10.6

Aa- *Aspergillus awamori* Af - *Aspergillus flavus*, Af - *A. fumigatus*, At - *A. terreus*, An - *A. niger*, Cu – *Curvularia lunata*  
 Me- *Memmoniella* sp., Pc - *Penicillium citrinum*, Tv - *Trichoderma viride*, Th - *T.harzianum*



Standard deviation ± Standard error

**Fig 1:** Colony interactions between *Fusarium oxysporum* and some soil fungi in dual culture experiments

**Table 2:** Effect of culture filtrates of some soil fungi on the growth of *F. oxysporum*

S.No.	Name of the fungi	Growth measurement (mm)			
		5%	10%	15%	20%
1	<i>Aspergillus awamori</i>	1.05±0.09	0.10±0.03	0.17±0.04	0.00±0.00

2	<i>A. flavus</i>	3.15±2.09	2.10±1.03	1.07±0.98	0.10±0.09
3	<i>A. fumigates</i>	1.10±1.03	0.00±0.00	0.00±0.00	0.00±0.00
4	<i>A. terreus</i>	2.05±1.01	1.21±1.10	1.16±0.80	0.13±0.10
5	<i>A. niger</i>	2.20±1.10	1.15±1.09	1.10±0.03	1.20±0.07
6	<i>Curvularia lunata</i>	1.05±1.07	0.09±0.05	1.07±0.98	0.00±0.00
7	<i>Memnoniella</i> sp.,	1.05±1.01	0.00±0.00	0.00±0.00	0.00±0.00
8	<i>Penicillium citrinum</i>	2.13±1.06	1.05±0.98	1.09±0.00	0.40±1.00
9	<i>Trichoderma viride</i>	3.25±1.15	2.20±1.10	1.95±0.50	1.12±1.20
10	<i>T.harzianum</i>	2.15±2.09	1.10±1.03	0.10±0.98	0.00±0.00

Standard deviation ± Standard error

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