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Acremonium kilense as an endophytic bioagent against turmeric (*Curcuma longa* L.) diseases and tolerance to plant protection chemicals

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Abstract
Turmeric (*Curcuma longa* L), "the golden spice of life" is one of most essential spice use as an important ingredient in culinary all over the world. Present investigation was done to understand the biocontrol potential of various endophytic microbes associated with turmeric against the pathogen causing rhizome rot disease of turmeric (*Pythium* sp.). A total of 15 endophytic fungi isolated from different locations of Andhra Pradesh state were evaluated. It was observed that Tc ed f 1(*Acremonium kilense*) was found to exert maximum antagonism against the pathogen. It was also observed that *Acremonium kilense* is incompatible with fungicides like Bordeaux mixture, Mancozeb 75%WP, Cyamoxanil 8% + Mancozeb 64%WP and Bordeaux mixture and higher concentrations of Fenamidone 10% + Mancozeb 50% WG and insecticides like Dimethoate, Malathion and Thiomethoxam showed 100 per cent inhibition indicating its poor level of compatibility.

Keywords: *Pythium* sp., endophyte, *Curuma longa*, antagonism, epiphyte

Introduction
Turmeric is an important spice crop being cultivated by majority of farmers owing to its remunerative prices. One of the constraints in the production and productivity of spices is the occurrence of diseases. The humid tropical conditions prevail in Andhra Pradesh state is conducive for the growth of many fungal pathogens. The production and productivity of the turmeric crop is crippled by many foliar and soil-borne diseases. Among them rhizome rot disease severe and occur in endemic proportion, causing severe damage to the crop which ultimately hampers the quality and quantity of the crop. Conventional control of diseases in turmeric cultivation often depends on chemical fungicides. The use of chemical pesticides has deleterious effect of environmental pollution thus leading to concern on food safety. Therefore, it is imperative to develop sustainable disease management strategy with emphasis on reducing the use of chemicals and to search benign alternatives like antagonistic microbes residing in and outside the plants. Association of endophytic microbes with turmeric have been documented by (Gary *et al.*, 2001) [4] and (Soapalun, *et al.*, 2003) [11]. So, in order to exploit the plant associated endophytic microbes in disease management of turmeric, the present investigations were carried out.

Materials and methods
The pathogen causing rhizome rot disease of turmeric was isolated from the infected rhizomes collected from different locations of Andhra Pradesh (Fig.1&2). The diseased rhizome samples were brought to the laboratory, washed under tap water and the infected area along with healthy portion were cut in to small bits. The bits were surface sterilized with one per cent sodium hypochlorite solution for one minute followed by washing in three changes of sterile distilled water. The surface sterilized bits were placed on Potato dextrose agar (PDA) medium in Petri dishes and incubated at room temperature (26 ± 2 °C). When the fungal growth was visible, small bits of mycelia were transferred to PDA mediated Petri dishes and the isolates were purified by hyphal tip method. These purified cultures were maintained in PDA slants for further studies. The cultural and morphological characters of the isolates were also studied. Pathogenicity of the isolates was established.

Endophytic microbes were isolated from healthy rhizome samples of turmeric collected from various locations of Andhra Pradesh (Table 1). Fresh rhizome samples brought in separate polythene bags were washed under tap water, and used for isolation within 48 h. of collection.

Surface sterilization

The surface of turmeric harbour a lot of epiphytes, thorough surface sterilization was needed to eliminate them. The concentration of sterilant and time of exposure were standardized so as to get the maximum number of endophytes with no growth on sterility check. Three different concentrations of sodium hypochlorite viz., one, two and three per cent were tried for three different exposure times viz. two, five and ten minutes. Further, three different weights of rhizome samples viz.0.5gm, 1.0gm and 2.0gm were also tried. Since the isolation from the 2gm of sample after surface sterilization with two per cent sodium hypochlorite for 10 min yielded good number of colonies with no growth in sterility check, it was selected for further studies.

For the isolation of endophytes from healthy rhizomes of turmeric samples were weighed out and exposed to sterilant followed by washing in three changes of sterile water and blot dried. The rhizome bits were then transferred to sterilized mortar containing 8 ml sterile Potassium phosphate buffer (PB 0.1M, pH) washed thoroughly in the buffer. From the final buffer wash, one ml was pipetted out and poured into sterile Petri plate. To this molten and cooled medium was added and this served as a sterility check. If microbial growth was observed in sterility check with in four days, the isolates obtained from particular samples were discarded. The surface sterilized leaves of nutmeg, clove and cinnamon were triturated (McInroy and Kloepper, 1995) [8] using sterile mortar and pestle with 8 ml of sterile buffer. The triturate was serially diluted in sterile PB up to 10^7. The dilution and medium used for enumeration of each group microorganism are furnished in (Table 2). One ml of diluted triturate was pipetted in to sterile Petri plate and suitable medium was poured. The plates were incubated at room temperature for various intervals.

The *in vitro* compatibility of the selected fungal antagonist *Acremonium kilense* was done with commonly using plant protection chemicals used in turmeric crop. The quantity of fungicides, insecticides needed to get the desired concentration was added to 100 ml sterilized, molten PDA medium, mixed well and poured in sterilized Petri dishes at the rate of 15-20 ml per plate. To avoid contamination, all ten fungicides were exposed to UV light for a period of 30 min before adding it into the medium. After solidification of the medium, mycelial discs of 8mm diameter from actively growing fungal antagonists were cut and placed at the centre of the each Petri dish. Control consisted of PDA medium alone inoculated with the antagonist. Three replications were maintained for each concentration. The inoculated plates were incubated at room temperature and observations on the mycelial growth of the fungal antagonists were taken when control plates showed full growth. Analysis of variance performed on the data collected in various experiments using the statistical package MSTAT (Freed, 1986) [3].

Results and discussion

Isolation of pathogen

The pathogen produced white cottony mycelium and attained 9 cm growth in PDA mediated Petri dish with in 5 to 6 days. Hypha is branched, hyaline, coenocytic. The average L/B ratio of sporangia of the isolate ranged from 19.8 to 9.9 µm (Fig.4) Based on the above characters the fungus was identified as *Pythium* sp. Similar findings was reported by the earlier workers (Lucas, 1975; Mehrotra and Aggrawal, 2004; Rangaswami and Mahadevan, 2005 and Gaur and Chauhan, 2007). These morphological characters are in conformity with those recorded by (Sparrow, 1960).

Fig 1: Healthy and diseased rhizome

Fig 2: Healthy and rhizome rot infected turmeric

Turmeric endophytes

Fifteen fungi of endophytic origin were isolated from turmeric, out of 15 endophytic fungi evaluated in the preliminary screening, only one exhibited antagonism towards P*ythium* sp. A perusal of observation revealed that the isolate *Acremonium kilense* showed more than 60 per cent inhibition over the pathogens and exhibited lysis and overgrowth type of antagonistic reaction. (Fig. 4) *Trichoderma viride* (DrYSRHU) culture was inoculated and maintained as control (reference culture).

Cultures grew rapidly in 12 days, hyphae thin walled, hyaline, conidiogenous cells, phialidic, mostly solitary, conidia produced singly at the tip of phialides and aggregating into slimy heads, ellipsoid to cylindrical with rounded ends. Straight or sometimes slightly curved single celled and hyaline. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T), New Delhi (Id no. 6762.15) the antagonist was identified as *Acremonium kilense.*

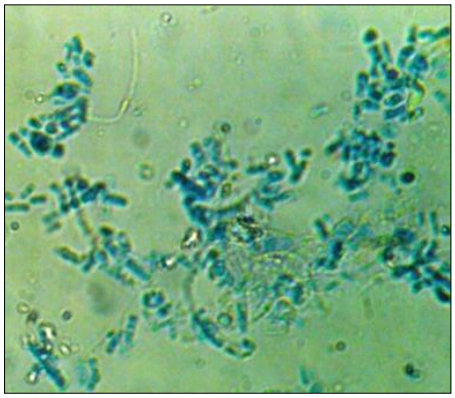

Fig 3: *Acremonium kilense*

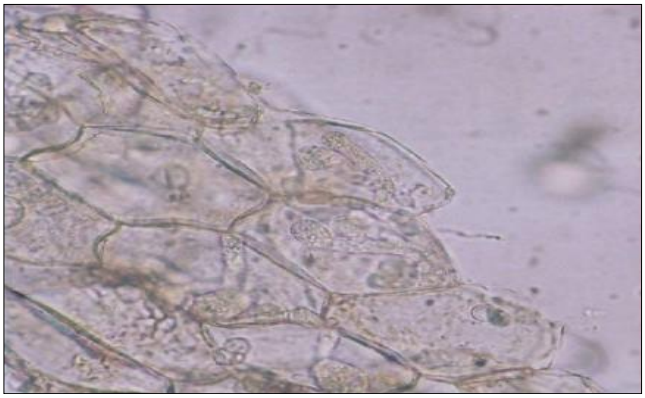

Fig 4: Perennating oospores in rhizomes

Compatibility with fungicides

Experiment revealed that *Acremonium kilense* was incompatible with fungicides like Copper oxychloride 50% WDP (Blitox), Mancozeb 4%+ Metalaxyl 64% W/W (Ridomil Gold MZ), Carbendazim 12%+Mancozeb 64% WP (Saaf), Bordeaux mixture, Mancozeb 75% WP (Indofil M-45), Fenamidone 10%+Mancozeb 50% WG (Sectin) and Cyamoxanil 8%+Mancozeb 64% WP (Curzate M-8) at all concentrations tested (Table. 3, Fig. 5). Higher concentrations of Sectin and all three concentrations of Curzate M-8, Indofil M-45 and Bordeaux mixture also recorded cent per cent inhibition of the endophytic fungi. The possible reason for its poor compatibility with copper-based fungicide like Bordeaux mixture is due to copper is a heavy metal, when it reacts with fungal mycelium it will react with (-SH) groups and forms Cupric ions which are toxic to the fungi. These findings are in tune with many workers like Mondal *et al.*, 1995 [9], Sarma and Anandaraj, 1999 [10]; Ahanger *et al.*, 2014 [1].

Compatibility with insecticides

The antagonists *Acremonium kilense* exhibited varying levels of sensitivity to different insecticides (Table. 4, Fig.6). The sensitivity of insecticides *viz.*, Thiomethoxam 25% WG (Cruiser), Chloropyrifos 20% EC (Dursban), Dimethoate 30 EC (Rogor), Malathion 50% EC (Malathion), Phosphamidon 40% SL (Demecron) each at three different concentrations was tested under *in vitro* conditions. The isolate showed incompatibility with insecticides like Dimethoate, Thiomethoxam and Malathion at all concentrations showing its poor level of compatibility Bhai and Thomas, (2010) [2] conducted similar studies.

Conclusion

The observed level of endophytic microorganism diversity is varying from location to location. The study revealed the antagonistic potential of endophytic fungus against *Pythium* sp creating a new eco friendly path in disease management.

Acknowledgement

The author is thankful to Dr. P. N. Chowdhary, N.C.F.T (National Centre for Fungal Taxonomy) Inderpuri, New Delhi for confirming the identity of fungus.

Table 1: Locations of collections of endophytic microflora of turmeric rhizome

S. No	Crop	District	Place of collection
1.	Turmeric	Kadapa	College of Horticulture, Anantharjupeta
			Rajampeta
		Kurnool	Nandyal, Allagadda
		Guntur	Tenali

Table 2: Dilution and media used for isolation and enumeration of endophytic microflora

S. No	Organism	Dilution	Medium	Period of incubation (Days)
1.	Fungi	10^{-4}	Potato dextrose agar (PDA)	2
2.	Bacteria	10^{-6}	Nutrient agar (NA)	2
3.	Fluorescent Pseudomonads	10^{-6}	King'S B agar	2
4.	Actinomycetes	10^{-1}	Ken Knight'S agar	7

Table 3: Compatibility of selected fungal antagonist with fungicides

S. No	Fungicides		Tc ed b 1	
		Concentration (Per cent)	Mean diameter of colony (mm)*	PIOC*
1	Blitox	0.1	40.00 (6.36)^{b}	55.55
		0.2	30.22 (5.54)^{c}	66.42
		0.3	25.00 (5.04)^{d}	72.22
2	Ridomil Gold MZ	0.05	30.66 (5.57)^{c}	65.93
		0.1	25.55 (5.04)^{d}	71.61
		0.2	10.00 (3.24)^{f}	88.88
3	Saaf	0.05	35.55 (6.00)^{c}	60.55
		0.1	25.55 (5.04)^{d}	71.61
		0.2	10.00 (5.52)^{c}	88.88
4	Bordeaux mixture	0.5	0 (0.71)^{g}	100
		1	0 (0.71)^{g}	100
		1.5	0 (0.71)^{g}	100
5	Indofil M-45	0.2	0 (0.71)^{g}	100

		0.25	0 (0.71)^{g}	100
		0.3	0 (0.71)^{g}	100
6	Sectin	0.05	22.22 (4.74)^{e}	75.31
		0.1	0 (0.71)^{g}	100
		0.2	0 (0.71)^{g}	100
7	Curzate M-8	0.1	0 (0.71)^{g}	100
		0.2	0 (0.71)^{g}	100
		0.3	0 (0.71)^{g}	100
8	Control	-	90 (9.51)^{a}	0
S.EM			0.236	0.037
CD at 5%			0.673	0.105
S. Ed.			0.333	0.052
C.V (%)			9.191	3.159

* Mean of three replications, Figures in parenthesis are ʀ√x+0.5 transformed values, in each column figures followed by same letter. PIOC = Per cent Inhibition Over Control

Table 4: Compatibility of selected fungal antagonists with insecticides

S. No	Insecticides	Concentration (per cent)	Tc ed b 1	
			Mean diameter of the colony (mm)*	PIOC*
1	Rogor	0.05	0 (0.71)^{f}	100
		0.1	0 (0.71)^{f}	100
		0.2	0 (0.71)^{f}	100
2	Dursban	0.05	36.00 (6.04)^{b}	60
		0.1	32.00 (5.70)^{c}	64.44
		0.2	25.00 (5.04)^{d}	72.22
3	Cruiser	0.1	0 (0.71)^{f}	100
		0.2	0 (0.71)^{f}	100
		0.3	0 (0.71)^{f}	100
4	Malathion	0.05	0 (0.71)^{f}	100
		0.1	0 (0.71)^{f}	100
		0.2	0 (0.71)^{f}	100
5	Demecron	0.05	31.00 (5.61)^{c}	65.55
		0.1	25.00 (5.04)^{d}	72.22
		0.2	24.00 (4.94)^{e}	73.33
6	Control	-	90 (9.51)^{a}	0
S.EM			0.388	0.044
CD at 5%			1.123	0.128
S. Ed.			0.549	0.063
C.V (%)			8.888	2.909

Figures in parenthesis are √0.5 transformed values

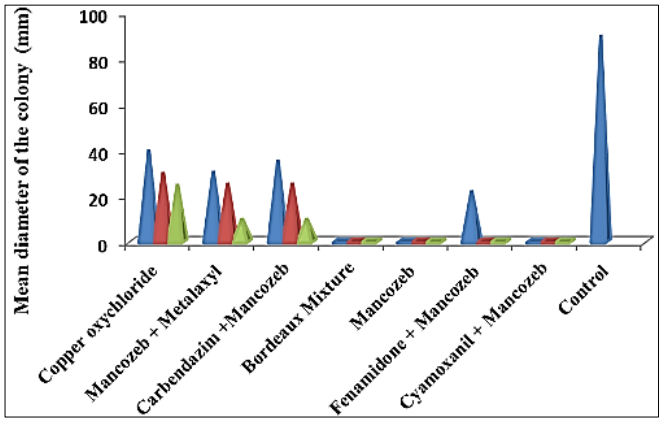

Fig 5: Compatibility of *Acremonium kilense* (Tc ed f 1) with fungicides

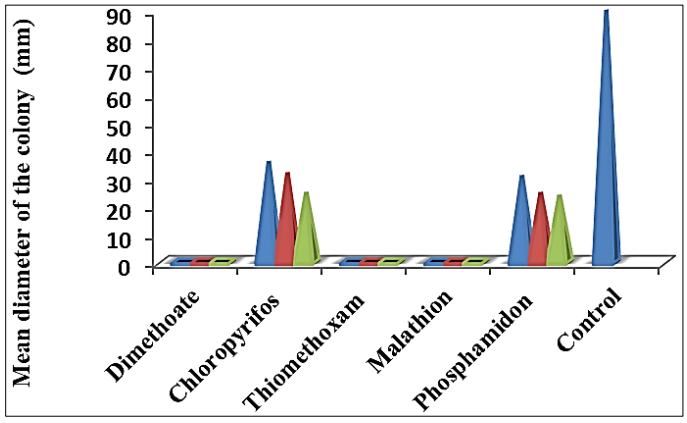

Fig 6: Compatibility of *Acremonium kilense* (Tc ed f 1) with insecticides

References

1. Ahanger RA, Gupta V, Razdan VK, Dar NA, Bhat HA. Compatibility studies of selected fungicides, insecticides and herbicides. Koul research foundation, Jalandar, India, Biopestcides International. 2014; 10:188-192.
2. Bhai RS, Joseph Thomas. Compatibility of *Trichodema harzianum* (Rifai) with fungicides, insecticides and fertilizers. Indian Phytopathology. 2010; 2:145-148.
3. Freed R. MSTAT version 1.2, Department of Crop and Soil Sciences, Michigan State University, 1986.
4. Gary A, Strobel, Emile D, Joe sears, Chris Markworth. Volatile antimicrobials from *Muscodor albus* a novel endophytic fungus. Microbiology. 2001; 147:2943-2950.
5. Karunakaran P, Nair MC. Twig blight and flower shedding diseases of clove trees. Agricultural Research Journal of Kerala. 1980; 18:130-131.

6. Karunakaran P. *Etiology and control of the diseases of tree spices (Clove, nutmeg and cinnamon) in Kerala.* Ph.D. thesis, Kerala Agricultural University, Thrissur, 1981, 197p.
7. Mathew SK, Beena S. A new record of *Phytophthora ramorum* causing leaf fall and shoot rot of nutmeg (*Myristica fragrans*). Indian. Journal of Mycology and Plant Pathology. 2012; 42:529-530.
8. McInroy JA, Kloepper JW. Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant and Soil. 1995; 173:337-342.
9. Mondal G, Srivastava KD, Aggarwal R. Antagonistic effect of *Trichoderma* spp.on *Ustilago segetum var. tritici* and their compatibility with fungicides and biocides. Indian Phytopathology. 1995; 48:466-470.
10. Sarma YR, Anandaraj M. Phytophthora foot rot of black pepper. Management of threatining plant disease of national Importance. Malhothra Publishing House, New Delhi, 1999, 237-248.
11. Sopalaun K, Strobel GA, Hess WM, Worapon J. A record of *Muscodor albus* an endophyte from *Myristica frgrans* in Thailand. Mycotaxon. 2003; 88:239-247.