BIOLOGICAL CONTROL OF BANANA WILT CAUSED BY *FUSARIUM SOLANI (Mart.) Sacc*

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ABSTRACT: The population dynamics of fungi in banana field soil, antibiotic potentiality of the soil fungi and antifungal ability of some plant extracts against *Fusarium solani* was studied. Maximum number of colonies were isolated during the month of November. The genus *Aspergillus, Pencillium, Trichoderma,* and *Fusarium* were isolated most frequently. Antibiotic interaction of the soil fungi against *F.solani* was performed. The test pathogen varied in its sensitivity to the metabolic growth products of antagonistic fungi. The maximum percentage of inhibition of pathogen was 85% against *T.viride* and 75% of inhibition was against *A.niger*. Culture filtrate of the soil fungi and the suppression of *F.solani* was observed. The maximum percentage of suppression of pathogen was observed against *T.viride* and *A.niger* at 20 per cent concentration level. The leaves of medicinal plant extract such as *Adathoda vasica, Azadirachta indica* and *Vitex negundo* was more effective at 20% concentration against the pathogen was reported. The leaf extracts (*Adathoda vasica, Azadirachta indica* and *Vitex negundo)* with culture filtrate of *Trichoderma viride* in combination of 1:1 ratio was more effective at 20 per cent level against the *F.solani*. However, the combined treatment of *A.vasica* and *T.viride* was more effective in controlling the pathogen *F.solani* causing wilt disease of banana.

Key words: Biological Control, Banana, *Fusarium solani*

INTRODUCTION

Biocontrol of plant pathogen involves the use of biological processes to reduce the inoculum density of pathogen and to maintain their soil population below the disease threshold level. This reduces crop losses while interfering minimally with the ecosystem and damaging the environment. The pathogen in the absence of their hosts survive either as dormant propagules or actively as saprophytes on dead organic substrates of the host in the soil. The survival structure of the pathogens in the soil are suppressed either due to natural suppressiveness of the soil or due to manipulation of the soil environment. The 'pathogen suppression' in the soil is considered as an important step in the control of disease as it involves the direct disinfestations of the soil.

Fusarium wilt (panama disease) of banana is reported from all banana growing areas in south pacific Islands and countries bordering and Mediterranean. The panama disease spread primarily by the planting infected and subsequently the pathogen persist in the soil for several years. Chemical control and flood of disease were proven ineffective in the managing banana wilt and host resistance was the most effectives were reported resistant to wilt of banana (Ramakrishna and Deodorant,1956),but very little work has been done on this disease.

MATERIALS AND METHODS

TEST ORGANISM

Fusarium solani

The fungus *F.solani*, one of the soil borne broad spectrum pathogen, causes a major disease in banana called *Fusarium* wilt. The pathogen was isolated from the banana field soil, and the pathogenecity was confirmed by adopting Koch's postulates. The pathogen was maintained in PDA(Potato Dextrose Agar) medium for further investigations.

Isolation of soil fungi

Collection of the soil sample

The soil samples were collected at the depth within 15cm from banana field. The collected soil samples were brought to laboratory in sterilized polythene bags and then stored in air dried containers for further use.

Serial dilution technique

One gram of the soil samples was taken in a 250 ml conical flask containing 100 ml sterile distilled water. The flask was shaken on the electric shaker to get a homogenous suspension and different dilution of the soil samples viz.,10^{-2} and 10^{-3} were prepared by transferring serially about 10 ml of the soil suspension to about 90 ml of sterile distilled water. One ml of 10^{-3} dilution was plated in Petri dishes containing Potato Dextrose Agar medium. Streptomycin sulphate (20 mg^{-1}) was added into the media to prevent the bacterial growth. The plates were incubated at 25±2° c for five days and the fungi appearing on the surface of the nutrient media were recorded.

$$\text{Population of fungi } g^{-1}\text{dry wt of the soil} = \frac{\text{Mean no of propagules in dilution plate}}{\text{Wt of the dry soil}} \times \text{Dilution factor}$$

$$\text{Percentage frequency} = \frac{\text{No of soil samples from which fungi were recorded}}{\text{No of soil samples}} \times 100$$

The fungi were identified by using standard manual of soil fungi (Gillman,1957),Dematiaceous Hyphomycetes (Ellis, 1971), More Dematiaceous Hyphomycetes (Ellis, 1976), Hyphomycetes (Subramanian, 1971).

Physico chemical properties of soil

Soil moisture, pH and temperature were determined as described by Mishra(1968).the total organic carbon total inorganic matter were estimated by rapid titration methods of Walkley and Black(1934).

The total organic matter was calculated by multiplying the organic carbon with constant factor 1.7241 as it is presumed that the organic matter of soil contains 58 percent carbon (Robinson,1969).Total organic nitrogen was estimated by the Micro-Kjeldhal distillation method(Jackson,1958)

Dual culture method

The test pathogen, *Fusarium solani* and soil fungi viz ., *Aspergillus flavus, A.fumigatus, A.niger, A.terreus, Penicillium chrysogenum, P.javanicum, T.harzianum, T.koeningii* and *T. viride* were maintained on PDA medium. Colony interaction study was performed by dual culture method (Skidmore and Dickinson,1976). The growth inhibition in the colony of the test pathogen and the antagonistic fungi was calculated and interaction grade have been determined as proposed by Porter(1924);and Skidmore and Dickinson,(1976).

$$\text{Percentage inhibition of growth} = \frac{r - r^1}{r} \times 100$$

r = growth of the fungus was measured from the center of the colony towards the center of the plate in the absence of antagonistic fungus.

r^1 = growth of the fungus was measured from the center of the colony towards the antagonistic fungus.

The colony interaction between the test pathogen and the soil fungi were assessed following the model proposed by Porter (1924) and Dickinson and Broadman (1971). Five type of interactions grade as proposed by Skidmore and Dickinson(1976) have been used.

They are as follows.

1. Mutual intermingling growth without any macroscopic sights of interaction - Grade 1.
2. Mutual intermingling growth where the growth of the fungus is ceased, and being over grown by the opposed fungus-Grade2.
3. Intermingling growth where the fungus under observation is growing into the opposed fungus either above (or) below – Grade 3.
4. Sight inhibition of both the interacting fungi with narrow demarcation line (1-2 mm) - Grade4.
5. Mutual inhibition of growth at a distance of >2mm – Grade 5

Culture filtrate method (Food poisoning)

Erlenmeyer flasks (250 ml) containing 100 ml of sterilized Potato Dextrose broth were taken. The broths were inoculated with three blocks (5mm diameter) cut from the actively growing margin of the individual antagonistic fungus and incubated at 28±2ºCfor 15 days, after which the hyphal mat of each fungus was filtered first through Whatman No.1 filter paper and finally through Millipore filter paper. Finally pH of each filtrate was determined. Culture filtrate of each fungus was added to the sterilized PDA medium in flask at different concentration 5,10,15 and 20%. The amended PDA medium dispersed in petri plates and allowed to solidify. There after 5 mm agar blocks cut from the activity growing margin of the test fungus, was inoculated at the center of the plates. The plates were incubated at 28±2ºC for five days and the radial growth was recorded periodically.

The percentage inhibition of growth was calculated as follows:

$$\text{Percentage of inhibition of growth} = \frac{\text{Growth in control - growth in treatment}}{\text{Growth in control}} \times 100$$

RESULTS AND DISCUSSION

22 species of fungi were isolated from the soil of banana field by using the conventional dilution plate technique. (Table 1)

Though the fungal species are cosmopolitan in distribution, their population in a particular habitat change is due to fluctuation in the physico-chemical parameters (Ambikapathy *et al*.,1994). In the present study, it was found that there was an increase in the number of colonies of fungi in the month of November. The moisture content varied from 17.6 to 40.0 per cent in the samples collected during May and December respectively. The range of pH of the soil was narrow. It ranged between 6.9 to 7.7. The minimum range of temperature between 18.1 to 24.3ºC during the study period October and March. The range of temperature between 26 to 36.1 ºC was in the month of August and February. Dwivedi (1966); Bissett and Parkinson,1979; has reported that environment factor such as pH, moisture, temperature, organic carbon, organic nitrogen play an important role in the distribution of mycoflora. Ambikapathy *et al* (1994), have also reported that fungal population in the particular habit, change is due to fluctuation in the physico-chemical parameters.

Antibiotic interaction between soil fungi and *Fusarium solani*

The types of interactions of the pathogen with soil fungi were as follows.

T.viride and T.harizanum	-	*Grade1*
A.fumigatus, A.niger and A.terreus	-	*Grade2*
P.chrysogenum	-	*Grade3*
A.flavus and P.javanicum	-	*Grade4*
T.koeningii	-	*Grade 5*

Table 1. Monthly variation in the population of soil fungi (number of colonies X 10^{-3} g^{-1} dry wt. of the soil) in the banana field

S.No	Name of the fungi	Month of soil sample collection												Frequency (%)
		Sep.	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	
1	*Aspergillus flavus*	1.6	-	2.2	1.0	2.2	-	2.3	-	1.6	1.8	1.4	-	66.66
2	*A.fumigatus*	1.0	1.8	1.6	1.6	2.4	-	2.3	2.8	1.0	-	1.0	1.0	83.33
3	*A.nidulans*	1.0	-	2.2	-	-	-	-	-	-	-	-	-	25.0
4	*A.niger*	3.1	2.0	3.0	2.8	2.3	4.1	2.8	3.1	3.2	1.2	1.0	3.0	100
5	*A.sulphureus*	-	-	-	-	-	-	1.0	-	-	-	-	-	16.66
6	*A.terreus*	1.5	3.1	1.4	-	1.3	1.8	-	2.0	-	2.0	1.6	-	66.66
7	*A.variecolor*	1.8	2.2	1.0	1.8	1.4	-	-	1.0	1.0	1.0	1.3	-	75.0
8	*Cunninghamella verticillata*	1.5	-	-	-	2.0	-	2.5	-	1.9	-	1.9	1.0	50.0
9	*Curvularia lunata*	1.5	1.3	-	1.8	2.0	1.7	1.6	-	2.4	-	1.0	1.5	75.0
10	*Fusarium solani*	2.1	1.0	0.5	1.3	1.0	1.4	0.8	2.0	1.0	1.6	1.8	1.0	100.0
11	*Helminthosporium oryzae*	-	0.5	1.8	1.6	2.0	1.2	-	1.8	-	-	1.0	-	58.33
12	*Mucor hiemalis*	1.0	-	1.6	-	1.0	-	1.0	-	-	0.8	-	-	41.66
13	*Neurospora crassca*	1.0	-	-	1.5	-	-	-	-	-	-	-	1.2	25.0
14	*Penicillium chrysogenum*	2.1	-	1.0	2.3	2.0	1.5	3.5	1.6	2.3	-	1.0	-	75.0
15	*P.citrinum*	1.3	-	2.1	-	1.0	2.0	-	4.0	1.8	1.5	1.3	-	66.66
16	*P.javanicum*	-	1.0	-	1.0	-	-	-	2.5	1.0	1.3	-	-	41.66
17	*P.lanosum*	-	1.0	-	-	-	-	-	1.8	1.6	-	0.2	-	33.33
18	*Rhizoctonia solani*	3.1	2.5	-	3.2	2.0	3.1	-	1.0	1.7	1.5	-	3.8	75.2
19	*Rhizopus nigricans*	-	-	5.0	1.0	1.3	1.2	-	1.6	1.0	-	-	-	50.0
20	*Trichoderma viride*	1.8	-	3.1	1.2	1.5	1.0	-	1.7	2.1	1.5	-	1.0	75.0
21	Black sterile mycelium	-	3.1	-	2.8	-	2.0	1.8	1.0	1.3	-	1.8	2.0	58.33
22	White sterile mycelium	-	1.2	-	1.5	-	1.0	-	2.1	1.0	2.1	-	-	41.6
	Total no.of colonies	25.4	20.7	29.2	26.4	25.4	22	24.1	1.3	26.4	16.3	19.3	19.8	---
	Total number of species	15	12	13	15	16	12	10	1.0	16	11	13	9	----

Table 2. Physico – chemical properties of the soil of banana field, 2007 – 2008

Month	Soil Moisture (%)	pH	Soil temperature		OC (%)	OM (%)	ON (%)	Annual Rain fall	
			Max.	Min.				R.F.(mm)	R.D
September	40.0	7.3	28.0	19.0	0.29	0.620	0.020	020.0	2
October	42.1	7.4	30.1	18.1	0.19	0.732	0.030	-	-
November	39.7	7.5	32.0	20.2	0.30	0.531	0.029	-	-
December	40.0	7.7	33.2	21.0	0.15	0.615	0.029	030.0	2
January	24.0	7.6	34.0	23.0	0.28	0.360	0.041	005.0	1
February	21.0	6.9	36.1	24.2	0.30	0.432	0.067	006.2	1
March	22.4	7.2	31.0	24.3	0.22	0.421	0.042	042.4	2
April	21.2	7.3	32.0	24.0	0.21	0.315	0.051	018.1	2
May	17.6	7.1	29.1	24.2	0.51	0.490	0.092	159.3	5
June	18.3	7.4	28.4	22.1	0.19	0.339	0.061	086.5	8
July	31.2	7.0	27.0	23.0	0.31	0.382	0.073	597.8	12
August	31.6	7.0	26.0	21.2	0.19	0.287	0.064	340.6	9

The maximum percentage inhibition of *Fusarium solani* with *T.viride* (85) followed by *A.niger* (75), *A.terreus* (65.05), *A.fumigatus* (60.33), *A.flavus* (55.50), *P.chrysogenum* and *P.javanicum* (44.66). The mycelium of *T.viride, T.harzianum* and *T.koeningii* were found growing over the pathogen (Table.3). The antagonistic properties of different species of *Aspergillus, Pencillium, Trichoderma* and *Drechslera* against different pathogens have also been reported (Panneerselvam and Saravanamuthu,1994,1996,1999; Ambikapathy *et al*., 2000; Madhanraj *et al.,* 2009;Muthukumar *et al*.,2006)(Table 3)

Table 3. Colony interaction between *Fusarium solani* and soil fungi in dual culture experiments

S.NO	Growth response of the antagonistic and test fungus (mm)	Antagonistic fungi tested (mm)								
		Af	*Afum*	*Anig*	*At*	*Pc*	*Pj*	*Tv*	*Th*	*Tk*
1	Colony growth of pathogen towards antagonist (mm)	15	18	15	18	20	16	8	10	18
2	Colony growth of pathogen away from the antagonist.	24	27	30	34	30	30	20	15	31
3	% growth inhibition of the pathogen in the zone of interaction (mm).	55.5	60.33	75.0	65.00	50	46.66	85.00	33.33	41.99
4	Colony growth of antagonist towards the centre pathogen. (mm)	20	20	32	15	16	5	32	18	19
5	Colony growth of antagonist away from the pathogen.(mm)	23	25	43	35	18	15	41	20	27
6	% of growth inhibition in the zone of interaction	13.04	20.0	25.58	57.14	66.66	66.66	21.95	10.0	29.62

Af – Aspergillus flavus, Afum – A.fumigatus, Anig – A.niger, At – A.terreus, Pc – Penicillium chrysogenum , Pj – P.javanicum, Tv – Trichoderma viride, th – T.harzainum, Tk – T.koeningii

Effect of culture filtrate of soil fungi on the growth of *Fusarium solani*

The maximum percentage inhibition of growth of *F.solani* in the Potato Dextrose Agar medium was amended with 20 percent of culture filtrate of *T.viride* (85) followed by the *P.chrysogenum* (70), *A.flavus* (73.25), *A.fumigatus* (60.50), *P.javanicum* (40.22), *A.niger*(70) and *A.terreus*(61). (Table 4).

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Table 4. Effect of culture filtrate of soil fungi on the growth of Fusarium solani

Name of the culture filtrate	pH	Concentration (%)	*Fusarium solani*	
			Growth rate (mm/day)	% inhibition
Control			30.0	
A.flavus	6.0	5	25.1	16.33
		10	23.5	21.66
		15	21.7	27.66
		20	20.0	73.25
A.fumigatus	6.8	5	22.5	25.0
		10	17.6	41.33
		15	15.3	49.00
		20	14.7	60.50
A.niger	5.8	5	28.6	4.66
		10	25.4	15.33
		15	23.1	23.00
		20	20.6	70.00
A.terreus	6.4	5	22.0	26.66
		10	17.8	40.66
		15	16.4	45.33
		20	14.6	61.00.
P.chrysogenum	6.5	5	18.5	38.33
		10	16.4	45.33
		15	14.0	53.33
		20	12.4	70.00
P.javanicum	6.2	5	24.7	17.66
		10	23.4	22.00
		15	20.6	31.33
		20	20.3	40.22
T.harzianum	5.5	5	24.2	19.33
		10	23.0	23.33
		15	20.6	31.33
		20	18.2	72.26
T.viride	6.0	5	24.5	18.33
		10	21.6	28.33
		15	19.8	34.00
		20	16.9	85.00
T.koeningii	5.6	5	23.7	21.00
		10	22.0	26.66
		15	18.6	38.00
		20	16.8	44.00

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