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RHIZOBACTERIA AND TRICHODERMA SPP - THE POTENTIAL BIO-CONTROL AGENTS AGAINST SUGARCANE WILT PATHOGENS.

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Abstract

Fusarium moniliforme and *Phytophthora infestance* are important soil borne pathogens that cause the major plant diseases along with sugar cane wilt. Different species of *Trichoderma* like *T. viride*, *T. harzianum* and *T. pseudockei* has been identified as potential biocontrol agent against *Phytophthora infestance* and *Fusarium moniliforme.* *T. viride* completely restricted the growth of the pathogens where as *T. harzianum & T. pseudockei* can inhibit up to 95%. Sixty four bacterial colonies were isolated from twenty rhizosphere soil samples out of which 35 cultures restricted the growth of pathogen and twenty four cultures showed the inhibitory activity against the above pathogens. Two cultures did not allow the pathogens to grow. Hence these potential rhizobacteria strains and T*richoderma* spp can be directly used to control the Sugarcane Wilt pathogens.

Key words: Rhizosphere, Rhizobacteria, Biocontrol agent, *Fusarium moniliforme, Trichoderma viride, Phytophthora infestance,* inhibitory activity.

INTRODUCTION

Sugarcane is the main source of sugar in India and it contributes mostly 90% of raw material to the sugar industries for production of sugar (Singh, 1996). The annual production decreases due to diseases and pests during the plantation, growth, harvest and storage stages of the canes. Sugar cane is seriously affected by the diseases like Ring spot, Red rot, Smut, Wilt, Red stripe, Grassy shoot, Root rot out of which Red rot and Wilt are most prevalent in sugar cane growing

areas of Orissa state. Wilt of sugar cane is also very common in other state like U.P., Bihar, Tamil Nadu; and it is caused by multiple activities of *Phytophthora infestance* and *Fusarium moniliforme* (Srinivasan, 1964).

The management of this disease has been tried by adjusting planting time and with the use of tolerant cultivars and fungicides. Even though the chemical control of these diseases is possible with fungicides like metalaxil or mancozeb, it is not eco-friendly as well as affordable for marginal and subsistent level farmers. Moreover, occurrence of the disease in rainy season makes the fungicidal spray ineffective. An attempt was made to isolate and explore the potential of native micro flora especially mycorhiza and rhizobacteria that are capable of controlling both the pathogens. Environment of the surface of the plant particularly of roots is known as the rhizosphere and the bacteria found in this region are known as rhizobacteria (Hiltner, 1904). Otherwise rhizosphere is the region of soil around the root directly influenced by the root exudates (Rovira, 1965). These root exudates attract the beneficial micro organisms and sometimes provide nutrients to them.

The bacteria predominate over other organisms and the growth of bacteria is enhanced by nutritional substances released from the plant tissue, which may be the amino acids, vitamins or other nutrients. The growth of the plant is influenced by the products of microbial metabolism that are released in to the soil. The microbes of rhizosphere are more active physiologically than that of non-rhizosphere soil. These species of bacteria are generally more attracted to this rhizosphere and grow in this environment. These rhizobacteria help the plant in various ways reducing the impact of pathogen in soil.

MATERIALS AND METHODS

An experimental plot (50'x20') at a suitable place was selected inside the sugar cane field of Sakthi Sugars Limited, Korian, Dhenkanal district of Orissa state. Root and soil samples were collected from different places of the experimental field. Roots along with closely adhering soil were collected from healthy plants growing in soil where the disease was present. The roots and soil were placed in sterile distilled water and rhizosphere associated bacteria and fungi were dislodged by continuous stirring. The sample flasks containing the materials were agitated for 10 minutes. The root fragments of all the plants were removed carefully using a sterile forceps. Serial dilutions were made up to 10^{-5} and the dilutions were plated on to Potato Dextrose Agar plates. The Petri plates were incubated for 3 to 7 days at 28°C. Single bacterial and fungal colonies emerging on the surface of the media were selected and a loopful of the culture was transformed to slants for purification.

The purified rhizobacteria and *Trichoderma* cultures were subjected to dual plating with *Phytophthora infestance* and *Fusarium moniliforme* to test their antagonism. *Phytophthora infestance and F. moniliforme* pathogens were incubated on PDA plates and grown in incubators at 28°C. Five mm diameter mycelial plug discs were cut from the juvenile pathogen colony and were

placed at the centre of the Potato Dextrose Agar medium plated on a Petri dish. Then two bacterial streaks of the test bacterial culture were placed one on either side of the mycelial plug of the pathogens. The diameter of the mycelial growth of the test pathogens was measured and area of the mycelial growth was calculated. For each rhizobacteria culture three replications were maintained. In the similar way the spore suspense of *Trichoderma* spp. were also incubated on both side of the pathogen disk.

Another experiment was also conducted to accesses the efficacy of the antagonism of *Trichoderma* spp. against the pathogens (*P. infestance* and *F. moniliforme*). Uniform quantity of inoculums (1g/kg dry soil) from 14 days old cultures of *P. infestance* and *F. moniliforme* were inoculated in sterile soil and maintained in sterilized plastic pots. Three species of *Trichoderma* viz., *T. viride, T. herzeanum* and *T. pseudockei* were inoculated (1 g/kg dry soil) separately to the pots previously inoculated with the test pathogens. Pots inoculated with test pathogens only were treated as control. Three replications were maintained for each set. The pots were kept in aseptic condition to avoid contamination. The population of the test pathogens were determined at 10 days interval starting from 0-30 days after inoculation by soil dilution plate method (Walks man 1922). The percent inhibition in the population of *P. infestance* and *F. moniliforme* over control was calculated.

RESULTS AND DISCUSSION

Twenty soil samples were taken for the experiment. From these twenty samples of rhizosphere regions of sugar cane plants, 64 bacterial colonies were isolated and purified. Many of them resembled actinomycetes, *Bacillus* spp and *Pseudomonas* spp. in dual plates. Twenty four cultures showed inhibitory activity against the pathogens. Two cultures e.g. R_1B_1 and R_2B_1 did not allow the pathogen (*Phytophthora infestance)* to grow at all. A few cultures formed inhibition zones with *P. infestance* as well as *F. moniliforme*. Even though 35 cultures restricted the growth of both the pathogens, the cultures R_1B_3, R_1B_4, R_3B_3, R_4B_1, R_4B_5, R_5B_1, R_6B_2, R_6B_3, $R_{10}B_1$, $R_{10}B_2$, $R_{11}B_3$, $R_{11}B_4$, $R_{12}B_2$, $R_{12}B_4$ $R_{13}B_1$, R_1B_4, $R_{13}B_5$, $R_{14}B_2$, $R_{15}B_2$, $R_{15}B_4$, $R_{16}B_3$ were selected as the best performing ones against *P. infestance*.

The same cultures were also treated against *Fusarium moniliforme*. All the above cultures except $R_{12}B_4$, $R_{13}B_5$ also showed inhibitory activity towards the *F. moniliforme*. The bacterial cultures like R_1B_3, R_1B_4, R_4B_1, R_4B_8, R_6B_2, R_6B_3, $R_{11}B_4$, $R_{15}B_2$, $R_{15}B_4$, $R_{16}B_3$ totally restricted the growth of *F. moniliforme* in the dual plates and did not allow it to grow. The other bacterial cultures like R_1B_3, R_1B_4, R_4B_1, R_4B_8, R_6B_2, R_6B_3, $R_{11}B_4$, $R_{14}B_2$, partially inhibited the growth of *F. moniliforme* but checked its growth successfully. The bacterial culture like $R_{10}B_1$, $R_{10}B_3$, $R_{12}B_2$, $R_{13}B_1$, $R_{13}B_4$, completely restricted the growth of both pathogens in dual plates (Table 1).

The inhibitory activities of the rhizosphere bacterial culture were also tested in soil against both the pathogens in 10 days interval up to one month. The same culture also able to inhibit the growth and multiplication of pathogens in soil. The percentage inhibition in population against

the pathogen was shown in table 2. The percentage inhibition in population of *P. infestance* by the rhizosphere bacteria varied from 69.83 to 92.98% and of *F. moniliforme* from 58.88 to 86.18 % at 30 days of soil inoculation. The rhizosphere bacterial culture like R_1B_3, R_3B_3, R_4B_1, R_4B_8, $R_{10}B_1$, $R_{10}B_3$, $R_{11}B_4$, $R_{11}B_3$, $R_{12}B_4$, $R_{13}B_1$, $R_{13}B_4$, $R_{15}B_4$, $R_{16}B_3$ showed more than 80% population inhibition against *P. infestance*. The culture $R_{12}B_2$ showed the maximum percent inhibition in population for *P. infestance.* Similarly the cultures like $R_{10}B_1$, $R_{10}B_3$, $R_{11}B_4$, $R_{12}B_2$, $R_{13}B_4$, $R_{15}B_4$ showed more than 80% inhibition in population of *F. moniliforme*. The rhizosphere culture $R_{10}B_3$ showed highest 86.18% population inhibition of *F. moniliforme* in soil and $R_{12}B_4$ showed the lowest 40.16%. The culture like $R_{10}B_1$, $R_{10}B_3$, $R_{11}B_4$, $R_{12}B_2$, $R_{13}B_4$, $R_{15}B_4$ were able to inhibit more than 80% in population of both the pathogens combindly (Table 2).

Three species of *Trichoderma* i.e. *T. viride, T. herzeanum, T. pseudockei* were isolated from the rhizosphere soil of sugar cane. *Trichoderma* which is one of the best biocontrol agents is also able to check the growth of sugar cane wilt pathogen, *P. infestance* and *F. moniliforme*. *Trichoderma viride* completely restricted the growth of *P. infestance* and *F. moniliforme* and did not allow them to grow. *T. herzeanum* and *T. pseudockei* also restricted the growth of the pathogens. The growth of *P. infestance* in dual plates, plated with *T. herzeanum* and *T. pseudockei* were 2.61 and 7.89 cm^2 against 136.58 cm^2 in control on 7^{th} day. Similarly the values for *F. moniliforme* were 5.31, 16.56 and 112.32 (Table 3).

The percentage inhibition in population of *P. infestance* and *F. moniliforme* in soil by *Trichoderma viride, T. herzeanum* and *T. pseudockei* were also studied and the inhibition percentage was 94.16, 82.96 and 93.16 respectively. Similarly the values for *F. moniliforme* were 80.71, 53.86 and 49.26 on 30^{th} day of inoculations (Fig. 1). *P. infestance* was more successfully controlled by *Trichoderma* species than *Fusarium moniliforme*.

The primary inoculums for sugar cane wilt pathogens survive in the infected setts used for planting and the secondary spread is by the sporangia produced in soil. If the inoculums are controlled at the germination stage in soil then the disease can be checked. The results of the present study showed the potentiality of using the rhizobacteria as biocontrol agent of the sugar cane wilt. This disease can be controlled by the use of various fungicides. But the fungicidal management of this disease is not eco-friendly and affordable by the marginal farmers of our country. The management of the disease caused by *Phytophthora* and *Fusarium* using bio control agents has been well studied in other crops. Park *et al.* (1995) found that the Rhizobacterium, Chromo bacterium, Violaceum (Strain c-61) had high chitinolytic activity and inhibited the growth of P*. capsice*, *F. moniliforme* and other phytopathogenic fungi. *B. bacillus* Sp. Str, C 18 inhibited mycelial growth of seven plant pathogenic fungi including *P. infestance* and *F. moniliforme* in vitro. In vivo this bacterium can control the infection of *Botrytis cineria* and *P. infestance* (Sadlers, 1996). Stirling et al., 1992 also isolated 164 bacteria from rhizosphere which showed in vitro antagonistic activities against *Phytophthora* spp. The bacteria were included 9 actinomycetes, 3 *Pseudomonas* spp and one *Sereratia* sp. We also have isolated the actinomycetes, *Pseudomonas* and *Bacillus*

species from the rhizosphere which showed antagonism towards *P. infestance* and *F. moniliforme*, in our present study.

Seedling wilt disease caused by *Phytophthora* can also be controlled by *Pseudomonas cepacia* and *P. fluorescens* (Myatt et al., 1993). Other works like Sriram *et al*. (2003), Ahn and Hwang (1992) also isolated actinomycetes, and *Bacillus* spp, that were antagonistic to *P. capsici,* P. *colocasiae*, and *Magonatorthe griseae* from rhizosphere soils in capsicum and colocasia growing areas.

Bacteria isolated from Srilankan cowpea fields significantly reduced in invitro mycelial growth and sporangial production of *P. vignae* by producing agar diffusible and volatile inhibitors. Soil and seed treatment with bacteria mainly with Bacillus species under green house conditions suppressed the disease on Cowpea caused by *P. vignae* (Fernando and Linderman, 1995). Number of workers had worked on antagonistic bacteria and was able to express the antimicrobial activity against *Phytophthora* species included with *P. infestance* (Li et al., 1995). In general, actinomycetes and spore forming bacteria were more antagonistic to *Phytophthora* spp. than other biocontrol fungal and bacterial agent (Duvenhage *et al.,* 1991).

Different species of *Trichoderma* have been identified as potential biocontrol agents of several plant pathogenic fungi (Papavizas, 1985). Earlier Pan et al. (1977) reported that *Trichoderma viride, T. herzeanum and Gloicladium virens* isolates were antagonistic to *P. colocasiae* and they also found that mycoparasitic or hyper parasitic activities of these fungal bio agent were brought about through several morphological changes like coiling of hyphae, formation of hausteria like structure, disorganization of host cell contents and penetration into host hyphae.

T. herzeanum culture filtrate caused 34.37 to 84.76% disease control in soil. High antagonistic activity of *T. herzeanum* also has been demonstrated against *P. nicotinae* and *Fusarium* spp. (Sawant and Mukhopadhyay, 1990).

The inhibition of pathogen growth in dual plate as well as in soil appears to be inhibited due to volatile compounds or agar diffusible compounds that may have antimicrobial activities. So the applications of rhizobacteria and *Trichoderma* are efficient bio control agents against sugar cane wilt pathogens.

CONCLUSION

Sugar producing industries commonly based on the cultivation and production of sugar cane which is the chief source of sugar. Keeping this in view, the pathogens associated in the sugar cane field which badly affect its production and quality, it is strictly needed the control of those harmful specific pathogens. Pathogens like *Phytophthora* and *Fusarium* abundantly present in the soil, cause a severe disease like wilt on the germination bed at the time of prime germination and the first growth stage. So instead of applying fungicides, it is efficient to apply *Trichoderma* and rhizobacteria, which show antagonistic effect towards both these pathogens, as they retains the fertility of the soil as such.

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Table 1. Growth (cm^2) of *Phytophthora infestance* and *Fusarium moniliforme* in dual plates co-inoculated with promising rhizobacteria.

Treatment/ Rhizosphere Bacteria	*P. infestance*				*F. moniliforme*			
	0 Day	10 Day	20 Day	30 Day	0 Day	10 Day	20 Day	30 Day
Control	0.00	20.20	118.12	136.58	0.00	13.72	77.88	112.32
R1B3	0.00	0.00	0.00	0.00	0.00	0.03	0.06	0.08
R1B4	0.00	0.06	0.09	0.11	0.00	0.18	0.21	0.21
R3B3	0.00	0.04	0.04	0.04	0.00	0.00	0.00	0.00
R4B1	0.00	0.01	0.00	0.00	0.00	0.01	0.04	0.06
R4B8	0.00	0.05	0.03	0.03	0.00	1.24	2.62	2.99
R5B1	0.00	0.05	0.71	0.85	0.00	0.00	0.00	0.00
R6B2	0.00	0.02	0.18	0.19	0.00	0.19	0.19	0.21
R6B3	0.00	0.00	0.09	0.12	0.00	0.07	0.12	0.15
R10B1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R10B3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R11B4	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.06
R12B2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R12B4	0.00	0.00	0.00	0.00	0.00	10.43	38.59	42.66
R13B1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R13B4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R13B5	0.00	0.06	0.09	0.16	0.00	9.58	18.69	25.32
R14B2	0.00	0.18	0.18	0.21	0.00	0.89	1.32	1.51
R15B2	0.00	0.44	0.44	0.48	0.00	0.00	0.00	0.00
R15B4	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
R16B3	0.00	0.06	0.03	0.03	0.00	0.00	0.00	0.00

Table 2. Percent inhibition in population over control of P. *infestance* and *F. moniliforme* in soil by rhizosphere bacteria.

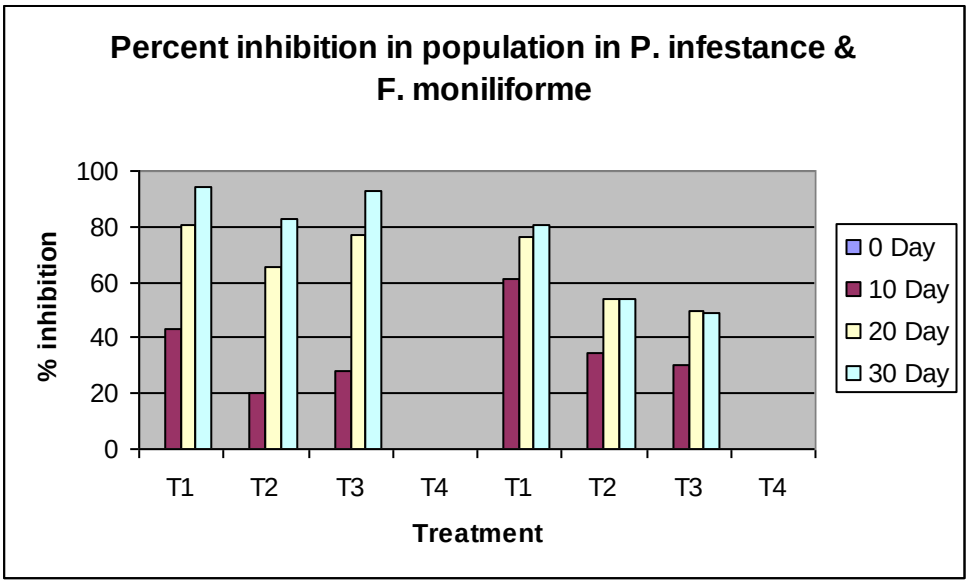
Treatment/ Rhizosphere Bacteria	*P. infestance*				*F. moniliforme*			
	0 Day	10 Day	20 Day	30 Day	0 Day	10 Day	20 Day	30 Day
R1B3	0.00	70.62	82.23	88.16	0.00	56.58	70.12	75.62
R1B4	0.00	68.45	78.13	79.14	0.00	61.82	68.31	71.63
R3B3	0.00	64.14	79.69	82.86	0.00	59.00	76.03	78.26
R4B1	0.00	73.34	85.63	84.84	0.00	59.82	71.00	71.60
R4B8	0.00	69.98	79.22	82.32	0.00	45.51	50.66	65.38
R5B1	0.00	65.26	71.80	76.86	0.00	68.81	75.34	76.63
R6B2	0.00	63.38	70.31	75.62	0.00	46.62	69.21	71.12
R6B3	0.00	60.14	76.66	75.83	0.00	53.53	68.19	79.62
R10B1	0.00	79.29	86.26	88.23	0.00	63.82	77.12	83.27
R10B3	0.00	79.89	85.11	87.76	0.00	69.36	75.56	86.18
R11B4	0.00	69.82	81.26	83.34	0.00	80.81	72.66	82.63
R12B2	0.00	82.66	89.48	92.98	0.00	68.63	76.71	81.83
R12B4	0.00	79.73	86.66	85.61	0.00	40.23	42.16	40.16
R13B1	0.00	75.21	84.88	86.24	0.00	66.83	74.43	76.33
R13B4	0.00	70.16	82.81	84.82	0.00	65.56	76.16	82.13
R13B5	0.00	68.11	72.22	76.76	0.00	42.03	55.22	56.88
R14B2	0.00	59.23	70.81	68.83	0.00	52.86	68.16	67.79
R15B2	0.00	50.69	68.66	71.66	0.00	70.67	75.92	76.25
R15B4	0.00	76.81	83.21	85.52	0.00	69.29	77.06	81.82
R16B3	0.00	75.58	81.33	82.81	0.00	70.83	76.19	75.80

Table 3. Growth of P. *infestance* and *F. moniliforme* in dual plates co-inoculated with Tr*ichoderma* spp.

Pathogen	Treatment	Growth of the pathogen (in cm^2)		
		3rd Day	5th Day	7th Day
Phytophthora infestance	T1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
	T2	0.48 (0.75)	0.58 (0.78)	2.61 (0.96)
	T3	5.21 (1.02)	6.68 (1.06)	7.89 (1.04)
	T4 (Control)	20.80 (4.60)	118.12 (10.83)	136.58 (12.80)
Fusarium miniliforme	T1	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
	T2	0.98 (0.69)	2.32 (0.82)	5.31 (1.02)
	T3	8.84 (1.23)	12.68 (2.16)	16.52 (2.96)
	T4 (Control)	13.72 (3.03)	77.88 (7.14)	112.32 (10.58)

T_1= *Trichoderma viride*, T_2= *T. hrzeanum*, T_3= *T. pseudockii*, T_4= Control

Fig. 1. Percentage inhibition in population in *P. infestance* (Left) and *F. moniliforme* (Right).



T_1= *Trichoderma viride*, T_2= *T. hrzeanum*, T_3= *T. pseudockii*, T_4= Control