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Biological control of taro leaf blight caused by *Phytophthora colocasiae* (Racib.) and storage losses with rhizobacteria

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ABSTRACT: Effect of seed treatment, soil application and foliar spray of rhizobacterial cultures that were isolated from *Colocasia esculenta* on *Phytophthora* blight was studied under polyhouse and field conditions. Under polyhouse conditions, when applied as seed tuber treatment, the antagonistic rhizobacterial cultures S1B3, S11B4, S13B5 and S23B5 reduced the *Phytophthora* blight disease severity. In these treatments there was no disease incidence compared to control where the disease severity was 2.92 on a 0-5 disease rating scale. In soil application, when rhizobacterial cultures S4B5, S13B5 and S23B5 were used, the disease incidence was nil compared to control where disease severity was 2.83 on a 0-5 disease rating scale. Foliar application with S1B4 and S11B3 reduced the disease severity to 0-0.33 rating compared to 2.66 in control. Under field conditions, tuber treatment with S1B3, soil application of S13B5 or foliar application with S1B4 and S11B3 reduced the disease severity and increased the yield compared to untreated pathogen-inoculated control plants. Seed treatment with S1B3 resulted in tuber yield of 255g/plant compared to 95.42g in control. Soil application with S13B5 resulted in 232.65g/plant, while in foliar application with S1B4 or S11B3, yield were 274g and 605g per plant, respectively. These treatments promoted the plant growth also. These treatments were tested in the field and it was found that application of bacteria in combination (seed treatment, soil treatment and foliar spray) helped in reducing the leaf area damaged due to blight by 41% during the first peak of the disease spread and by 28% during the second peak of the disease spread. Rhizobacteria treatment also helped in reducing the storage losses. The storage loss of tubers harvested from rhizobacteria treated plots ranged from 4.14 to 21.24% compared to 26.02 and 21.78% in fungicide treated and control plots, respectively, resulting in 18 to 36% increased yield in the field trials.

KEY WORDS: Biological control, *Colocasia esculenta*, *Phytophthora colocasiae*, rhizobacteria

INTRODUCTION

Leaf blight of taro, *Colocasia esculenta* (L.) Schott, caused by *Phytophthora colocasiae* Racib. is a most devastating disease in many parts of the taro growing areas causing heavy yield loss (25 to 50%) every year (Jackson *et al.*, 1980, Misra, 1993). The primary inoculum of taro leaf blight pathogen survives in the infected seed tubers and the secondary spread is by the sporangia produced on

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the leaf surface during the blight phase. Fungicides, namely, mancozeb and ridomil, are primarily used for control of the disease. However, the waxy layer on the surface of the leaf and incessant rainfall during the crop growth period make the fungicidal application less effective (Misra, 1999). Moreover, chemical control of this disease is not affordable for marginal and subsistence level farmers. Besides causing leaf blight, it causes corm rot too. Therefore, management of this disease has been tried by adjusting planting time and with the use of tolerant cultivars and fungicides. The potential of biological control agents such as *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus* spp. has been utilized in the management of many diseases caused by *Phytophthora* spp. on many horticultural crops (Sadlers, 1996; Stirling *et al.*, 1992). Earlier, Pan and Ghosh (1997) reported that *Trichoderma viride*, *T. harzianum* and *T. virens* (= *Gliocladium virens*) isolates were not only antagonistic to *P. colocasiae*, but also mycoparasitic or hyper-parasitic brought through several morphological changes like coiling of hyphae, formation of haustoria-like structures, disorganization of host cell contents and penetration into host hyphae. However, not much effort has been made to explore the potential of the microflora available in the rhizosphere of taro, especially rhizobacteria, as they have been utilized for managing many diseases on other crops.

Rhizobacteria had been isolated from rhizosphere of taro and screened *in vitro* against *P. colocasiae* (Sriram *et al.*, 2003). In the present study, we report the relative efficacy of native rhizobacteria in the management of taro leaf blight under polyhouse and field conditions.

MATERIALS AND METHODS

All experiments reported in the present study were taken up at the Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar, Orissa, India. *Phytophthora* blight susceptible cultivar of *C. esculenta*, 'Telia', was used in the pot culture and field experiments.

Testing antagonistic activity under polyhouse conditions

Seed tubers of *C. esculenta* cv Telia were collected from plots that were earlier infected by leaf blight. The effects of antagonists were tested as seed tuber treatment, soil application and foliar application. For each treatment, three replications each with 25 plants were maintained. For seed tuber treatment, the bacterial cultures grown on nutrient agar were used (10^9 cfu per ml). The tubers were soaked in bacterial suspension (one litre suspension for 1kg of seed tuber) for 30 minutes. The treated seed tubers were shade-dried and planted in pots. For soil application, the bacterial cultures were applied at the root zone 15 days after planting at the rate of 100 ml suspension (1×10^9cfu/ml) per plant. For foliar application, the cultures were diluted in water to have a final population of 10^8 cfu/ml at the rate of 800 litres/ha and sprayed 30 days after planting. The plant height and disease severity ratings (0-5 scale) were recorded during the peak of the crop growth and disease development, respectively. For recording disease severity, the scale developed by British Mycological Society and later used by James *et al.* (1971, 1972) and adopted after suitable modifications by Prasad (1982) was used. The yield data were also recorded for each treatment after harvest.

Plant growth promotion and rhizosphere colonization

Plant growth promotion due to potential rhizobacterial cultures (S1B3, S13B5, S1B4 and S11B3) was studied in sterile sand bed in trays. Fifty seed tubers of colocasia cv. Telia were treated with the bacterial suspension (1×10^9 cfu/ml). The plant height, root length, fresh and dry weights of the root and shoots were recorded after 30 days. Five replications each with 10 plants were maintained for all the treatments. Seed tubers without any treatment served as control. Rhizosphere colonization by these cultures was studied by counting the bacterial population using serial dilution method.

Field evaluation

The field trials were taken up for two years (2002 and 2003) at the Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar, Orissa, India. Selected treatments, *i.e.*, tuber treatment with S1B3, soil application with S13B5 and foliar application with S1B4 and S11B3 that reduced the disease severity and increased the yield compared to the untreated control were taken up for the field trial. These treatments were tested both as individual treatments and in combination for biomass production and rhizosphere colonization after 30 days. The size of the plot for each treatment was 5 x 3 m^2 and three replications were maintained for each treatment, with spacing of 40cm x 30 cm. For seed treatment, the seed tubers were treated with bacterial suspension (1 x 10^9 cfu/ml) for 30 min and seed tubers were shade dried. The soil application was done as drenching (100ml per plant) with bacterial suspension 15 days after planting. For foliar spray, the bacterial suspension was sprayed (1.2 litre per plot, *i.e.*, at the rate of 800L/ha using high volume sprayer) 30 days after planting. The germination percentage, leaf area damaged and yield of tubers were recorded. For calculating the leaf area damaged, the number of plants infected per plot, number of infected leaves per plant and number of spots per leaf were recorded during the peak of the disease spread at weekly interval. Then five leaves from five infected plants were selected randomly and average size of the blighted area was recorded in terms of diameter of the spots. For blighted area, five readings were taken for the determining diameter of the spot or blighted area. From these observations, leaf area damaged per plant was calculated (Birader *et al.*, 1978). The yield data were recorded at harvest of the crop. The harvested tubers were stored separately treatment wise in sand bed and percentage loss during storage was recorded after four months.

RESULTS AND DISCUSSION

In the present study, cultivar 'Telia' susceptible to taro leaf blight was used in polyhouse conditions, which resulted in plants showing disease severity nearer to 3.0 rating on a 0-5 scale. When 26 rhizobacterial cultures were used for seed treatment, plants obtained from the tubers treated with cultures S1B2, S11B4, S13B5 and S23B5 did not develop any symptoms of taro leaf blight in the sick soil. The disease incidence was nil in all these treatments. Similarly, when soil application was given, the plants treated with S4B5, S13B5 and S23B4 did not develop any symptoms. When the bacterial cultures were used for foliar application, treatment with cultures S1B4 and S11B4 were found to be very effective with a maximum disease severity of 0.33 on 0-5 disease rating scale (Table 1).

When the rhizobacterial cultures were used for seed treatment, S15B4, S16B3, S4B5 and S23B4 affected the growth of the plants in terms of plant height (Table 2). Seed treatment with S1B3, S11B4 and S14B2 rhizobacterial cultures helped in getting better tuber yield (255g, 172.56g, and 254g per plant, respectively) compared to control where it was 129.45g/plant only (Table 2). With S12B3 and S24B1, the yield was either less than control or did not significantly differ from untreated control. These cultures may not be plant growth promoting rhizobacteria. They may be producing secondary metabolites that are inhibitory to root growth and tuber development. Similarly, with soil application under polyhouse conditions, the yield was higher with the cultures S1B3, S1B4, S4B1, S10B1, S13B4, S1B5, S15B2 and S16B3 (133.75, 192.5, 288.33, 278.75, 210.0, 232.7, 232.5 and 252.0 g/plant, respectively) than that of control (133.75 g/plant) while with other cultures the yields were either less than or on par with control. Foliar spray of rhizobacterial cultures S1B4, S11B3 and S15B2 resulted in better yield (274.9, 605.0, 368.3 g/plant respectively) than control (128.45).

Tuber treatment with S1B3, soil application with S13B5, and foliar application with S1B4 or S11B3 reduced the disease severity and increased the yield compared to the untreated control. These cultures were selected and used for testing under field conditions.

Table 1. Effect of rhizobacteria on the disease severity (0-5 scale) in polyhouse conditions

Rhizobacterial culture	Disease severity (0-5 scale)		
	Tuber treatment	Soil treatment	Foliar application
S1B3	0.00	1.33	1.00
S1B4	1.50	0.25	0.00
S3B3	1.25	1.00	0.50
S4B1	0.80	0.83	0.75
S4B5	0.67	0.00	0.67
S5B1	0.50	0.50	0.80
S6B2	1.00	1.00	0.33
S6B3	0.80	0.67	1.00
S10B1	0.50	1.25	0.60
S10B2	1.00	0.60	0.75
S11B2	0.33	0.67	1.00
S11B3	1.00	0.50	0.33
S11B4	0.00	0.33	0.00
S12B2	0.40	0.25	0.33
S12B4	0.50	0.67	0.33
S13B1	1.00	0.75	0.50
S13B4	0.80	0.33	0.50
S13B5	0.00	0.00	1.00
S14B2	1.00	1.00	0.20
S15B2	1.33	2.00	1.33
S15B4	1.00	0.80	0.33
S16B3	0.40	2.40	1.40
S23B4	2.00	0.00	1.25
S23B5	0.00	1.00	1.20
S24B1	1.17	0.67	1.00
S27B3	1.00	1.00	0.67
Control	2.92	2.83	2.66
CD (P = 0.05)	0.59	0.52	0.61

Table 2. Effect of antagonist application on tuber yield under polyhouse conditions

Rhizobacterial cultures	Plant height (cm)			Yield (g per plant)		
	Tuber	Soil	Foliar	Tuber	Soil	Foliar
Control	49.10	51.73	50.30	129.45	133.75	128.45
S1B3	58.20	36.63	80.88	255.00	192.50	85.00
S1B4	76.63	18.55	35.40	129.00	163.00	274.90
S3B3	38.30	35.60	42.00	55.00	82.50	85.00
S4B1	42.68	72.47	33.58	142.05	288.83	86.16
S4B5	26.68	23.63	26.27	79.17	103.30	67.50
S5B1	43.65	42.80	47.92	71.00	54.00	127.50
S6B2	49.05	45.03	35.66	123.33	116.00	45.00
S6B3	31.80	38.92	60.20	32.50	110.00	29.00
S10B1	46.20	64.73	28.10	52.50	278.75	100.00
S10B2	48.57	33.54	35.88	71.66	86.25	122.50
S11B2	44.30	10.53	45.20	90.00	20.00	50.00
S11B3	78.95	26.45	62.90	68.33	26.00	605.00
S11B4	37.75	37.23	34.20	172.56	105.00	47.50
S12B2	41.06	35.35	34.23	28.50	68.50	109.00
S12B4	42.90	58.67	38.43	56.67	70.00	57.50
S13B1	50.13	43.53	50.05	163.75	28.75	103.33
S13B4	43.54	67.53	61.15	125.00	210.00	125.00
S13B5	48.60	49.03	36.17	149.00	232.67	160.00
S14B2	65.02	24.95	77.24	254.00	143.00	140.00
S15B2	91.12	68.00	39.80	115.00	232.50	368.33
S15B4	12.50	24.98	37.70	110.00	34.50	42.50
S16B3	23.92	85.52	65.10	190.00	252.00	147.50
S23B4	37.72	7.20	20.35	50.00	65.00	51.00
S23B5	26.75	35.27	68.94	98.00	52.50	110.00
S24B1	57.27	30.50	30.20	27.50	75.00	174.80
S27B3	53.65	47.88	33.30	95.42	103.75	90.80
CD (P = 0.05)	4.49	5.58	5.11	14.38	15.2	16.78

Plant growth promotion and rhizosphere colonization

Plant growth promotion due to S1B3, S13B5, S1B4 and S11B3 rhizobacterial cultures was studied in sterile sand bed in trays. The results showed that the rhizobacterial treatment helped in plant growth promotion, in terms of plant height as well as fresh and dry weights of biomass of root and shoot (Table 3). Seed treatment with S13B5 increased the plant height significantly (46.55cm), followed by treatment with S1B4 (43.86 cm) compared to control (40.76 cm). Treatment with S13B5, S1B4, S11B3 increased root length significantly (436.20, 426.00, 501.44 cm) compared to control (259.0 cm). Root weight also increased significantly in plants treated with S13B5, S1B4 and S11B3 (18.0g, 21.67g, 13.89g per plant) compared to control (10.7g/ plant).

Field evaluation

For the field trial, planting was done in the first week of July in 2002 and 2003. In the first year (2002), because of the weather conditions the disease incidence did not reach more than 1.0 on the 0-5 point scale. Therefore, the trial was repeated in 2003.

The leaf area infected during the second year (2003) of the field trial is given in Table 4. It was observed that the application of bacteria in combination, *i.e.*, seed treatment, soil treatment and foliar application, reduced the leaf area infected compared to individual application of biological control agents. During the first peak of the disease spread, leaf area infected per plant was 56.0, 57.52, 56.42 cm^2 in seed, soil and foliar applications, respectively, when applied individually and they did not differ significantly compared to control (59.42cm^2). The leaf area damaged in seed + soil, seed + foliar and soil + foliar treatment combinations were 49.14, 48.66 and 44.70 cm^2, respectively. Combination of all the three treatments reduced the leaf area infection to 34.81 cm^2 compared to 59.42cm^2 in control, while in fungicide treatment, it was 21.33cm^2.

During the second peak of the disease spread, the combination of all the three treatments was found to be on par with chemical spray and significantly lower than that of control. In individual treatment, the leaf area infection ranged from 443.83 to 447.67 cm^2, whereas in combination treatments it ranged from 347.93 to 380.60 cm^2 compared to fungicide treatment (324.63 cm^2) and untreated control (488.18cm^2). It was felt that the application of soil and foliar treatments should be repeated since the incessant rain made it difficult to increase the antagonist population in the field. The biological control treatments could reduce the leaf area damaged by 28%, while chemical control (mancozeb 0.2% spray followed by ridomil 0.2% spray) reduced it by 33% during the second peak of the disease spread (Table 4). The first specks of blight disease started appearing in the middle of August. The disease spread generally reached two

Table 3. Plant growth promotion and rhizosphere colonization in sterile sand bed in polyhouse conditions

Treatment	Plant height (cm)	Root length (cm)	Fresh weight of root (g/plant)	Fresh weight of shoot (g/plant)	Colonization (cfu/g)
S1B3	35.25	272.10	14.80	16.60	1.3 x 107
S13B5	46.50	436.20	18.00	18.30	1.8 x 107
S1B4	43.86	426.00	21.67	20.50	2.3 x 106
S11B3	37.11	501.44	13.89	17.56	3.9 x 106
Control	40.76	259.00	10.70	15.30	1.7 x 105
CD (P = 0.05)	3.01	56.23	2.67	1.90	

peaks, first on 19th September with blighted leaf area of 59.42 cm^2 /plant and second on 26th September with 488.18 cm2 / plant in untreated control plot. Appearance of two peaks was due to complete blighting of the leaves followed by defoliation and second flush coming after the defoliation. During the first peak of the disease, the effect of chemical treatment was very conspicuously evident and statistically also more significant than other treatments. However, since the cultivar used was susceptible and disease spread was a function of weather parameters and chemical control was hindered by rainy season, there was no significant difference between the rhizobacterial combination treatment and chemical application during the second peak. Tuber bulking stage (45-90 days) is a very important stage for the short duration taro cultivar. The combination of rhizobacterial treatment reduced leaf area infection and thereby increased the yield compared to the untreated plots (Table 4). There was no significant difference between control plot and chemical treated plots in yield (3720 and 3746 kg / ha, respectively) also. However, the plots with soil application of rhizobacteria with seed treatment significantly recorded higher yield compared to the other treatments. In the second year, the combination tuber, soil and foliar application of rhizobacteria was as effective as chemical control in terms of yield (5153 and 4823 kg / ha, respectively) and reduced leaf area infection.

The rhizobacterial treatments helped in delaying the storage losses too (Table 4). In the first year, seed treatment with S1B3 was effective in reducing the storage loss significantly. In the second year, all the treatments were effective in reducing the storage loss. The rhizobacterial treatments, especially tuber treatment, seed and soil treatment or combination of tuber, soil and foliar treatments reduced the storage losses by 8.53, 4.14 and 10.28%, respectively. In chemical treated plots, the storage loss was 26.02% as compared to control that recorded 21.78% loss. Chemical application was only on foliar region. It did not help in reducing storage rot, probably due to low level of penetration

Table 4. Effect of application of rhizobacteria individually or in combination on leaf area damaged due to *Phytophthora* leaf blight, tuber yield and storage loss

Treatment	Leaf area damaged / plant (Sq.cm)		Yield (kg / ha)		Storage loss (%)	
	19-Sep 2003	26-Sep 2003	2002-03	2003-04	2002-03	2003-04
Seed tuber treatment with S1B3	56.00	447.64	4300.00	4340.76	20.62	8.53
Soil application of S13B5	57.52	455.36	4500.00	4339.93	41.93	18.11
Foliar application of S1B4 + S11B3	56.42	443.83	4600.00	4406.57	34.49	21.24
Seed tuber treatment + Soil application	49.14	380.60	5300.00	4489.87	32.08	4.14
Seed tuber treatment + Foliar application	48.66	362.31	3966.67	4506.53	49.41	13.37
Soil and foliar applications	44.70	367.90	4333.33	4839.73	47.54	15.59
Seed tuber treatment + Soil and foliar application	34.81	347.93	5113.33	5156.27	33.12	10.28
Mancozeb + Ridomil spray	21.33	324.63	3720.00	4823.07	39.96	26.02
Control	59.42	488.18	3746.67	4348.26	37.19	21.78
CD (P = 0.05)	5.66	24.60	223.40	165.30	4.62	3.67

of chemicals in soil, especially around tuber surface. Besides taro leaf blight, storage rot causes significant losses to farmers. Since loss prevented in storage is equal to gain in yield from field and considering the loss during storage and yield protection by rhizobacteria in spite of taro leaf blight spread during favourable season for the disease, we conclude that use of biological control agents, especially rhizobacteria, in taro will be much beneficial, not only in reducing the leaf blight disease and tuber rot, but also it will be commercially more viable since cost of the chemicals is higher than that of biocontrol agents.

The results of the present study show the potential of rhizobacteria to reduce disease incidence and promote the growth of the plant. The ability of the rhizobacteria in reducing storage loss can be exploited to reduce the corm rot incited by *P. colocasiae*, followed by tuber rot incited by various pathogens like *Fusarium* spp. and *Botrydiplodia* spp.

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