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In vivo effect of antagonistic treatments on red rot disease incidence, yield and quality parameters of sugarcane

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ABSTRACT: Experiments were carried out to develop an effective biocontrol method against sugarcane red rot disease under field conditions in sugarcane *var*. CoC-671. Integrated approach as sett treatment + foliar spray + soil application of the antagonistic organism *Pseudomonas fluorescens* recorded the lowest disease incidence (10.37 per cent), higher germination (77.67 per cent), more number of tillers (2.67/ha), economic shoots (1.95/ha) and increased yield (47.80tonnes/ha), highest brix value (21.27 per cent), sucrose (18.67 per cent) and commercial cane sugar (10.86 per cent) in the sugar cane var. CoC-671.

KEY WORDS: *Colletotrichum falcatum*, sugarcane, red rot, sett treatment, foliar spray

INTRODUCTION

Red rot (*Colletotrichum falcatum* Went) is one of the most devastating and serious diseases responsible for heavy losses, both qualitative and quantitative, to the profitable cash crop of sugarcane (*Saccharum officinarum* L). Continuous cultivation of highly susceptible cultivars resulted in epiphytotic nature of the disease and most of the cultivars were found susceptible both in epiphytotic and epiphytic conditions (Viswanathan *et al.,* 1998). The yield loss due to red rot was estimated from 28 to 82 per cent (Ahmad *et al*., 1986). Red rot infection on sugarcane not only reduces its yield attributes but also the juice qualities such as brix, sucrose, purity and commercial cane sugar (Bakshi Ram *et al.,* 2006). Among the different management practices, planting of resistant variety is the best way to overcome this problem. However, due to the development of new variants of this fungus, newly released resistant varieties often become susceptible after some years of cultivation (Padmanaban *et al*., 1996) and make breeding resistant varieties a routine process. Although several fungicides have been found effective *in vitro,* they failed in the field because of the impervious nature of the cane rind (Anilkumar and Satyavir, 1998). Viswanathan and Samiyappan (2006) stated that *P. fluorescens* application as sett treatment followed by soil application not only reduced red rot disease development in crops grown in pathogen infected soil but also improved the germination of setts. Experiments were conducted to assess the efficacy of biocontrol agents which were effective against red rot disease in sugarcane *var*. CoC-671 and to evaluate the improvement of various yield attributes and juice qualities of sugarcane.

MATERIALS AND METHODS

The field trials were laid out at MRK Co-operative Sugar factory, Sethiyathoppu, Chidambaram district. Each plot had five rows of 6 m length with 90 cm between rows. The treatments were replicated thrice in a randomized block design. Thirty healthy two–budded setts were planted in each row. The details of the treatments are given below.

The treatments such as sett treatment, soil application and foliar spray of antagonist and carbendazim were given. The crop was irrigated and fertilized normally as per blanket recommendations. The germination and number of tillers produced per clump were recorded 45 days after planting (DAP) and the incidence of red rot was recorded at 60, 90, 120, 150, 180 and 120 DAP. The biometric observations *viz.,* germination percentage, number of tillers produced/ha, economic shoots produced/ha, yield/ha and juice quality parameters, *viz.,* brix, sucrose and commercial cane sugar content in each treatment, were recorded just before harvest.

Treatment details	
T_{1-}	Sett treatment with *Pseudomonas fluorescens* @ 0.2%
T_{2-}	Sett treatment with *Trichoderma viride* @ 0.2%
T_{3-}	Sett treatment with *Trichoderma harzianum* @ 0.2%
T_{4-}	T_1 + *P. fluorescens* spray @ 0.2% on 30, 60, 90, 120, 150 and 180 DAP*
T_{5-}	T_2 + *P. fluorescens* spray @ 0.2% on 30, 60, 90, 120, 150 and 180 DAP
T_{6-}	T_3 + *P. fluorescens* spray @ 0.2% on 30, 60, 90, 120, 150 and 180 DAP
T_{7-}	T_4 + Soil application of *P. fluorescens* @ 2.5 kg/ha on 0, 45, 90, 135 and 180 DAP
T_{8-}	T_5 + Soil application of *P. fluorescens* @ 2.5 kg/ha on 0, 45, 90, 135 and 180 DAP
T_{9-}	T_6 + Soil application of *P. fluorescens* @ 2.5 kg/ha on 0, 45, 90, 135 and 180 DAP
T_{10-}	Sett treatment with carbendazim @ 0.05% + Urea (1%)
T_{11-}	Control

Estimation of sugarcane juice parameters

The cane stalks were cut at ground level from different treatment plots in the field under naturally infected condition. The canes were cleaned and crushed immediately in a 3 - roller power crusher and juice was strained through a muslin cloth to remove suspended impurities and used for analysis. Brix value was recorded in a brix hydrometer and the correction was applied using the correction table depending on the juice temperature. Purity of juice was determined by following the methods described by Chen (1985). To assess sucrose per cent, 2 gram of lead acetate was added to 100 ml of juice, shaken well and five minutes later the juice mixture was passed through filter paper. The filtrate was taken in polariscope observation tube (200 mm) and reading was recorded. The sucrose per cent was determined from the brix and corresponding polariscope reading by referring to the Schmitz table. The commercial cane sugar (CCS) per cent was calculated with the help of winter's formula (Chen, 1985), i.e., CCS % = [S – {(B-S) x 0.4}] x 0.73 where, S = Sucrose per cent of juice and B = Brix per cent of juice.

RESULTS AND DISCUSSION

Effect of antagonist on red rot disease incidence

The results of the field experiment on the management of red rot in CoC 671 variety are presented in Table 1. The least red rot incidence (10.37 per cent) was recorded in (T_7), sett treatment with *P. fluorescens* @ 0.2% and P. *fluorescens* spray @ 0.2% on 30, 60, 90, 120, 150 and 180 DAP in combination with soil application of *P. fluorescens* @ 2.5 kg ha^{-1} on 0, 45, 90, 135 and 180 DAP, followed by (T_4) sett treatment with *P. fluorescens* @ 0.2% in combination with *P. fluorescens* spray @ 0.2% on 30, 60, 90, 120, 150 and 180 DAP with 18.13 per cent red rot incidence. Sett treatment with *P. fluorescens* @ 0.2% (T_1) recorded 21.46 per cent red rot incidence, which was on par with carbendazim treated plots (24.22 per cent). The maximum red rot incidence (53.20 per cent) was recorded in control (T_{11}). There is no appreciable disease reduction in other treatments (Table 1).

The present result is in agreement with the findings of Singh (1994) that sett treatment with *T. harzianum* and *Chaetomium* sp. significantly improved germination in the infected setts apart from reducing the disease build up. The laboratory and field experiments of Revathi *et al.* (1997) indicated that *T. viride* was effective in inhibiting the growth of *C. falcatum*, whereas its efficacy increased in the field when combined with *T. harzianum*. Natarajan *et al.* (1997) reported no significant difference in reduction of red rot incidence even when *T. viride* and *T. harzianum* were combined with *P. fluorescens* either as spray or soil application. Senthil *et al.* (2000) reported that sett treatment combined with soil application of *P. fluorescens* reduced the sugarcane red rot disease development. Viswanathan and Samiyappan (2000) tested the efficacy of different species of *P. fluorescens* and found that sett treatment followed by soil application of bacterial strains reduced the red rot development in sugarcane grown in pathogen-infected soil. They also concluded that the inhibitory action of bacterial strains was due to direct antagonistic activity.

Efficacy of *P. fluorescens* both *in vitro* and in the field was due to its direct action on pathogen as well as induction of disease resistance in the host. The mechanism of action of *P. fluorescens* includes competition for nutrient and space (Elad and Chet, 1987), antibiosis by producing antibiotics (Pierson and Thomashow, 1992), production

Table 1. Field evaluation of antagonists against red rot disease of sugarcane

Treatments	Red rot percent (Days after planting)*							
	30DAP	60DAP	90DAP	120 DAP	150DAP	180DAP	210DAP	Mean
$T_{1.}$	-	0.16 (2.31)	14.65 (22.52)	33.79 (35.44)	33.02 (35.05)	35.57 (36.62)	37.04 (37.63)	21.46 (27.56)
$T_{2.}$	-	0.30 (3.30)	13.83 (21.83)	32.72 (34.75)	33.12 (35.13)	34.42 (35.92)	38.04 (38.03)	21.87 (27.83)
$T_{3.}$	-	0.33 (2.94)	16.52 (23.69)	38.36 (38.06)	38.11 (38.13)	38.79 (38.52)	41.94 (40.33)	24.86 (29.87)
$T_{4.}$	-	0.29 (2.94)	15.92 (23.63)	26.75 (30.75)	26.46 (30.95)	27.65 (31.72)	29.89 (32.93)	18.13 (25.18)
$T_{5.}$	-	0.46 (3.02)	17.70 (24.45)	43.07 (41.22)	43.48 (41.26)	44.49 (41.84)	46.74 (43.12)	27.99 (31.88)
$T_{6.}$	-	0.31 (3.11)	16.77 (24.31)	39.36 (38.92)	39.45 (38.91)	41.69 (39.44)	44.31 (42.13)	25.98 (30.59)
$T_{7.}$	-	0.00 (0.00)	7.22 (15.78)	15.65 (23.25)	15.57 (23.24)	16.52 (23.21)	17.62 (24.29)	10.37 (18.72)
$T_{8.}$	-	0.31 (2.94)	18.08 (25.26)	38.81 (38.31)	38.44 (38.44)	39.33 (37.25)	41.80 (40.16)	25.25 (30.13)
$T_{9.}$	-	0.61 (4.79)	19.03 (26.04)	38.64 (38.15)	38.10 (38.10)	39.51 (38.95)	40.75 (39.67)	25.23 (30.20)
$T_{10.}$	-	0.29 (3.60)	16.65 (24.05)	37.24 (37.73)	37.27 (37.61)	38.39 (37.95)	39.72 (39.07)	24.22 (29.47)
$T_{11.}$	-	0.77 (15.43)	35.95 (37.06)	80.85 (63.81)	80.67 (63.65)	85.58 (69.42)	88.64 (70.32)	53.20 (46.83)
CD (P=0.05)	-	0.09	2.26	1.54	2.52	2.05	2.20	

*Mean of three replications; data in parentheses are arc sine transformed values

of siderophores (Kloepper *et al.,* 1980), secretion of lytic enzymes such as β-1, 3 glucanase (Velazhahan *et al.,* 1999) and degradation of toxin produced by pathogen (Duffy and Defago, 1997). The induction of systemic resistance in host plant by *P. fluorescens* inoculation reduced the disease incidence in many host-pathogen interactions (Wei *et al.,* 1996). Induction of pathogenesis related proteins, phenolics and phenylalanine ammonia lyase by *P. fluorescens* has been reported in other crops also (Meena *et al.,* 2000). Plants have endogenous defense mechanisms that can be induced in response to attack by insects and pathogens (Heil, 2001).

Effect of antagonists on plant biometrics and yield parameters

The results of field experiments clearly indicated that in general antagonist application improved the plant biometrics and juice quality (Table 2). Among the antagonists, *Pseudomonas fluorescens* played a major role. Sett treatment with *P. fluorescens* @ 0.2% and P. *fluorescens* spray @ 0.2% on 30, 60, 90, 120, 150 and 180 DAP in combination with soil application of *P. fluorescens* @ 2.5 kg/ha on 0, 45, 90, 135 and 180 DAP (T_7) ranked first in the improvement of plant biometrics and juice quality followed by sett treatment with *P. fluorescens* @ 0.2% in combination with *P. fluorescens* spray @ 0.2% on 30, 60, 90, 120, 150 and 180 DAP. Sett treatment with *P. fluorescens* @ 0.2% (T_1) ranked third.

The highest germination (77.67 per cent) was recorded in *P. fluorescens* treated plots as sett + spray + soil treatment (T_7) followed by plots treated with *P. fluorescens* as sett and foliar spray (T_4) (75.78 per cent), which was on par (75.56 per cent) with *P. fluorescens* sett treatment (T_1) alone. The germination of sugarcane setts in carbendazim treated plot (T_{10}) was 71.89 per cent. The germination of sugarcane setts in control plot (T_{11}) was only 57.89 per cent. The tiller production was significantly higher (2.67 tillers/ ha) in *P. fluorescens* treatment (T_7) as sett + spray + soil. The tillers produced in plots treated with *P. fluorescens* (T_4) as sett + spray treatment (2.60 tillers/ ha), was on par with plots treated with *P. fluorescens* as sett (T_1) treatment (2.58 tillers/ha) alone and sett treatments with *T. viride* (T_2),

T. harzianum (T_3) and Carbendazim (T_{10}) produced 2.53 tiller/ha. The control plot (T_{11}) recorded only 2.25 tillers/ ha.

Significant increase in the number of economic shoot production (1.95 shoots/ ha) was seen in plots treated with *P. fluorescens* as sett + spray + soil (T_7) treatment and it was followed by *P. fluorescens* treatment as sett +spray (T_4) with 1.27 shoots/ ha. The shoot production (1.10 shoots/ ha) in sett treatment with *P. fluorescens* (T_1) was on par with Carbendazim treated plants (T_{10}) with 1.07 shoots/ ha. The control plots (T_{11}) registered only 0.68 shoots/ ha. The cane yield of 47.80 t/ha was recorded in plots treated with *P. fluorescens* as sett + spray + soil (T_7) and it was significantly higher than the yield of 46.77 t/ha in plots of sett + spray treatment (T_4). The plots treated with *P. fluorescens* as sett treatment (T_1) registered a cane yield of 45.46 t/ha. The other sett treatments with *T. viride* (T_2) and Carbendazime (T_{10}) also performed better in reducing the incidence of red rot and promoted the yield up to 43.53 and 43.46 t/ha, respectively. The yield registered in the control plot (T_{11}) was only 16.37 t/ha. Red rot disease in India was reported to cause a loss of more than $ 500 million every year (Viswanathan *et al*., 1997). The reduction in cane production due to infection by *C. falcatum* ranged from 28 to 82 per cent in the cultivars L 116 and B 4360. Around 90 per cent reduction in juice extraction was recorded with 50 per cent red rot incidence (Ahmad *et al.,* 1986).

Sugarcane juice quality parameters

The quality parameters of cane juice also improved considerably due to the application of antagonist, the trend prevailed in plant biometric was continued in the juice quality also. The brix per cent of juice was higher (21.27 per cent) in plots treated with *P. fluorescens* as sett + spray + soil (T_7) treatment followed by canes from plots treated with *P. fluorescens* as sett + spray (T_4) treatment (15.20 per cent) and it was on par (14.57 per cent) with *P. fluorescens* sett (T_1) treatment alone.

The brix value in plots of carbendazim sett treatment (T_{10}) was also found to be encouraging with 14.40 per cent,

Table 2. *In vivo* Effect of antagonists on plant biometrics and sugarcane juice parameters

Treatments	Germination per cent (35 DAP)*	No. of tillers/ha (‘000)	Economic shoots/ha (‘000)	Yield/ha (tonnes)	Brix (%) *	Sucrose (%) *	CCS (%) *
T_1.	75.56 (60.35)	2.58	1.10	45.46	14.57 (22.41)	13.03 (22.10)	8.27 (16.70)
T_2.	74.67 (59.79)	2.53	1.08	43.53	14.50 (22.35)	12.90 (20.43)	8.17 (16.59)
T_3.	71.78 (57.76)	2.53	1.06	41.97	14.17 (22.07)	12.17 (20.34)	8.07 (16.49)
T_4.	75.78 (60.50)	2.60	1.27	46.77	15.20 (22.86)	13.47 (21.48)	8.60 (17.04)
T_5.	67.44 (55.20)	2.50	1.02	33.27	12.43 (20.16)	10.61 (18.91)	7.23 (15.60)
T_6.	68.11 (55.61)	2.50	1.02	37.76	12.46 (20.60)	10.73 (19.06)	7.77 (16.17)
T_7.	77.67 (61.79)	2.67	1.95	47.80	21.27 (27.46)	18.67 (25.57)	10.86 (9.24)
T_8.	69.11 (56.17)	2.50	1.06	39.67	13.53 (21.54)	11.47 (20.29)	7.90 (15.67)
T_9.	71.33 (57.54)	2.51	1.04	41.23	13.67 (21.55)	11.77 (20.64)	7.97 (16.39)
T_{10}.	71.89 (57.97)	2.53	1.07	43.46	14.40 (24.27)	12.80 (21.27)	8.10 (16.53)
T_{11}.	57.89 (49.40)	2.25	0.68	16.37	8.47 (16.84)	7.00 (15.19)	5.63 (13.72)
CD. (P=0.5)	2.98	0.12	0.03	2.27	2.52	1.71	1.13

*Mean of three replications; data in parentheses are arc sine transformed values

but it was very less (8.47 per cent) in control (T_{11}). Red rot infection on sugarcane not only reduces its yield attributes but also the juice qualities such as brix, sucrose, purity and commercial cane sugar (Bakshi Ram *et al.,* 2006).

The sucrose content is an important quality parameter of sugarcane juice. It was found significantly higher in juices obtained from the plots treated with *P. fluorescens* as sett + sprays + soil (T_7) treatment canes with a level of 18.67 per cent. The canes of *P. fluorescens* treatment as sett + sprays (T_4) and sett treatment (T_1) alone contain 13.47 and 13.03 per cent sucrose content respectively. The juices of other treatment plots had moderate to poor sucrose content. The least sucrose content of 7.00 per cent was recorded in control (T_{11}). The red rot infection caused almost complete depletion of sucrose in the cane (Agnihotri *et al.,* 1992). Pathogen infection also resulted in increased levels of total soluble salts, acidity, reducing sugars and gum with simultaneous decrease in pH, sucrose and purity of the cane juice (Singh and Waraitch, 1977).

Commercial cane sugar (CCS), the output of cane cultivation, was significantly higher in plots treated with *P. fluorescens* as sett + sprays + soil (T_7) treatment with 10.86 per cent followed by sett + sprays of *P. fluorescens* treated (T_4) plots with 8.60 per cent. The next higher CCS content was registered in plots of sett treatments with *P. fluorescens* (T_1) *T. viride* (T_2) and Carbendazime (T_{10}) alone, with 8.27, 8.17 and 8.10 per cent CCS respectively. The control plot has registered only 5.63 per cent of CCS. Agnihotri *et al.* (1989) reported that the infection reduced total carbohydrates, brix value and nucleic acids in the canes and it was more in highly susceptible varieties.

The above result of present investigation clearly indicate that the integrated application of the antagonist, Pseudomonas fluorescens on sugar cane variety CoC-671 as sett treatment, foliar spray and soil application ensure low red rot disease incidence, higher germination per cent, increased no of tillers, economic shoots/ha, yield and juice qualities as compared to other treatments.

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