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Talc Formulated Fluorescent Pseudomonads for Sugarcane Red Rot Suppression and Enhanced Yield Under Field Conditions

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Plant growth promoting rhizobacteria (PGPR) belonging to fluorescent pseudomonads group were isolated from sugarcane rhizosphere and sugarcane stalk tissue. Induction of systemic resistance against *Colletotrichum falcatum* Went causing red rot disease in sugarcane by PGPR strains were studied under field conditions. The PGPR formulation was applied three times, sett treatment while planting and soil application twice in the field. Talc formulation of five PGPR strains significantly reduced red rot disease incidence when the treated canes were challenge inoculated with pathogen. When PGPR strains were evaluated for their efficacy against the disease in endemic locations, strains of *Pseudomonas fluorescens* such as EP1, Pf1 and CHAO and *P. putida* KKM1 strongly suppressed the red rot disease development in two field trials. In addition to their efficacy against red rot disease in sugarcane, the strains significantly improved sett germination, number of millable canes (NMC) and cane yield in the field. Analysis of cane juice characters revealed that PGPR treatments have positively improved juice characters *viz.*, sucrose %, brix % and commercial cane sugar % as compared to the control in the field. The efficacy of PGPR strains against red rot pathogen, enhanced cane and sugar yields suggest that these bacterial strains could be exploited for management of red rot disease in sugarcane.

KEY WORDS : Sugarcane, induced systemic resistance, growth promotion, *Pseudomonas fluorescens*, red rot disease

Red rot of sugarcane caused by the fungus *Colletotrichum falcatum* Went (Perfect state: *Glomerella tucumanensis* (Speg.) Arx & Muller) is one of the oldest recorded diseases and has caused significant losses both to the cane growers and to sugar factories in India and other countries. The disease is considered as the major debilitating malady to sugarcane in India and several epidemics have occurred in India over the decades (Alexander and Viswanathan, 1996). Various fungicides were tried for the management of the disease, but limited success was achieved under field conditions (Singh and Singh, 1989). Hence, plant protection chemicals are not useful for managing the red rot disease in India. Further the newly released varieties to replace the susceptible varieties succumbed to the pathogen due to the frequent emergence of new variants of the pathogen (Viswanathan *et al.*, 1997). Similarly, use of disease free seed canes for planting was not successful since failure in diagnosing the dormant infections of the fungal pathogen in seed canes in the field under Indian conditions (Viswanathan and Samiyappan, 2000). It is therefore important to explore other avenues for the management of red rot in sugarcane. In this context, management of red rot disease through biocontrol agents is increasingly capturing the attention of scientists as an alternative strategy for the disease management, which is also ecologically sound and environmentally safe.

Plant growth promoting rhizobacteria (PGPR) are root colonizers and belong to different genera and most reported strains are from *Pseudomonas* spp. Many of the fluorescent pseudomonads are plant growth promoting rhizobacteria, which are known to colonize many crop plant parts. Plant growth promoting effect of these bacteria has enhanced final yield in many crops in addition to their effect on plant diseases caused by fungi, bacteria and viruses have well been documented for the past two decades. Induced systemic resistance (ISR) by PGPR strains has been studied mainly in the laboratory and greenhouse conditions. However, few literatures indicate that ISR by PGPR can protect the

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plants under field conditions (Wei *et al.*, 1996, Nandakumar *et al.*, 2001). The PGPR strains native to sugarcane rhizosphere and internal stalk tissue have been isolated and their efficacy against the pathogen was demonstrated under laboratory, green house and limited field studies (Viswanathan and Samiyappan, 1999). Further studies were conducted to assess the efficacy of talc formulated *Pseudomonas* spp. against red rot disease development under disease endemic locations.

MATERIALS AND METHODS

Bacterial strains and the formulation

The PGPR strains were isolated from sugarcane rhizosphere soil samples obtained from different parts of Tamil Nadu state, India on King's B (KB) medium (King *et al.*, 1954) and incubated at room temperature (28 ± 2 °C) for 24 h. For the isolation of endophytes, internal stalk tissues of CoC 671 was made into small pieces and put them in 250 ml conical flasks containing sterile water and kept in a shaker for 15 min. A loopful of the suspension was later streaked over the KB medium and plates were incubated for about 48 h at 28 ± 2 °C. The fluorescent colonies were viewed under UV light at 366 nm and the bacterial strains were characterized (Hildebrand *et al.*, 1992). In addition to the strains isolated for the study, the PGPR strains *viz.*, Pf1, CHAO and *Trichoderma viride* were obtained from the bacterial culture collection of Department of Plant Pathology, TNAU, Coimbatore, India were also used for comparative study in field studies. The talc-based formulation of fluorescent pseudomonads, which were found to improve sugarcane growth, was prepared by following the method of Vidhyasekaran and Muthamilan (1995). At the time of application, the population of bacteria in talc formulation was 2.5 to 3 × 10^8 cfu g^{-1}.

Effect of PGPR strains on red rot disease development under field condition – Artificial inoculation

Initially a field experiment was conducted to test the efficacy of talc powder formulation of PGPR strains against red rot pathogen at Research Farm, Tamil Nadu Agricultural University, Coimbatore. In the field experiment, four disease susceptible varieties such as Co 997, Co 6304, CoC 671, and CoC 92061 were planted in the field. Approximately 150 two budded setts were used for planting in each plot to a size of 20 m^2 which had 5 rows of 4 m length. The trials were replicated three times in a randomized block design. Talc formulated five PGPR strains *viz.*, EP1, KKM1, Pf1, VPT4 and VPT10 were applied 3 times (sett treatment and 2 soil applications on 60th and 120th day after planting) in the field. For sett treatment, the formulations were mixed with water at the rate of 20 g L^{-1} and two budded setts (vegetative stalk cuttings) were soaked in it for 1 h. After soaking, the treated setts were incubated overnight (18 h) in the field before planting. For soil application, the formulations were applied at the rate of 25 kg ha^{-1}. The recommended dose of formulations were mixed with 250 kg of farmyard manure and applied to base of the crop. The effect of induced systemic resistance by PGPR strains was evaluated by artificially inoculating the pathogen. Conidial suspension was prepared from the pathogen mass multiplied in oatmeal agar medium after 8 d and adjusted the inoculum was adjusted to 10^6 conidia mL^{-1}. The pathogen conidial suspension was inoculated by the standard plug method at 3rd internode from the base. About one ml of the suspension was placed into the bore hole (12 mm) made using the red rot inoculator and the borehole was sealed with plastic clay. The inoculated canes were split open on 30 days after pathogen inoculation and disease intensity was assessed on 0-9 scale (Srinivasan and Bhat, 1961). The pathogen inoculation was done 30 days after the last PGPR application.

Efficacy of PGPR strains on red rot disease development in endemic locations

The field trials were conducted to find the efficacy of effective PGPR strains against natural red rot disease incidence in three sugar factory areas, where the disease is endemic. The first trial was located at Thirukandeswaram village in EID Parry sugar factory areas, Nellikuppam, Cuddalore District, Tamil Nadu, India during 1999 –2000, second trial in Sozhatharam village in M. R. K. cooperative sugar mills limited, Sethiathope, Cuddalore District, Tamil Nadu, India during 2000 – 2001 and the third in Thirumandankudi village in Ambiga sugar mills ltd, Kumbakonam, Tanjore District, Tamil Nadu, India during 2001 – 2002. The selected fields had previous history of red rot disease incidence ranging from 10 to 18 %. In all the three trials, disease free canes (sett) of disease susceptible varieties such as cv. CoC 90063 (trial I and II) and cv. Co 97009 (trial III) were used. The five PGPR strains *viz.*, CHAO, EP1, KKM, Pf1 and VPT4 and fungal biocontrol agent, *T. viride* were used in trial I. In addition to the five strains used in the trial I, a formulation containing Pf1 + KKM1 mixture and fungicide carbendazim for comparison were used in trial II and III. As a check to the bacterial strains, carbendazim (2 – benzimidazole carbamic acid methyl ester) was used as sett treatment at the rate of 2.5 g L^{-1} of water. An untreated control was also maintained in each trial. The plot size for each treatment was 200 m^2 and two budded setts of 1500 was required for planting in the plots. The treatment were replicated thrice using Randomized Block Design. The PGPR strains were applied as described earlier. Normal crop cultivation practices were followed (Sundara, 1998). The trial plots were irrigated once in a week. Germination data was recorded on 30 days after planting. Natural incidence of red rot disease was recorded at monthly interval till harvest at 12th month. Number of millable canes (NMC) was recorded on 5th and 12th month and expressed as

NMCs ha^{-1}. The cane yield from different treatments was recorded after harvest and expressed as tones ha^{-1}.

Cane juice quality analysis

The cane stalks were cut at ground level at the time of harvest, cleaned and crushed immediately in a 3-roller power crusher giving about 60 % juice extraction. The juice was then strained through a muslin cloth to remove suspended impurities and used for further analysis (Rao, 1986). Brix (total soluble solids) was recorded in a brix hydrometer and the correction was applied using the correction table depending on the juice temperature. To assess sucrose percentage, 2 g of lead acetate was added to 100 ml of juice and shaken well. Five min later it was passed through filter paper, filtrate taken in a Polaris cope observation tube (200 mm) and polariscope reading was recorded. The sucrose % was determined from the brix and corresponding polariscope reading by referring to the Schmitz table.

The juice purity % was calculated using the following formula :

$$\text{Purity} = \frac{\text{Sucrose \%}}{\text{Corrected brix \%}} \times 100$$

The commercial cane sugar (CCS) % was calculated using the following formula :

$$CCS\ \% = [(S-(B-S) \times 0.4] \times 0.73$$

Where,

S= sucrose per cent of juice

B= brix per cent of juice

Statistical analysis

Statistical analyses of the experiments were performed using the IRRISTAT modules of the International Rice Research Institute Biometrics Unit, Manila, The Philippines and the treatment means were compared by Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

Efficacy of *Pseudomonas* strains on disease development

Results of the first field experiment revealed the induction of systemic resistance by five PGPR strains against *C. falcatum* in disease susceptible varieties, which were challenge inoculated with the pathogen. A reduced disease development was observed in most of the treatments. The strain KKM1 and VPT10 significantly reduced red rot disease development in cv. CoC 671 whereas, Pf1 and VPT4 were more effective in case of another susceptible variety CoC 92061. However, all the PGPR strains effectively restricted the disease intensity in other two varieties *viz.*, Co 997 and Co 6304 (Table 1).

Efficacy of powder formulation of PGPR strains against red rot disease incidence under endemic areas in coastal and deltaic zones of Tamil Nadu, India revealed that all the PGPR strains were effective in restricting the disease development. Combination of *P. fluorescens* strain Pf1 + *P. putida* strain KKM1 also showed higher reduction in disease levels however the suppression was equal to Pf1 and higher than KKM1. The endophytic strain EP1 was superior against red rot disease development in both field locations where disease appeared. The PGPR strains KKM1 and VPT4 were comparatively better in trial II as compared to trial I. The results have clearly proved that the PGPR strains were more effective than the *T. viride* and fungicide Carbendazim, in controlling the red rot disease in the disease susceptible variety CoC 90063. No incidence of red rot disease was noticed in the variety Co 97009 in trial III and this might be due to extraneous factors, which were unamenable for red rot disease development. In general, PGPR treatments decreased red rot disease incidence by 50 % (Table 2). *Pseudomonas* spp. have been reported to induce systemic resistance (ISR) against fungal, bacterial and viral plant pathogens by triggering defense genes in response to infection by plant pathogens (Van Peer *et al.* 1991, Maurhofer *et al.* 1994, M'Piga *et al.*, 1997, Van Loon *et al.* 1998). Detailed studies conducted earlier by Viswanathan and Samiyappan (2000) revealed that the PGPR strains triggered defense mechanisms in different sugarcane varieties to varying levels. The PGPR mediated ISR was more pronounced in disease susceptible varieties as compared to moderately susceptible or moderately resistant varieties. Physiology of PGPR mediated ISR indicated that bacterial treatment results in enhanced induction of pathogenesis – related proteins such as chitinases, b-1,3-glucanases, thaumatin like-proteins, peroxidases and phenylalanine ammonia lyase in disease susceptible varieties (Kloepper *et al.* 1993; Viswanathan and Samiyappan 1999, 2001). Induced state in sensitized plants restricts colonization and spread by challenging pathogen in the stalk

Table - 1 : Evaluation of PGPR – mediated resistance in different red rot susceptible sugarcane varieties against *C. falcatum* under field conditions

PGPR strains	Disease intensity in 0-9 scale*			
	Co 997#	Co 6304#	CoC 671#	CoC 92061#
Pf1	5.60^{c}	6.17^{b}	7.93^{ab}	4.67^{b}
VPT4	5.93^{c}	5.87^{b}	7.77^{ab}	4.57^{b}
EP1	6.23^{c}	6.07^{b}	7.69^{ab}	5.90^{a}
KKM1	6.13^{c}	5.57^{b}	7.42^{b}	5.92^{a}
VPT10	7.05^{b}	5.77^{b}	7.23^{b}	6.05^{a}
Control	7.83^{a}	7.24^{a}	8.33^{a}	6.43^{a}

*Mean of three values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

#The pathogen was artificially inoculated by plug method at 3rd inter node from the base of the cane and disease intensity assessed 60 days later.

Table - 2 : Effect of PGPR strains on the disease incidence in field conditions at endemic locations

PGPR strains	Per cent incidence	
	Trial I*	Trial II*
Pf 1	6.6[b]	2.9[c]
VPT 4	8.2[b]	4.1[bc]
CHAO	7.1[b]	4.6[bc]
EP 1	5.2[b]	3.3[c]
KKM 1	8.4[b]	3.6[c]
Pf 1 + KKM 1	-	2.9[c]
T. viride	12.9[a]	-
Carbendazim	-	6.8[b]
Control	14.3[a]	10.4[a]

*Mean of three replications
- : Indicates non inclusion of the treatment
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

resulting in protection in the disease susceptible varieties. Previous studies of Senthil *et al.* (2000) also showed suppression of red rot disease incidence by application of *P. fluorescens* as sett treatment and soil application in a susceptible variety CoC 671 in an endemic location.

In the field studies at endemic locations, the mixture of strains Pf1 + KKM1 have exhibited suppression of red rot disease development. Application of a mixture of introduced biocontrol agents would more closely mimic the natural situation and might broaden the spectrum of biocontrol activity and enhance the efficacy and reliability of control (Duffy and Weller 1995). Strains of *P. fluorescens* mixture (Pf1 + FP7) recorded lesser sheath blight disease of rice compared to the strains applied singly (Nandakumar *et al.* 2001). The PGPR strains *viz.*, Pf1 and KKM1 were found to be more efficient in protecting the sugarcane crop from the red rot disease and highly restrictive to fungal pathogen propagules surviving in the soil/debris than the other strains such as VPT4 and CHAO. The endophytic strain EP1 showed strong antagonism towards the pathogen and its ISR efficacy in sugarcane against *C. falcatum* was highly effective against red rot disease incidence in the field. Endophytic bacteria belonging to *Pseudomonas* and other genera are involved in plant growth promotion and disease suppression in many crops has been reported (Hallmann *et al.* 1997). Vegetative propagated crop like sugarcane offers potential use of endophytes because of their capability to colonize and persist in the intercellular space of epidermal cells thereby reducing the need for further application since the same vegetative parts are used as propagative material.

The PGPR strains were found better for red rot disease management than *T. viride* in the trial I. Earlier Singh (1994) reported that application of *Trichoderma* spp. and *Chaetomium* sp. effectively reduced red rot disease under greenhouse conditions. However, these agents may effectively reduce soil-borne propagules of the pathogen but the pathogen infection mostly comes through the inocula carried through irrigation/flood water and air. Hence, the above said biocontrol agents failed to control the pathogen infection under field conditions. In coastal areas of Tamil Nadu, sugarcane is mostly cultivated under irrigated condition. There is lesser or flooding the field brought out no survival of *Trichoderma* spp. population in rhizosphere (Jeyarajan *et al.* 1994). Hence, application of *Trichoderma* in sugarcane may not reduce the disease in field as compared to PGPR strains.

***Pseudomonas* spp. on cane growth:** Sett treatment with PGPR strains has resulted in better germination of setts in the fields. The PGPR strain Pf1 showed highest germination in the treated plots and other strains *viz.*, VPT4, EP1 and KKM1 also exhibited significantly higher germination as compared to untreated plots in trial I. In subsequent trials strain VPT4 treatment recorded maximum germination than other strains. The standard fungicide carbendazim treatment showed germination almost equivalent to control (Table 3). All the five PGPR strains have improved cane growth significantly in both the varieties CoC 90063 and Co 97009 in the field. Influence of sugarcane plant growth in terms of shoot and number of millable canes assessed on 5th and 12th respectively revealed that plots treated with bacterial strains recorded higher number of millable cane (NMCs) populations and increased cane yield at the time of harvest. The strain Pf1 showed higher NMC population followed by EP1 and KKM1 recorded higher NMC and increased cane yield in CoC

Table - 3 : Effect of PGPR strains on sugarcane sett germination

PGPR strains	Per cent germination*		
	Trial I	Trial II	Trial III
Pf 1	40.2[a]	75.9[a]	50.3[ab]
VPT 4	39.7[ab]	78.3[a]	58.2[a]
CHAO	37.7[ab]	67.0[a]	54.5[a]
EP 1	39.0[ab]	70.3[a]	52.7[ab]
KKM 1	38.5[ab]	76.2[a]	57.8[a]
Pf 1 + KKM 1	-	77.8[a]	47.0[ab]
T. viride	36.0[ab]	-	-
Carbendazim	-	54.5[b]	41.8[b]
Control	32.7[b]	52.8[b]	42.4[b]

*Mean of three replications
- : Indicates non inclusion of the treatment
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

90063 in trial I. The PGPR strains *viz.*, KKM1, EP1 and Pf1 treatments and strain mixture Pf1 + KKM1 recorded maximum NMC and cane yield in trial II and III (Table 4).

Sugarcane juice quality parameters recorded at the time of harvest in the field showed that PGPR treatments recorded significantly higher juice parameters *viz.*, brix (%), sucrose (%) and commercial cane sugar (%) as compared to *Pseudomonas* untreated plots. Juice characters recorded at the time of harvest showed that more than 18 % brix with all PGPR strains. Sucrose content of both varieties varied between 14 to 20 %. Recovery of commercial cane sugar from cane stalks was more than 10 percent when compared to control treatment in all the three field locations (Table 5). The PGPR have been reported to enhance plant growth by several ways. They can fix atmospheric nitrogen and supply it to plants, synthesize siderophores that can solubilize and sequester iron from the soil and provide it to plant cells. They synthesize phytohormones such as gibberellins, cytokinins and indole acetic acids that promote plant growth at various stages, solubilize minerals such as phosphorus and synthesize enzymes/ low molecular mass compounds that can modulate plant growth and development (Glick 1994, Kloepper *et al.* 1986). In the fields the PGPR strains have improved the vegetative sett germination and growth especially the number of millable canes leading to higher cane yield. The PGPR strains have been demonstrated previously on their positive role in improving sugarcane true seed germination, sett germination and cane growth (Viswanathan and Samiyappan 1999, 2002).

Usually pathogen infection in the cane stalks causes higher inversion of sugar by invertase enzyme, which results, in poor juice quality. Further, mixing of inverted juice with good quality juice, affects sugar recovery significantly during milling process in sugar mill (Singh and Waraitch 1977) and such loss, in sugar recovery usually occurs in the red rot endemic areas in the country. The increased sucrose content in PGPR treated canes might be due to reduced damage by the pathogen induced invertase enzyme and which leads to better recovery of commercial cane sugar from cane juice.

PGPR treatment from planting onwards showed comparatively higher resistance and this may be due to the early establishment of the bacterial population in the rhizosphere and showed better sett germination and stalk growth later. Sett treatments followed by soil application are highly effective in inducing resistance against *C. falcatum* and ISR persisted upto 90 days in sugarcane (Viswanathan and Samiyappan 1999). Normally the pathogen infects the crop at 6 to 8 months of age that coincides with the active monsoon periods in the country, which favours pathogen spread and disease outbreak. By scheduling the PGPR application at 4 to 5 months stage of the crop the resistance can be maintained up to the maturity of the crop. Once the matured crop in the field is either partially or fully immunized, we can expect improved crop yield and higher sugar recovery in the red rot endemic areas. The reduced pathogen load in the PGPR treated canes as compared with the untreated canes. The reduction in the colonization was more evident in the upper nodes and it might be due to the PGPR induced systemic protection against the red rot pathogen. Remarkable reduction in the disease, high cane yield and more sucrose in the PGPR treated plots indicate that these strains need to be popularized for the management of red rot disease under field condition in sugarcane growing areas.

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Table - 4 : Effect of PGPR strains on sugarcane growth and cane yield under field conditions

PGPR strains	Population at 5th month (x 1000)			NMC ha^{-1}at 12th month (x 1000)			Cane yield in t ha^{-1} 12th month		
	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III
Pf 1	424.7^{a}	249.2^{ab}	132.2^{b}	94.4^{a}	93.6^{cd}	94.9^{ab}	50.7^{d}	114.3^{d}	134.3^{a}
VPT 4	244.0^{d}	209.5^{cd}	136.8^{b}	70.0^{c}	104.9^{abc}	91.1^{b}	74.0^{ab}	117.5^{cd}	130.4^{a}
CHAO	369.6^{b}	219.8^{c}	133.2^{b}	71.0^{bc}	95.6^{cd}	94.3^{ab}	76.8^{a}	118.9^{bcd}	132.2^{a}
EP 1	358.9^{b}	237.5^{bc}	129.8^{b}	90.1^{a}	104.8^{abc}	95.8^{ab}	69.9^{b}	123.6^{ab}	135.6^{a}
KKM 1	329.8^{bc}	228.9^{bc}	164.1^{a}	76.2^{bc}	115.2^{a}	92.7^{ab}	61.6^{c}	125.5^{a}	134.5^{a}
Pf 1 + KKM 1	-	267.2^{a}	161.9^{a}	-	109.1^{ab}	97.2^{a}	-	120.0^{bc}	137.4^{a}
T. viride	145.8^{e}	-	-	72.7^{bc}	-	-	42.9^{e}	-	-
Carbendazim	-	171.1^{e}	126.1^{b}	-	91.6^{d}	84.5^{c}	-	104.8^{e}	117.6^{b}
Control	296.8^{c}	186.1^{de}	121.3^{b}	79.5^{b}	99.8^{bcd}	85.5^{c}	62.3^{c}	107.8^{e}	121.3^{b}

- : Indicates non inclusion of the treatment
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table - 5 : Effect of PGPR strains application on cane juice parameters of sugar cane in the field trials

PGPR strains	Brix (%)			Sucrose (%)			Purity (%)			CCS (%)		
	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III
Pf 1	22.1^{a}	19.4^{a}	18.3^{b}	19.8^{b}	16.3^{ab}	15.8^{c}	90.1^{a}	84.3^{b}	86.4^{bc}	13.5^{b}	11.0^{bc}	11.1^{d}
VPT 4	20.2^{bcd}	18.7^{a}	18.0^{b}	18.4^{ab}	15.8^{ab}	15.4^{c}	91.3^{a}	84.2^{b}	85.7^{c}	12.9^{ab}	10.6^{c}	10.8^{d}
CHAO	20.1^{cd}	19.3^{a}	21.8^{a}	18.3^{ab}	16.2^{ab}	19.1^{a}	89.7^{a}	83.5^{b}	87.6^{abc}	12.8^{ab}	10.7^{c}	13.5^{a}
EP 1	20.3^{bc}	19.3^{a}	19.8^{ab}	18.4^{ab}	16.5^{ab}	17.5^{b}	90.7^{a}	85.3^{a}	88.3^{ab}	12.9^{ab}	11.3^{ab}	12.4^{bc}
KKM 1	21.1^{b}	20.2^{a}	22.3^{a}	19.0^{ab}	17.2^{a}	19.1^{a}	90.2^{a}	85.3^{a}	85.7^{c}	13.3^{ab}	11.8^{a}	13.4^{a}
Pf 1 + KKM 1	-	20.1^{a}	20.1^{ab}	-	17.2^{a}	18.0^{b}	-	85.3^{a}	89.8^{a}	-	11.7^{a}	12.9^{ab}
T. viride	21.1^{b}	-	-	19.0^{ab}	-	-	90.0^{a}	-	-	13.2^{ab}	-	-
Carbendazim	-	18.7^{a}	19.4^{ab}	-	15.3^{bc}	17.1^{b}	-	82.2^{c}	88.1^{ab}	-	10.0^{d}	12.1^{c}
Control	19.3^{d}	17.2^{a}	18.5^{b}	16.9^{a}	14.0^{c}	16.2^{b}	89.9^{a}	81.3^{c}	87.5^{abc}	11.6^{a}	8.9^{c}	11.1^{d}

-: Indicates non inclusion of the treatment
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

REFERENCES

Alexander, K.C. and Viswanathan, R. (1996). Major diseases affecting sugarcane production in India and recent experiences in quarantine. In : Croft, B.J., Piggin, C.M., Wallis, E.S., Hogarth, D.M. (ed.) *Sugarcane Germplasm Conservation and Exchange*. p. 46-48. Proc. ACIAR **67**, Canberra, Australia.

Duffy, B.K. and Weller, D. M. (1995). Use of *Gauemannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. *Pl. Dis.* **79** : 907-911.

Glick, B.R. (1995). The enhancement of plant growth by free living bacteria. *Can. J. Microbiol.* **4** : 109-117.

Hallmann, J., Quadt-Hallmann, A., Mahaffeee, W.F. and Kloepper, J.W. (1997). Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* **43** : 895-914, 1997.

Hildebrand, D.C., Schroth, M.N. and Sands, D.C. (1992). *Pseudomonas* – In : Schaad, N.W. (ed.) *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. 2nd edition. American Phytopathological Society. St. Paul, USA.

Jeyarajan, R., Ramakrishnan, G., Dinakaran, D. and Sridar, R. (1994). Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot diseases. In : Dwivedi (ed.): *Biotechnology in India*, p. 25-36, Bioved Research Society, Allahabad.

King, E.O., Ward, M.K. and Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* **44** : 301-307.

Kloepper, J. W., Tuzun, S., Liu, L. and Wei, G. (1993). Plant growth promoting rhizobacteria as inducers of systemic resistance in plants. In : Lumsden, R.D., Vaughn, J. L. (ed.) *Pest Management : Biologically Based Technologies*, p156-165. Am. Chem. Soc., Washington.

Kloepper, J.W., Scher, F.M., Laliberte, M. and Tipping, B. (1986). Emergence promoting rhizobacteria: Description and implications for agriculture. In : Swinburne, T.R. (ed.). *Iron, Siderophore and Plant Diseases*. p 155-164. Plenum Pub. Co., New York.

Maurhofer, M., Hase, C., Meuwly, P., Metraux, J.P. and Defago, G. (1994). Induction of systemic resistance of tobacco to tobacco necrosis virus by the root colonizing *Pseudomonas fluorescens* strain CHAO: Influence of the *gacA* gene and of pyoverdine production. *Phytopathology*, **84** : 139-146.

M'Piga, P., Belanger, R.R., Paulitz, T.C. and Benhamou, N. (1997). Increased resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomato plants treated with endophytic bacterium *Pseudomonas fluorescens* strain 63-28. *Physiol. Mol. Pl. Pathol.* **50**: 301-320.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T., and Samiyappan, R. (2001). Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. *Soil Biology and Biochemistry*, **33** : 603-612.

Rao, K.C. (1986). Analysis of sugarcane for maturity and quality. In : *Selection procedures in sugarcane varietal testing*. p 12-14. Sugarcane Breeding Institute, Coimbatore, India.

Senthil, N., Jayaraj, J. and Shanmugam, V. (2000). Evaluation of fungal and bacterial antagonists against *Colletotrichum falcatum* Went, causing red rot of sugarcane. *Indian Sugar* **50** : 423-432.

Singh, K. and Singh, R.P. (1989). Red rot. In : Ricaud, C., Egan, B.T., Gillaspie, Jr.A.G., Hughes, C.G. (ed.) : *Diseases of Sugarcane: Major Diseases*. p 169-188. Elsevier, Amsterdam.

Singh, N. (1994). *Trichoderma harzianum* and *Chaetomium* sp. as potential biocontrol fungi in management of red rot disease of sugarcane. *J. Biol. Control.* **8** : 65-67.

Singh, O.N. and Waraitch, K.S. (1977). Metabolic changes induced by *Colletotrichum falcatum* Went in sugarcane. *Sugarcane Pathologist's Newsl.* **19** : 7-9.

Srinivasan, K.V. and Bhat, N.R (1961). Red rot of sugar cane: Criteria for grading resistance. *J. Indian Bot. Soc.* **40** : 566-577.

Sundara, B. (1998). Sugarcane cultivation. p. 392. - Vikas Publishing House Pvt Ltd, New Delhi. India.

Van Loon, L.C., Bakker, P.A.H.M. and Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. *Ann. Rev. Phytopathol.* **36** : 453-483.

Van Peer, R., Niemann, G.J. and Schippers, B. (1991). Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417. *Phytopathology,* **81** : 728-743.

Vidhyasekaran, P. and Muthamilan, M. (1995). Development of formulation of *Pseudomonas fluorescens* for control of chickpea wilt. *Pl. Dis.* **79** : 782-780.

Viswanathan, R., Padmanaban, P. and Mohanraj, D. (1997). Growing virulence of red rot pathogen of sugarcane in Tamil Nadu. *Indian Sugar,* **37** : 23-30.

Viswanathan, R. and Samiyappan, R. (1999). Induction of systemic resistance by plant growth promoting bacteria against red rot disease caused by *Colletotrichum falcatum* Went. in sugarcane. Proc. of Sugar Technology Association of India, **61** : 24-39.

Viswanathan, R. and Samiyappan, R. (2000a). Red rot disease in sugarcane : Challenges and Prospects. *Madras Agric. J.* **87** : 549-559.

Viswanathan, R. and Samiyappan, R. (2001). Role of chitinases in *Pseudomonas* spp. induced systemic resistance against *Colletotrichum falcatum* in sugarcane. *Indian Phytopath.,* 54 : 418-423.

Viswanathan, R. and Samiyappan, R. (2002). Induced systemic resistance by fluorescent pseudomonads against red rot disease of sugarcane caused by *Colletotrichum falcatum. Crop Protection,* **21** : 1-10.

Wei, G., Kloepper, J. W. and Tuzun, S. (1996). Induction of systemic resistance in cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. *Phytopathology,* **86** : 221-224.