

A comparative evaluation of bioagents and chemicals for the control of stalk rot of Sorghum caused by *Dickeya dadantii*

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Abstract

Stalk rot sorghum caused by *Dickeya dadantii* (syn. *Erwinia chrysanthemi*) is a devastating sorghum disease and is highly detrimental to sorghum cultivation in tarai region of India. The bacterium disrupts and disintegrates vascular bundles of sorghum stem manifesting slimy soft rot symptom. *In vitro* studies on bioagents and chemicals revealed that among bioagents assessed *Pseudomonas fluorescens* strain Psf-173 and *Trichoderma harzianum* strain Th-14 surpassed the other biological control agents whereas among chemicals, oxytetracycline and tetracycline were outstanding than other chemicals and their combination products used for the control of stalk rot of Sorghum caused by *D. dadantii*. Field trial with pre-plant soil application had maximum reduction in disease severity in treatment with antibiotic oxytetracycline (28.18%) whereas trial with pre-plant soil application with one (34.49%) and two foliar spray (37.03%) showed maximum reduction in disease severity in treatment with *P. fluorescens* strain Psf-173. All the three field trials involving pre-plant soil application, pre-plant soil application and one foliar spray and trial with pre-plant soil application and two foliar spray revealed that biological control agent *P. fluorescens* strain Psf-173 alleviates symptom of stalk rot of sorghum and stimulates seed germination and plant growth.

Keywords: *Dickeya dadantii*, oxytetracycline, *Pseudomonas fluorescens*, Stalk rot, Sorghum

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INTRODUCTION

Sorghum stalk rot is a destructive sorghum disease caused by *Dickeya dadantii* (syn. *Erwinia chrysanthemi*). Stalk and top rot of sorghum caused by *Erwinia* in 1987-88, at Pantnagar, Uttarakhand, destroyed 60-80% plants of distinct sorghum genotypes (Saxena *et al.* 1991). The infected stem pith disintegrates with slimy soft-rot symptom and emits foul-smell, eventually leading to wilting of whole plant (Zummo, 1969; Hepperly and Ramos-Davila, 1987; Hseu *et al.*, 2008). The entire length of the stalk or few internodes may rot followed by drying and maceration of internal tissues. Leaves and leaf sheath covering the rotting tissues turn chlorotic with pale-straw colour. Hepperly and Davila (1987) evaluated twenty-two antibiotics against *E. chrysanthemi* pv. *zeae* under *in*

vitro and *in vivo* conditions and reported bacterium to be sensitive to tetracycline, chloramphenicol, kanamycin and gentamycin and insensitive to penicillin group of antibiotics. Bleaching powder of 1000ppm concentration was reported to suppress growth of *Ecarotovora* ssp. *carotovora* both *in vitro* and *in vivo* application by soil drenching as well enhanced tuber germination (Saini and Parashar, 1981). Chemicals such as streptomycin, streptomycin, terramycin, agrimycin-100, Blitox-50, Dithane M-45, Dithane Z-78, bleaching powder, captan and ziram has been reported to have inhibitory effect on *Erwinia* (Rangarajan and Chakravarti, 1969; Sinha and Prasad, 1977; Thind and Payak, 1985; Kumar *et al.*, 2016). Biological control agents as *Bacillus subtilis*, *P. fluorescens*, *actinomyces* and VAM fungi has been studied to provide significant control of *E. chrysanthemi*

(Karkouri et al., 2010 and Nagaraj et al., 2012). The aim of the present work was to study– i) *In vitro* assessment of antagonistic potential of bio-control agents and chemicals against *D. dadantii*. ii). Field trail to assess the efficacy of biocontrol agents and chemicals tested *in vitro* against the bacterium.

MATERIALS AND METHODS

In vitro assessment of antagonistic potential

Biocontrol agents by Dual culture assay: Biological control agents as isolates of *Trichoderma*, Th-14, UKT-22 and UKT-40 and *P. fluorescens* isolate, Psf-173 were procured from Biological control laboratory, Pantnagar, and were examined against *D dadantii* for their antagonistic potential by dual culture technique (Morton and Stroube, 1955). Under aseptic conditions, nutrient agar medium (NAM) was poured in a sterilized petri plates and left to solidify. The cell suspension of conc. 10^4 cell /ml was prepared from 24h old culture of test bacterium and five mm disk dipped in bacterial suspension and 4 days old culture of biocontrol agents was cut into disk with sterilized cork borer, and were placed on solidified NAM in a manner that they lied just opposite to each other along the diameter of the petri plate. The discs were placed at distance of 2cm from the periphery of petri plates in a straight line followed by incubation at $28 \pm 1^\circ\text{C}$. The disk dipped in bacterial suspension, inoculated centrally on NAM amended Petri plate served as control. The experiment was performed in completely randomized design (CDR) with three replications. Percent inhibition of the test pathogen was calculated 3-5 days after incubation at 28°C by comparing the growth of the test pathogen (after parasitization) with the control. Inhibition zone was calculated on the basis of formula, per cent inhibition (PI) = $C-T/C \times 100$ Where, PI = per cent inhibition, C = Radial growth (cm) of test pathogen in absence of antagonist (cm), T = Radial growth (cm) of test pathogen in presence of antagonist (Skidmore and Dickinson, 1976).

Chemicals by disc diffusion method: Antibiotic susceptibility test was performed by Kirby-Bauer and Stroke's method. The paper discs of 5 mm diameter were prepared from whatman filter paper no.1 were placed in glass vial and sterilized in autoclave at 121°C for 15 minutes. Nutrient agar plate was seeded with 100 μl of the test bacterium under aseptic condition. The discs dipped in different antibiotics concentrations 100, 200, 400, 500 and 1000 ppm were placed under aseptic condition on the seeded nutrient agar plate. Each petri plate should have only 4 discs, 3 of different concentration and one of control (dipped in sterile water), placed precisely ensuring contact of disc with medium. Plates are examined for zone of inhibition after incubation at 28°C for 48 hours (2-3 days). The inhibition zone diameter was measured including disc diameter (as judged by the unaided eye). Calculation of percent inhibition was

done on basis of formula: Percentage of inhibition = $R_1 - R_2/R_1 \times 100$. Where, R_1 = Radius of the radial growth of the pathogen towards opposite side in control plate, R_2 = Radius of the radial growth of the pathogen towards the opponent antagonist in test plate (Hedges, 1999).

Field Assessment: The field experiment was designed and conducted at Livestock Research centre, amid 2014 *Kharif* season at G.B. Pant University of Agriculture and Technology, Pantnagar to compare the potentiality of bioagents and chemicals screened by *in vitro* assay against *D dadantii*. Experiment was performed in Randomized Block Design (RBD) with 3 replications of each treatment. The sorghum seedlings of variety SPV 2128 was artificially inoculated with bacterial suspension of *D dadantii*, Pantnagar isolate containing 0.7% of Tween-80 (v/v) + 1×10^7 cells/ml (cfu) by leaf whorl inoculation method. The sterilized water was used to inoculate control plants. The treatments for evaluation of bioagents and chemicals for field experiment were designed as follows:-

- Pre- plant soil application
 - Pre-plant soil application + one foliar spray
 - Pre-plant soil application + two foliar spray
- Disease severity was recorded 40 and 60 days after sowing (DAS). The observations on growth parameters, seed germination (%), plant height (cm) and stem diameter (cm) was first recorded 20 DAS from all the plots and then again at 40 DAS from each plot separately by taking random samples. Statistical analysis of data was carried using SPSS software.

RESULTS AND DISCUSSION

In vitro results

Screening of biocontrol agent: The antagonistic potential of biological control agents was assessed against the pathogen by dual culture assay. The *P. fluorescens* strain Psf-173 was par excellence among bioagents with maximum (71.75%) reduction of radial growth (Table 1). The competent biocontrol agent, recorded under *in vitro* conditions were further evaluated for competence under field conditions.

Xu and Gross (1986) found that Fluorescent Pseudomonads showed high level of antagonistic activity against *E. carotovora*. The *P. fluorescens* has been reported by various researchers to restrict the growth of disease causing microorgan-

Table 1. Dual culture assay for efficacy of biocontrol agents against *D dadantii*.

Treatment	Colony diameter (cm)	Reduction in colony diameter (%)
Th – 14	1.26	68.50
UKT – 22	2.16	46.00
UKT – 40	2.30	42.50
Psf – 173	1.13	71.75
C(Control)	4.00	0.00
CD at 5% sem	0.22 5.97	

Table 4. Pre-plant soil application and one foliar spray with bioagents and chemicals and its effect on stem diameter, plant height, disease severity and yield in field trial.

Treatment	Stem diameter (cm)	Plant height (cm)	Disease severity (%)		Mean	Decrease in disease severity (%)	Yield (Biomass) Q/ha
			40 DAS	60 DAS			
Tetracycline	1.60	233.00	29.76	31.22	30.49	26.81	176.5
Oxytetracycline	1.80	235.26	28.00	29.42	28.71	31.08	188
Blitox -50	1.51	229.33	29.67	36.33	33.00	20.78	142.5
Bleaching powder	1.50	227.00	30.77	36.75	33.76	18.96	180.5
Streptocycline	1.31	227.87	26.03	34.27	30.15	27.62	112
Blitox-50 + Streptocycline	1.42	227.00	24.36	35.35	29.85	28.34	125
Blitox-50 + oxytetracycline	1.64	230.18	24.23	34.54	29.38	29.47	185
Psf - 173	1.82	235.51	24.50	30.08	27.29	34.49	170
Th- 14	1.74	231.46	25.56	30.13	27.84	33.17	109.8
UKT-22	1.61	227.11	28.80	33.55	31.17	25.18	110.5
UKT-40	1.57	225.45	32.41	38.41	35.41	15.00	125
Control	1.03	214.80	34.13	49.20	41.66	-	77.08
CD at 5%	0.109	1.605	2.125	1.827	-	-	2.048
SE (m)	0.037	0.544	0.720	0.619	-	-	0.694

Table 5. Pre-plant soil application and two foliar spray with bioagents and chemicals and its effect on stem diameter, plant height, disease severity and yield in field trial.

Treatment	Stem diameter (cm)	Plant height (cm)	Disease severity (%)		Mean	Decrease in disease severity (%)	Yield (Biomass) Q/ha
			40 DAS	60 DAS			
Tetracycline	1.50	236.73	27.61	29.63	28.62	31.30	171.5
Oxytetracycline	1.90	235.73	25.50	27.63	26.56	36.24	184.68
Blitox -50	1.70	234.41	27.75	34.21	30.98	25.63	173.4
Bleaching powder	1.50	227.36	30.77	35.76	33.26	20.16	139.47
Streptocycline	1.75	229.67	25.42	32.03	28.72	31.06	124.12
Blitox-50 + Streptocycline	1.40	236.67	25.57	34.41	29.99	28.01	171.2
Blitox-50 + oxytetracycline	1.60	231.58	23.06	32.00	27.53	33.91	138.42
Psf - 173	1.65	237.65	24.46	28.00	26.23	37.03	184.88
Th- 14	1.80	235.74	24.00	30.00	27.00	35.18	184.66
UKT-22	1.60	225.80	27.92	30.74	29.33	29.59	111.22
UKT-40	1.55	224.40	31.17	35.05	33.11	20.52	105.42
Control	1.03	214.80	34.13	49.20	41.66	-	75.24
CD at 5%	0.122	1.66	1.454	1.690	-	-	2.661
SE (m)	0.041	0.42	0.493	0.572	-	-	0.901

bition was observed with oxytetracycline (3.50 cm) among all the chemicals used.

Christensen and Wilcoxan (1966) reported control of soft rot erwinia by chemical treatment of seed, soil or foliage of corn. Antibiotics such as streptomycin, streptomycin and terramycin has been reported to be effective against *E. chrysanthemi* even at lower concentration (Chakravarti and Rangarajan, 1966; Rangarajan and Chakravarti, 1969). Saini and Parashar (1981) recorded inhibitory effect of bleaching powder at 1000 ppm concentration against *E. carotovora* ssp. *carotovora*. *E. carotovora* ssp. *carotovora* has been reported to be sensitive to streptomycin in banana and potato (Mahmood et al., 1981; Parashar and Sindhan, 1988). Antibiotics streptomycin/tetracycline 100 ppm mixed with 20 ppm copper fungicides may be used for seed treatment to control soft rot of vegetables by *E. carotovora* pv. *carotovora*, pv. *atroseptica* (Verma, 2012).

In vivo results: The result of treatment with only pre-plant soil application is presented in Table 3, clearly demonstrating that disease severity was reduced in all the treatments significantly over control. Maximum seed germination, stem diameter and plant height was observed in treatment with oxytetracycline (78.58%), Th-14 (1.70cm), Th-14 (233.13cm) respectively. Whereas maximum reduction in disease severity was observed with oxytetracycline (28.18%) followed by those in treatment with Psf-173 and maximum yield was observed for treatment with oxytetracycline (157.5 Q/ha). It is evident from result of treatment with pre-plant soil application and one foliar spray, presented in the Table 4, that disease severity was significantly reduced in all the treatments over control. The treatment with Psf-173 showed maximum reduction in disease severity of 34.49% followed by Th-14 and oxytetracycline, thickest stem diameter was observed in treatment with Psf-173 (1.82cm), plant height was found to be maximum in treatment with Psf-173 (235.51cm) and maximum yield was obtained from plot treated with oxytetracycline (188 Q/ha). The result of treatment with pre-plant soil application and two foliar spray are presented in Table 5 indicating that maximum increase in stem diameter was with oxytetracycline (1.90cm), maximum increase in plant height was recorded with Psf-173 (237.65cm), disease severity was reduced maximum by Psf-173 (37.03%) followed by oxytetracycline and Th-14 and maximum yield was obtained in plot treated with Psf-173 (184.88 Q/ha). In field experiment, *P. fluorescens* strain Psf-173 was at par with antibiotic oxytetracycline as far as reduction in disease severity was concerned.

Several other workers as Cronin et al. (1997) while working with soft rot of potato obtained similar result and reported that *P. fluorescens* F113 has ability to produce DAPG, which plays a crucial role in inhibition of *E. carotovora* ssp. *atroseptica*. Nagaraj et al. (2012) reported use of *Bacillus subtilis* and *P. fluorescens* for management of *E. ca-*

rotovora subsp. *carotovora* and *E. chrysanthemi* causing tipover disease of banana. The strains of *P. fluorescens* and *P. putida* has been reported to inhibit soft rot causing bacteria, *E. carotovora* pv. *carotovora* and enhances plant growth and yield. The Pseudomonas strains were also found compatible with fungicides used against potato soft rot (Burr et al., 1978). Hajhamed et al. (2007) found that the disease severity of soft rot of potato caused by *E. carotovora* ssp. *carotovora* was reduced significantly by all tested bioagents (upto 83.5%). The biological control agent, *P. fluorescens* has been reported by above workers to inhibit erwinia and enhance crop growth and yield in similar manner to present work. Christiano et al. (2010) found that the foliar spray of oxytetracycline under varying temperature, sunlight and stimulated rainfall condition on peach was effective in controlling *Xanthomonas arboricola* pv. *pruni* on stone fruits and *E. amylovora* on pome fruits. Trunk-injection of apple with oxytetracycline reduces incidence of fire blight of apple upto 60%, which was exceptionally higher than those provided by kasugamycin and copper chelates (Aćimović et al., 2014). Efficacy of different antibiotics as oxytetracycline and kasugamycin have been reported in various crops to control erwinia similar to present work in which oxytetracycline was reported to reduce disease severity to considerable extent.

Conclusion

From both *in vitro* and *in vivo* study performed on bioagents and chemicals to control *D. dadantii* it can be concluded that among bioagents used, Psf-173 and Th-14 was par excellence with other biological control agents used. Whereas among chemicals, oxytetracycline and tetracycline were found effective in reducing disease severity and were far much better than the fungicide and bleaching powder used alone and there combination product with antibiotics. It was revealed that from all the three field trials, biological control agent Psf-173 was at par with oxytetracycline as far as reduction in disease severity was concerned. So we can emphasize upon safer use of biological control agents as pseudomonas rather than chemicals or antibiotics as oxytetracycline for management of stalk rot of sorghum. Biological control agent as pseudomonas alleviated stalk rot of sorghum as well augmented plant growth of sorghum. Use of biological control agents protects environment by avoiding superfluous accumulation of chemicals, preventing residue accretion and pollution.

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