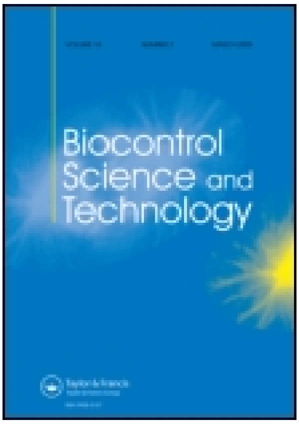

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Efficacy of combined applications of antagonist bacteria and chemical resistance inducers for the management of Fusarium solani causing root rot in Withania somnifera

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SHORT COMMUNICATION

Efficacy of combined applications of antagonist bacteria and chemical resistance inducers for the management of *Fusarium solani* causing root rot in *Withania somnifera*

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Application of Thiosalicylic acid + *Bacillus cereus*; O-Acetylsalicylic acid + *Pseudomonas fluorescens* reduced root rot severity by 85 and 88% and enhanced root yields by 358 and 419%, respectively, against *Fusarium solani* induced root rot disease in *Withania somnifera*. Reduction in disease severity was correlated with defence-related enzymes peroxidase, polyphenol oxidase and phenyl ammonium lyase.

Keywords: *Fusarium solani*; *Withania somnifera*; chemical resistance inducers; PGPR

Withania somnifera (L.) Dunal (ashwagandha) has been an integral part of the ayurvedic medical systems (Dhuley 2001) renowned as the best rejuvenative herb promoting energy and vitality. *Fusarium solani* causes root rot in *W. somnifera* leading to enormous yield losses (Gupta, Misra, Kalra, and Khanuja 2004). Reducing the impact of *Fusarium* on *Withania* root yields and quality remains an intractable problem because of lack of effective fungicides and their restrictive use.

Biological control and induced systemic resistance are important components of integrated disease management approach. Salicylic acid, its functional analogues and β-amino-n-butyric acid (BABA) and its derivatives have been established as potential systemic resistance inducers in innumerable plant diseases (Pandey and Kalra 2005) but a very few have been developed commercially due to their potential phytotoxicity to some crops (Siegrist, Orober, and Buchenauer 2000) and energetic cost involved in induction of resistance resulting in crop yield losses (Smedegård-Petersen and Tolstrup 1985). *Pseudomonas* spp. and other siderophore producing rhizobacteria have been demonstrated to play an active role in resistance mechanism in diseases associated with *Fusarium* spp. (Gade and Armarkar 2011). Peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonium lyase (PAL), and total phenols may serve as markers for resistance of plants to diseases (Mandal, Mitra, and Mallick 2009). The present study aims at exploring the possibility of enhancing disease control potential of

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chemical resistance inducers and plant growth promoting rhizobacteria (PGPR) when applied together.

Thirty-day-old seedlings raised from surface sterilised seeds of *W. somnifera* cv. Poshita in nine inches earthen pans containing sterilised soil and vermicompost (2:1) were subjected to various treatments before transplanting into pots with the similar mixtures of soil and vermicompost. For fungal inoculations *F. solani*, obtained from root rot affected *W. somnifera* tissues (Gupta et al. 2004), was grown on Sand Maize Meal Media (Sand:Maize:Water = 3:1:9) for 15 days and evenly mixed with soil and allowed to establish for three days prior to transplanting of *Withania* seedling.

Aqueous solutions (2 mM) of chemical resistance inducers namely [Thiosalicylic acid (TSA), O-acetyl salicylic acid (O-ASA) and DL-2-amino butyric acid (DL-2-ABA)], and two siderophore producing PGPRs namely, Sd23 (*Bacillus cereus* MTCC 430) and DPF (*Pseudomonas fluorescens* MTCC 103) showing antagonism against *F. solani* were used for subsequent experiments. The seedlings were dipped in the rhizobacterial suspensions (10^8 CFU ml^{-1}) and aqueous solutions of chemical resistance inducers for 1 hour before transplantation. The plants applied with the combined applications were dipped in the mixture of the cell suspensions and chemical inducers (Kataria, Wilmsmeier, and Buchenauer 1997).

The plants were harvested after 90 days. The average plant height, shoot dry weight and root dry weight were determined. The disease severity on each plant was rated using a scale of 0–4, where 0 = no disease and 4 $\geq$ 75% roots affected. For estimating phenols and enzyme activities fourth and fifth leaves from top were taken, one month after the treatments. Activity of PAL was determined as described by Dickerson, Pascholati, Hagerman, Butler, and Nicholson (1984). Peroxidase activity was estimated following the procedure of Hammerschmidt, Nuckles, and Kuć (1982), whereas PPO activity was determined as described by Mayer, Harel, and Saul (1965). Total phenols were estimated as per the procedures described by Zeislin and Ben-Zaken (1993).

The collected data were subjected to statistical analysis suitable to completely randomised design (CRD) for pot experiment, with the help of software IBM SPSS PASW Statistics 20. Significant differences among treatment means were based on analysis of variance using Duncan's multiple range test ($p \leq 0.05$). There were three trials conducted and as the trials had a similar variance value; the data were combined for further analysis.

Fusarium causes severe root rot in *Withania* leading to the severe reductions in shoot and root dry weight and the plant height of the untreated *Fusarium* challenged plants. *Withania* seedlings challenged with *Fusarium* showed 54.35 and 55.66% reductions in the shoot and root dry weights, respectively, as compared to the plants not subjected to the fungal infection (Table 1). The non-treated *Fusarium* infected plants showed stunted growth with a 48.38% decrease in the plant height. The *Fusarium* challenged plants also recorded a marked increase in the activity of the enzymes and phenol, the biochemical markers of plants inherent disease resistance mechanisms, suggesting activation of pathogen mediated resistance mechanisms commonly termed as Systemic Acquired Resistance.

All the resistance inducers provided significant protection of *Withania* seedlings against root rot, a reduction in root rot disease severity by 40–50% relative to the untreated *Fusarium* infested plants (Figure 1), with increase in root dry weight and plant height by more than 80 and 70%, respectively. The plants treated with O-ASA

Table 1. Analysis of *Fusarium* infected *Withania somnifera* plant growth and biochemical parameters under various chemical resistance inducer and PGPR treatments.

Treatments	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Plant height (cm)	PO (abs change min^{-1} g^{-1} plant material)	PPO (abs change min^{-1} g^{-1} plant material)	PAL (µg cinnamic acid min^{-1} g^{-1} plant material)	Phenol (mg catechol g^{-1} plant material)
Non-inoculated	7.177f	2.237h	31.00de	56.00g	0.688f	138.33d	0.235h
F.s.	3.277i	0.992j	16.00f	80.34de	0.365g	200.00c	0.529e
F.s. + TSA	4.073h	1.798i	29.33de	85.93c	0.801de	207.00c	0.636d
F.s. + O-ASA	4.687gh	1.847i	27.33e	84.90c	0.802de	203.33c	0.665c
F.s + DL-2-ABA	5.037g	2.560g	35.67cde	83.86cd	0.735ef	204.00c	0.244h
F.s. + TSA + Sd23	14.003a	4.551b	51.67b	178.20a	1.377b	258.67a	0.764b
F.s. + O-ASA + Sd23	7.070f	3.458de	34.67cde	86.33c	1.059c	216.67bc	0.675c
F.s. + DL-2-ABA + Sd23	10.280e	3.393e	42.33c	87.00c	0.851d	211.00bc	0.497f
F.s. + TSA + DPF	12.207b	4.405b	40.33c	87.33c	0.875d	212.68bc	0.508ef
F.s. + O-ASA + DPF	13.263a	5.149a	59.33a	124.33b	1.606a	226.67b	0.842a
F.s. + DL-2-ABA + DPF	10.807de	3.115f	37.67de	124.00b	1.123c	227.33b	0.489f
F.s. + Sd23	11.313cd	3.703d	40.00c	67.66df	0.786de	201.00c	0.456g
F.s. + DPF	11.640bc	4.030c	40.67c	79.00e	0.737ef	202.33c	0.439g

Non-inoculated, No *Fusarium* infection; F.s., *Fusarium solani*; TSA, Thiosalicylic acid; O-ASA, O-acetylsalicylic acid; DL-2-ABA, DL-2-aminobutyric acid; Sd23, *Bacillus cereus*; DPF, *Pseudomonas fluorescens*; PO, Peroxidase; PPO, Poly polyphenol oxidase; PAL, Phenylalanine ammonium lyase.
Means in the same column followed by different letters differ significantly at $p < 0.05$.

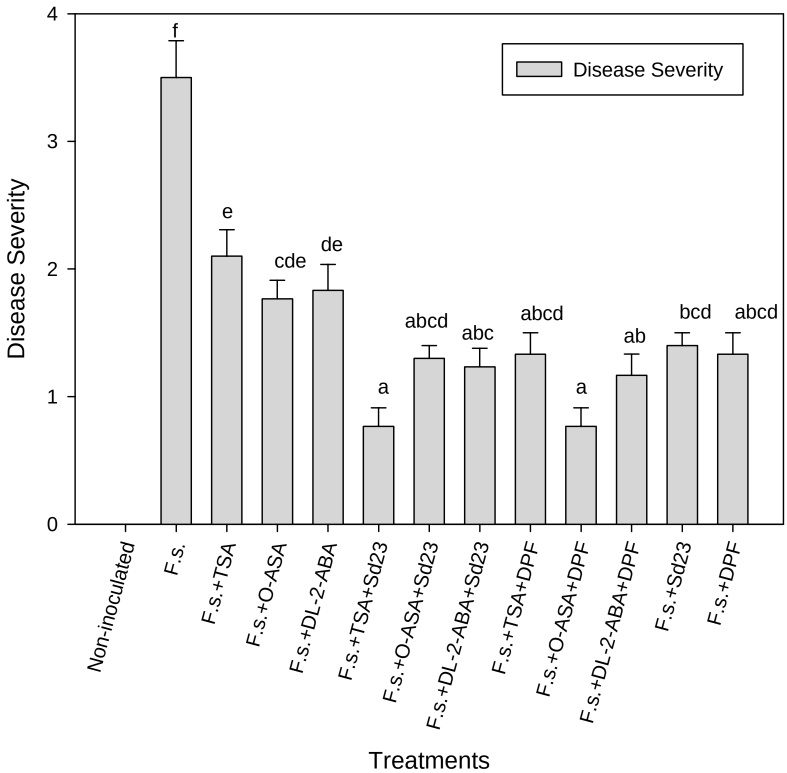

Figure 1. Effects of single and combined applications of chemical resistance inducers and inoculated with the PGPRs on the root rot disease severity in *Withania somnifera* plants. Non-inoculated, No *Fusarium* infection; F.s., *Fusarium solani*; TSA, Thiosalicylic acid; O-ASA, O-acetylsalicylic acid; DL-2-ABA, DL-2-aminobutyric acid; Sd23, *Bacillus cereus*; DPF, *Pseudomonas fluorescens*. Bars are means of three replicates ± Standard Error of Means. Columns with different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

showed maximum reduction in the disease severity but the highest root and shoot weight recorded in DL-2-ABA treated plants (Table 1), suggesting its probable role in improving growth and yields beyond disease control.

Although, the chemical resistance inducers reduced disease severity to a significant level but were less efficient in comparison to the PGPRs (Figure 1). *P. fluorescens* (DPF) and *B. cereus* (Sd23) were found to be effective in reducing the severity by 60%. The plants inoculated with DPF recorded an increase of more than twofold in the shoot and root dry weight and also the plant height in comparison to the untreated as well as the chemical inducer treated plants (Table 1). The reduction in disease severity in PGPR treated plants could be attributed to their direct antagonism and their capability to produce siderophores not only useful in iron acquisition but also their known role to interfere in the establishing a pathogenic relationship of *Fusarium* with host plant (Sayyed and Chincholkar 2009).

The combined applications were more effective in reducing the disease severity (Figure 1). Application of TSA + Sd23 and O-ASA + DPF reduced the root rot severity by 85 and 88% and enhanced root yields (root being the economic part of the plant) by 358 and 419%, respectively. Among all the treatments, the maximum growth was observed in plants treated with TSA + Sd23 followed by O-ASA + DPF and DL-2-ABA + DPF suggesting the specificity of interaction between the chemical inducers and the PGPRs. The combined effects of the chemical resistance inducers and *P. fluorescens* have been found to provide better disease control in bean plants against *Rhizoctonia solani* (Kataria et al. 1997).

The reduced disease severity by the combined treatments was correlated to its ability to induce defence enzymes such as PO, PPO and PAL. The plants treated with TSA + Sd23 showed maximum PO, PPO and PAL content followed by the plants treated with O-ASA + DPF and DL-2-ABA + DPF; whereas the maximum phenolics accumulation was observed in the plants treated with O-ASA + DPF over *Fusarium* inoculated plants (Table 1).

The data from our experiments do not discount the possibility of enhancing control of *Fusarium* root rot of *Withania somnifera* by combined application of chemical inducers and the PGPR for higher crop yields. Application of TSA + *B. cereus* and O-ASA + *P. fluorescens* may prove to be a helpful tool for managing root rot disease in *W. somnifera* where use of chemical fungicides is restricted because of the direct consumptions of its roots for health benefits.

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