Biocontrol Science and Technology

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbst20

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To cite this article: Nidhi Bharti, Purvi Agrawal, Bishal Misra, Arpita Tripathi, Rakshpal Singh, Deepamala Maji, Hemendra Pratap Singh & Alok Kalra (2013): Efficacy of combined applications of antagonist bacteria and chemical resistance inducers for the management of Fusarium solani causing root rot in Withania somnifera, Biocontrol Science and Technology, 23:2, 239-244

To link to this article: http://dx.doi.org/10.1080/09583157.2012.755611

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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...
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 (10⁸ 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 ≥ 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 Kuč (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* ≤ 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.

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

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 $p < 0.05$. 
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 \textit{P. fluorescens} have been found to provide better disease control in bean plants against \textit{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 \textit{Fusarium} inoculated plants (Table 1).

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

\textbf{Acknowledgement}

The authors wish to thank the Director, CSIR- Central Institute of Medicinal and Aromatic Plants, Lucknow, India, for providing necessary facilities and encouragement during the course of investigation.

\textbf{References}


