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# Improvement of bio-efficacy of bacterial antagonists by using bleaching powder and resistant cultivars to control bacterial wilt of tomato

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## ABSTRACT

**In present investigation, two bacterial antagonists *Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were tested for their bio-efficacy against *Ralstonia solanacearum* causing bacterial wilt of tomato in combination with chemicals, *viz*., bleaching powder, calcium chloride, sodium carbonate and sodium bicarbonate under *in vitro* conditions. Sodium carbonate (0.1%) with *P. fluorescens* inhibited the maximum growth of *R. solanacearum* (10.4 cm² inhibition zone) followed by sodium bicarbonate with *B. subtilis* (7.23 cm² inhibition zone). Bleaching powder and calcium chloride in combination with the bioagents significantly increased growth inhibition of *R. solanacearum* as compared to bioagents alone. Bleaching powder along with resistant cultivar Arka Abha and susceptible cultivar Pusa Ruby were taken under this investigation to improve the bio-efficacy of bacterial antagonists under glasshouse conditions. Six treatments, *viz*., bleaching powder (0.01%) *B. subtilis* DTBS-5, *P. fluorescens* DTPF-3, bleaching powder (0.01%) + *B. subtilis* DTBS-5, bleaching powder (0.01%) + *P. fluorescens* DTPF-3 and control (only *R. solanacearum*) without chemical and bioagents were taken. Minimum wilt disease incidence 19.0 and 29.6% was found in combination of bleaching powder (0.01%) + *B. subtilis* DTBS-5 followed by 19.6 and 31.6% in bleaching powder + *P. fluorescens* DTPF-3 after 30 days of inoculation of *R. solanacearum* in Arka Abha and Pusa Ruby tomato, respectively. Integration of antagonistic bacteria, bleaching powder (0.01%) and Arka Abha reduces bacterial wilt incidence and improved bio-efficacy under glasshouse conditions.**

**Key words:** *Bacillus subtilis,* bacterial wilt, *Pseudomonas fluorescens*, *Ralstonia* sp., rhizobacteria, tomato.

## INTRODUCTION

Bacterial wilt in tomato caused by *Ralstonia solanacearum* (Smith) Yabuuchi is a serious problem in coastal, hilly and foot hill areas including Goa, Karnataka, Kerala, Maharashtra, Odisha, Jharkhand, West Bengal and states of north-eastern hills like Himachal Pradesh, Jammu & Kashmir, Uttarakhand (Singh *et al.*, 13) and causes heavy loss to the tomato crop, which vary from 2- 90% under different climates and seasons (Singh *et al.*, 13). Since, the disease is soil-borne and has wide host range (450 species and 54 families) including tomato, potato, eggplant, pepper, groundnut, tobacco, weeds and also roots of non-host plants (Allen, 1), hence it is very difficult to control the disease. There are no antibiotics or other group of chemicals available to effectively control the bacterial plant diseases particularly bacterial wilt of solanaceous crops. However, some chemicals like bleaching powder and calcium chloride were used for controlling the bacterial wilt disease of tomato and other solanaceous crops (Sharma *et al.*, 12; Singh *et al.*, 14); but these chemicals are not much effective and also phytotoxic to the plants at higher doses particularly bleaching powder (Singh *et al.*, 14). Moreover, these chemicals may cause soil and water pollution. Hence, alternative approaches of non-chemical methods such as cultural methods, resistant cultivars and biocontrol with antagonistic bacterial agents have been used to manage bacterial diseases of plants successfully (Almoneafy *et al.*, 2). In bio-control method, various fungal and bacterial antagonists are used to control plant diseases. Among these, bacterial antagonist has become good candidate as an agent of biocontrol plant growth promoting bacteria (Chung *et al.*, 4; Tan *et al.*, 16).

Biological control of bacterial wilt solanaceous crops particularly tomato has been done through the antagonistic bacteria, which reduces the incidence of bacterial wilt disease in great extent (Almoneafy *et al.*, 2; Aiye *et al.*, 3; Tan *et al.*, 16). The rhizobacteria are found quite effective to suppress the bacterial pathogen as antagonists and promote plant growth (Rajendran *et al.*, 10; Singh *et al.*, 15) due to well-developed secretary system and produces structurally diverse secondary metabolites with a wide spectrum of antibiotic activity and their fast growth ability. However, biocontrol agents have their own limitations to control the plant diseases and it is difficult task to manage such deadly soil-borne diseases by using only microbes. Hence, it is a good option to integrate

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other control methods like cultural practices, resistant or tolerant varieties and chemicals at lower doses to improve bio-efficacy of antagonistic bacteria to control bacterial wilt disease of tomato.

## MATERIAL AND METHODS

*Ralstonia solanacearum* UTT-25 was obtained from the Division of Plant Pathology, ICAR-IARI, New Delhi, which was isolated from wilted tomato plants from farmer's field at Haldwani (Village: Chorgaliya), Nainital, Uttarakhand. The culture of bacteria was maintained in 20% glycerol and stored at -80°C for further study. Bacterial antagonist *Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were obtained from Plant Bacteriology Laboratory, Division of Plant Pathology, ICAR-IARI, New Delhi. Bacterial cultures were maintained on the respective slants and stored at 4°C for further use.

Dual culture method was used for the screening of antagonistic properties of bacteria against *R. solanacearum*. Bacterial antagonist *Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were selected for this study based on preliminary experiments *in vivo*. Four chemicals such as bleaching powder, calcium chloride, sodium carbonate and sodium bicarbonate were taken and concentration was decided based on earlier studied reported by Singh *et al.* (14). Chemical @ 0.1% concentration was added in CPG agar medium and poured into the petriplates. 100 µl of 48-h-old culture of *R. solanacearum* UTT - 25 ($10^{10}$ cfu/ ml) was spread onto the petri plates to make a lawn of the bacteria. Three wells of 0.5 cm diameter in each petri plate were made and 40 µl of 48-h-old culture of both the antagonistic bacteria *B. subtilis* DTBS-5 and *P. fluorescens* DTPF-3 at inoculum load of $10^8$, $10^9$ and $10^{10}$ cfu/ ml was poured in each well separately. The plates were incubated at 28 ± 1°C for 48 h with three replications. Inhibition zone formed by bacteria was recorded after 24 h of inoculation and converted into the area of inhibition zone using $\pi r^2$ formula.

The tomato cultivars Pusa Ruby (susceptible) and Arka Abha (resistant) were grown in nursery tray at National Phytotran Facility, ICAR-IARI, New Delhi. Seedlings (25 days) of both the cultivars were transplanted in autoclaved pots (6 inch dia.) having 1 kg of soil mixture. Six treatments, *viz.*,1. Bleaching powder (0.01%), 2. *B. subtilis* DTBS-5, 3. *P. fluorescens* DTPF-3, 4. Bleaching powder (0.01%) + *B. subtilis* DTBS-5, 5. Bleaching powder (0.01%) + *P. fluorescens* DTPF-3, 6. control (only *R. solanacearum*) without chemical and bioagent. The *Bacillus* was grown on TSA medium, *Pseudomonas* on King's B medium and *R. solanacearum* on CPG medium and after 48 h, bacterial growth was harvested and inoculum load of *R. solanacearum* was maintained 0.1 OD at 600 nm by using spectrophotometer (Schaad *et al.*, 13). Before transplanting, 0.01% concentration of bleaching powder was mixed in 1 kg of sterilized soil and then 50 ml of 48-h-old biocontrol agents *B. subtilis* DTBS-5 and *P. fluorescens* DTPF-3 was added as per treatment and thoroughly mixed into the soil. Then 25 ml of 48-h-old culture of *R. solanacearum* UTT-25 containing ($10^{11}$ cfu/ ml) poured in each pot without making injury to the tomato plants. Bacterial wilt incidence was recorded at 30 days of inoculation. Biological control efficacy (BCE) was calculated by using formula BCE = $[(Dc-D_T)/Dc \times 100$ as given by Guo *et al.* (6). Where Dc is disease of control and $D_T$ is disease of the treatment group. Population of *R. solanacearum* was recorded at initial stage and after 30 days of inoculation into the soil and converted into log value as described earlier (Schaad *et al.*, 11). The analysis of variance for antagonistic ability was performed by using factorial CRD as standard procedure (Gomez and Gomez, 5). Mean comparisons were conducted using a least significant difference (LSD) test (P = 0.05). Standard error and a LSD result were recorded.

## RESULTS AND DISCUSSION

To improve the efficacy of antagonistic bacteria bleaching powder, calcium chloride, sodium carbonate and sodium bicarbonate @ 0.1% concentration with bioagents was tested to improve their bio-efficacy against *R. solanacearum in vitro*. In case of *B. subtilis*, maximum inhibition zone (7.23 $cm^2$) was formed by sodium carbonate (0.1%) and *B. subtilis* ($10^{10}$cfu/ ml population) followed by sodium bicarbonate (Table 1). In case of *P. fluorescens*, maximum inhibition zone (10.4 $cm^2$) was formed by sodium carbonate with *P. fluorescens* ($10^{10}$ cfu/ml) followed by sodium carbonate and *P. fluorescens* (Table 2). The bio-efficacy of both the bioagents was reduced significantly by decreasing the population $10^{10}$ cfu/ ml to $10^8$ cfu/ml from 7.08 to 4.90 $cm^2$ and from 4.65 to 2.77 $cm^2$ against *R. solanacearum* in *P. fluorescens* DTPF-3 and *B. subtilis* DTBS-5, respectively. Bio-efficacy of both the bioagents was enhanced by combining with the chemicals. Improvement of bio-efficacy of both the bioagents *B. subtilis* and *P. fluorescens* might be due to antibacterial property of these chemicals, which reduces the inoculum of *R. solanacearum*, thus bioagents were able to form more inhibition zone (Sharma *et al.*, 12).

Minimum disease incidence 19.0 and 29.6% was found in bleaching powder (0.01%) + *B. subtilis* followed by 19.6 and 31.6% in bleaching powder + *P. fluorescens* after 30 days of inoculation in Arka Abha and Pusa Ruby cultivars, respectively. Biological

**Table 1.** Improvement of bio-efficacy of *P. fluorescens* against *R. solanacearum in vitro.*

| Chemical (0.1% conc.) | Inhibition zone (area $cm^2$) | | | Mean |
|---|---|---|---|---|
| | $10^{10}$ (cfu/ ml) of *B. subtilis* DTBS-5 | $10^{9}$ (cfu/ ml) of *B. subtilis* DTBS-5 | $10^{8}$ (cfu/ ml) of *B. subtilis* DTBS-5 | |
| Bleaching powder | 4.90 | 4.52 | 3.25 | 4.22 |
| Calcium chloride | 6.90 | 5.31 | 3.91 | 5.38 |
| Sodium carbonate | 9.25 | 8.05 | 7.39 | 8.23 |
| Sodium bicarbonate | 10.40 | 9.31 | 7.22 | 8.99 |
| Control | 3.96 | 3.14 | 2.74 | 3.28 |
| Mean | 7.08 | 6.07 | 4.90 | |
| $CD_{0.05}$ | | | Treatment = 0.46 | |
| | | | Population of bacteria = 0.36 | |
| | | | Treatment × Population of bacteria = 0.80 | |

**Table 2.** Improvement of bio-efficacy of *B. subtilis* against *R. solanacearum in vitro.*

| Chemical | Inhibition zone ($cm^2$) | | | Mean |
|---|---|---|---|---|
| | $10^{10}$ (cfu/ ml) of *P. fluorescens* DTPF-3 | $10^{9}$ (cfu/ ml) of *P. fluorescens* DTPF-3 | $10^{8}$ (cfu/ ml) of *P. fluorescens* DTPF-3 | |
| Bleaching powder (0.1%) | 3.96 | 2.93 | 2.52 | 3.13 |
| Calcium chloride (0.1%) | 4.16 | 3.14 | 2.05 | 3.12 |
| Sodium carbonate (0.1%) | 7.23 | 6.31 | 5.18 | 6.24 |
| Sodium bicarbonate (0.1%) | 5.45 | 2.18 | 2.04 | 3.23 |
| Control | 3.14 | 2.45 | 1.83 | 2.48 |
| Mean | 4.65 | 3.33 | 2.77 | |
| $CD_{0.05}$ | | | Treatment = 0.45 | |
| | | | Population of bacterium = 0.37 | |
| | | | Treatment × Population of bacteria = 0.81 | |

control efficacy of both the biocontrol agents was found comparatively better and it was maximum in bleaching powder + *B. subtilis* 37.29 and 36.06% followed by bleaching powder + *P. fluorescens* 35.31 and 31.75% in Arka Abha and Pusa Ruby cultivars, respectively. Bleaching powder ($CaOCl_2$) also reduced the wilt incidence in both the cultivars with the biological control efficacy of 7.59% in Arka Abha and 29.92% in Pusa Ruby. The reduction of bacterial wilt incidence by using bleaching powder may be due to reduction in *R. solanacearum* population by releasing chlorine, which acted as bactericide and also Ca accumulation in leaf tissue of tomato plant and soil, which reduces the rate of bacterial development (Sharma *et al.*, 12). Although, bleaching powder (0.01%) along with bioagents performed better than the applied separately. Resistant cultivar Arka Abha has lower wilt incidence as compared to susceptible cultivar in all the treatments (Table 3). It might be due to resistant gene found in Arka Abha against *R. solanacearum.* Yamazati *et al.* (17) reported that bacterial population was negatively correlated with Ca concentration and increased Ca content in plant tissues induced resistance to some disease by inhibition of polygalacturonase, increase resistance in cell walls (Padmaja and Jayaram, 8) and inhibition of ethylene production.

Minimum population of *R. solanacearum* was found in bleaching powder (0.01%) + *B. subtilis* DTBS-5 in the soil rhizospheric of Pusa Ruby (4.2 log value / g soil) and Arka Abha (4.3 log value/ soil) followed by after 30 day of inoculation. It was also noticed that population of *R. solanacearum* was significantly declined from initial level in due course of time, which was significantly lower in treated soil either bioagents or in combination with bleaching powder as compared to control (Table 4). Similar reports of bacterial pathogen decline in the soil due to antagonistic, and bacterial wilt reduction have been reported by Ran *et al*. (9), Lamessa and Zeller (7) and

**Table 3.** Effect of bleaching powder and antagonists on bacterial wilt incidence in tomato cultivars after 30 days of inoculation.

| Treatment | Wilt incidence (%) | | Biological control efficacy (%) | |
|---|---|---|---|---|
| | Arka Abha | Pusa Ruby | Arka Abha | Pusa Ruby |
| Bleaching powder (0.01%) | 28.0$^{ab}$ | 34.3$^{ab}$ | 7.59 | 28.92 |
| *P. fluorescens* DTPF-3 | 21.0$^{bc}$ | 32.0$^{ab}$ | 30.69 | 31.75 |
| *B. subtilis* DTBS-5 | 25.0$^{abc}$ | 37.3$^{b}$ | 17.67 | 19.40 |
| Bleaching powder (0.01%) + *P. fluorescens* DTPF-3 | 19.6 $^{c}$ | 31.6$^{bc}$ | 35.31 | 31.75 |
| Bleaching powder (0.01%) + *B. subtilis* DTBS-5 | 18.6$^{c}$ | 29.6$^{c}$ | 37.29 | 36.06 |
| Control | 30.3$^{a}$ | 46.3$^{a}$ | - | - |

Values are means of three replications. Data followed by the same letter(s) in a column are not significantly different from each other according to DMRT at P = 0.05.

**Table 4.** Effect of bleaching powder and biocontrol agents on population of *R. solanacearum* and antagonistic bacteria after 30 days in tomato cultivars.

| Treatment | Initial population of *R. solanacearum* (log value/ g of soil) | Population of *R. solanacearum* (Log value/ g of soil) after 30 days of inoculation | |
|---|---|---|---|
| | | Arka Abha | Pusa Ruby |
| Bleaching powder (0.01%) | 5.6$^{b}$ | 4.4$^{ab}$ | 4.6$^{b}$ |
| *P. fluorescens* DTPF-3 | 6.0$^{ab}$ | 4.5$^{a}$ | 4.6$^{bc}$ |
| *B. subtilis* DTBS-5 | 6.0$^{ab}$ | 4.2$^{ab}$ | 4.4$^{d}$ |
| Bleaching powder (0.01%) + *P. fluorescens* DTPF-3 | 6.1$^{a}$ | 4.3$^{ab}$ | 4.4$^{cd}$ |
| Bleaching powder (0.01%) + *B. subtilis* DTBS-5 | 6.1$^{a}$ | 4.3$^{ab}$ | 4.2$^{c}$ |
| Control | 6.2$^{a}$ | 5.2$^{b}$ | 5.9$^{a}$ |

Values are means of three replications. Data followed by the same letter in a column are not significantly different from each other according to DMRT at p = 0.05.

Singh *et al.* (15). However, no significant variation in declining the population of *R. solanacearum* in soil was noted either treated with the bio-control agent or bleaching powder alone or in combination. But, resistant cultivar (Arka Abha) and susceptible cultivar (Pusa Ruby) showed variation in their rhizospheric population of *R. solanacearum.*

Integration with bleaching powder, biocontrol agent and resistant variety reduced wilt incidence significantly and these combination has potential to control bacterial wilt disease in tomato under the field conditions.

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