



## Assessment of fungal and bacterial bioagents against *Bipolaris sorokiniana* inciting spot blotch on barley (*Hordeum vulgare*)

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### ABSTRACT

In India, spot blotch disease caused by *Bipolaris sorokiniana* is one of the major constraints in barley (*Hordeum vulgare* L.) production. The present study was carried out during 2022–23 at ICAR-Indian Agriculture Research Institute, New Delhi to identify promising biocontrol agents, which can act as eco-friendly alternatives to chemicals against *Bipolaris sorokiniana* inciting spot blotch on barley (*Hordeum vulgare* L.). In this study, 18 isolates of *Trichoderma asperellum*, 15 isolates of *Trichoderma harzianum* and 5 bacterial species were tested against *B. sorokiniana* under *in vitro* and *in planta* conditions. In the dual culture assays, *Trichoderma asperellum* 8686 and *Trichoderma asperellum* 8687 showed significantly highest per cent disease inhibition of 71.73% and 71.37% respectively. Among bacterial strains, *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* showed significantly good per cent disease inhibition of 64.09% and 57.09% respectively. *B. subtilis* and *Pantoea* spp. did not show any pathogen inhibition. In addition, the superior bioagents were also screened out. In the studies on *in planta* assays, *Bacillus amyloliquefaciens* (per cent disease control-55.19%) was found most effective for seed treatment against *B. sorokiniana*. Post-inoculation with biocontrol agents revealed that *Bacillus amyloliquefaciens* was at par with *Trichoderma asperellum* and *Pseudomonas fluorescens* against *B. sorokiniana*. Additionally, a combination of seed treatment, pre and post-inoculation treatment of biocontrol agents revealed that *Bacillus amyloliquefaciens* and *Trichoderma asperellum* 8686 were at par with *Trichoderma harzianum* (Pusa Th3) against *B. sorokiniana*. Overall, *Bacillus amyloliquefaciens* was more effective and consistent to manage spot blotch disease.

**Keywords:** Bacterial antagonist, Barley, Biocontrol, *Bipolaris sorokiniana*, Spot blotch, *Trichoderma* spp.

Barley (*Hordeum vulgare* L.) is an important cereal crop belonging to Gramineae family. It is the 4<sup>th</sup> largest cereal crop grown worldwide after wheat, maize, and rice. The crop exhibits high tolerance to adverse conditions such as drought, salinity and alkaline soil. However, the cultivation of barley is challenged by several diseases like leaf rust, stripe disease, spot blotch and smut diseases. Among these, spot blotch caused by *Bipolaris sorokiniana* is pronounced as the most important fungal disease of barley (Valjavec-Gratian and Steffenson 1997, Arabi and Jawhar 2004). This disease is one of the foremost problems in South Asian regions and in Indian context it causes significant loss in the states of Uttar Pradesh, Bihar, West Bengal, Orissa, and Assam (Chaurasia *et al.* 2000, Kumar *et al.* 2007, Chand *et al.* 2008, Vaish *et al.* 2011). Yield loss of 25–45% has been reported in barley due to spot blotch pathogen, which

can further increase under conducive environments (Iftikhar *et al.* 2009). This highlights the need for control measures to combat the losses caused by the pathogen. Spot blotch can be most effectively managed through integrated pest management (Mehta and Igarashi 1985, Dubin and Duveiller 2000). Triazole group of fungicides like tebuconazole, epoxiconazole and propiconazole are known to effectively control this pathogen (Acharya *et al.* 2011, Yadav *et al.* 2015). However, the use of these chemicals has adverse impact on the environment by effecting non-target organisms and contaminating the soil (Li *et al.* 2015). Therefore, the harmful effect of these chemicals on the environment emphasizes the need of alternative management practices. Biocontrol agents include beneficial microorganisms, which inhibits the plant pathogens and enhances plant immunity (Nakkeeran *et al.* 2018, Yang *et al.* 2019). They acts as environmentally safe and economically feasible option to manage the plant diseases. Until now, very limited number of biocontrol agents have been identified against spot blotch of barley, which necessitates more comprehensive studies. Keeping this in view, the present study was carried out to

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find out the promising biocontrol agents against *Bipolaris sorokiniana* inciting spot blotch of barley both *in vitro* and *in planta* assays.

## MATERIALS AND METHODS

**Biocontrol agents used for dual culture assays:** The study was carried out during 2022–2023 at ICAR-Indian Agriculture Research Institute, New Delhi. All the fungal isolates used in the study were procured from Indian type culture collection (ITCC), ICAR-Indian Agricultural Research Institute, New Delhi. The fungal isolates included 15 *Trichoderma harzianum* isolates, (ITCC No-8617, 8016, 8103, 8361, 8681, 8621, 7349, 7357, 7338, 6914, 7838, 7230, 7077, 6276, 8366) and 18 *Trichoderma asperellum* isolates (ITCC no-8549, 8619, 8369, 8541, 8547, 8518, 7903, 8516, 8607, 8687, 8686, 8614, 7041, 6585, 7828, 8272, 6413, 7885). Five bacterial isolates, viz. *P. fluorescens*, *P. chlororaphis*, *B. amyloliquefaciens*, *B. subtilis* and *Pantoea* spp. were procured from Plant Bacteriology Laboratory, ICAR-Indian Agriculture Research Institute, New Delhi. Highly virulent isolate of *Bipolaris sorokiniana* (BS-2) isolated from infected barley leaves obtained from Pusa, Bihar region was used in our study. To study the inhibition of *B. sorokiniana* with different strains of *Trichoderma* dual culture method was used (Fokkema 1973). Several strains of *Trichoderma* were grown in petri plates containing PDA media and incubated at 25°C for 6 days. *B. sorokiniana* was grown in petri plates containing PDA medium for 7 days. Mycelial discs of 5 mm diameter were taken from the margins of *Trichoderma* colony of 6-day old culture and placed 15 mm away from the periphery on fresh PDA plates. Similarly, mycelial discs of 5 mm diameter were taken from the margins of *B. sorokiniana* (BS) colony of 7-days old culture and placed 15 mm away from the periphery on the same plate. Control plates contained only the mycelial disc (5 mm diameter) of pathogen placed in centre of the plates. These plates were then sealed with parafilm and kept at 25°C for 10 days. Three replications were maintained for each strain of *Trichoderma* evaluated.

For evaluating bacterial strains against *B. sorokiniana* the procedures given by Ganesan and Gnanamanickam (1987) were followed. The bacterial strains were grown in petri plates containing nutrient agar media and kept at 28°C for 2 days. The pathogen *B. sorokiniana* was grown in petri plates containing PDA medium for 7 days. Mycelial discs of 5 mm diameter were taken from the margins of *B. sorokiniana* colony of 7-days old culture and placed at the centre of fresh petri plates containing PDA media. Bacterial strains of 48 h old culture were streaked in straight lines on both sides 30 mm away from the periphery on the same PDA plates. Control plates contained only the mycelial disc (5 mm diameter) of pathogen placed at the centre of the petri plate. These plates were then sealed with parafilm and kept at 25°C for 10 days. Three replications were maintained for each bacterial strain evaluated. The per cent inhibition of the pathogen was calculated after 10 days for both treatment and control plates. The per cent inhibition

was calculated as Vincent (1947):

$$PI = [C-T/C] \times 100$$

where PI, Per cent inhibition on the growth of pathogen; C, Radial growth of pathogen (mm) in control; T, Radial growth of pathogen (mm) in treatment.

**In planta assay using selected biocontrol agents:** *In planta* assay was conducted to find out promising bioagents against *B. sorokiniana* at, ICAR-Indian Agricultural Research Institute, New Delhi (2022–23). Four different assays namely seed treatment, pre-inoculation treatment with biocontrol agents, post-inoculation treatment with biocontrol agents and combination of all treatments were conducted under net house conditions. Highly susceptible barley genotype, viz. EC0578292 was taken for this experiment. Mass culturing of the potential *Trichoderma* strains was done on autoclaved sorghum grains followed by preparation of spore suspension and adjusting the concentration to  $10^8$  conidia/ml. For potential bacterial bioagents talc-based formulations were used @10 g/kg of seed for seed treatment and 15 g/L of water for foliar treatments, which were procured from, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi. Pusa Th3 (*T. harzianum*) was kept as positive control which was used @3 ml/kg of seed for seed treatment and 3 ml/L of water for foliar treatments.

For seed treatment, the barley seeds were soaked with the biocontrol agents and for control, seeds were soaked in sterile distilled water for 1 h. Thereafter, 4-inch pots containing sterilized soil were mixed with mass culture of *B. sorokiniana* on sorghum seeds @10 g/kg of soil. A total of 8–10 treated barley seeds were sown per pot and were maintained under the net house conditions. After 30 days, the per cent disease index (PDI) was measured (Fetch and Steffenson 1999). Apart from, plant height was also taken. For pre inoculation treatment, 30-days old barley plants grown in 4-inch pots containing sterilized soil were sprayed with spore suspension of *Trichoderma* and bacterial biocontrol agents using the hand atomizer. The control plants were sprayed with sterile distilled water. After 24 h, the plants were spray inoculated with *B. sorokiniana* spore suspension @ $10^4$  conidia/ml and kept in moisture chamber with dark conditions for initial 24 h. After 10 days of pathogen inoculation, diseases scoring was observed, and per cent disease index (PDI) was calculated. For post inoculation treatment, 30-days old barley plants grown in 4-inch pots containing sterilized soil were sprayed with spore suspension of *B. sorokiniana* @ $10^4$  conidia/ml and kept in moisture chamber with dark conditions for initial 24 h. After 24 h, the plants were treated with spore suspension of *Trichoderma* and bacterial biocontrol agents using the hand atomizer. The control plants were sprayed with sterile distilled water. After 10 days of pathogen inoculation, disease scoring of plants was recorded and per cent disease index (PDI) was calculated.

The combination of all treatments (seed treatment, pre and post inoculation treatment with biocontrol agents)

Table 1 Bio efficacy of *Trichoderma* species and bacterial strains against *B. sorokiniana* *in vitro* conditions in barley

| Treatment                                            | Radial growth (mm)       | Per cent inhibition (%)  |
|------------------------------------------------------|--------------------------|--------------------------|
| T <sub>1</sub> , <i>Trichoderma asperellum</i> 8549  | 7.88 <sup>bcdef</sup>    | 55.19 <sup>defg</sup>    |
| T <sub>2</sub> , <i>Trichoderma asperellum</i> 8619  | 6.88 <sup>cdefghi</sup>  | 60.82 <sup>abcdef</sup>  |
| T <sub>3</sub> , <i>Trichoderma asperellum</i> 8369  | 8.43 <sup>bcde</sup>     | 52.24 <sup>efg</sup>     |
| T <sub>4</sub> , <i>Trichoderma asperellum</i> 8541  | 8.37 <sup>bcde</sup>     | 52.46 <sup>efg</sup>     |
| T <sub>5</sub> , <i>Trichoderma asperellum</i> 8547  | 6.43 <sup>defghi</sup>   | 63.50 <sup>abcdef</sup>  |
| T <sub>6</sub> , <i>Trichoderma asperellum</i> 8518  | 7.78 <sup>bcdef</sup>    | 56.16 <sup>cdefg</sup>   |
| T <sub>7</sub> , <i>Trichoderma asperellum</i> 7903  | 6.22 <sup>efghi</sup>    | 64.76 <sup>abcde</sup>   |
| T <sub>8</sub> , <i>Trichoderma asperellum</i> 8516  | 6.33 <sup>defghi</sup>   | 64.09 <sup>abcde</sup>   |
| T <sub>9</sub> , <i>Trichoderma asperellum</i> 8607  | 8.17 <sup>bcde</sup>     | 53.72 <sup>efg</sup>     |
| T <sub>10</sub> , <i>Trichoderma asperellum</i> 8687 | 5.05 <sup>hi</sup>       | 71.37 <sup>a</sup>       |
| T <sub>11</sub> , <i>Trichoderma asperellum</i> 8686 | 4.98 <sup>i</sup>        | 71.73 <sup>a</sup>       |
| T <sub>12</sub> , <i>Trichoderma asperellum</i> 8614 | 6.50 <sup>defghi</sup>   | 63.13 <sup>abcdef</sup>  |
| T <sub>13</sub> , <i>Trichoderma asperellum</i> 7041 | 6.55 <sup>cdefghi</sup>  | 62.76 <sup>abcdef</sup>  |
| T <sub>14</sub> , <i>Trichoderma asperellum</i> 6585 | 7.05 <sup>cdefghi</sup>  | 59.93 <sup>abcdefg</sup> |
| T <sub>15</sub> , <i>Trichoderma asperellum</i> 7828 | 8.57 <sup>bcd</sup>      | 51.53 <sup>efg</sup>     |
| T <sub>16</sub> , <i>Trichoderma asperellum</i> 8272 | 9.38 <sup>b</sup>        | 46.44 <sup>g</sup>       |
| T <sub>17</sub> , <i>Trichoderma asperellum</i> 6413 | 6.95 <sup>cdefghi</sup>  | 60.75 <sup>abcdef</sup>  |
| T <sub>18</sub> , <i>Trichoderma asperellum</i> 7885 | 5.25 <sup>hi</sup>       | 70.24 <sup>ab</sup>      |
| T <sub>19</sub> , <i>Trichoderma harzianum</i> 8617  | 6.78 <sup>cdefghi</sup>  | 61.50 <sup>abcdef</sup>  |
| T <sub>20</sub> , <i>Trichoderma harzianum</i> 8016  | 8.12 <sup>bcde</sup>     | 54.29 <sup>defg</sup>    |
| T <sub>21</sub> , <i>Trichoderma harzianum</i> 8103  | 7.83 <sup>bcdef</sup>    | 55.91 <sup>defg</sup>    |
| T <sub>22</sub> , <i>Trichoderma harzianum</i> 8361  | 5.68 <sup>fghi</sup>     | 67.75 <sup>abcd</sup>    |
| T <sub>23</sub> , <i>Trichoderma harzianum</i> 8681  | 7.07 <sup>cdefghi</sup>  | 59.86 <sup>abcdefg</sup> |
| T <sub>24</sub> , <i>Trichoderma harzianum</i> 8621  | 5.33 <sup>ghi</sup>      | 69.71 <sup>abc</sup>     |
| T <sub>25</sub> , <i>Trichoderma harzianum</i> 7349  | 7.28 <sup>bcdefgh</sup>  | 58.76 <sup>abcdefg</sup> |
| T <sub>26</sub> , <i>Trichoderma harzianum</i> 7357  | 7.73 <sup>bcdef</sup>    | 56.21 <sup>cdefg</sup>   |
| T <sub>27</sub> , <i>Trichoderma harzianum</i> 7338  | 7.22 <sup>bcdefghi</sup> | 59.12 <sup>abcdefg</sup> |
| T <sub>28</sub> , <i>Trichoderma harzianum</i> 6914  | 7.67 <sup>bcdef</sup>    | 56.53 <sup>cdefg</sup>   |
| T <sub>29</sub> , <i>Trichoderma harzianum</i> 7838  | 8.38 <sup>bcde</sup>     | 52.48 <sup>efg</sup>     |
| T <sub>30</sub> , <i>Trichoderma harzianum</i> 7230  | 7.55 <sup>bcdefg</sup>   | 57.31 <sup>bcdefg</sup>  |
| T <sub>31</sub> , <i>Trichoderma harzianum</i> 7077  | 6.90 <sup>cdefghi</sup>  | 60.89 <sup>abcdef</sup>  |
| T <sub>32</sub> , <i>Trichoderma harzianum</i> 6276  | 5.33 <sup>ghi</sup>      | 69.73 <sup>abc</sup>     |
| T <sub>33</sub> , <i>Trichoderma harzianum</i> 8366  | 7.72 <sup>bcdef</sup>    | 56.18 <sup>cdefg</sup>   |
| T <sub>34</sub> , <i>Pseudomonas chlororaphis</i>    | 8.83 <sup>bc</sup>       | 49.86 <sup>fg</sup>      |
| T <sub>35</sub> , <i>Bacillus amyloliquefaciens</i>  | 7.55 <sup>bcdefg</sup>   | 57.09 <sup>bcdefg</sup>  |
| T <sub>36</sub> , <i>Pseudomonas fluorescens</i>     | 6.33 <sup>defghi</sup>   | 64.09 <sup>abcde</sup>   |
| T <sub>37</sub> , Control (BS)                       | 17.67 <sup>a</sup>       | 0 <sup>h</sup>           |
| CD (P=0.05)                                          | 1.859                    | 11.042                   |
| SE (m)                                               | 0.658                    | 3.91                     |

consisted of growing of treated barley seeds in autoclaved soil containing mass inoculum of *B. sorokiniana*. At 30-days old barley plants, pre-inoculation of biocontrol agents was performed. First pre-inoculation with biocontrol agents was done then after 24 h, *B. sorokiniana* was inoculated. After 24 h pathogen inoculation, post-inoculation of biocontrol agents was conducted. Spot blotch disease scoring was performed and per cent disease index was calculated. For all the conducted assays, three replications were maintained.

The laboratory and pot experiments were performed in completely randomized design and three replications were maintained for each *in vitro* and *in planta* treatments. All the data were statistically analysed based on standard procedure given by Panse and Sukhatme (1967). The critical difference (5% level of significance) and standard error was obtained for the experiments conducted. The Duncan's multiple-range test (DMRT) was used to find significance level. The statistical analysis was performed on the OPSTAT web server.

## RESULTS AND DISCUSSION

*In vitro studies of biocontrol agents:* Among *Trichoderma* spp., the highest per cent inhibition was showed by *Trichoderma asperellum* 8686 (T<sub>11</sub>) with 71.73% followed by *Trichoderma asperellum* 8687 (T<sub>10</sub>) with 71.37%, which were found statistically similar to inhibit *B. sorokiniana*. The next best result was shown by *Trichoderma asperellum* 7885 (T<sub>18</sub>) with per cent inhibition of 70.24%. Among the five bacterial strains, *Bacillus subtilis* and *Pantoea* spp. did not show bio efficacy against *B. sorokiniana*. *Pseudomonas fluorescens* (T<sub>36</sub>) and *Bacillus amyloliquefaciens* (T<sub>35</sub>) showed 64.09% and 57.09% inhibition of *B. sorokiniana* respectively (Table 1 and Fig. 1). Earlier reports revealed *Trichoderma viride* as the most effective biocontrol agent in inhibiting *Bipolaris sorokiniana* amongst other strains of *Trichoderma* under *in vitro* inhibition studies (Singh *et al.* 2018). Similarly, *Trichoderma* spp. was previously identified as a promising biological solution to manage foliar blight disease incited by *B. sorokiniana* (Singh *et al.* 2019, Kaur *et al.* 2021). *T. asperellum* was also found effective against various phytopathogens using *in vitro* studies (Rai and Singh 2023, Sehim *et al.* 2023). *Pseudomonas* spp. and *Bacillus* spp. were also found effective against *B. sorokiniana* infecting wheat (Minaeva *et al.* 2018, Harba *et al.* 2020, Kang *et al.* 2023). The culture filtrate of *B. amyloliquefaciens* was highly effective *in vitro* against *Bipolaris sorokiniana* infecting wheat and other phytopathogens (Yi *et al.* 2021).

*In planta studies of biocontrol agents:* The fungal (*Trichoderma asperellum* 8686, *Trichoderma asperellum* 8687) and bacterial (*Pseudomonas fluorescens*, *Bacillus amyloliquefaciens*) biocontrol strains that showed good bio-efficacy *in vitro* were subjected to *in planta* studies. In seed treatment, the highest per cent disease control of 55.19% was shown significantly in *Bacillus amyloliquefaciens* (T<sub>3</sub>) followed by *Trichoderma asperellum* 8686 (T<sub>1</sub>) which showed per cent disease control of 41.43%. The highest plant height of 18 cm was observed in *Bacillus*



Fig. 1 Dual culture assay using various strains of *Trichoderma* and biocontrol bacteria against *B. sorokiniana* of barley.

*amyloliquefaciens* treatment ( $T_3$ ) followed by *Trichoderma asperellum* 8686 ( $T_1$ ) and *Pseudomonas fluorescens* ( $T_4$ ) treatments both of which showed plant height of 15 cm (Table 2). In pre-inoculation treatment with biocontrol agents, the *Trichoderma harzianum* Pusa Th3 ( $T_5$ ) gave highest per cent disease control (58.36%) followed by *Trichoderma asperellum* 8686 (50.12%) ( $T_1$ ) (Table 2). In post-inoculation treatment with biocontrol agents, the *Bacillus amyloliquefaciens* ( $T_3$ ) gave highest per cent disease control (45.31%) which was found to be statistically at par with *Trichoderma asperellum* 8686 ( $T_1$ ) and *Pseudomonas fluorescens* ( $T_4$ ) (Table 2). In the combined treatment with biocontrol agents, *Trichoderma harzianum* Pusa Th3 ( $T_5$ ) and *Trichoderma asperellum* 8686 ( $T_1$ ) were found statistically at par in percent disease control. *Bacillus amyloliquefaciens* ( $T_3$ ) showed 45.58% per cent disease control (Table 2).

Overall, *Bacillus amyloliquefaciens* ( $T_3$ ) treatment showed significantly consistent and better results in all four assays to manage spot blotch of barley followed by *Trichoderma asperellum* 8686 ( $T_1$ ) treatment (Fig. 2). *Bacillus amyloliquefaciens* also showed plant growth promoting activity. In earlier studies, *Bacillus amyloliquefaciens* was reported as an effective biocontrol agent against several plant pathogens (Kruichkova 2017, Yadav et al. 2023). PGPR like activity has also been reported in *Bacillus amyloliquefaciens* (Kim et al. 2017, Yi et al. 2021, Luo et al. 2022). The present investigation highlights that *Trichoderma asperellum* 8686 and *Trichoderma asperellum* 8687 showed significantly maximum inhibition of *B. sorokiniana* in vitro. *Bacillus amyloliquefaciens* showed nearly consistent results for the management of spot blotch of barley. In addition, plant growth promoting activities of *B. amyloliquefaciens* will be explored.

Table 2 Effect of seed treatment, pre-inoculation, post-inoculation with biocontrol agents against spot blotch of barley

| Treatment                                                | Average per cent disease index (%) | Average per cent disease control (%) | Average plant height (cm) |
|----------------------------------------------------------|------------------------------------|--------------------------------------|---------------------------|
| T <sub>1</sub> , <i>Trichoderma asperellum</i> 8686      | 58.57 <sup>d</sup>                 | 41.43 <sup>b</sup>                   | 15.00 <sup>b</sup>        |
| T <sub>2</sub> , <i>Trichoderma asperellum</i> 8687      | 65.74 <sup>c</sup>                 | 34.26 <sup>c</sup>                   | 14.33 <sup>b</sup>        |
| T <sub>3</sub> , <i>Bacillus amyloliquefaciens</i>       | 44.81 <sup>e</sup>                 | 55.19 <sup>a</sup>                   | 18.00 <sup>a</sup>        |
| T <sub>4</sub> , <i>Pseudomonas fluorescens</i>          | 66.66 <sup>c</sup>                 | 33.34 <sup>c</sup>                   | 15.00 <sup>b</sup>        |
| T <sub>5</sub> , <i>Trichoderma harzianum</i> (Pusa Th3) | 87.37 <sup>b</sup>                 | 12.63 <sup>d</sup>                   | 13.33 <sup>b</sup>        |
| T <sub>6</sub> , Control                                 | 100 <sup>a</sup>                   | 0 <sup>c</sup>                       | 13.33 <sup>b</sup>        |
| CD ( <i>P</i> =0.05)                                     | 5.751                              | 5.751                                | 2.203                     |
| SE(m)                                                    | 1.846                              | 1.846                                | 0.707                     |

Effect of pre-inoculation spray with biocontrol agents against spot blotch of barley

| Treatment                                                | Average per cent disease index (%) | Average per cent disease control (%) |
|----------------------------------------------------------|------------------------------------|--------------------------------------|
| T <sub>1</sub> , <i>Trichoderma asperellum</i> 8686      | 49.88 <sup>c</sup>                 | 50.12 <sup>b</sup>                   |
| T <sub>2</sub> , <i>Trichoderma asperellum</i> 8687      | 52.93 <sup>bc</sup>                | 47.07 <sup>bc</sup>                  |
| T <sub>3</sub> , <i>Bacillus amyloliquefaciens</i>       | 52.83 <sup>bc</sup>                | 47.17 <sup>bc</sup>                  |
| T <sub>4</sub> , <i>Pseudomonas fluorescens</i>          | 56.33 <sup>b</sup>                 | 43.67 <sup>c</sup>                   |
| T <sub>5</sub> , <i>Trichoderma harzianum</i> (Pusa Th3) | 41.64 <sup>d</sup>                 | 58.36 <sup>a</sup>                   |
| T <sub>6</sub> , Control                                 | 100 <sup>a</sup>                   | 0 <sup>d</sup>                       |
| CD ( <i>P</i> =0.05)                                     | 5.576                              | 5.576                                |
| SE(m)                                                    | 1.79                               | 1.79                                 |

Effect of post-inoculation spray with biocontrol agents against spot blotch of barley

| Treatment                                                | Average per cent disease index (%) | Average per cent disease control (%) |
|----------------------------------------------------------|------------------------------------|--------------------------------------|
| T <sub>1</sub> , <i>Trichoderma asperellum</i> 8686      | 58.86 <sup>bc</sup>                | 41.14 <sup>ab</sup>                  |
| T <sub>2</sub> , <i>Trichoderma asperellum</i> 8687      | 63.71 <sup>b</sup>                 | 36.29 <sup>b</sup>                   |
| T <sub>3</sub> , <i>Bacillus amyloliquefaciens</i>       | 54.69 <sup>c</sup>                 | 45.31 <sup>a</sup>                   |
| T <sub>4</sub> , <i>Pseudomonas fluorescens</i>          | 58.63 <sup>bc</sup>                | 41.37 <sup>ab</sup>                  |
| T <sub>5</sub> , <i>Trichoderma harzianum</i> (Pusa Th3) | 66.56 <sup>b</sup>                 | 33.44 <sup>b</sup>                   |
| T <sub>6</sub> , Control                                 | 100 <sup>a</sup>                   | 0 <sup>c</sup>                       |
| CD ( <i>P</i> =0.05)                                     | 7.644                              | 7.644                                |
| SE(m)                                                    | 2.454                              | 2.454                                |

Effect of seed treatment, pre inoculation, and post inoculation spray of biocontrol agents against spot blotch of barley

| Treatment                                                | Average per cent disease index (%) | Average per cent disease control (%) |
|----------------------------------------------------------|------------------------------------|--------------------------------------|
| T <sub>1</sub> , <i>Trichoderma asperellum</i> 8686      | 50.06 <sup>c</sup>                 | 49.94 <sup>a</sup>                   |
| T <sub>2</sub> , <i>Trichoderma asperellum</i> 8687      | 64.54 <sup>b</sup>                 | 35.46 <sup>b</sup>                   |
| T <sub>3</sub> , <i>Bacillus amyloliquefaciens</i>       | 54.42 <sup>c</sup>                 | 45.58 <sup>a</sup>                   |
| T <sub>4</sub> , <i>Pseudomonas fluorescens</i>          | 65.62 <sup>b</sup>                 | 34.38 <sup>b</sup>                   |
| T <sub>5</sub> , <i>Trichoderma harzianum</i> (Pusa Th3) | 47.84 <sup>c</sup>                 | 52.16 <sup>a</sup>                   |
| T <sub>6</sub> , Control                                 | 100 <sup>a</sup>                   | 0 <sup>c</sup>                       |
| CD ( <i>P</i> =0.05)                                     | 7.31                               | 7.31                                 |
| SE(m)                                                    | 2.346                              | 2.346                                |

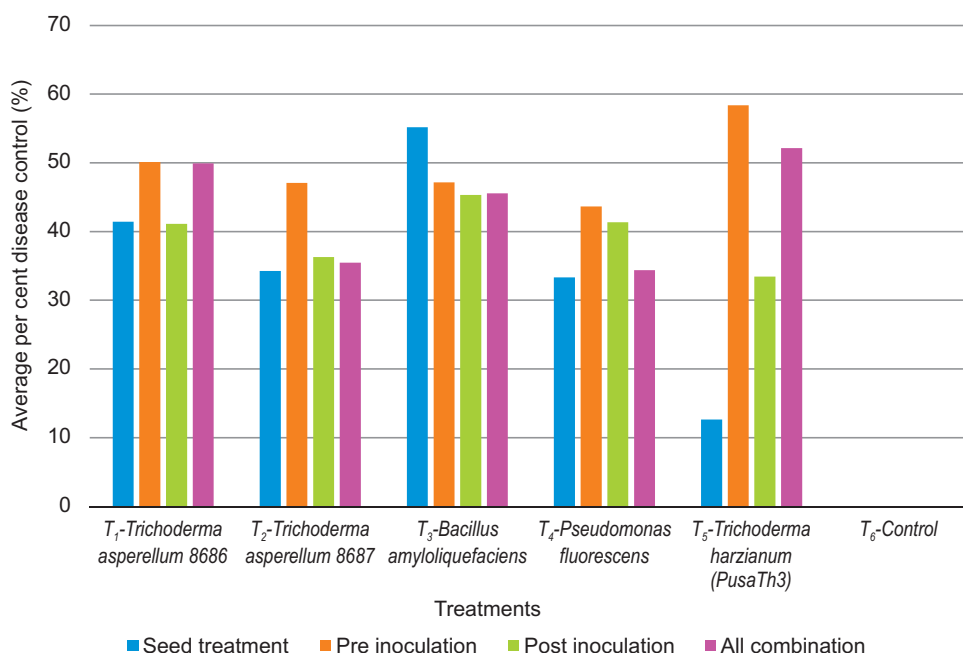


Fig. 2 Effect of seed treatment, pre inoculation spray, post inoculation spray and all combination of treatments of biocontrol agents against spot blotch of barley.

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