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# Enzymatic Effect of Different Host Defense Induction against *Alternaria tenuissima* (Kunze ex pers.) Wiltshire causing Dieback Disease of Chilli

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**ABSTRACT**

*Dieback disease, caused by Alternaria tenuissima, (Kunze ex pers.) Wiltshire, is one of the most important disease that affecting all the plant parts of chilli. Among different methods of bioagent application, combination of both soil and root dip application was significantly superior over soil and root dip application. The accumulation of enzymes i.e. polyphenol oxidase (PPO), peroxidase (PO) and total phenol significantly increased upto 72 hours. Among bioagents highest significantly increase in PPO, PO and total phenol activity were recorded in Trichoderma harzianum PBAT-21 + Pseudomonas fluorescens PBAP-27 i.e. 0.034, 0.381 and 8.58 µmol/min/mg protein respectively. The no. of spot/leaf (2.33%), no. of infected leaves/plant (1.44%) and disease severity (13.33%) of dieback disease of chilli were minimum in combination of soil and root dip inoculation of bioagents Trichoderma harzianum PBAT-21 and Pseudomonas fluorescens PBAP-27 as compare to control.*

**Keywords:** *Alternaria tenuissima, Dieback, Bioagents, polyphenol oxidase (PPO), peroxidase (PO) and total phenol*

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## INTRODUCTION

Chilli (*Capsicum annum* L.) is cultivated over an area of 775 thousand hectares with an annual production of 1492 thousand tonnes and productivity of 1.9 metric tonnes per hectare in India (NHB, 2014). Chilli is affected by 750 pathogens of different origins, reported from different part of the world, but only few are responsible for considerable loss of production and productivity. Among the fungal diseases, dieback, leaf spot and fruit rot is caused by *Alternaria spp.*, damping off caused by *Pythium* spp., *Phytophthora* spp. and other fungi, seedling blight caused by *Rhizoctonia* spp., wilt caused by *Fusarium* spp., anthracnose and dieback caused by *Colletotrichum capsici* are major diseases. Among all these *Alternaria* spp. are responsible for dieback, leaf spot and fruit rot have been identified as major limiting factor in chili cultivation is very common problem in fields and greenhouse. Use of host defence biotic agents has been considered an alternate and effective approach for the control of pathogenic microbes. Plants can be sensitized for a more rapid and intense mobilization of defence responses leading to enhanced resistance to biotic stresses [1] (Beckers and Conrath, 2007). Induction of systemic resistance is associated with gene induction, activation of a wide range of resistance mechanisms and the production of variety of defence compounds. It is a race non-specific and is often effective against a broad spectrum pathogenic agents [2] (Walters and Fountaine, 2009). Thus, study on induction of host defence through biotic and abiotic factors can be considered as one of the effective sustainable approaches in disease management.

## METHODOLOGY

Soil was collected from the upper 0-15 cm layer from Vegetable Research Centre (VRC), GBPUA&T Pantnagar and was sterilized by autoclaving at 20 lb psi (121.6°C) for one hour on three consecutive days. The sterilized soil was filled in 1.5 kg capacity plastic pots and kept in glasshouse. Thirty days old seedlings of chilli cultivar Pant C1 grown in portrays under polyhouse was used in this experiment. Pots were watered regularly as and when required to maintain optimum moisture. Five bioagents viz.

*Trichoderma harzianum* PBAT-21 @ 10g/kg soil, *Pseudomonas fluorescens* PBAP-27 @ 10g/kg soil, *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27 @ 10g/kg soil, *Trichoderma viride* $G_2$ mutant @ 5g/kg soil and *Bacillus subtilis var. amyloliquefaciens* FZB24 @ 4g/kg soil were applied in pots by thorough mixing with soil before planting the seedling.

For root dip treatment seedling roots were dipped for thirty minutes in each bioagents at concentration separately. A combination of above two treatments (both) was also taken. Experiment was laid out in a completely randomized block design with three replications. After 30 days of transplanting plants were inoculated by spraying with spore suspension of $1x10^6$ spores/ml by sterilized atomizer of the *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire.

**Effect of bioagents on enzymatic activity after post inoculation of *Alternaria tenuissima* on chilli:**

Top five leaves per treatments were harvested 24, 48, 72, 96 and 120 hours after inoculation and brought to the laboratory in an ice box for analysis of the enzymes as mentioned below.

**Polyphenol oxidase (PPO) activity**

PPO activity was determined as per the procedure given by [3] Mayer *et al*. (1965). Leaf samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µl of 0.01M catechol was added and the activity was expressed as µmol/min/mg protein.

**Peroxidase (PO) activity**

Assay of Peroxidase (PO) activity was carried by [4] Hammerschmidt *et al*. (1982) method. Enzymes extract was prepared by homogenizing one gram of leaf samples in 0.1M sodium phosphate buffer (pH 6.0). It was centrifuged at 10,000 rpm for 20 minutes. The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01 M sodium phosphate buffer (pH 6.0) and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction, which was followed colorimetrically at 480 nm. The boiled enzyme preparation served as blank. Activity was expressed as µmol/min/mg protein.

**Total phenolics**

Total phenolics content was determined as per methodology given by Swain and Hills (1959). One gram leaves were homogenized in 10 ml of 80 per cent methanol and agitated for 15 minute at 70°C. One milliliter of methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-ciocalteu reagent, after this the solution was kept at 25°C. After 3 minutes, 1 ml of a saturated solution of $Na_2CO_3$ and 1 ml of distilled water were added, and the reaction mixture was incubated for 1 hr at 25°C. The absorbance of the developed color was measured using spectrophotometer at 725 nm. The total soluble phenolic content was calculated by comparison with a standard curve obtained from Folin-Ciocalteu reaction with catechol.

**Effect of bioagents on dieback disease of chilli**

The effect of bioagents on no. of spots/leaf and disease severity of dieback disease of chilli was observed. Observations on number of spots/leaf, number of infected leaves/plant were recorded 15 days after inoculation. Disease severity was also recorded 15, 30, 45 and 60 days after inoculation on 0-5 scale suggested by [5] Vishwakarma and Sitaramaiah (1986) and percent disease index (PDI) was calculated as described by [6] McKiney (1923).

$$\text{PDI} = \frac{\text{Sum of all disease ratings}}{\text{Total number of plants observed x maximum rating value}} \times 100$$

**RESULT**

**Effect of bioagents on Polyphenol oxidase (PPO) activity in chilli**

A significant increased in polyphenol activity was recorded in chilli plants treated with biotic elicitor as compared to control (Table 1). An increased in PPO activity was recorded at 24 hour interval and reached its higher value at 72 hours after inoculation of *Alternaria tenuissima* while after 72 hrs PPO activity decreased significantly in all the treatments and methods of application. Among method of bioagent application, both soil application + root dip was found significantly superior over root dip followed by soil application at every 24 hrs interval. The interaction between treatments, methods of application and time interval was found none significant. This trend was observed at all time durations at its peak *i.e.* 72 hours after treatment, in case of soil and root dip application of *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27 PPO activity was 80.46% while in case of *Trichoderma harzianum* PBAT-21 (78.26%), *Trichoderma viride* G2 mutant BARC strain (73.12%), *Pseudomonas fluorescens* PBAP-27 (66.66%), and *Bacillus subtilis* var. *amyloliquefaciens* FZB24 (48.98%) respectively higher than initial value (0 hour). Among bioagents highest mean increase in PPO activity was recorded in *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27 followed by *Trichoderma harzianum* PBAT-21, *Trichoderma viride* G2 mutant BARC strain, *Pseudomonas fluorescens* PBAP-27, and *Bacillus subtilis* var. *amyloliquefaciens* FZB24 *i.e.* 0.0340, 0.0305, 0.025 and 0.0249 and 0.013 µmol/min/mg protein respectively in combination of both soil and root dip method of application.

**Effect of bioagents on Peroxidase (PO) activity in chilli**

The peroxidase activity was increased significantly as compared to control in chilli plants treated with biotic elicitor *Alternaria tenuissima*. An increased in PO activity was recorded at 24 hour and reached its higher value at 72 hours after inoculation of *Alternaria tenuissima* in all the treatments. However after 72 hrs, there has been decrease in polyphenol activity in all the different methods of application. Data pertaining to effect of different bioagents on peroxidase activity in chilli presented in Table 2 revealed that all the bioagents increased the peroxidase accumulation as compared to control. Among bioagents highest increase in PO activity was recorded in *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27 followed by *Trichoderma harzianum* PBAT-21, *Trichoderma viride* G2 mutant BARC strain, *Pseudomonas fluorescens* PBAP-27, and *Bacillus subtilis* var. *amyloliquefaciens* FZB24. Among method of bioagent application, both soil application + root dip proved to be the best as compared to root dip and soil application alone. In case of soil and root dip application of *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27, PO activity was 88.32% followed by *Trichoderma harzianum* PBAT-21 (86.58%) and *Trichoderma viride* G2 mutant BARC strain (83.91%) respectively higher than initial value after 72 hours after treatment.

**Effect of bioagents on total phenol accumulation in chilli**

The effect of bioagents on total phenol accumulation in chilli after 120 hours of inoculation of *Alternaria tenuissima* was recorded at every 24 hours interval. Among method of bioagent application root dip was found superior over soil application, but combination of both, soil application + root dip proved to be the best. The data revealed in Table 3 showed that the total phenol accumulation increases upto 72 hours but later on total phenol activity decreases in all the three methods of application. Among bioagents highest increase in total phenol content was recorded in *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27 followed by *Trichoderma harzianum* PBAT-21, *Trichoderma viride* G2 mutant BARC strain, *Pseudomonas fluorescens* PBAP-27, and *Bacillus subtilis* var. *amyloliquefaciens* FZB24. In case of soil and root dip application of *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27, total phenol content was 94.07% higher than initial value (0 hour). In case of *Trichoderma harzianum* PBAT-21, *Trichoderma viride* G2 mutant BARC strain, *Pseudomonas fluorescens* PBAP-27 and *Bacillus subtilis* var. *amyloliquefaciens* FZB24 it was 93.18, 91.84, 89.77 and 84.91 per cent, respectively.

**Effect of bioagents on leaf infection of dieback disease of chilli**

The bioagents reduced the no. of spot/leaf and no. of infected leaves/plant significantly as compared to control ( Table 4). Among the different methods of application the combination of both soil and root dip showed significantly minimum no. of spot/leaf and no. of infected leaves/plant as compared to other methods. The minimum no. of leaf spots/ leaf and no. of infected leaves/plant was recorded 2.33 and 1.44 respectively in both soil and root dip methods of application of *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27followed by *Trichoderma harzianum* PBAT-21 *i.e.* 3.11 and 2.00 respectively.

**Effect of bioagents on severity of dieback disease of chilli**

The results of the experiment presented in Table 5 revealed that 15, 30, 45 and 60 days after inoculation (DAI), all the bioagents reduced the disease severity of *Alternaria tenuissima* as compared to control. Among treatments having various bioagents, combination of *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27 was found best followed by fungal bioagents while bacterial bioagents were found least effective. In case of method of bioagent application root dip was found better than soil application, but when both the methods were used in combination proved to be the best. The percent disease severity was found minimum in *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluorescens* PBAP-27 *i.e.* 0.00, 1.33, 9.33 and 13.33 followed by *Trichoderma harzianum* PBAT-21 *i.e.* 4.00,8.00, 20.00 and 24.00 in both soil and root dip method at 15, 30, 45 and 60 DAI respectively.

**DISCUSSION**

The fungus causing leaf spot, fruit rot and early dieback of chilli under *tarai* conditions of Uttarakhand was established as *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire which is probably the first report from India so very few works were done with the same crop.

The present study clearly showed increased activity of PO, PPO and Phenol in treated plants as compared to control. These finding are in accordance of Mayer [3], who observed induction of Poly phenol oxidase (PPO) in plants, particularly under condition of stress and pathogen attack. Shoresh *et al*. [7] reported the induction of PO, PPO and phenol in response to *T. harzianum* against *A. alternata.* Karthikeyan *et al.* (2006) observed that soil application of *T. viride* and *T. harzianum* triggered systemic resistance in coconut by inducing the increased activity of peroxidase (PO), polyphenol oxidase (PPO) whose

concentration reached to the maximum level within three days. Paul and Sharma [8] tested strains of *Pseudomonas fluorescens* for their efficacy in inducing defense enzymes in black pepper and observed increased levels of peroxidise, polyphenol oxidase and total phenol in leaves and roots of treated plants, indicating systemic protection in black pepper caused *by Phytopththora capsici*.
Efath *et al*. [9] have reported efficacy of *Trichoderma viride* ($Tv^{-1}$) and *Trichoderma harzianum (*$Th^{-1}$), each at $1x10^9$ spores/ml for effective management of foliar blight of onion caused by *Alternaria tenuissima.* Lal and Upadhyay [10] have reported suppression of leaf blight in pigeon pea caused by *A. tenuissima* when it was treated with *T. viride*. Petrescu and Sesan [11] also recorded the efficacy of strains of *Trichoderma* sp. for effective management of *Alternaria tenuissima* and *Botrytis cinerea* Pers. on *Ribes nigrum* L. Reduction in disease severity by the use of *Trichoderma harzianum* have also been reported by Perazzolli *et al*. [12] in downy mildew of grape, Harman *et al*. [13] in anthracnose of maize and Sharma *et al.* [14] in tomato and cauliflower diseases. Efficacy of several fluorescent *Pseudomonas* in controlling leaf blight (*Alternaria alternata*) of groundnut Chitra *et al*., [15] and *Colletotrichum capsici* on chilli. Dadwal and Singh [16] reported that *Bacillus firmus* and *B. amyloliquefaciens* were effective in reducing the disease severity of *Rauvolfia serpentina* caused by *A. tenuissima.*

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**Table 1: Effect of Bioagents on Polyphenol oxidase (PPO) activity in chilli**

| Treatments | POLYPHENOL OXIDASE (µmol/min/mg protein) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 Hr | | | 24 Hrs | | | 48 Hrs | | | 72Hrs | | | 96 Hrs | | | 120 Hrs | | | Mean | | |
| | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both |
| *Trichoderma harzianum* PBAT-21 | 0.0177 | 0.0203 | 0.0237 | 0.0200 | 0.0233 | 0.0273 | 0.0233 | 0.0273 | 0.0317 | 0.0283 | 0.0327 | 0.0383 | 0.0240 | 0.0283 | 0.0327 | 0.0223 | 0.0257 | 0.0293 | 0.0226 | 0.0263 | 0.0305 |
| *Trichoderma viride* $G_2$ mutant *(*BARC strain ) | 0.0133 | 0.0167 | 0.0197 | 0.0153 | 0.0190 | 0.0223 | 0.0180 | 0.0217 | 0.0253 | 0.0217 | 0.0263 | 0.0310 | 0.0190 | 0.0227 | 0.0267 | 0.0143 | 0.0207 | 0.0243 | 0.0170 | 0.0212 | 0.0249 |
| *Bacillus subtilis* var. *amyloliquefaciens* FZB24 | 0.0087 | 0.0097 | 0.0107 | 0.0113 | 0.0110 | 0.0120 | 0.0130 | 0.0127 | 0.0137 | 0.0150 | 0.0160 | 0.0163 | 0.0123 | 0.0127 | 0.0140 | 0.0120 | 0.0120 | 0.0133 | 0.0121 | 0.0123 | 0.0133 |
| *Pseudomonas fluoresens* PBAP-27 | 0.0113 | 0.0130 | 0.0153 | 0.0133 | 0.0150 | 0.0177 | 0.0150 | 0.0177 | 0.0207 | 0.0180 | 0.0220 | 0.0250 | 0.0157 | 0.0183 | 0.0215 | 0.0143 | 0.0170 | 0.0192 | 0.0146 | 0.0172 | 0.0199 |
| *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluoresens* PBAP-27 | 0.0197 | 0.0227 | 0.0267 | 0.0223 | 0.0257 | 0.0303 | 0.0257 | 0.0300 | 0.0350 | 0.0320 | 0.0363 | 0.0427 | 0.0270 | 0.0310 | 0.0363 | 0.0243 | 0.0283 | 0.0330 | 0.0252 | 0.0290 | 0.0340 |
| Control | 0.0083 | 0.0083 | 0.0083 | 0.0097 | 0.0097 | 0.0097 | 0.0117 | 0.0117 | 0.0117 | 0.0137 | 0.0137 | 0.0137 | 0.0113 | 0.0113 | 0.0113 | 0.0110 | 0.0110 | 0.0110 | 0.0109 | 0.0109 | 0.0109 |
| Mean | 0.0132 | 0.0151 | 0.0174 | 0.0153 | 0.0173 | 0.0199 | 0.0178 | 0.0202 | 0.0230 | 0.0215 | 0.0245 | 0.0278 | 0.0182 | 0.0207 | 0.0238 | 0.0164 | 0.0191 | 0.0217 | 0.0171 | 0.0195 | 0.0223 |
| CD at 1 % | a=0 .0003 b=0.0002 c=0.0003 a x b= 0.0006 b x c=0.0008 c x a =0.0006 a x b x c=0.0013 | | | | | | | | | | | | | | | | | | | | |
| CV | 4.8591 | | | | | | | | | | | | | | | | | | | | |
| | a = Treatments b = Application c = Hours SA= Soil Application RD = Root dip Both = SA + RA | | | | | | | | | | | | | | | | | | | | |

**Table 2: Effect of Bioagents on Peroxidase (PO) activity in chilli**

| Treatments | PEROXIDASE (µmol/min/mg protein) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 Hr | | | 24 Hrs | | | 48 Hrs | | | 72Hrs | | | 96 Hrs | | | 120 Hrs | | | Mean | | |
| | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both |
| *Trichoderma harzianum* PBAT-21 | 0.1967 | 0.2387 | 0.2597 | 0.2240 | 0.2720 | 0.2960 | 0.2603 | 0.3157 | 0.3433 | 0.3120 | 0.3787 | 0.4123 | 0.2680 | 0.3257 | 0.3547 | 0.2443 | 0.2963 | 0.3223 | 0.2509 | 0.3045 | 0.3314 |
| *Trichoderma viride* $G_2$ mutant *(*BARC strain ) | 0.1587 | 0.1873 | 0.2167 | 0.1807 | 0.2140 | 0.2470 | 0.2097 | 0.2480 | 0.2867 | 0.2520 | 0.2977 | 0.3440 | 0.2163 | 0.2560 | 0.2957 | 0.1967 | 0.2330 | 0.2690 | 0.2023 | 0.2393 | 0.2765 |
| *Bacillus subtilis* var. *amyloliquefaciens* FZB24 | 0.0657 | 0.1057 | 0.1163 | 0.0748 | 0.1203 | 0.1330 | 0.0867 | 0.1397 | 0.1543 | 0.1043 | 0.1683 | 0.1850 | 0.0897 | 0.1443 | 0.1593 | 0.0813 | 0.1313 | 0.1447 | 0.0837 | 0.1349 | 0.1488 |
| *Pseudomonas fluoresens* PBAP-27 | 0.1313 | 0.1493 | 0.1713 | 0.1500 | 0.1703 | 0.1957 | 0.1740 | 0.1980 | 0.2270 | 0.2090 | 0.2373 | 0.2723 | 0.1797 | 0.2043 | 0.2340 | 0.1633 | 0.1857 | 0.2133 | 0.1679 | 0.1908 | 0.2189 |
| *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluoresens* PBAP-27 | 0.2163 | 0.2537 | 0.2983 | 0.2470 | 0.2890 | 0.3400 | 0.2867 | 0.3350 | 0.3947 | 0.3440 | 0.4023 | 0.4740 | 0.2960 | 0.3460 | 0.4077 | 0.2690 | 0.3150 | 0.3707 | 0.2765 | 0.3235 | 0.3809 |
| Control | 0.0553 | 0.0553 | 0.0553 | 0.0630 | 0.0630 | 0.0630 | 0.0733 | 0.0733 | 0.0733 | 0.0883 | 0.0883 | 0.0883 | 0.0760 | 0.0760 | 0.0760 | 0.0683 | 0.0683 | 0.0683 | 0.0707 | 0.0707 | 0.0707 |
| Mean | 0.1373 | 0.1650 | 0.1863 | 0.1566 | 0.1881 | 0.2124 | 0.1818 | 0.2183 | 0.2466 | 0.2183 | 0.2621 | 0.2960 | 0.1876 | 0.2254 | 0.2546 | 0.1705 | 0.2049 | 0.2314 | 0.1753 | 0.2106 | 0.2379 |
| CD at 1 % | a=0 .0024 b=0.0015 c=0.0022 a x b= 0.0038 b x c=0.0054 c x a =0.0038 a x b x c=0.0095 | | | | | | | | | | | | | | | | | | | | |
| CV | 2.8413 | | | | | | | | | | | | | | | | | | | | |
| | a = Treatments b = Application c = Hours SA= Soil Application RD = Root dip Both = SA + RA | | | | | | | | | | | | | | | | | | | | |

**Table 3: Effect of Bioagents on total phenol accumulation in chilli**

| Treatments | Total Phenols (mg/gm of fresh leaf) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 Hr | | | 24 Hrs | | | 48 Hrs | | | 72Hrs | | | 96 Hrs | | | 120 Hrs | | | Mean | | |
| | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both |
| *Trichoderma harzianum* PBAT-21 | 4.4377 | 5.3721 | 5.8467 | 5.0620 | 6.1237 | 6.6607 | 5.8723 | 7.1037 | 7.7263 | 7.0467 | 8.5247 | 9.2717 | 6.0600 | 7.3307 | 7.9737 | 5.5147 | 6.6710 | 7.2563 | 5.6656 | 6.8543 | 7.4559 |
| *Trichoderma viride* G2 mutant *(*BARC strain ) | 3.5973 | 4.2423 | 4.8877 | 4.1010 | 4.8360 | 5.5723 | 4.7573 | 5.6097 | 6.4640 | 5.7090 | 6.7320 | 7.7570 | 4.9097 | 5.7893 | 6.6707 | 4.4677 | 5.2680 | 6.0703 | 4.5903 | 5.4129 | 6.2370 |
| *Bacillus subtilis* var. *amyloliquefaciens* FZB24 | 1.5127 | 2.3930 | 2.6420 | 1.7250 | 2.7283 | 3.0117 | 2.0013 | 3.1653 | 3.4940 | 2.4013 | 3.7987 | 4.1927 | 2.0650 | 3.2667 | 3.6057 | 1.8780 | 2.9723 | 3.2810 | 1.9306 | 3.0541 | 3.3712 |
| *Pseudomonas fluoresens* PBAP-27 | 2.9887 | 3.3860 | 3.8950 | 3.4077 | 3.8603 | 4.4403 | 3.9527 | 4.4783 | 5.1507 | 4.7437 | 5.3740 | 6.1810 | 4.0793 | 4.6217 | 5.3153 | 3.7127 | 4.2057 | 4.8373 | 3.8141 | 4.3210 | 4.9699 |
| *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluoresens* PBAP-27 | 4.8877 | 5.7193 | 6.7243 | 5.5720 | 6.5200 | 7.6657 | 6.4640 | 7.5633 | 8.8923 | 7.7570 | 9.0760 | 10.6707 | 6.6707 | 7.8053 | 9.1767 | 6.0703 | 7.1030 | 8.3507 | 6.2369 | 7.2978 | 8.5801 |
| Control | 0.6323 | 0.6323 | 0.6323 | 0.7210 | 0.7210 | 0.7210 | 0.8367 | 0.8367 | 0.8367 | 1.0041 | 1.0041 | 1.0041 | 0.8630 | 0.8630 | 0.8630 | 0.7857 | 0.7857 | 0.7857 | 0.8071 | 0.8071 | 0.8071 |
| Mean | 3.0094 | 3.6242 | 4.1047 | 3.4314 | 4.1316 | 4.6786 | 3.9807 | 4.7928 | 5.4273 | 4.7770 | 5.7516 | 6.5129 | 4.1079 | 4.9461 | 5.6008 | 3.7382 | 4.5009 | 5.0969 | 3.8408 | 4.6245 | 5.2369 |

CD at 1 % a=0 .0504 b=0.0356 c=0.0504 a x b= 0.0874 b x c=0.1237 c x a =0.0872 a x b x c=0.2146

CV 2.9137

a = Treatments b = Application c = Hours SA= Soil Application RD= Root dip Both = SA + RA

**Table 4: Effect of bioagents on leaf infection of chilli by *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire.**

| Treatments | No. of spots/leaf | | | No. of infected leaves/plant | | |
|---|---|---|---|---|---|---|
| | SA | RD | Both | SA | RD | Both |
| *Trichoderma harzianum* PBAT-21 | 5.44 | 3.78 | 3.11 | 3.44 | 2.44 | 2.00 |
| *Trichoderma viride* G2 mutant (BARC strain) | 6.11 | 4.67 | 3.78 | 4.11 | 3.00 | 2.44 |
| *Bacillus subtilis* var. *amyloliquefaciens* FZB24 | 8.33 | 6.89 | 6.11 | 5.67 | 4.56 | 4.00 |
| *Pseudomonas fluoresens* PBAP-27 | 7.44 | 5.56 | 4.56 | 4.89 | 3.56 | 3.00 |
| *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluoresens* PBAP-27 | 3.78 | 3.00 | 2.33 | 2.56 | 2.00 | 1.44 |
| Control | 10.50 | 10.5 | 10.50 | 6.67 | 6.67 | 6.67 |
| CD at 1 % | a=0.61 b= 0.43 axb=1.06 | | | a=0.45 b= 0.32 axb=0.78 | | |
| CV | 14.61 | | | 16.71 | | |

a = Treatments b =method of application SA= Soil Application RD = Root dip Both = SA + RA

**Table 5: Effect of bioagents on severity of *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire.**

| Treatments | PDI at 15 DAI* | | | PDI at 30 DAI | | | PDI at 45 DAI | | | PDI at 60 DAI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SA | RD | Both | SA | RD | Both | SA | RD | Both | SA | RD | Both |
| *Trichoderma harzianum* PBAT-21 | 5.33 | 4.00 | 4.00 | 12.00 | 9.33 | 8.00 | 22.67 | 21.33 | 20.00 | 32.00 | 28.00 | 24.00 |
| *Trichoderma viride* G2 mutant (BARC strain) | 9.33 | 4.00 | 4.00 | 17.33 | 16.00 | 12.00 | 26.67 | 25.33 | 25.33 | 40.00 | 36.00 | 32.00 |
| *Bacillus subtilis* var. *amyloliquefaciens* FZB24 | 20.00 | 17.33 | 12.00 | 28.00 | 26.00 | 24.00 | 36.00 | 34.00 | 32.00 | 57.33 | 52.00 | 48.00 |
| *Pseudomonas fluoresens* PBAP-27 | 13.33 | 8.00 | 8.00 | 25.33 | 20.00 | 16.00 | 32.00 | 29.33 | 26.67 | 49.33 | 44.00 | 40.00 |
| *Trichoderma harzianum* PBAT-21 + *Pseudomonas fluoresens* PBAP-27 | 1.33 | 0.00 | 0.00 | 6.67 | 4.00 | 1.33 | 16.00 | 12.00 | 9.33 | 20.00 | 16.00 | 13.33 |
| Control | 21.33 | 21.33 | 21.33 | 28.00 | 28.00 | 28.00 | 36.00 | 36.00 | 36.00 | 61.33 | 61.33 | 61.33 |
| CD at 1 % | a=1.47 b=1.04 axb=2.54 | | | a=1.16 b=0.82 axb=2.01 | | | a=1.47 b=1.04 axb=2.54 | | | a=1.27 b=0.90 axb=2.20 | | |
| CV | 15.86 | | | 6.93 | | | 5.74 | | | 3.35 | | |

DAI* = Days after inoculation, a= treatments b= method of application, SA= Soil Application, RD = Root Dip, Both = SA + RA

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