***Original Research Article***

***In Vitro* Efficacy of Different Bioagents Against *Alternaria porri* Causing Purple Blotch in Onion (*Allium cepa* L.)**

**Abstract**

India, a primarily agrarian country, is experiencing significant changes in land use and cropping patterns due to rapid urbanization, alongside a growing population that demands increased agricultural productivity. Vegetables, especially onion (*Allium cepa* L.), play a crucial role in enhancing nutritional security and contributing to the national economy. Despite being the second-largest onion producer globally, India faces considerable yield losses from biotic stresses, notably purple blotch disease caused by *Alternaria porri*. This foliar disease severely affects onion crops during the kharif season, causing yield reductions up to 80% under epidemic conditions. Conventional chemical control methods pose environmental and resistance challenges, necessitating sustainable alternatives. This study evaluated the in vitro antagonistic potential of seven bioagents against *A. porri* using the dual culture technique. Among them, *Trichoderma asperellum* and *Pseudomonas fluorescens* showed the highest efficacy, inhibiting the pathogen’s radial growth by 88.14% and 83.14%, respectively. *T. harzianum* followed with 76.85% inhibition. Moderate to low antagonistic activity was observed in *Pseudomonas striata* (67.96%), *Bacillus subtilis* (65.92%), *Paecilomyces lilacinus* (60.18%), and *Metarhizium anisopliae* (54.25%). These findings underscore the potential of *T. asperellum* and *P. fluorescens* as promising candidates for integration into sustainable and environmentally sound management strategies for purple blotch disease in onion.

**KEYWORDS:** *In vitro,* Onion, *Allium cepa*, Purple blotch, *Alternaria porri*

***Introduction***

India, a predominantly agrarian nation, is undergoing significant transitions in its land use and cropping patterns driven by rapid urbanization and an expanding population. This growing demand for food has placed increased emphasis on enhancing agricultural productivity in a sustainable manner. Vegetables, in particular, play a dual role in this transformation supporting the nutritional needs of the population while also contributing substantially to the nation's agricultural economy.

Among vegetable crops, onion (*Allium cepa* L.) is a key horticultural commodity, cultivated extensively for its culinary and medicinal properties. Recognized as one of the oldest cultivated vegetables, onion is grown in over 175 countries (Mehta, 2017), with its primary center of origin being Central Asia and secondary centers in the Mediterranean and Near East (Vavilov, 1951). In India, onion cultivation spans diverse agro-climatic zones, with major producing states including Maharashtra, Madhya Pradesh, and Karnataka. As per recent statistics, the country ranks second globally in onion production, with 12.85 lakh hectares under cultivation and an annual yield of approximately 232 lakh tonnes (Anonymous, 2020).

Despite its economic and nutritional significance, onion cultivation faces persistent challenges from a range of biotic stresses. One of the most destructive diseases affecting onions is purple blotch, caused by the fungal pathogen *Alternaria porri* (Ellis) Cif. The disease primarily affects the foliage, manifesting as purplish concentric lesions that expand rapidly under favourable environmental conditions typically 25–30°C temperature and relative humidity above 90% (Shahanaz et al., 2007; Kumar et al., 2021). In severe cases, purple blotch can lead to necrosis and premature death of the plant, resulting in yield losses ranging from 30% to as high as 80% under epidemic conditions (Dar et al., 2020).

Chemical fungicides are commonly employed for managing *Alternaria porri*, the causal agent of purple blotch in onions. Fungicides such as mancozeb, chlorothalonil, propiconazole, hexaconazole, and azoxystrobin have demonstrated effectiveness in suppressing the pathogen under field conditions (Meena et al., 2017; Sharma et al., 2013). However, the indiscriminate and prolonged use of these chemicals has resulted in several issues, including the emergence of fungicide-resistant *A. porri* strains, environmental pollution, and the accumulation of harmful residues in consumable produce.

These concerns highlight the need for eco-friendly and sustainable disease management alternatives. Among these, the use of biological control agents (bioagents) has gained considerable attention. Several bioagents have shown significant antagonistic activity against *A. porri* in vitro and in vivo. Notably, species of *Trichoderma* such as *T. harzianum*, *T. viride*, and *T. asperellum* exhibit mycoparasitic behaviour and produce antifungal metabolites that inhibit the growth of *A. porri* (Prasad et al., 2017; Jagtap et al., 2012). Likewise, bacterial bioagents like *Pseudomonas fluorescens* and *Bacillus subtilis* are known to produce antibiotics, siderophores, and lytic enzymes that contribute to the suppression of this pathogen (Niranjana et al., 2009; Chattopadhyay et al., 2014).

In this context, the present study was undertaken to evaluate the *in vitro* antagonistic efficacy of different bioagents against *Alternaria porri*, the causal organism of purple blotch in onion. The objective was to identify potent biocontrol agents that could be integrated into Integrated Disease Management (IDM) strategies for the effective and sustainable control of this economically significant disease.

**MATERIALS AND METHODOLOGY**

The present investigation was carried out in the laboratory of the Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani, with the primary objective of assessing the in vitro efficacy of selected bioagents against *Alternaria porri*, the causal organism of purple blotch in onion. The experimental materials, including bioagents, Potato Dextrose Agar (PDA) medium, laboratory-grade glassware, and other essential apparatus, were procured from the departmental facilities. Diseased onion plants exhibiting characteristic symptoms of purple blotch such as elliptical to oval lesions with concentric rings surrounded by chlorotic halos were collected from the field for pathogen isolation. The pathogen was successfully isolated on PDA medium under aseptic conditions and was identified as *Alternaria porri* based on morphological characteristics.

For the *in vitro* antagonistic assay, seven bioagents, namely *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Pseudomonas striata*, *Metarhizium anisopliae*, *Paecilomyces lilacinus*, and *Bacillus subtilis* were procured from the Biomix research unit, Department of Plant Pathology, VNMKV, Parbhani. These bioagents were evaluated using the dual culture technique. In this method, a 5 mm mycelial disc of *A. porri* was placed at one end of the Petri dish containing PDA medium, while an equal-sized disc or loopful of the respective bioagent was inoculated at the opposite end, equidistant from the center. The experiment was laid out in a Completely Randomized Design (CRD) with three replications for each treatment. The plates were incubated at a temperature of 28±2°C and observed daily. The radial growth of the pathogen was recorded once the control plates (without bioagents) were fully colonized by *A. porri*. The percent inhibition of mycelial growth in the presence of bioagents was calculated using Vincent’s formula:

C - T

Percent Inhibition= **………...**  x 100

C

where **C** represents the colony diameter in the control, and **T** is the colony diameter in the treatment. This methodology facilitated a comparative evaluation of the antagonistic potential of the selected bioagents against *A. porri*, thereby providing insight into their suitability for integrated disease management strategies in onion cultivation.

**RESULTS AND DISCUSSION**

The *in vitro* evaluation of different bioagents against *Alternaria porri*, the pathogen responsible for purple blotch in onion, yielded significant variation in their antagonistic efficacy. The detailed results are presented in Table 1, Plate 1 and graphically illustrated in figure 1. The experiment revealed marked differences among treatments in their ability to suppress the radial growth of the pathogen, indicating variable levels of bioefficacy.

Among the tested bioagents, *Trichoderma asperellum* (T₁) demonstrated the highest antagonistic potential. It significantly reduced the mycelial growth of *A. porri*, with a minimum mean colony diameter of 10.66 mm and a maximum percent inhibition of 88.14%, thereby emerging as the most effective treatment. This was closely followed by *Pseudomonas fluorescens* (T₃), which recorded a colony diameter of 15.16 mm and an inhibition percentage of 83.14%. The performance of T₃ was statistically at par with T₁, underscoring its comparable efficacy in inhibiting pathogen growth.

*Trichoderma harzianum* (T₂) also exhibited notable antagonism, resulting in a colony diameter of 20.83 mm and percent inhibition of 76.85%. Although statistically lower than T₁ and T₃, its performance remained significantly superior to the remaining treatments. *Pseudomonas striata* (T₄) and *Bacillus subtilis* (T₇) displayed moderate efficacy, registering colony diameters of 28.83 mm and 30.66 mm, and inhibition percentages of 67.96% and 65.92%, respectively. These two treatments were statistically at par.

**Table No. 1.** ***In vitro* bio-efficacy of the bioagents against *Alternaria porri* causing onion purple blotch**

|  |  |  |  |
| --- | --- | --- | --- |
| **Tr. No** | **Treatment details** | **Colony Diam. of test pathogen \* (mm)** | **% Inhibition** |
| T₁ | *Trichoderma asperellum* | 10.66 | 88.14  (69.84) |
| T₂ | *Trichoderma harzianum* | 20.83 | 76.85  (61.23) |
| T₃ | *Pseudomonas fluorescens* | 15.16 | 83.14  (65.75) |
| T₄ | *Pseudomonas striata* | 28.83 | 67.96  (55.51) |
| T₅ | *Metarhizium anisopliae* | 41.16 | 54.25  (47.42) |
| T₆ | *Paecilomyces lilacinus* | 35.83 | 60.18  (50.85) |
| T₇ | *Bacillus subtilis* | 30.66 | 65.92  (54.26) |
| T₈ | Control (untreated) | 90.00 | 100  (90.00) |
|  | **S.E. (m) ±** | **0.81** | **0.90** |
|  | **C.D. at 1%** | **2.47** | **2.74** |

*\*Mean of three replications. Figure in parenthesis are arcsine transformed values.*

Among the entomopathogenic fungi, *Paecilomyces lilacinus* (T₆) and *Metarhizium anisopliae* (T₅) recorded relatively higher colony diameters of 35.83 mm and 41.16 mm, with corresponding inhibition percentages of 60.18% and 54.25%, respectively. These values were statistically lower compared to those recorded for *Trichoderma* and *Pseudomonas* species, indicating comparatively weaker antagonistic interactions with *A. porri*.

As expected, the untreated control (T₈) displayed the maximum radial mycelial growth of 90.00 mm, with 0% inhibition, confirming the virulence of the pathogen in the absence of any antagonistic organism.

The statistical analysis confirmed the high significance of treatment differences, with a Critical Difference (CD) at 1% level of 2.47 mm for colony diameter and 2.74% for percent inhibition. The Standard Error of the Mean (SEm ±) was 0.81 mm and 0.90%, respectively, indicating a high level of precision in the experimental design and data collection.

These results clearly establish that *Trichoderma asperellum* and *Pseudomonas fluorescens* are the most potent antagonists against *Alternaria porri* under in vitro conditions, followed closely by *T. harzianum*. The remaining bioagents, though exhibiting some inhibitory effect, were significantly less effective and could be considered of moderate to low bioefficacy. These findings support the potential integration of specific bioagents, particularly *T. asperellum* and *P. fluorescens*, into sustainable and eco-friendly management strategies for controlling purple blotch disease in onion.

**Conclusion**

The present in vitro study clearly demonstrated significant variation in the antagonistic potential of bioagents against *Alternaria porri*, the pathogen causing purple blotch in onion. *Trichoderma asperellum* emerged as the most effective, showing 88.14% inhibition of mycelial growth, followed closely by *Pseudomonas fluorescens* (83.14%) and *T. harzianum* (76.85%). These results are consistent with earlier findings by Hariprasad et al. (2021), Jagtap et al. (2012), and Niranjana et al. (2009), who reported similar antagonistic efficacy of these agents against *A. porri*. Moderate inhibition was observed with *P. lilacinus* and *M. anisopliae*, suggesting potential as secondary biocontrol options in integrated management strategies. The untreated control confirmed full pathogen virulence with 0% inhibition. Overall, *T. asperellum* and *P. fluorescens* stand out as potent biocontrol agents and could be effectively integrated into eco-friendly disease management practices for onion. These findings support and expand upon previous research advocating the shift from chemical fungicides to sustainable biological alternatives.

**Disclaimer (Artificial intelligence)**

**Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.**

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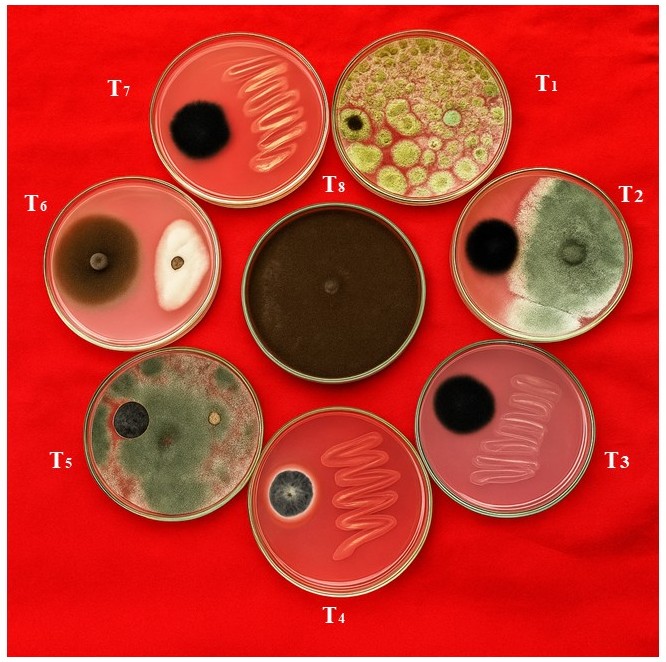
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**PLATE 1. Inhibition of *Alternaria porri by* different bioagents**

|  |  |  |  |
| --- | --- | --- | --- |
| T₁ | *Trichoderma asperellum* | T₅ | *Metarhizium anisopliae* |
| T₂ | *Trichoderma harzianum* | T₆ | *Paecilomyces lilacinus* |
| T₃ | *Pseudomonas fluorescens* | T₇ | *Bacillus subtilis* |
| T₄ | *Pseudomonas striata* | T₈ | Control (untreated) |

**Fig 1. Graphical representation of the Colony diameter of the pathogen and per cent inhibition achieved by each bioagent.**