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Evaluation of Bioagents against *Stemphylium vesicarium* Inducing Stemphylium Blight in Onions (*Allium cepa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Stemphylium blight of onion incited by *S. vesicarium* is one of the economically important diseases, causing severe yield losses in onion crop. The present investigation was carried out to evaluate efficacy of different bioagents against *S. vesicarium* under *in vitro* conditions. The experiment was laid out in a Completely Randomized Design (CRD) with three replications per treatment. Five fungal biocontrol agents *viz., Trichoderma harzianum, T. viride, T. hamatum, T. virens* and a fungal

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consortium from MPKV, Rahuri along with two bacterial biocontrol agents namely *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their effectiveness against *S. vesicarium.* The results showed significant variation in the inhibitory effects of bioagents on the mycelial growth of the test pathogen with percent growth inhibition ranging between 53.70 to 85.19 per cent. *T. harzianum* was reported to be most effective, showing a minimum colony diameter of 13.33 mm with the highest growth inhibition (85.19%) thus reported as superior to all other treatments whereas, *Pseudomonas fluorescens* was reported with a least percent growth inhibition (53.70%).

Keywords: Bioagents; growth inhibition; Stemphylium vesicarium; Trichoderma.

1. INTRODUCTION

The onion (*Allium cepa* L.) is the important commercially cultivated vegetable crop all over the world belonging to family *Alliaceae*. In India, onions are extensively cultivated for both domestic consumption and export purpose. India is one of the largest producers of onions, with Maharashtra is the leading state in production, contributing about 43% of the nation's total onion output, which producing 13,301.7 tons. Madhya Pradesh, Karnataka, and Gujarat follow as the next leading producers. In the 2022/23 season, India’s onion production reached an estimated 318 lakh metric tons (LMT), slightly surpassing the previous year's 316.98 LMT [1].

Onions are susceptible to a range of diseases incited by specific pathogens causing severe impact on health and yield of the crop. Stemphylium leaf blight caused by *Stemphylium vesicarium* is one of the economically important diseases causing severe losses in onion production. For the first time, Stemphylium blight of onion was documented in India by Rao and Pavgi [2], who reported that the yield of onion bulbs was reduced by nearly 90% due to disease incidence.

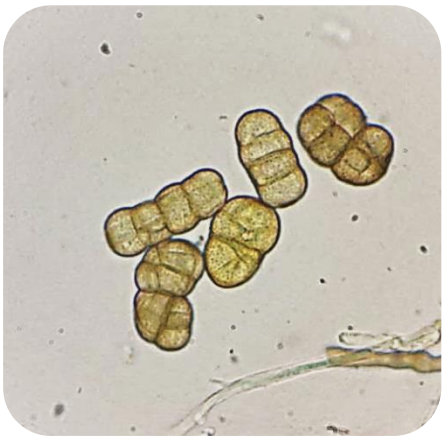
Spread of Stemphylium blight is occur by infected seed material and through infested soil. Initial symptoms of Stemphylium blight appear on the foliar plant parts. The symptoms of Stemphylium blight include small, water-soaked lesions that develop into dark brown to black spots on the leaves, often leading to complete blighting [3]. These lesions then develop into elongated spots, eventually turning dark olive brown to black due to spore development. The center of the lesions turns brown and thus produces conidiophore and conidia which gives blighted appearance to infected plant. Severe infestation may result in complete blighting of the leaves.

Eco-friendly, non chemical management techniques are becoming more prominent as an alternative to chemical management practices. It is feasible to control the Stemphylium blight of onions with the application of fungicides, but prolonged use of chemicals has a harmful environmental impact and has caused pathogens to become resistant to them. So, keeping in view the commercial importance of the crop in India and the magnitude of losses caused by Stemphylium blight in onion. The present study was planned to evaluate efficacy of different bioagents against *S. vesicarium* under *in vitro* conditions.

2. MATERIALS AND METHODS

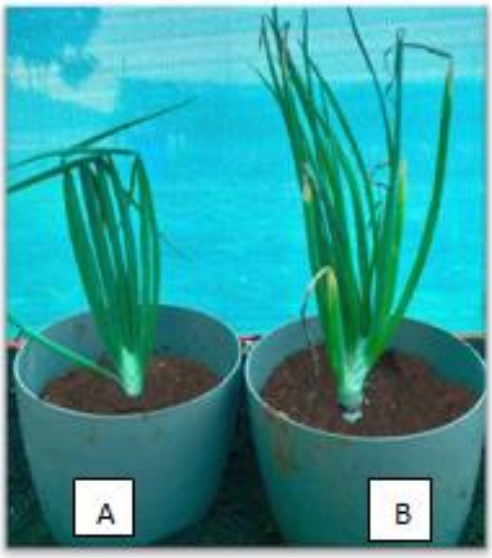
Isolation, purification and identification of the pathogen associated with onion leaf blight: Naturally infected onion leaves showing characteristic symptoms of Stemphylium blight were collected from the experimental plot located at Krishi Vigyan Kendra, Baramati. Collected samples were put in paper bag and brought to Plant Pathology laboratory. The infected onion leaves were washed thoroughly with distilled water. Leaf samples were blot dried and cut with sharp sterilized blade into small bits (5 mm), keeping half healthy and half diseased portion intact. These pieces were surface sterilized with 0.1% aqueous solution of sodium hypochlorite for two minutes followed by giving three consecutive washes with sterile distilled water to remove the residues of sodium hypochlorite. The surface sterilized diseased leaf bits were transferred into sterilized Petri-dishes contained PDA medium under aseptic conditions of laminar air flow cabinet. Inoculated plates were incubated in BOD incubator at 24±1°C temperature for 7 days. The emerged fungus was picked up and sub culture was carried out until the pure isolate of *Stemphylium vesicarium* was obtained. The isolated test pathogen was identified based on colony characters, spore morphology referring to the description by Chowdhury *et al.* [4].

Pic. 1 A. Pure culture of *S. vesicarium*



B. Conidia of *S. vesicarium*

Pathogenicity test: Onion variety Agrifound Dark Red was used to perform the pathogenicity test. Potting mixture of soil: sand: FYM were sterilized and filled in earthen pots in 2:1:1 ratio. Seeds were sown in pots and healthy onion seedling per pot was maintained, watered regularly and kept in the polyhouse for further growth. The test pathogen (*Stemphylium vesicarium*) was mass multiplied on the culture medium of PDA in petri dishes. Spore cum mycelial suspension of the test pathogen was prepared from 7 to 8 days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore mycelial suspension was filtered through double layered muslin cloth and filtrate obtained was suitably diluted with sterile distilled water. Spore concentration was adjusted to 5×10^4 conidia per ml with hemocytometer. 2 months old seedlings of onion which were already grown in earthen pots were artificially inoculated by spraying spore suspension of the test pathogen with hand automizer. Onion seedling of uninoculated control was maintained in earthen pots and sprayed with sterile water i.e., without inoculum. Both inoculated and uninoculated pots in the polyhouse were maintained with 80 - 90% humidity and 24±1°C optimum temperature by covering them with clear polyethylene bags for 72 hours. After 3 days, polythene bag was removed and disease development was assessed until the development of characteristic symptoms of Stemphylium blight. Pathogen was reisolated from the developed symptoms on artificially inoculated leaves, and the resulting cultures were compared with the original inoculants to fulfil Koch's postulates.



Pic. 2. A. Uninoculated healthy B. infected plant after inoculation

Antimicrobial efficacy of bioagents: The antimicrobial efficacy of various biocontrol agents, including *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma viride*, *Trichoderma virens*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and MPKV fungal consortium, was evaluated against *Stemphylium vesicarium* using the dual culture technique [5] on PDA medium.

Dual Culture Technique: PDA medium was prepared and sterilized in an autoclave at 15 psi pressure at 121°C for 20 minutes. Sterilized medium (20 ml) was poured into sterilized Petri-dishes (90 mm diameter). After solidification, each Petri-dish was inoculated with a 5 mm mycelial disc from an actively growing 7-day-old culture of *S. vesicarium* placed at the periphery of one side of the plate. The biocontrol agent disc was inoculated on the opposite side of the same plate, equidistant from the pathogen. For bacterial bioagents, the inoculum was streaked on opposite sides of the pathogen. PDA plates inoculated solely with *S. vesicarium* served as untreated controls.

Experimental Procedure: All inoculated petri plates were incubated at 24 ± 1°C for seven days. Each treatment was replicated three times. After the incubation period, observations were carried out when the fungal growth in the control plates reached a maximum diameter of 90 mm. Radial growth of the test pathogen was measured, and the percentage of growth inhibition relative to the control was calculated using Vincent's [6] formula,

$$\text{Percent Inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Radial growth of fungus on control plate
T = Radial growth of fungus on treated plate

3. RESULTS AND DISCUSSION

The results show significant variation in the inhibitory effects of bioagents on the mycelial growth of the test pathogen with per cent growth inhibition ranging between 53.70 and 85.19 per cent. *T. harzianum* was reported to be most effective, showing a minimum colony diameter of 13.33 mm with the highest growth inhibition of 85.19 per cent and being reported as superior to all other treatments. The mechanism behind *T. harzianum's* effectiveness can be attributed to the secretion of cell-wall degrading enzymes such as chitinases and glucanases, alongside its ability to outcompete pathogens for space and nutrients [7].

The second most effective treatment was *T. viride,* with a colony diameter of 16.83 mm and growth inhibition of 81.30 per cent. *T. hamatum* and *T. virens* were, also, notably effective, with colony diameters of 22.50 mm and 29.17 mm with inhibition rates of 75.00 and 67.59 per cent, respectively. The *Trichoderma* species causes enzyme production and secondary metabolite synthesis thus have competitive abilities against other pathogens in the rhizosphere [8].

The MPKV fungal consortium showed significant effectiveness, with a colony diameter of 27.50 mm and growth inhibition of 69.44 per cent. The use of fungal consortia has been shown to produce synergistic effects, enhancing overall biocontrol potential against diverse pathogens [9]. This suggests that combining multiple strains may offer a broader spectrum of protection and resilience in integrated pest management (IPM) system.

On the other hand, the bacterial bioagents *B. subtilis* and *P. fluorescens*, showed comparatively moderate inhibition rates. *B. subtilis* was found to be comparatively less effective with colony diameter of 36.33 mm and 59.63 per cent growth inhibition. whereas, *P. fluorescens* was reported with a least per cent growth inhibition (53.70%) and a colony diameter of 41.67 mm. Although *Bacillus* and *Pseudomonas* species are widely recognized for their antimicrobial activities producing secondary metabolites such as lipopeptides and siderophores. The moderate performance of these bacterial agents suggests they may be better suited for suppressing bacterial pathogens or used in combination with fungal bioagents for enhanced its control.

The effectiveness of *T. harzianum* and *T. viride* had been reported earlier by Kumar *et al*. [10], Mishra and Gupta [11], Shahnaz *et al.* [12], Nainwal and Vishunavat [13], Abo Elyousr [14], Kamal *et al.* [15], Gondaliya *et al.* [16], Devi *et al.* [17] that is in accordance with present findings.

The findings are exactly matching with the report of Kumar *et al*. [10] who conducted an *in vitro* experiment to evaluate the efficacy of fungal bioagents *viz., T. harzianum* and *T. viride* against the Stemphylium pathogen. Among the bioagents tested, *T. harzianum* showed the highest inhibition of colony growth (81.2%) followed by *T. viride* (74.5%)

Table 1. Antimicrobial efficacy of different bioagents against the *S. vesicarium* under *in vitro* condition

Tr. No.	Treatments	Colony diameter of the pathogen* (mm)	Per cent growth inhibition *(%)
T_1	*Trichoderma harzianum*	13.33	85.19
T_2	*Trichoderma viride*	16.83	81.30
T_3	*Trichoderma hamatum*	22.50	75.00
T_4	*Trichoderma virens*	29.17	67.59
T_5	*Pseudomonas fluorescens*	41.67	53.70
T_6	*Bacillus subtilis*	36.33	59.63
T_7	MPKV fungal consortium	27.50	69.44
T_8	Control	90.00	-
	SE(m)±	**0.46**	
	C.D. at 1%	**1.92**	

**Mean of three replications*

Plate 1. *In vitro* efficacy of various bioagents against S. vesicarium

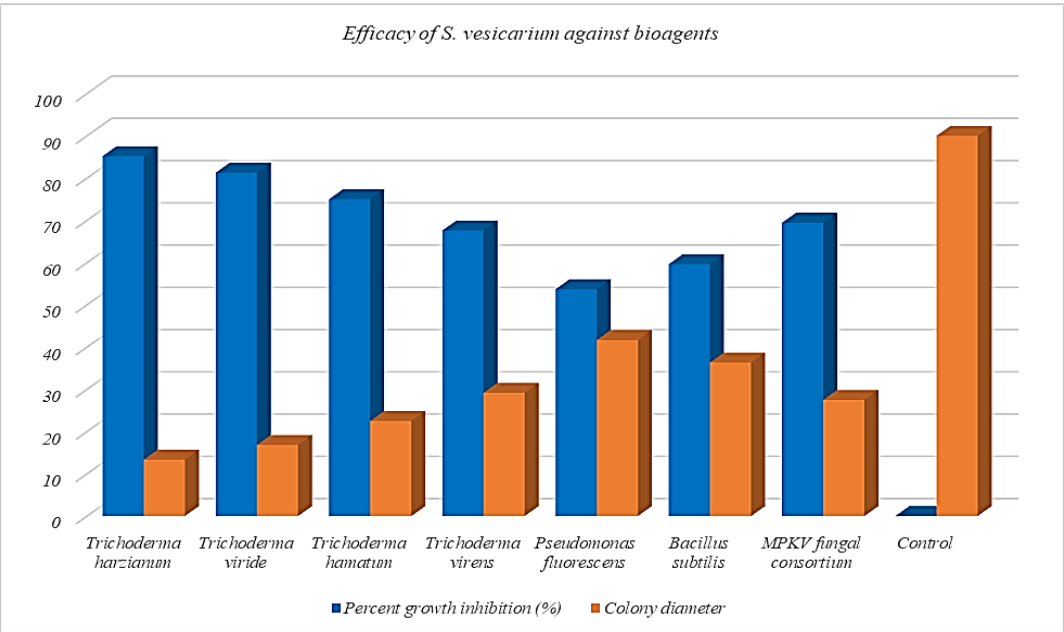


Fig. 1. *In vitro* efficacy of various bioagents against Stemphylium vesicarium

The results obtained of present investigation are in close conformity with Mishra and Gupta [11], who tested the efficacy of fungal biocontrol agents *viz.*, *T. viride*, *T. harzianum*, *T. hamatum*, *A. niger*, *T. koningii, T. virens* and bacterial antagonists *P. fluorescens* and *B. subtilis*. All antagonistic organisms demonstrated inhibitory effects on the growth of *S. vesicarium* with inhibition ranging from 19.20 per cent to 55.95 per cent. *T. viride* exhibited the highest level of inhibition about 56.15 per cent against *S. vesicarium*. This was followed by *T. harzianum* with inhibition of about 51.95 per cent and *T. koningii* with inhibition of 45.25 per cent, respectively. Conversely, *P. fluorescens* displayed the least effectiveness with inhibition rate 20.17 per cent.

Gondaliya *et al.* [16] reported the *in vitro* efficacy of various isolates of bioagents against the *S. vesicarium* and *Alternaria porii* and resulted that *T. viride* shows the highest growth inhibition followed by *T. harzianum*, *longibrachiatum*, *P. fluorescens* and *B. subtilis*. furthermore, Hussein *et al.* [18] also evaluated the *in vitro* efficacy of different bioagents against the *S. vesicarium* and reported that *T. harzianum* shows 78.1 per cent mycelial growth inhibition when dual cultured with *S. vesicarium* under *in vitro* condition.

4. CONCLUSION

The *in vitro* evaluation of seven biocontrol agents revealed that, *T. harzianum* exhibited effective antagonism and maximum mycelial growth inhibition against the test pathogen.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I hereby declare that no generative AI technologies, including but not limited to Large Language Models (such as ChatGPT, Copilot, etc.) or text to image generators, were utilized in the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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