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Morphological, Cultural Characteristics of Post-Harvest Diseases in Onion and its Management through Bio-Agents

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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

The onion (*Allium cepa* L.) is a biennial herb characterized by its bulbous structure, and it stands as one of the vital vegetable crops cultivated in India. Its origins can be traced back to the central Asian region. This underground bulbous vegetable crop is cultivated on a commercial scale due to its extensive adaptability and the significant potential for high production. Classified under the Amaryllidaceae family and Allium genus, the onion holds a pivotal role in the world of vegetables. The primary culprits responsible for onion bulb rot after harvesting were identified as *Aspergillus niger*, *A. flavus*, and *Fusarium oxysporum* f. sp. *cepae*. It was determined that the media PDA, SDA, MEA, and YDA were conducive to the growth and sporulation of *A. niger* mycelium. Conversely, MEA, OM, PDA, and YDA were found to be favorable for the development and sporulation of *A.*

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flavus. Lastly, PDA, CDA, OMA, and RA culture media demonstrated suitability for promoting mycelial growth and sporulation of *F. oxysporum* f. sp. *cepae*.

Keywords: Onion; fusarium; aspergillus; morphology; management.

1. INTRODUCTION

Onion (*Allium cepa* L.) is a bulbous, biennial herb and one of the most important vegetable crops grown in India. It was originated in the region of central Asia. It is a commercially grown underground bulbous vegetable crop with an extended range of adaptations and a relatively high production potentiality. It belongs to the family *Amaryllidaceae* and genus *Allium* is an important vegetable. Onion bulbs are rich in proteins, minerals and ascorbic acid [1]. It is used throughout the year in the form of salad and for cooking with other vegetables, as well as it has many medicinal uses. Thus, it is known as Queen of the kitchen [2].

Kharif, Rabi and summer are the three seasons, onions are grown. During the *kharif* season, seeds are sown in May-June, transplanted in July-August and onions are ready for harvesting in October-November [3]. The *kharif* season covers about 20% of the total area. Onions cultivated in the *kharif* season are mostly grown in the major countries i.e., China, India, France, USA, Japan, Brazil, Korea, Pakistan and Spain. Among the onion producing countries, India is the world's second-largest onion producer after China with an area of about 480.6 thousand hectares with a production of 5466.7 thousand tonnes which accounts for 14 % and 12 % of the area and production of the world respectively. Onion is a bulbous, biennial herb and one of the most important vegetable crops grown in India. The reddish colour of the outer peel of the onion is due to catechuic acid, protocatechuic acid and phenolic factors which are present in red onions and they have antifungal properties also [4]. The nutritional value of onion is very high where onion (per 100 g) edible portion contains carbohydrates (9.34 g), protein (1.1 g), calcium (23 g), phosphorus (29 mg), iron (0.21 mg) and riboflavin (0.027 mg). It also contains a volatile oil called Allyl propyl disulfide due to which the onion smell is pungent. Its food value per 100 g is 49 calories [5]. Onion is prone to many of diseases, among these, *Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum* f. sp. *cepae* which causes post-harvest losses [6]. Considering the onion's economic importance and yield losses due to post harvest diseases, the study on symptomology, morphology, cultural studies and invitro evaluation of bio agents against the pathogens.

2. MATERIALS AND METHODS

2.1 Isolation of the Pathogens

The pathogens were isolated utilizing tissue isolation procedures on PDA. The diseased onion bulbs were carefully rinsed with clean water to remove the dirt and chopped into little pieces using a sterile sharp blade. Surface sterilization was performed for one minute with 0.1% $HgCl_2$ solution, followed by three washes with sterile distilled water to eliminate residues of $HgCl_2$ and blot and dry with sterile Whatman No.1 filter papers. In each petri plate, two to three dried sick scale fragments were placed aseptically on sterilized PDA media under aseptic circumstances in a Laminar air flow cabinet. The petri plates were incubated at room temperature 28 ±1 ºC in a BOD incubator. After four days of incubation, a well-developed, pure culture was obtained using hyphal tip isolation method and maintained by repeated subculturing on sterile PDA and PDA slants.

2.2 Morphology of the Pathogens

Morphological characteristics of the fungus were investigated using a compound microscope at 100X magnification with cotton blue dye. The form of conidia, conidiophore and hyphae in *A. niger* and *A. flavus*, as well as septation, shape, colour, mycelium, micro and macro-conidia, and chlamydospores in *F. oxysporum* f. sp. *cepae*, were recorded from a seven-day-old culture cultivated on PDA media using the slide culture technique.

2.3 Cultural Studies of *A. Niger*, *A. Flavus*, and *F. Oxysporum* f. sp. *Cepae* by Using Various Artificial Media

The fungal cultures were purified and grown on various mediums to evaluate their growth characteristics and sporulation nature. The various mediums employed for this investigation were Sabouraud's agar, Czapeck's dox agar,

Malt extract agar, Richard's agar, Oat meal agar, PDA and Yeast Extract agar.

2.4 *In vitro* Evaluation of Bioagents

Trichoderma harzianum, *T. viride*, *T. hamatum*, and *T. koningii* isolates were evaluated *in vitro* against *A. niger*, *A. flavus*, and *F. oxysporum* f. sp. *cepae* using a dual culture approach [7]. Seven days old culture was used in dual culture assay with biocontrol agent and test pathogen on opposite side in petri plate. As a control, a plate with test pathogen only without biocontrol agent was used and incubated for seven days at 28±1˚ C.

3. RESULTS

3.1 Symptomatology and Morphology of *Aspergillus Niger*, *Aspergillus Flavus* and *Fusarium Oxysporum* f. sp. *Cepae*

3.1.1 Symptomatology and morphology *Aspergillus niger*

Aspergillus niger is distinguished by the presence of a black powdery mass of spores on the scale's exterior and interior surfaces. Powdery black spore masses are often visualized as streaks along veins on and between outer dry scales. Infection can spread from the neck into the centre fleshy scales, causing bulb decomposition and emitting a foul odour. Colony was carbon black in colour. The hyphae are hyaline, septate, and yellow. Conidiophores are long, smooth, septate structures growing straight from the substratum. Vesicles are globose or subglobose in form, with blackish-brown to purple-brown conidial heads and an almost columnar mass of a few conidial chains to the 28 common globes or radiating heads.

3.1.2 Symptomatology and morphology *Aspergillus flavus*

Aspergillus flavus produces white mycelium on the surface of bulb. After sporulation, it produced faint green coloured patches on onion bulb. Later, water-soaked lesions are formed on onion bulbs and led to partial decay of inoculated bulbs.

The *A. flavus* colony was greenish in colour and propagated radially from the spot of inoculation. As the colony expanded, it became somewhat elevated in the centre, floccose, rough and sporulation commenced after five days from the centre and proceeded radially covering the colony's surface. Conidia ranged from yellowish to olive in colour. However, when sporulation extended, it left a distinctive white border enclosing the sporulating mycelia and generate additional conidia. On the back, colonies exhibited clear exudates and a cream colour. Colonies were either uniseriate, biseriate or both. The phialides were carried on the metuale in biseriate cells and directly connected to the vesicles in uniseriate cells. Metulae carried by globose vesicles covered almost the whole surface of the vesicles and radiated in all directions.

3.1.3 Symptomatology and morphology *Fusarium oxysporum* f. sp. *cepae*

The white mycelial mat was developed all over the surface of the bulb. Semi soft rotting was spreading from the stem plate to the leaf scales and bulbs starts to rot in upward scales and few days later began to dry. When infected onion bulbs were cut vertically, the outermost layer of the stem plate showed watery brown discoloration leading to partial decay of bulbs.

The fungus produced chlamydospores, macroconidia, and microconidia. The aerial mycelium was white, hyaline, cottony to slightly pink in colour. Sporulating cultures of the test pathogen were observed under a compound microscope showing, microconidia were kidney-shaped, with one or two cells, while macroconidia were curved shape with three to four septa. Chlamydospores were often vacuolated, septate, amorphous, colourless and comprised of 1 or 2 spherical cells with strong cell walls, generated alone or in groups, which can also be created intercalary or terminally (Fig. 1).

3.2 Cultural Study of *Aspergillus Niger*, *Aspergillus Flavus* and *Fusarium Oxysporum* f. sp. *Cepae* on Different Media

A. niger cultural features, including as mycelial growth, sporulation, and colony characteristics were examined in vitro using seven different culture mediums. Mycelial growth measured in all test medium varied from 86 mm to 90 mm. Richard's agar (90.00 mm), PDA (90.00 mm), Yeast dextrose agar (90.00 mm), Malt extract agar (90.00 mm), Sabouraud's dextrose agar

(90.00 mm), and Czapek's dox agar all showed outstanding mycelial growth (88.33 mm). In oat meal agar, the least amount of mycelium development was detected (86.00 mm). Richard's agar has a black core with a white and uneven edge. On oat meal agar, the colour was light yellow with a regular margin, while on Yeast dextrose agar, the colour was light brown with a white margin and flat growth. PDA, Sabouraud's dextrose agar and malt extract agar showed excellent sporulation, whereas Yeast dextrose agar showed good sporulation followed by Richard's Agar, oat meal agar, and Czapek's dox agar.

The cultural features of *A. flavus* were examined in vitro using seven culture mediums. Mycelial growth measured in all test medium varied from 73.67 mm to 90 mm. The growth characteristics of *A. flavus* in diverse solid medium revealed that malt extract agar, oat meal agar and PDA promoted the most fungal colony development. On oat meal agar, Sabouraud's dextrose agar, PDA, malt extract agar and Yeast dextrose agar, light or dark green centres with white uniform margins and sparse flat growth were seen. Czapek's dox agar and Richard's agar had a dark green colour, an uneven border, and flat growth. PDA, oat meal agar and malt extract agar showed excellent sporulation followed by Sabouraud's dextrose agar, Richard's agar, and Yeast dextrose agar showed good sporulation with minimal sporulation in Czapek's dox agar.

F. oxysporum f. sp. *cepae*, also studied with a mycelial growth measured in all test medium varied from 40.00 mm to 90 mm. PDA (90.00 mm) showed the best mycelial growth, followed by Czapek's dox agar (81.33 mm), Sabouraud's dextrose agar (78.33 mm), oat meal agar (76.67 mm), Richard's agar (63.33 mm), and Yeast dextrose agar (63.33 mm). Malt extract agar had the least amount of mycelium development (40.00 mm). The growth characteristics of *F. oxysporum* f. sp. *cepae* in different solid medium revealed that PDA, Czapek's dox agar and Sabouraud's dextrose agar supported the most fungal colony development. On oat meal agar, white cottony growth with a golden serrated edge was detected. On Yeast dextrose agar, PDA, malt extract agar and Sabouraud's dextrose agar, however white cottony growth with regular or smooth margins was seen. Richard's agar revealed white cottony pluffy growth with an irregular margin. PDA and Sabouraud's dextrose agar showed excellent sporulation, whereas Czapek's dox agar, oat meal agar, and Richard's agar showed good sporulation. However, significant sporulation was seen in Yeast dextrose agar and sparse sporulation in malt extract agar (Fig. 2).

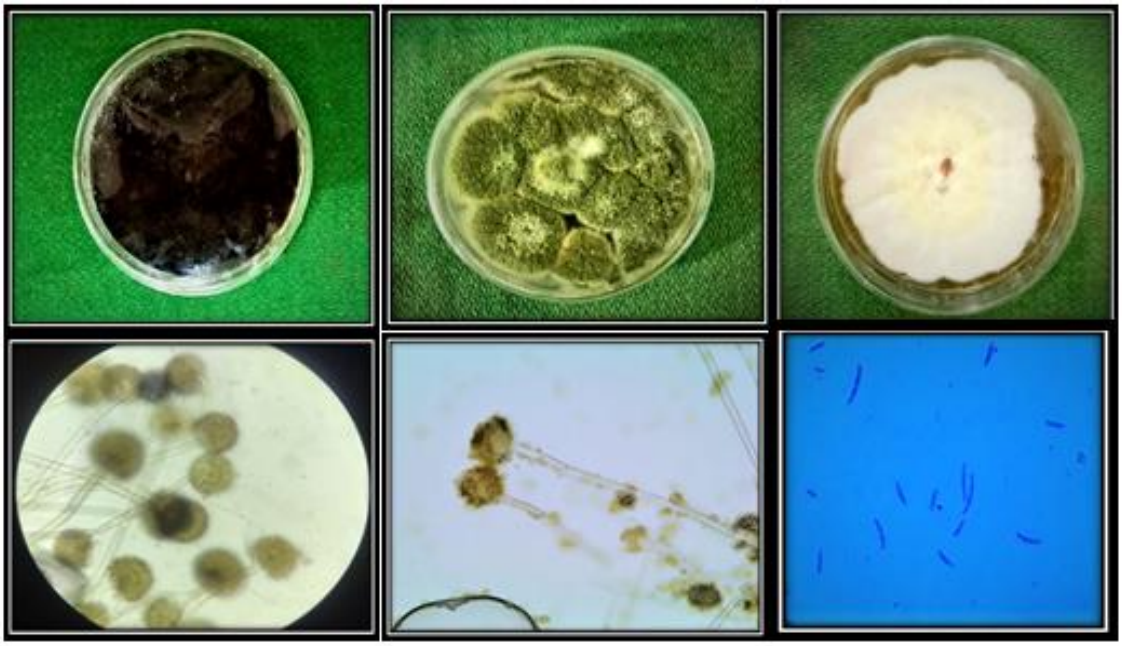

Fig. 1. Morphological and Microscopy of *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *cepae*

Fig. 2. Cultural variability of *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *cepae* in different media respectively

3.3 *In vitro* Evaluation of Bioagents against *A. Niger*, *Aspergillus Flavus* and *Fusarium Oxysporum* f. sp. *Cepae*

Trichoderma viride, *T. harzianum, T. hamatum* and *T. koningii* were tested *in vitro* against *A. niger* using a dual culture approach, with observations made after 7 days. All the bioagents tested showed antifungal action and suppress *A. niger* mycelial growth. *T. viride* was found to be the most effective bioagent, with the least linear mycelial growth (37.75 mm) and the highest mycelial growth inhibition (58.06%). *T. koningii* and *T. harzianum* were the second and third best bioagents, with mycelial growth of 55.00 and 56.50 mm and inhibition of 38.89 and 37.22%, respectively.

T. hamatum, on the other hand, was found to be the least effective, with the largest linear mycelial growth (72.50 mm) and the lowest mycelial growth inhibition (19.44%). Thus, all of the fungal bioagents tested in vitro were antifungal against *A. niger* and significantly reduced its growth compared to control. Several investigators previously found that bioagents such as *T. viride, T. harziamim, T. hamatum* and *T. koningii* inhibited *A. niger* mycelial growth significantly. Ramakrishna *et al.* [8] investigated the effect of bioagents against *A. niger in vitro. T. viride*, on the other hand, was shown to be the most effective in reducing *A. niger* mycelial growth (63.13%). Raju [9] tested seven bioagents and *T. viride* demonstrated 69.90% inhibition.

All of the bioagents tested showed antifungal action and suppressed *A. flavus* mycelial growth. *T. harzianum* was found to be the most effective of the four bioagents tested, with the least linear mycelial growth (29.75 mm) and the highest mycelial growth inhibition (66.19%). *T. viride* and *T. hamatum* were the second and third best bioagents, with mycelial growth of 33.50 mm and 63.75 mm with inhibition of 61.93% and 27.56%, respectively.

T. koningii, on the other hand, was found to be the least effective, with the largest linear mycelial growth (67.50 mm) with lowest mycelial growth inhibition (23.30%). Several workers previously found that bioagents such as *T. viride, T. harziamim, T. hamatum* and *T. koningii* greatly suppressed the mycelial growth of *A. flavus*. Ranganathswamy *et al.* [10] used a dual culture to test bioagents for their ability to prevent the mycelial growth of *A. flavus. T. harzianum*, on the other hand, inhibited mycelial development the most. Bhosale *et al.* [11] evaluated bioagents against *A. flavus in vitro. T. harzianum*, on the other hand, was determined to be the most effective, with the highest mycelial growth inhibition (77.78%).

All of the bioagents tested showed antifungal action and inhibited *F. oxysporum* f. sp. *cepae* mycelial growth. *T. viride* was shown to be the most effective of the four bioagents tested, with the least linear mycelial growth (16.25 mm) and the highest mycelial growth inhibition (81.74%). *T. koningii* and *T. hamatum* were the second and third best bioagents, with mycelial growth of 38.00 mm and 42.00 mm, and inhibition of 57.30% and 52.53%, respectively. *T. harzianum*, on the other hand, was found to be the least effective, with the maximum linear mycelial growth (42.25 mm) and the lowest mycelial growth inhibition (52.81%) (Fig. 3).

Fig. 3. Inhibition of A. niger, A. flavus and F. oxysporum f. sp. cepae by different biocontrol agents

4. DISCUSSION

The phialides were usually biseriate (two series), coating the vesicles heavily. Conidia have a smooth, globose form Walker, [12]. Conidiophores had thick walls and were hyaline, coarsely roughened or pitted, whereas conidia were globose and somewhat roughened [13,14]. Visual observation was used to study symptomatology based on the typical symptoms produced on infected bulbs. Several researchers Holz and Knox-Davies, [15] Padule *et al.,* [16] Mishra *et al.,* [17]. Sinclair and Letham, [18] obtained comparable results.The effect of different culture medium on cultural features and sporulation of *A. flavus* is consistent with Thathana *et al.,* [13] who found that PDA provided the optimum growth and sporulation of *A. flavus*. Pooja [19] Kawade [20] showed that *F. oxysporum* f. sp. *cepae* grew and sporulated best on PDA (90.00 mm) and Czapek's dox agar (78.97 mm). Ramakrishna *et al.* [8] investigated the effect of bioagents against *A. niger in vitro. T. viride*, on the other hand, was shown to be the most effective in reducing *A. niger* mycelial growth (63.13%). Raju [9] tested seven bioagents and *T. viride* demonstrated 69.90% inhibition. T. viride was reported to be most efficient against F. oxysporum f. sp. cepae by El-mougy and Abdel-Kader [21] Jagtap and Suryawanshi [22] and decreased radial mycelial development by 78.88 %. [23,24].

5. CONCLUSION

The major pathogens such as *A. niger, A. flavus* and *F. oxysporum* f. sp. *cepae* were found to be the cause of post-harvest bulb rot of onion. The media PDA, SDA, MEA and YDA were shown to be good for *A. niger* mycelial development and sporulation studies. MEA, OM, PDA and YDA, on the other hand, were shown to be great for *A. flavus* mycelial development and sporulation. PDA, CDA, OMA, and RA culture media were shown to be good for mycelial development and sporulation of *F. oxysporum* f. sp. *cepae*. The current study's findings on the effect of various culture media on cultural characteristics and sporulation are consistent with the findings of several scientists, including Nalawade et al., 2019; Sharma, 2010, who found that maximum growth and sporulation of *Aspergillus niger* was best on PDA and Sabouraud's dextrose agar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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