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**ISOLATION OF *Aspergillus niger* FROM *Allium cepa* BULB AND PRODUCTION OF CITRIC ACID FROM IT**

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**ABSTRACT**

The fungal pathogen, *Aspergillus niger* is known to cause decaying in storage onion bulbs and causes black mold not only in *Allium cepa* but also fungal disease in *Arachis* and *Vitis* spp. Post harvest disease of onion causes by *Aspergillus niger* may be observed between bulb scales. Infection of onion seedlings by *A. niger* can become systemic, manifesting only when conditions are conducive. In present studies is focused on the citric acid producing organisms were isolated from *Allium cepa* bulb and identified morphological characteristics by the microscopic and identified by amylase production test. The isolated fungal species were screened on the acid production using Czapek - dox agar medium and citric acid produced by using *Aspergillus niger*.

**KEYWORDS:** *Aspergillus niger*, citric acid, Starch hydrolysis test, Onion.



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## 1. INTRODUCTION

Fungi are used widely in biotechnology for many processes, including the production of antibiotics, enzymes, food products, industrial acids, and alcohol. *Aspergillus* mold have a powdery texture. However the colour of the mold's surface differs from species to species and can be used to identify the type of *Aspergillus*. The *Aspergillus niger* or *Aspergillus niger* is a fungus and one of the most common species of the genus, *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called black mould [1]. These fungi are seed- and soil-borne and are generally present in soils where onion is grown extensively [2, 3, 4]. The Citric acid worldwide demand is about  $6.0 \times 10^5$  tons year<sup>-1</sup> [5]. Approximately 75% commercial use of citric acid is for food and 12% for pharmaceutical industries [6]. Commercial production of citric acid is generally by submerged fermentation of sucrose or molasses using the filamentous fungus *A. niger* or synthetically from acetone or glycerol [7, 8, 9, 10].

## 2. MATERIALS AND METHODS

The Investigation was done during 2012-13 in Department of Life Science, Dr. C.V. Raman University, Kota, Bilaspur to identify the seed borne fungi of onion (*Allium cepa* L.) from various locations of Maharashtra Fungal Isolation. The *Aspergillus niger* isolated from naturally infected onion seeds were used [11-12].

### 2.1 Inoculum and Identification

The identified colonies were streaked on the PDA (Potato dextrose Agar) plated medium and incubated, after incubation the loop full colonies were inoculated into the PDA broth and incubated at 25°C for 2-3 days, after then observed Black mold.

### 2.2 Lactophenol cotton blue method

Lactophenol Blue Solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue. The *Aspergillus niger* is reacted the solution and Delicate blue hyphae and fruiting structures with a pale blue background [13].

### 2.3 By Amylase production Test

Amylase production is known in some bacteria while well-known in fungi. Amylases commercially produced from various aspergilla are used in the initial steps in several food fermentation processes to convert starch to fermentable sugars. Starch is the presence of iodine produces dark- blue coloration of the medium and a yellow zone around a colony in an otherwise blue medium indicates amylolytic activity [14].

### 2.4 Screening of the fungal cultures

*Aspergillus niger* cultures were screened qualitatively for the production of citric acid [15]. Czapek-Dox agar medium (10 ml) was poured into individual sterile petri plates and allowed to cool at room temperature. Approximately 0.5 ml of the of *Aspergillus niger* broth was transferred to each of the petri plates. The plates were incubated at 30 °C for 3-5 days and plates were observed after incubation for yellow zones due to citric acid formation.

## 3. RESULTS AND DISCUSSION

*Aspergillus niger* culture were showed in PDA (Potato Dextrose Agar) medium and it is growed black colour mold, (Figure 1). The fungi were staining and identified by lacto phenol blue method and the result is delicate blue hyphae and fruiting structures with a pale blue background of *Aspergillus niger*. The *A. niger* was growed in Starch agar medium and incubated in 25°C for 2-3 days, it showed black mold, and their appeared yellow zone around the colony in presences of iodine solution (Figure 2). After then again

*Aspergillus niger* sample streaked were Czapek-Dox agar medium and incubated in 25°C for 3-5 days. Then observed yellow zone showed in *Aspergillus niger*. The *Aspergillus* cultures were screened quantitatively for the production of citric acid as described by Ali (2004). According to them explained that the nature of sugar source has a marked effect on citric acid production by *Aspergillus niger* [16]. In present study the *Aspergillus niger* were showed yellow zone in Czapek dox agar

medium, and *A. niger* is produced citric acid (Figure 3). So *Aspergillus niger* is cultured for the industrial production of many substances. Various strains of *A. niger* are used in the industrial preparation of Citric acid have been assessed as acceptable for daily intake by the World Health Organization. Nagerbi and Abdalla [17] detected that *Aspergillus* was the most prevalent genus among all the seed borne fungi of onion.



(Figure 1)

(Figure 2)

(Figure 3)

**Figures of *Aspergillus niger* growing different tested medium.**

(1) *Aspergillus niger* growth in PDA

(2) *Aspergillus niger* (Amylase Production Test)

(3) Czapek - dox agar medium

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