

Journal of Advances in Microbiology Research



E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2024; 5(1): 178-180
© 2024 JAMR
www.microbiojournal.com
Received: 05-12-2023
Accepted: 11-01-2024

Dr. Mahendra Pal
Professor, Founder and
Managing Director, Narayan
Consultancy Veterinary Public
Health and Microbiology,
Sapphire Lifestyle,
Maktampur, Bharuch,
Gujarat, India

Correspondence
Dr. Mahendra Pal
Professor, Founder and
Managing Director, Narayan
Consultancy Veterinary Public
Health and Microbiology, B-
103, Sapphire Lifestyle,
Maktampur, Bharuch,
Gujarat, India

Isolation and identification of *Aspergillus niger* from the onions in Bharuch, Gujarat, India

Dr. Mahendra Pal

Abstract

A mycological study was undertaken to know the natural occurrence of *Aspergillus niger*, a saprobic filamentous fungus, on the raw onions. A total of 47 onions collected from vegetable markets and street vendors were examined by swab technique. The sterile cotton swab dipped in glucose solution and chloramphenicol solution was rubbed on the bulb of each onion, and streaked on to the plates of Pal sunflower seed medium (Pal medium) and APRM agar. The inoculated plates were incubated at 30 °C, and examined daily for the fungal growth. The fungal growth resembling *Aspergillus* was observed in 21 out of 47 onion samples. The cultural growth and detailed microscopic morphology in Narayan stain confirmed the identity of all the 15 fungal isolates as *Aspergillus niger*. Since *A. niger* has the potential to infect the humans, the housewives and others who work in kitchen should take great precautions to avoid the transmission of this medically important fungus. To the authors knowledge, this seems to be first study on the isolation of *A. niger* from onions on APRM agar and Pal medium.

Keywords: APRM agar, *Aspergillus niger*, Narayan satin, Onion, pal medium

Introduction

Onion (*Allium cepa* L.) also known as bulb onion or common onion is an important root vegetable that is frequently consumed daily in the kitchen both in developing and developed nation of the world. It possesses several health benefits as it can lower the blood pressure, improves bone health, and also decrease the risk of cancer development (Corzo-Martinez and Villamiel, 2012) [3]. The onion is usually eaten as raw by many people in the form of food like salads and soups. Presently, India is the largest producer of onions in the world. The onion is produced in many States of India, such as Maharashtra, Rajasthan, Haryana, Gujarat, Karnataka, Telangana, Madhya Pradesh etc. In Maharashtra, Nasik city is very famous for growing onions that supplies onion to other cities of Maharashtra.

Aspergillus niger is a ubiquitously prevalent soil borne fungus (Pal and Matsusaka, 1999; Pal, 2007) [20, 16] that can cause black mould rot in onion, which is the main pre-harvest and post-harvest disease (Wani and Taskeen, 2011) [22]. It is implicated in the etiology of onychomycosis (Pal *et al.*, 2002) [17], dermatitis (Pal, 2007; Pal and Kerosa, 2020) [16, 19], otomycosis (Kurnatowski and Kilipiak, 2001; Mishra *et al.*, 2004) [8, 10], oculomycosis (Pal, 2007) [16], and pneumonia in the susceptible individuals (Kierownik, 1990; Nagakawa *et al.*, 1999) [7, 11]. *Aspergillus niger* can also cause otitis in dogs (Pal, 1982) and abortion in buffalo (Pal, 1988) [13]. In addition, it produces several mycotoxins like aflatoxin, ochratoxin A, fumonisin, and 3-nitropropionic acid (Frisvad *et al.*, 2011) [6]. These toxins are known to produce acute to chronic toxicity in humans and animals (Schuster *et al.*, 2002) [21]. The ingestion of *A. niger* contaminated raw onion in salads and soups give an opportunity to the mycotoxins in the body. An experiment conducted by Devi and Rajini (2021) [5] indicated that eugenol fumigation checked the germination of conidia and mycelial growth of *A. niger*. It is important to mention that eugenol fumigation can significantly control the incidence of black mould in onions during storage and also maintained post-harvest quality (Devi and Rajini, 2021) [5]. The objective of this study was to isolate and identify *Aspergillus niger* from onion obtained from city of Bharuch, Gujarat, India. In addition, the efficacy of APRM agar and Pal medium is also assessed for the recovery of *A. niger* from onions.

Materials and Methods

A total of 47 onions obtained from the vegetable markets and street vendors were examined for the presence of *A. niger*. Two sterilized cotton swabs moistened in 1% glucose and chloramphenicol (0.5 µg/ml) solution were used to swab surfaces of each onion.

One swab was then inoculated onto the plates of Pal medium (pulverized seed of sunflower 45 g, Agar 20 g, distilled water 1000 ml and chloramphenicol 100 mg) (Pal, 1997) [14] and another on APRM (Anubha, Pratibha, Raj and Mahendra) agar (Dave and Pal, 2015) [4]. This medium contained 4 g of dried marigold flower, 2.0 g agar, 50 mg of chloramphenicol, and 100 ml of distilled water. In order to avoid aerial contamination, cello-tape was applied on each inoculated plate. The plates were kept in the incubator at 30°C for one week, and examined daily for the fungal growth. The colonies appearing on each plate of Pal medium and APRM agar were subjected to examined gross and microscopical examination (McClenny, 2005; Pal, 2007) [9, 16]. The morphology of each fungal isolate was studied macroscopically by observing the colour and texture of the colony, and microscopically in Narayan stain (Pal, 2004) [15] for the vesicle, hyphae, conidia, conidiophores, and phialides. The composition of Narayan stain includes 0.5 ml of methylene blue (3% aqueous solution), 6 ml of dimethyl sulfoxide (DMSO) and 4 ml of glycerin (Pal, 2004) [15].

Results

Out of 47 swabs cultured from the same numbers of onion bulbs, only 21 showed the presence of fungal growth resembling to *Aspergillus*. The remaining plates were contaminated with fast growing environmental moulds/bacteria. The detailed macroscopic and microscopic features of fungal colonies developed from 21 onions could be identified as *A. niger*. The colonies grew on the plates of both inoculated media i.e. Pal medium and APRM agar. Initially, the fungal colonies appeared as white to yellow and then turning to dark brown to black coloured (Pal, 2007) [16]. Microscopic examination of fungal isolates in Narayan stain revealed typical conidiophores, septate hyphae, phialides, vesicles, and conidia morphologically resembling to *A. niger* (Pal, 2007) [16].

Discussion

Onion is one of the most widely consumed vegetables in the homes, restaurants, and hotels throughout the world. The isolation of *A. niger* from the onions goes parallel with the observations of earlier researchers who also recovered *A. niger* from the onions (Ara *et al.*, 2008; Chandraker *et al.*, 2014; Devi and Rajini, 2021) [1, 2, 5]. In the present study, the isolation of *A. niger* on Pal medium and APRM agar from 21 out of 47 raw onions is an important finding as this filamentous fungus is known to produce several mycotoxins (Frisvad *et al.*, 2011) [6], which can cause deleterious effects on human and animal health (Schuster *et al.*, 2002) [21]. It is, therefore, essential that onion must be carefully examined for black colour, and such onion should not be eaten raw. It is advised that house wives and other persons working in the kitchen should avoid any injury on the skin and nail as the spores of *A. niger* from onion can enter through minor trauma causing infections of the skin and nail. Furthermore, the conidia of *A. niger* can also be inhaled through respiratory tract causing pneumonia (Kierownik, 1990) [7].

The efficacy of APRM agar for the isolation of *A. niger* from soil, pigeon excreta, milk, ear swab, conjunctival swab, and nasal swab have been studied by Dave and Pal (2015) [4]. Pal medium was developed in 1980 for the rapid isolation and presumptive identification of *Cryptococcus neoformans* from clinical and environmental sources

(Pal, 1997; Pal, 2007). The isolation of *A. niger* from onion on APRM agar and Pal medium was attempted for the first time for the isolation of *A. niger* from onion. This findings indicate that both media can be used for the isolation of *A. niger* from onion and also from vegetables and fruits.

Conclusion

Aspergillus niger is attributed to cause otitis, nail infection, keratitis, dermatitis and pneumonia in the vulnerable subjects. The observation of the present study revealed that *Aspergillus niger* occurs naturally in the onions that are widely used as vegetable in kitchen. Interestingly, both media i.e. Pal medium and APRM agar supported the growth of *A. niger*, and therefore, these media, which are easy to prepare, stable at room temperature for about 7 days, and low cost compared to Sabouraud dextrose agar, can be routinely employed for the isolation of *A. niger* from environmental as well as clinical specimens. Furthermore, detailed morphology of fungal isolates can be easily studied in Narayan stain, which is cheaper than the other stains like lactophenol cotton blue stain. The persons who are dealing with onions are advised to take all necessary precautions to prevent the transmission of *A. niger* as this fungus has the potential to cause variety of infections in human beings.

Acknowledgements

Thanks are due to the laboratory staff for technical support. This paper is dedicated to all the scientists who did pioneer work in the field of *Aspergillus* and Aspergillosis.

Financial support

There was no financial support from any organization.

References

1. Ara MAM, Khatun ML, Ashrafuzzaman M. Fungi causing rots in onion at storage and market. Journal of Bangladesh Agricultural University. 2008;6(2):245-251.
2. Chandraker D, Sao S, Deshmukh YK, Verma L, Sahu PK. Isolation of *Aspergillus niger* from *Allium cepa* bulb and production of citric acid from it. Indian Journal of Pharma and Bio Sciences. 2014;5(1):144-147.
3. Corzo-Martínez M, Villamiel M. An overview on bioactivity of onion. Onion Consumption and Health. 1^a Ed Nueva York: Nova Science Publishers Inc.; c2012. p. 1-48.
4. Dave P, Pal M. New medium APRM for isolation of medically important fungi from clinical and environmental samples. International Journal of Livestock Research. 2015;5:10-18.
5. Devi SS, Rajini P. First report of post-harvest management of black mold of onion by eugenol. South Asian Experimental Biology. 2021;11(1):759-767.
6. Frisvad JC, Larsen TO, Thrane U, Meijer M, Varga J, Samson RA, *et al.* Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. PLoS one. 2011;6:e23496.
7. Kierownik. Pulmonary aspergillosis caused by *Aspergillus niger*. Pneumonol. Pol. 1990;58:328-333.
8. Kurnatowski P, Kilipiak A. Otomycosis: Prevalence, clinical symptoms, therapeutic procedure. Mycoses. 2001;45:472-479.
9. McClenny. Laboratory detection and identification of

- Aspergillus* species by microscopic observation and culture: The traditional approach. *Medical Mycology*. 2005;43:125-128.
10. Mishra GS, Mehta N, Pal M. Chronic bilateral otomycosis caused by *Aspergillus niger*. *Mycoses*. 2004;47:82-84.
 11. Nakagawa Y, Shimazu K, Ebihara M, Nakagawa K. *Aspergillus niger* pneumonia with fatal pulmonary oxalosis. *Journal of Infections Chemotherapy*. 1999;5:97-100.
 12. Pal M. Isolation of *Aspergillus niger* from otitis in a dog. *Veterinary Research Journal*. 1982;5:62-63.
 13. Pal M. *Aspergillus niger* associated with mycotic abortion in a buffalo (*Bubalus bubalis*). *Mycoses*; c1988. p. 17-19.
 14. Pal M. Use of Pal's sunflower seed medium for an early diagnosis of cryptococcosis. *The Antiseptic*. 1997;95:175.
 15. Pal M. Efficacy of Narayan stain for morphological studies of moulds, yeasts and algae. *Revista Iberoamericana De Micologia*. 2004;21:218.
 16. Pal M. *Veterinary and Medical Mycology*. First Edition. Indian Council of Agricultural Research, New Delhi, India; c2007.
 17. Pal M, Dave PS, Ahmed K. Onychomycosis caused by *Aspergillus niger*. *The Antiseptic*. 2002;99:363-364.
 18. Pal M, Dave P, Dave K, Gutama KP, Thangavelu L, Paula CR, *et al*. Etiology, clinical spectrum, epidemiology, new developments in diagnosis and therapeutic management of onychomycosis: An update. *American Journal of Microbiological Research*. 2022;11(1):19-24.
 19. Pal M, Kerorsa GB. Growing significance of non-dermatophytic fungi in cutaneous disorders of humans and animals. *Journal of Microbiology, Immunology, and Biotechnology*. 2020;7:27-32.
 20. Pal M, Matsusaka N. Studies into prevalence of pathogenic fungi in wildlife environment. *Verh. ber. Erkr. Zootier*. 1999;32:143-146.
 21. Schuster E, Dunn-Coleman N, Frisvad J, Van Dijck P. On the safety of *Aspergillus niger* - A review. *Applied Microbiology and Biotechnology*. 2002;59:426-435
 22. Wani AH, Taskeen UN. Management of black mould rot of onion. *Journal of Mycology and Plant Pathology*. 2011;9:43-49.

How to Cite This Article

Pal M. Isolation and identification of *Aspergillus niger* from the onions in Bharuch, Gujarat, India. *Journal of Advances in Microbiology Research*. 2024;5(1):178-180.

Creative Commons (CC) License

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.