

Studies on Microbial Contaminants Associated with Arecanut Leaf Sheath Plates

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Abstract

Areca is being grown in an area of 2.71 lakh ha in Karnataka and contributes 65 per cent area to the country. Areca sheds the leaf sheath and they are widely used for consumption and packing of food in various occasions. The survey was conducted to know the microbial contaminants associated with leaf sheath plates and during the survey visited to 30 plates manufacturing units situated in Shivamogga and Davanagere districts in Karnataka. Randomly collected areca plates were used for laboratory study to know the contaminants. From these, fungal and bacterial contaminants were isolated and identified by cultural, morphological and biochemical studies. The result revealed that *Rhizopus stolonifer*, *Penicillium citrinum*, *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma harzianum* and prevalence of *Pseudomonas* sp. (32×10^6 cfu/ml), *Bacillus* sp. (19×10^6 cfu/ml), *Micrococcus* sp. (12×10^6 cfu/ml) and *E. coli* (4×10^6 cfu/ml) were observed in contaminated plates under laboratory studies. The study concluded that the prevalence of fungi and bacteria may cause deleterious effects on human health is a chance factor. In future, it is required to concentrate on the sterilizing process which may prevent the health hazardous on human beings.

Keywords: Areca sheath plate, contaminants, Human health

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INTRODUCTION

Areca nut palm (*Areca catechu* L.) leaf sheath sheds naturally which have been used as an ecofriendly food serving and filling material for centuries (Kalita et al)¹. Nowadays with modern technology, this natural and rigid material is compressed into different shapes and used to serve and pack food. The micro enterprise units manufacturing plates from areca leaf sheath are concentrated in major areca nut growing districts in Karnataka viz., Shivamogga, Davanagere, Chikkamagalur, Chitradurga, Dakshina Kannada, Udupi and Uttar Kannada. Leaf Sheath goes waste if not properly utilized for making usable commodities which may helps to small and marginal entrepreneurs for better prize (Adivappar et al)². The prevalence of contaminants in leaf materials may cause food spoilage and also results in reduction in quality (Omidbeygi et al)³. Some of the fungal species are toxic and may produce mycotoxins in food packing and serving material cause a hazard to human health.

MATERIAL AND METHODS

In present investigation, contaminated plates were randomly collected from 30 plate making units from different places of Shivamogga and Davanagere districts of Karnataka. The main objective of investigation is to find out the contaminants which are present in the plates under in vitro. Contaminated leaf plate samples were chopped into small pieces and it was aseptically surface sterilized by immersing in sodium hypochlorite (1 %) for 30 seconds then poured in a conical flask containing sterile distilled water.

Isolation of bacterial contaminants

The procedure was followed as per serial dilution technique and pour plate procedure

were followed to identify the bacterial colonies then plates were kept at 27°C for 24 to 36 hrs. After incubation, the colonies on nutrient agar plate were counted from different dilutions such as 10⁻⁶, 10⁻⁷. Bacterial morphology was studied microscopically by Gram staining technique and recognized by following biochemical tests.

Biochemical characterization of bacteria

Biochemical tests viz., Gram reaction, KOH solubility, catalase test, starch hydrolysis, gelatine liquefaction, H₂S production, protein digestion, ammonia production, methyl red tests were carried out as per the methods described in laboratory guide for Identification of Bacteria (Schaad)⁴.

Isolation of fungal contaminanats

The samples were taken into laboratory for surface sterilization then made into small pieces and kept for incubation at 27°C for three days. Fungi which are present in the sample were cultured on PDA plates and observed under microscope.

Cultural and morphological characterization of fungi

The randomly collected areca leaf sheath plates were used for microbial studies. A 5 mm diameter disc of sterile cork borer was used to cut the vigorously growing edge of a fresh culture then placed on Petri plates containing medium (PDA). The set of cultured Petri dishes were incubated at 27°C then fungal growth was examined. For morphological studies isolated fungal culture slides were prepared then observed under microscope.

RESULTS

Survey was conducted to know the microbial contaminants associated with leaf sheath plates and during the survey visited



Fig. 1 & 2. Manufacturing units drying the leaf sheath and plates in open condition

to 30 plates manufacturing units situated in Shivamogga and Davanagere districts in Karnataka (Fig. 1, 2). In this study, the contaminated areca

leaf plate (Fig. 3) was used for microbial analysis under laboratory condition. Prevalence of fungal contaminants *Rhizopus stolonifer*, *Penicillium*

Table 1. Prevalence of fungal contaminants in areca leaf sheath plate

Sl.No.	Fungal contaminants	Culture	References
1	<i>Rhizopus stolonifer</i>	Dense cottony growth first white then become grey or yellowish brown with spore production	Dolatabadi et al ⁹
2	<i>Penicillium citrinum</i>	Shades of green, sometimes white	Visagie et al ¹⁰
3	<i>Aspergillus niger</i>	Black cottony growth	Gams et. al ¹¹
4	<i>Aspergillus flavus</i>	Granular growth, bright to dark yellow-green	Hedayati et al ¹²
5	<i>Trichoderma harzianum</i>	Initially white and downy, later it developing deep green compact mass, look like concentric ring-like zones	Sandoval-denis et al ¹³



Fig. 3. Contaminated sample

citrinum, *Aspergillus niger*, *A. flavus*, *Trichoderma harzianum* were grown in the culture plate (Table 1), among the fungal contaminants most of the samples were contaminated with *Aspergillus niger* (Fig. 4,5) and *A. flavus* (Fig. 6,7). Biochemical characters to determine the colonies of bacteria among that highest prevalence were observed in *Pseudomonas* sp. (32×10^{-6} cfu/ml) followed by *Bacillus* sp. (19×10^{-6} cfu/ml), *Micrococcus* sp. (12×10^{-6} cfu/ml) and *E. coli* (4×10^{-6} cfu/ml) and following were given in Table 2.

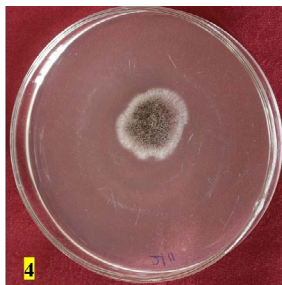


Fig. 4 & 5. Cultural and morphological characteristics of *Aspergillus niger*

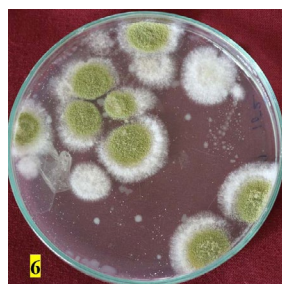


Fig. 6 & 7. Cultural and morphological characteristics of *A. flavus*

Table 2. Prevalence of bacterial contaminants in areca leaf sheath plate

SL.No.	Prevalent contaminants	Bacterial count (10 ⁻⁶ cfu/ml)	Colony characters
1.	<i>Bacillus</i> sp.	19	Colonies are rough, opaque, fuzzy white or slightly yellow with irregular edges and circular in shape. Gram positive
2.	<i>Micrococcus</i> sp.	12	Colonies are circular, yellow, convex and smooth. Gram positive
3.	<i>Pseudomonas</i> sp.	32	Colonies are large, opaque, flat colonies with irregular margins and distinctively fruity odour. Gram negative
4.	<i>E. coli</i>	4	Off-white or beige in color with a shiny texture slightly raised colonies. Gram negative

DISCUSSION

Areca leaf plates were used for microbial analysis under laboratory condition. Biochemical characters determine the colonies of bacteria among that highest prevalence was observed in *Pseudomonas* sp. (32x10⁻⁶ cfu/ml) followed by *Bacillus* sp. (19x10⁻⁶ cfu/ml), *Micrococcus* sp. (12x10⁻⁶ cfu/ml) and *E. coli* (4x10⁻⁶ cfu/ml). Similar studies were carried out by Janaki et al⁵. reported that areca leaf sheath plates were easily get contaminated by bacteria when compared to other microbes. In case of fungal contaminants prevalence of *Rhizopus stolonifer*, *Penicillium citrinum*, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harzianum* were grown in the culture plate, Matan et al⁶ also identified mold growth on areca leaf sheath such as *Mucor dimorphosporus*, *Penicillium* sp., *Aspergillus niger* and *Rhizopus* sp. etc. The contaminants are usually attached on the surface of plates after the process of manufacture in unit under unhygienic condition was resulted the molds could be observed on plates within a week (Nitimongkonchi)⁷. *Lyratzopoulos* et al⁸, reported that several species of *Penicillium* are common contaminants on diverse substrates and are called as potential mycotoxin producers. Identification of *Penicillium* contamination in food is more important where human pathogenic species are rare, however in favorable condition infections leading to mycotic keratitis, otomycosis and endocarditis. So there is a need to concentrate on sterilization techniques like UV irradiation treatment to avoid any serious health issue on human beings by using different by products of arecanut leaf sheath viz., plates, cups and food packing materials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors listed have made substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Kalita P, Dixit US, Mahanta P, Saha UK. A novel energy efficient machine for plate manufacturing from areca palm leaf sheath. *J. Sci. Indu. Res.* 2008; 67:807-811.
2. Adivappan N, Hanumanthaswamy BC, Shashidhara KC. Conversion of Areca leaf sheath into eco-friendly plates: A case study of remunerative micro enterprise. *Inter. J. Trade & Global Bus. Pers.* 2014;1(3):2319-9059.
3. Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. *Food Control.* 2007;18:

- 1518–1523. doi: 10.1016/j.foodcont.2006.12.003
4. Schaad NW. Laboratory guide for the identification of plant pathogenic bacteria, 2nd Ed. *American Phytopathol. Soc.* 1992:138pp.
5. Janaki S, Thenmozhi S, Mythili KK, Janani R. Bactericidal effect of various sterilizing sources on bacteria isolated from contaminated areca leaf plates in Tamil Nadu, *J. Biol. Chem. Res.* 2018;35(1):103-108.
6. Matan N, Warasri S, Nirundorn Matan. Antifungal activities of essential oils applied by dip-treatment on areca palm leaf sheath and persistence of their potency upon storage. *Inter. Biodeterioration and Biodegradation.* 2011;65:212-216. doi: 10.1016/j.ibiod.2010.09.009
7. Nitimongkonchi N. Utilization of cinnamon oil as antifungi in durian Paste, M.Sc. Thesis, Graduate School, Kasetsart University, Thailand 1999.
8. Lyratzopoulos G, Ellis M, Nerringer R. Invasive infection due to *Penicillium* species other than *P. Marneffeii*. *J. Infect.* 2002;45(3):184-195. doi: 10.1053/j.jinf.2002.1056
9. Dolatabadi S, Walther G, Ende AHGG, GS De Hoog. Diversity and delimitation of *Rhizopus microsporus*. *Fung. Divers.* 2014;64: 145-163. doi: 10.1007/s13225-013-0229-6
10. Visagie CM, Houbraken J, Frisvad J.C, et al. Identification and nomenclature of the genus *Penicillium*. *Stud. Mycol.* 2014;78: 343-371. doi: 10.1016/j.simyco.2014.09.001
11. Gams W, Christensen M, Onions AH. Infrageneric taxa of *Aspergillus*. In advances in *Penicillium* and *Aspergillus* systematics. Samson RA, Pitt JI, eds. New York: Plenum Press: 1985:55-62. doi: 10.1007/978-1-4757-1856-0_5
12. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiol.* 2007;153:1677-1692. doi: 10.1099/mic.0.2007/007641-0
13. Sandoval-Denis M, Sutton DA, Cano-Lira JF, et al. Phylogeny of the clinically relevant species of the emerging fungus *Trichoderma* and their antifungal susceptibilities. *J. Clin. Microbiol.* 2014;52:2112-2125.