

Screening of Post- Harvest Disease Caused by *Aspergillus flavus* in Coconut, *Cocos nucifera*

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ABSTRACT

Coconut (Cocosnucifera) is a significant green manor crop that assumes a significant part in the monetary, social and refined exercises of individuals of India. The current investigation is an endeavor to appraise the misfortunes because of post collect and capacity decay in coconut under various capacity conditions in East Godavari area of Andhra Pradesh, India. For compelling administration of different post gather infections and to have clear comprehension on the *Aspergillusflavus*, it was screened, secluded and described till the atomic level.

Keywords:Coconut, *A. flavus*, Post-harvest loss

Mathematics Subject Classification : 62P10

Journal of Economic Literature (JEL) Classification Number: Q56, Q57

1. INTRODUCTION

Coconut (*Cocosnucifera* Linn.) is the most financially significant types of the palm family Arecaceae (Jonathan et al 2012), in the agrarian economy of numerous countries around the world (Nair et al 2003). The coconut is found in numerous pieces of the world wherein, Indonesia, the Philippines, and India are the three driving makers followed by Brazil (Abiva 2011, IBGE 2015). Different types of microorganisms, growths infections and different vermin assault the plants at various phases and lessens their usefulness promoting colossal food and monetary misfortune to man. A very little is known about the species of main fungal pathogens that cause the postharvest diseases. The diseases caused by fungal pathogens in harvested fresh fruits are considered as one of the most serious losses of production at the postharvest and consumption levels (Karabulut et al 2002, Vitoratos et al 2013, Spadaro and Droby, 2016). The main worldwide postharvest diseases caused by fungi are brown rot caused by *Moniliniafructicola* or *M. laxa*, *Rhizopus*; rot caused by *R. stolonifer*; grey mold caused by *Botrytis cinerea* (Arrebola et al 2010), and other economically important fungal diseases caused by *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp. and *Aspergillus* spp. (Usall et al, 2013, Usall et al, 2015, Mari et al 2016). The longer the storage conditions expand the phytosanitary issues and postharvest stem-end decay on organic products brought about by the

growth *Lasiodiplodiatheobromae* (Pat.). It spreads promptly through the dirt by root contact among palms and most likely via air-borne basidiospores (Jonathan et al 2012). The coconut bole decay infection is brought about by the growth of *Ceratocystisparadoxa* (Ojomo et al 2010). Diagrammatic scales are vital for influence the epidemiological comprehension of way systems (Vivas et al 2010, Lima et al 2011). Diagrammatic scales additionally contribute for surveying the forcefulness of the microorganism (Djocgoue et al 2010) among secludes from various geographic areas and their hosts (Dugan et al 2016); contribute for evaluating the productivity of items to be utilized in infectious prevention (Locatelli et al 2015), just as empower examination between this illness and different sorts of wounds brought about by different microbes *Ceratocystisparadoxa* (Dade) and nuisances (*AceriaguerreronisKeifer*) (Galvão et al 2008) that happen on the basal segment of coconut natural products. Parasitic growths cause the best contact concerning illness and yield creation misfortunes. The examination is pointed toward assessing the misfortunes because of post reap and capacity decay in coconut under various capacity conditions in East Godavari locale of Andhra Pradesh, India and to screen, segregate and portray the *Aspergillusflavus* till the sub-atomic level.

2. MATERIAL AND METHODS

2.1 Distribution of Post-harvest diseases of coconut in East Godavari district

Data was collected in the Konaseema region of East Godavari District of Andhra Pradesh. Copra tests were gathered from various dealers under differed capacity conditions. Ten samples were gathered from every dealer and a sum of 100 samples were gathered from ten brokers in Amalapurammandal of East Godavari District, as Amalapuram turns out to be the potential exchange place for copra Andhra Pradesh. Data was acquired from brokers with respect to their mindfulness on copra decaying growths and ensuing aflatoxin defilement. The gathered copra were cut into pieces of 1.0 cm size and surface sanitized in 0.1% mercuric chloride for 3 minutes, trailed by 3 washes in cleaned refined water prior to plating them on to potato dextrose agar plates. Ten pieces were kept up with in each plate and 100 pieces for every replication. The plates were then hatched for 4 days at 25°C and number and kind of contagious states got were recorded and specified by taking the normal of the relative multitude of three replications for every each.

2.2 Confinement of Mycoflora related with Copra

The gathered copra tests were cut into little pieces of one centimeter size. These samples were surface disinfected with 0.1% mercuric chloride arrangement followed by three washings with sterile refined water. The pieces were set on PDA and brooded at 35°C. The mycoflora related with copra tests were recorded occasionally.

2.3 Seclusion and Purification of *Aspergillusflavus*. Thom and Raper

The copra gathered during the survey period was cut into little pieces of 0.1 cm size and surface sanitized with 0.1 percent mercuric chloride for a moment. Then, at that point copra pieces were washed completely for multiple times by changing refined water threefold $\pm$ and plated on potato dextrose agar and brooded at $29 \pm 10^\circ\text{C}$ temperatures for time of 3 days.

The way of life in this way got was seen under compound magnifying instrument and decontaminated by single hyphal tip technique (Florian 1996).

2.4 Recognizable proof of *Aspergillus flavus*

The disengage *Aspergillus* was recognized to the species level dependent on the mycelial and social characters with the assistance of standard portrayals (Martin et al 1983).

2.5 Pathogenicity test

The microorganism assumed to be connected from the rottened copra was tried later for pathogenicity. At first, the microorganism was developed on potato dextrose agar (PDA) plate. Microbe spore suspension was ready by utilizing sterile refined water and showered on copra and brooded for the illness improvement. Untreated copra was kept up with at the same time as a control. The microbe was reisolated from the tainted copra and contrasted and unique culture and afterward kept up with by regular subculturing (Christensen et al 1988).

2.6 Effect of physical parameters on the growth of *A. flavus*

2.6.1 Impact of media on mycelial development of *A. flavus*

Eight millimeters measurement circles of multi day old societies of *A. flavus* were immunized in petri dishes containing Potato Dextrose Agar (PDA) and *Trichoderma* explicit media. The Petri plates were vaccinated at $29 \pm 10^{\circ}\text{C}$ for 3 days. The mycelial development of *A. flavus* on PDA and *Trichoderma* explicit media was recorded by periodical perception.

2.6.2 Impact of pH on mycelial development of *A. flavus*

Wide scope of pH viz., 4.5, 5.5, 6.5, 7.0, 7.5 and 8.5 were tried against *A. flavus* by changing pH of the PDA media prior to autoclaving by utilizing pH cushions and changing the ideal pH by utilizing 0.1 N HCl or 0.1N NaOH by and large. What's more, 8 mm measurement plates of multi day old societies of *A. flavus* were vaccinated on PDA plates at pH viz., 4.5, 5.5, 6.5, 7.0, 7.5 and 8.5 and brooded at $29 \pm 10^{\circ}\text{C}$ temperature. The impact of pH on mycelial development of *A. flavus* was recorded.

2.6.3 Impact of light on mycelial development of *A. flavus*

By taking 8 mm breadth circles of multi day old societies of *A. flavus* were immunized on the plates and presented to finish light (36h), substitute light (18h) and haziness (18h) and complete dimness (36h). Mycelial development of *A. flavus* was recorded following 3 days of brooding at $29 \pm 10^{\circ}\text{C}$ temperature.

2.6.4 Impact of temperature on mycelial development of *A. flavus*

By taking 8 mm measurement plates of multi day old societies of *A. flavus* was immunized on the plates and brooded at different temperatures viz., 25, 30, 35 and 40°C . Mycelial development of *A. flavus* was recorded following 3 days of brooding.

2.6.5 Examining Electron Microscopy of *A. flavus*

For Scanning Electron tiny investigations, multi day old mycelium of *A. flavus* was fixed in 6% cushioned glutaraldehyde followed by post obsession in Osmium tetroxide (5%) and afterward got dried out in expanding grouping of ethyl liquor. The examples were mounted on copper hits with twofold sided sticky tape, covered with gold polaron, AU/PD falter coater and examined in SEM (Jeol JSM 5600 Japan) and captured. The SEM examines were directed at Ruska labs, Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad.

2.7 Molecular identification

2.7.1 DNA confinement and enhancement of 18s RNA quality of *Aspergillusflavus* by PCR

The format genomic DNA from *Aspergillusflavus* was disengaged following the convention portrayed (Volossiuk et al 1995, Sambrook 1989). In Polymerase Chain Reaction, the particular groundworks Forward and Reverse (Institute of Biological Sciences, Vijayawada) were utilized to enhance the genomic succession of the open understanding edge (ORF) of the quality. PCR conditions were 94°C for 2 min, and afterward 94°C for 1 min, 60°C for 1 min, 72°C for 3 min for a sum of 30 cycles, with the augmentation at 72°C for 10 min.

2.7.2 Groundwork plan

The preliminary grouping addressing *Aspergillus* species were recovered from GenBank (National Center for Biotechnology data; <http://www.ncbi.nlm.nih.gov>) adjusted utilizing Claustal X (Tsai and Olson 1992), and the *Aspergillus* class explicit preliminaries (forward: 5' - TTTTTCCTT-3' and opposite: 5'- TTTCTCCGC - 3') were planned dependent on the homologous areas explicit to *Aspergillus* family.

2.7.3 Agarose gel electrophoresis

Required measure of agarose (w/v) was gauged and softened in 1X TBE support (0.9M Tris-borate, 0.002 M EDTA, pH 8.2). Then, at that point, 1-2 µl ethidium bromide was added from the stock (10 mg/ml). Subsequent to cooling, the combination was filled a projecting plate with a suitable brush. The brush was eliminated after cementing and the gel was set in an electrophoresis chamber containing 1X TBE cradle. The items were blended in with 6X stacking support (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water) at 5:1 proportions and stacked into the well. Electrophoresis was done at 60V (Cole et al 2003).

2.7.4 Eluting DNA from agarosegel sections

Ethidium bromide stained agarose gel was pictured under a transilluminator. The part of interest was extracted with a spotless extremely sharp edge. Subsequent to eliminating the overabundance fluid, the agarose piece was set in the twist segment. The cylinder was centrifuged at 5500 rpm for not over

45 seconds for the elution of DNA. The eluent was checked by running on an agarose gel and saw on a transilluminator for the presence of ethidium bromide stained DNA. The eluted DNA was utilized straightforwardly in control responses. This DNA division was oppressed for sequencing (Institute of Biological Sciences, Vijayawada).

2.7.5 Sequencing and fabrication checking

The eluted PCR item was straightforwardly sequenced utilizing *Aspergillus* variety explicit preliminaries without GC-clip at Institute of Biological Sciences, Vijayawada. Sequencing responses were completed with ABI PRISM Dye Terminator Cycle Sequence Ready Reaction Kit (Applied Biosystems Inc., USA). All arrangements displaying fewer than 95% grouping similitude to existing successions in GenBank were checked utilizing CHIMERA-CHECK program at the Ribosomal Database Project (RDP) utilizing default settings (Altschul et al 1997). All agent groupings comparing to groups were *Aspergillus* species.

3. RESULTS AND DISCUSSION

3.1 Distribution of Post-harvest diseases of coconut in East Godavari District

The market review was led during the year 2005-2006 in different business sectors of East Godavari region for deciding the power of sickness rate under changed capacity states of coconut (Table1). The sickness occurrence was around 5.0 percent in concrete godowns type stockpiling and it recorded to be around 20.0 percent in open stockpiling conditions. The mean Copra decay occurrence was discovered to be most elevated (20.0%) in outdoors type stockpiling conditions followed by cottage type stockpiling conditions (19.9%). Minimal frequency of Copra decay was recorded in concrete godowns type stockpiling conditions.

Table1. Distribution of Copra rot in East Godavari district of Andhra Pradesh

S.No.	Type of Storage	Percent Mean Disease incidence
1	Open storage	20
2	Hut storage	10
3	Cement Godown storage	5

3.2 Isolation of Mycoflora associated with Copra

Confinement considers visualized that *Aspergillus flavus*, *A. niger*, *Rhizopus* spp., *Drechslera* spp., *Botryodiplodia* spp., *Penicillium* spp. are the regularly related mycoflora on copra during capacity (Table.2; Fig.1). *Aspergillus flavus* was prevalent among the mycoflora with percent settlements going from 68 to 92 for every each example. This is trailed by *Penicillium* spp. with a scope of 61 to 69 percent states. *Aspergillus niger* was recorded to a tune of 46 to 64 percent provinces while different

types of *Aspergillus* were recorded to a degree of 47 to 57 percent. It's obviously true that copra put away under various dampness conditions show diverse mycoflora on their surface.

Table 2. Extent of fungal infection on copra collected from Traders

Fungus	Number of colonies /sample (%)
<i>Aspergillusflavus</i>	68-92
<i>Penicillium</i> spp	61-69
<i>Aspergillusniger</i>	46-64
<i>Aspergillus</i> spp	47-67
<i>Rhizopus</i> spp	33-44
<i>Drechslera</i> spp	20-31
<i>Botryodiplodiaspp</i>	11-18



Fig.1. Micoflora associated with copra

3.3 Isolation, Purification and Identification of *Aspergillusflavus*

Isolation of the microorganism was produced using the ailing copra tests gathered during study. The mycelia on minute assessment uncovered the presence of septate mycelium. The parasite related with the illness was segregated and sanitized by single agar media (PDA). On the side of this, announced that Copra quality is discovered to be decreased by numerous organisms and significant of them appending Copra are, *Botryodiplodiatheobromae*, *Aspergillus* spp. what's more, *Rhizopusoryzae* (Rao et al 1971). When working on nature and degree of contagious contamination on Copra gathered from merchants of East Godavari locale, Andhra Pradesh, India, *Aspergillusflavus*

was prevalent among the mycoflora with percent provinces went from 68 to 92 percent for every each example and is trailed by *Penicillium* spp. whose reach was 61 to 69 percent settlements.

3.4 Pathogenicity test

The pathogenicity test directed by utilizing copra vaccination technique and percent disease was recorded by immunizing the Copra with *A. flavus* culture. Immunized copra showed spoiling with foul smell and states of *A. flavus* were noticed while the control didn't display any manifestations of contamination. The microbe was reisolated from contaminated copra tests and reinoculated on to new copra to build up Koch proposes to see the rate of the sickness by and by. Illness side effects were seen indeed and comparative indications were likewise recorded.

3.5 Effect of physical parameters on the growth of *A. flavus*

3.5.1 Impact of temperature on mycelial development of *A. flavus*

The mycelial development of *A. flavus* was discovered most extreme whose state breadth was 90 mm when developed on Potato Dextrose Agar plates under in vitro conditions at temperatures viz. 25°C, 30°C and 35°C. The mycelial development of *A. flavus* at a temperature of 40°C was moderate which was controlled by estimating the province width and discovered to be 75 mm. This demonstrates that the development was delayed above 40°C when contrasted and the typical encompassing temperature (Table 3).

Table3. Effect of media, pH, light and temperature on *A. flavus* under *in vitro* conditions

Cultural Characteristics	Variables	Mycelial growth of <i>A.flavus</i> after 3 days	
		Colony Diameter (mm)	Growth pattern
Media	Potato Dextrose Agar (PDA)	90	Normal growth
	<i>Trichoderma</i> specific media	90	Normal growth
pH	4.5	90	Normal growth
	5.5	90	Normal growth
	6.5	90	Normal growth
	7	90	Normal growth
	7.5	90	Normal growth
	8.5	90	Normal growth
Time of light exposure	Complete light (36h)	90	Normal growth
	Alternating light (18h) and darkness(18h)	90	Normal growth
	Complete darkness(36h)	90	Normal growth
Temperature	25°C	90	Normal growth
	30°C	90	Normal growth
	35°C	90	Normal growth
	40°C	75	Slow growth

3.5.2 Impact of media on mycelial development of *A. flavus*

The mycelial development of *A. flavus* was discovered most extreme on both potato dextrose agar and Trichoderma explicit media plates. This was finished up by deciding the development in plates and estimating the province width. The province breadth of the growth in both the plates was discovered to be 90 mm (Table 4).

3.5.3 Impact of pH on mycelial development of *A. flavus*

A. flavus was found to develop ordinarily at wide scope of pH viz. 4.5, 5.5, 6.5, 7.0, 7.5 and 8.5 under in vitro conditions on Potato Dextrose Agar plates (Table 3). There was no distinction in mycelial development in every one of the plates at the pH viz. 4.5, 5.5, 6.5, 7.0, 7.5 and 8.5 as the breadth of the provinces in every one of the plates was 90 mm. These discoveries close the presence of *A. flavus* in various soil states of Andhra Pradesh.

3.5.4 Impact of light on mycelial development of *A. flavus*

The force of light showed no impact on mycelial development of *A. flavus* on Potato Dextrose Agar plates under in vitro conditions (Table.3). The mycelium development was uniform when presented to finish light, substituting light and haziness and complete dimness where the width of the state was 90 mm. This affirms that the growth is a dirt borne microbe.

3.5.5 Scanning Electron Microscopy of *A. flavus*

Scanning electron microscopy examines uncovered the atomic mycelial characters of *A. flavus* (Fig.2). The hyphae are all around grown, bountifully spread, septate and hyaline. The cells are multinucleate. The conidiophores are long and erect. Conidium bearing sterigmata are bottle formed. The conidia are globose and unicellular.



Fig.2. Scanning Electron microphotograph of *A. flavus*

3.5 Amplification of DNA coding for 18s r RNA

The genomic DNA of the organic entity screened was oppressed for the seclusion of the DNA coding for 18s rRNA by utilizing Polymerase chain response (Fig.3). The groups were cut and eluted and the DNA so acquired was oppressed for sequencing. The succession examination exhibited that every one of the comparing groups on agarose gel had a place with *Aspergillusflavus*.

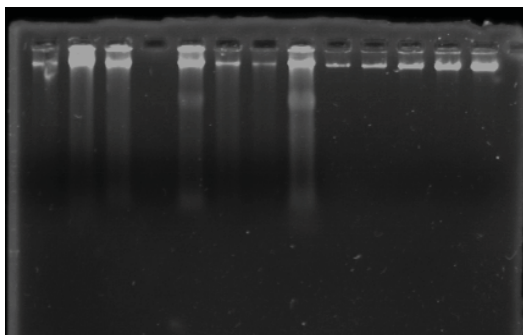


Fig.3. DNA Profile of *A.flavus* isolates

4. DISCUSSION

Aspergillusflavus is a saprophytic and pathogenic organism which sullies an assortment of practical yields (like peanuts and maize) with mycotoxins, causing gigantic financial misfortunes (Amaiike and Keller 2011, Lim et al 2014, Bhatnagar-Mathur et al 2015). The relationship of *Aspergillus flavus*, *Pencillium* spp. *Aspergillus niger*, *Rhizopus* spp., *Drechslera* spp. *Botryoelipsoidia* spp. other *Aspergillus* spp as the normal occupants of copra during surrounding stock piling states of Andhra Pradesh (Srinivasulu et al 2003). Studies led on nut decaying showed that spoiling was discovered to be 1% in un-husked nut and about 30% in de-husked nuts individually in customary godowns (Anon 2004). Copra containing 5% dampness and put away in gunny sacks fixed with polythene sheet was liberated from disease (Susamma and Menon 1983).

Studies directed on nut decaying demonstrated that spoiling was seen in the middle 1% to 30% in unhusked nuts and dehusked nuts individually under regular godowns (Anon 2004). The nut spoiling noticed most extreme during the long stretches of September to December and yield misfortune was recorded up to 10% of nuts because of decaying (Anon 2004). Review led comparable to microflora answerable for Copra decaying and conceivable aflatoxin defilement uncovered that microflora assumed an indispensable part in spoiling of Copra (Srinivasulu et al 2003). From the assessment overview led among brokers, obviously 72% of the dealers are uninformed of the aflatoxin tainting of Copra and then again, the excess (28%) merchants credited the harshness in taste of Copra might be because of aflatoxin pollution because of molds (Srinivasulu et al 2003). Under high dampness conditions, *Aspergillus* spp. quickly infiltrate into the copra meat causing it's staining, along these lines brings about an expansion in free unsaturated fat levels. Then again, copra put away at 5%

dampness levels remained reasonably liberated from disease (Susamma and Menon 1983). Storm periods favor the advancement of parasitic populace in copra (Susamma 1980). Numerous growths have been found to assault copra in this manner decaying its quality and hence exchange *Aspergillusniger*, *A.glaucus*, *A.flavus*, *A.fumigatus* and *A.ochraceous*, *Ceratostomellaadiposum* (Thompson 1933), *Botryodiplodiatheobromae*, *Aspergillus* spp. what's more, *Rhizopusoryzae* (Rao et al 1971) and *Penicilliumfrequentas* (Nair and Nathan 1971) are the regularly related mycoflora on copra under capacity conditions. In the current examination, the commonness of *Aspergillusflavus*, the lone aflatoxin delivering parasite in all the copra tests under investigation is of concern. Itemized examinations are vital in contemplating the mycoparasitic collaborations between the related mycoflora and misusing the possibility of the conceivable adversary to *A. flavus* assuming any, to lessen the development and increase of this aflatoxin delivering parasite. The evil impacts of devouring aflatoxin polluted cake and oil has been basically seen in poultry and milch steers (Bhat et al 1978). It is even perceived that when creatures devour feed sullied with aflatoxins, the poison is used in the body and potentially get into the milk as aflatoxin M1, or might be found in creature items like meat, eggs and so on, which are devoured by man. Along these lines, developing mindfulness among brokers, retailers' seeing aflatoxin pollution as a negative normal property asset is fundamental. Severe authoritative measures must be upheld to make the dealers store their gathered copra under legitimate conditions subsequently keeping the decay from these aflatoxin-creating molds.

5. CONCLUSION

Isolation of the infectious agent was made of the unhealthy coconut samples and therefore the microscopic examination discovered the presence of septate mycelium. The fungus related to the copra rot was isolated and sublimated by single hyphal tip methodology and plated on potato glucose agar media. *Aspergillus flavus* was predominant among the mycoflora whose percentage colonies ranged from 68 to 92 and to a lesser extent, the incidence was noticed by *Pencillium* spp. whose range is in between 61 to 69% colonies. These observations were recorded while working on morphological characterization of the fungi and extent of fungal infection on copra collected from traders. The mycelial growth of *A. flavus* at temperature of 40°C was slow which was determined by measuring the colony diameter and found to be 75 mm. This indicates that the growth was slow above 40°C when compared with the normal ambient temperatures in between 25- 35°C. The mycelial growth of *A. flavus* was found maximum and similar on both potato dextrose agar and *Trichoderma* specific media. These findings indicate the existence of *A. flavus* in numerous soil conditions of Andhra Pradesh. The mycelial growth was uniform when exposed to complete light, alternating light and darkness and complete darkness where the diameter of the colony was 90 mm. This indicates that the fungus is a soil borne pathogen.

Scanning microscopy studies discovered the molecular mycelial characters of *A. flavus*. The hyphae are well developed, abundantly branched, septate, and hyaline. The cells are multinucleate. The conidiophores are long and erect. Conidium-bearing sterigmata are bottle-shaped. The conidia are

globose and unicellular. Inoculated copra showed rotting with foul smell and colonies of *A. flavus* were discovered whereas there were no symptoms in the control. The pathogen was reisolated from infected Copra sample and was reinoculated on to fresh Copra and the disease was once again noticed. Mycoflora related with copra during inappropriate capacity assume a significant part in the misfortunes, accordingly influencing the economy and furthermore human wellbeing. In the current examination, an endeavor was made to assess the misfortunes because of post reap and capacity decay in coconut under various capacity conditions in East Godavari locale of Andhra Pradesh, India. For powerful administration of different post collect sicknesses and to have clear comprehension on the different illnesses seen in various territories was assessed till the sub-atomic characterization using scanning electron microscopy and molecular screening.

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