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ISOLATION AND MAINTENANCE OF FUNGAL PATHOGENS *ASPERGILLUS NIGER* AND *ASPERGILLUS FLAVUS*

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ABSTRACT

In an Agriculture different plants are the source of organism (pathogen) host or colony are present in the seed, root, leaf, and stem on such part different species of fungi are found that of the one are Aspergillus. This pathogen causes diseases or do harmfull effect such reduction in crop yields, loss in germination percentage, development of plant disease, discoloration and shrivelling, biochemical change in seed. For example Soybean, Maize, Gram seeds mostly growth of Aspergillus are common .In this Investigation we collection different plant material with infected of fungus aspergillusspecies. Isolation of Aspergillus niger and Aspergillus flavus from seeds. Then after identification will done microscopic structure of spore the identified species of Aspergilus are Aspergillus niger and Aspergillus flavatus. Such Aspergillus niger and Aspergillus flavatus culture are made from the collected material .Then isolatation of fungal spciemens from seed then that grown on PDA medium .Then on potato dextrose agar medium are use as nutrient medium for the growth of the both species. Firstly from the potato dextrose agar slant are prepare in aseptic condition. Then some culture are add then see the growth of Aspergillus niger and Aspergillus flavatus. Then some patato dextrose agar are made on petriplate and we add after some time these spp shows growth and the growth of species are collected and preserved as well as maintained

***KEYWORDS**: Aspergillusniger, Aspergillusflavatus, Isolation, Cultured, PDA and Maintenance*

Article History

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INTRODUCTION

In an Agriculture different plants are the source of organism (pathogen) host or colony are present in the seed, root, leaf, stem on such part different species of fungi are found that of the one are *Aspergillus*. These pathogen causes diseases or do harmfull effect such reduction in crop yields, loss in germination percentage, development of plant disease discoloration and shriveling, biochemical change in seed. *Aspergillus niger* is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual although perfect forms (forms that reproduce sexually) have been found. *Aspergillus* are geographically widely distributed and have been observed in a broad range of habitats, because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation. The spores are wide spread, and are often associated with organic materials and soil. Introduction to history of mycology cambridge university press, Cambridge was identified A. flavus on the basis of morphology and growth pattern. (Ainsworth, G.C. 1976)The genus *Aspergillus* is usually defined as asexual saprophytic

fungi that are arranged in a globose head radiating from spherical conidiophores. It was based on general examination of mycelia and spore under microscope**.** *A.niger* is a fungus but it is specified as a mold. It is an ascomycetous fungus that produced microscopic spores inside elongated cells called asci. *A. niger* is known to reproduce through asexual spores only, no sexual reproduction as this microbe is an important host for production of various proteins and metabolites, it would be beneficial for strain improvement if there was an available sexual reproduction cycle. Aspergillusis one of the oldest named genera of fungi. *Aspergillus* had become one of the best known and most studied mould groups. *A. niger* was identified on the basis of morphology and growth. *Aspergillus niger* can produce a variety of fungal metabolites, termedmycotoxins depending upon growth condition and the strain of the organisms .The mycotoxins include oxalic acid crystals *Aspergillus niger* capable of producing several mycotoxin show ever, mycotoxin production appears to be controlled by the conditions of fermentation. *Aspergillus flavus* is a saprophytic and pathogenic fungus. The cultured A. solani on PDA medium for study of biology and pathogenicity on tomato plants (Tong-Yunhui et.al. 1994).With a cosmopolitan distribution it is best known for its colonisation of cereals grains, legumes & tree nuts. Postharvest rot typically develops during. Harvest storage and / or transit. *A.flavus*infections can occur while hosts are still in the field (preharvest) but often show no symptoms. Until postharvest storage and transport. In addition to causing preharvest**.***A. flavus*infection. Many strains produce significant quantities of toxic compounds known as mycotoxins. *A. flavus* is found globally as a saprophytic in soils and causes disease on many important agricultural crops common host of pathogen are cereal grains, legumes. Infection causes ear rot in corn and yellow mold in peanuts. Infection can be present in the field preharvest, post harvest, during storage and during transit it is common for the pathogen to originate while host crops are still in the field, however, symptoms and sign of the pathogen are often seen. *Aspergillus flavus* has the potential to infect seedlings by sporulation on injured seeds generally excessive moisture conditions and high temp of storage grains and legumes increase the occurrence of *A. flavus*aflotoxin production.

Aspergillus flavus colonies are commonly powdery masses of yellow green spores on the upper surface and reddish gold on the lower surface. In both grains and legumes infection is minimized to small areas and discoloration and dullness of affected areas is often seen growth is rapid and colonies appear downy or powdery in texture. Hyphal growth usually occurs by thread like branching and produced mycelia once, established the mycelium secretes derivative enzymes or proteins which cam break down complex nutrients. However conidia producing thick mycelia mats are often seen the conidiophores are asexual spores produced by *A.flavus* during reproduction. The conidiophores of *A. flavus* are rough and colorless recently, petromyces was identified as the sexual reproduce stage of *A. flavus* where the ascospores develop within sclerotial. *Aspergillus flavus* is unique in that it is a tolerant disease, so can survive at temperature that other diseases cannot *A. flavus* can contribute to the storage rots, especial when the plant material are stored at high moisture levels. A .*flavus* grows and thrives in hot and humid climates. *A. flavus* has a minimum growth temperature of 12^0C and a maximum growth temperature 48^oc *A. flavus* has rapid growth at 30-35^oc, slow growth at 12.15^oc and almost cases growth at 5-8 +/- ^{0}C The growth and sporulation of A.tenuis was sporse in Czapek'sdox synthetic medium, but was high in semi-synthetic and natural carrot leaf media.(Fencelli and Kimati 1990)*.A.flavus* growth occurs at different moisture levels for different crops for starchy cereals, growth occurs at 13.0-13.2% for soybean growth occurs at 11.5-11.8% for other crops growth occurs at 14%..The *cultural* characters for the *Aspergillus niger* and *Aspergillus flavus* were studies on the following solid media viz PDA [potato Dextrose Agar] media. Mximum growth of A. gomphrenae on both potato dextrose agar and Richard's agar (Joshi 1981). Potato dextrose agar and lima bean agar were the best media for growth and sporulation of A. solani. (Barksdale 1968). Among three media (MSTOM, V8 ,and PDA) tested, MSTO media was found

to be best for mycelial growth and sporulation of Colletorichum, and Alternaria species(Pria et.al, 1997).The induced sporulation of A. solani by growing the fungus on potato dextrose agar for 10-12 days. A. flavus human pathogen allergen and mycotoxin producer, microbiology .A flavus one of the major storage fungi found regularly in important cereals (Rands 1917) Potato Dextrose Agar[PDA media]. Several reports in the literature showed PDA as good medium for the growth and sporulation of A. niger and A.flavus (Bonde 1929, Neergaard 1945). Potato Dextrose Agar are common microbiological growth media from potato infusion, and dextrose potato dextrose agar (abbreviated PDA) it is most widely used media for growing fungi and bacteria which attack living plants or decaying dead plant matter. Potato Dextrose Agar is used for the cultivation of fungi. The isolation of A.citri grows mostly rapidly on PDA followed by yeast extract agar and Czapek'sDox agar (Cheema et.al. 1976). Maximum growth of A. solani was recorded on potato dextrose broth followed by Richard's medium, Czapek'sdox and oat medium (Mohapatra et.al.1977) Potato dextrose agar (PDA) is a general purpose media for yeast and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. The cultural variability of A. solani isolates on potato dextrose agar and classified them into four different cultural groups based on type of growth, colony colour of the substrate and growth rate (Kaul and Sexena 1988) It is recommended for plat count method for goods, dairy products and testing cosmetic.

MATERIAL AND METHOD

Material

Seed sample of Soybean, Maize, Gram was collected from Local farmers. Seed samples were then brought immediately to the laboratory for further study. Potato Dextrose Agar medium, were used for the cultural studies of the pathogen dextrose etc. were used during the experimentation. Petriplate, conical flask, culture tube, measuring cylinder, glass rod, beaker etc. Autoclave, laminar air flow, digital balance, Incubator etc.

Methods

Isolation of Pathogen

Pathogen was isolated by standard tissue isolation technique **(**Tatiana 2010).Infected Seeds of Soybean, Maize, Gram showing brown to dark leathery necrotic spots were collected from the susceptible. The infected seeds along with some healthy portions were surface sterilized using 0.1% mercuric chloride solution for 2 minutes. The bits were then washed thoroughly in sterilized distilled water for three times to remove traces $HgCl_2$. Further those bits were aseptically transferred to sterilized potato dextrose agar (PDA) plates, incubated at room temperature (27+2^oC) and observed periodically for fungal growth. Pure colonies which developed from the bits were transferred to PDA slants and incubated at (27+2^oC) for sporulation for 15 days.

Single Spore Isolation

Clear, filtered 10ml 2% water agar was poured in sterile petri-plates and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from15 day's old culture. 1 ml of such suspension was spread uniformly on agar. The growing hyphal tip portion was transferred to PDA slants with the help of a cork borer under aseptic conditions and incubated at (27+2^oC). Such cultured tubes were used for further studies.

Cultural Studies

The cultural characters for the pathogen were studied on the following solid media (Tuite, J. 1969) *viz*.200 grams of peeled

potatoes were cut into small pieces and boiled in 250 ml distilled water and then filtered to get the extract. 20 grams of agar powder was boiled separately in 100 ml distilled water. Both were mixed together and to it 20 grams of dextrose was added and final volume was made up to 1000 ml by distilled water.

Maintenance of Pure Culture of Pathogen on PDA Media

The fungus isolated from seeds was observed under research microscope for identification, on the basis of morphological characters. The fungus was sub-cultured on PDA slants and allowed to grow at (27+2^{o}C) for 15days. Such slants were preserved in a refrigerator at 5-10^{o}C and renewed once in a month. This pure culture was used for further study.

RESULT AND DISCUSSION

Standard tissue isolation technique was followed to obtain *Aspergillus niger* and *Aspergillus flavus* from PDA medium. The result of experiment will observe the growth of fungus on PDA medium was seen the first collection of culture from infected seed or plant material it will isolate as seen in Fig no Seed borne pathogen association with the first result of experiment is isolation. Isolation was repeated several times to obtain pure culture. After the isolation of culture of the inoculant Aspergillus we have to prepare media for the growth of inculum the aspergillus growth are mostly on PDA medium For that we have see by preparing the slant and see the growth of aspergillus Fig no 2 *Aspergillus niger* on PDA and Fig no 4 *Aspergillus flavus* on PDA we can see the slant of *Aspergillus niger* and *A. flavus* growth will occurs the next we see the Microscopic structure of *A. flavus and A. niger*in Fig 6 Spores of *Aspergillus niger* and Fig 3 Spores of *Aspergillus flavus.* The final result of growth both spp of aspergillus on PDA mediumon the petriplate we have see that in Fig: 5 *Aspergillus niger* on PDA and Fig: 7 *Aspergillus flavus* on PDA.

The description of the fungus & isolated is as follows. The *A. niger* showing large, dark brown conidial heads, which become radiate, conidiophores are smooth-walled, hyline or turning dark towards the vesicle. Conidial heads are globose to subglobose dark brown to black and rough walled. The description of this fungus agreed with the description gives for *Aspergillus niger* was identified on the basis of morphology and growth pattern, according to method recommended by Harrigen(1998).

The *A. flavus* conidia producing thick mycelia mats are often seen. The conidiophores are asexual spores producing by *A. flavus* during reproduction. The conidiophores of *A. flavus* are rough and colourless. Fungus agreed with description gives for *A. flavus* was identified on the basis of morphology and growth recommended by Ainsworth, G.C. (1976).

Figure 1: Seed Borne Pathogen Associated with Seed

Figure 2: Aspergillusniger on PDA

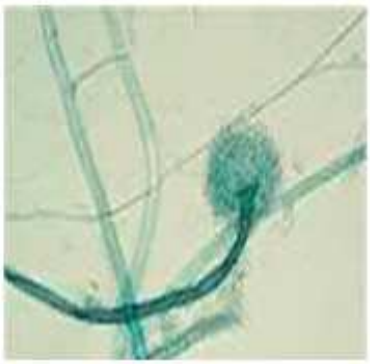

Figure 3: Spores of Aspergillusflavus

Figure 4: Aspergillusflavus on PDA

Figure 5: Aspergillusnigeron PDA

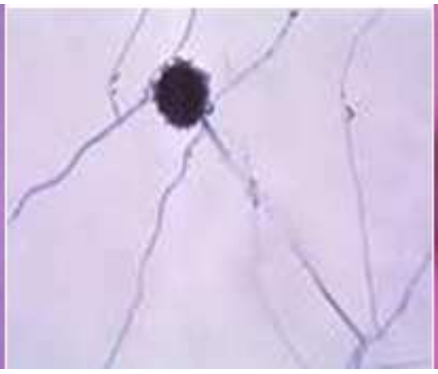

Figure 6: Spores of Aspergillusniger

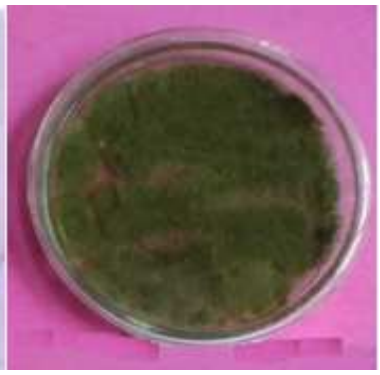

Figure 7: Aspergillusflavus on PDA

CONCLUSIONS

Aspergillus niger is conidial heads are large dark brown to black, conidia are globose to subglobose dark brown to black and rough walled. Growth on PDA media and cultural characteristics are often taken *A. niger* and *A. flavus* from solid media to study the growth and cultural characteristics. Among the PDA media tested for growth of *A. niger* and *A. flavus* the maximum growth was observed on PDA. After 9 day of inoculation which may be attributed to complex nature of natural media supporting good fungal growth. The solid PDA media used for *A.niger* and *A. flavus*, PDA showed good growth with excellent sporulation after 10 days of inoculation. The survivability of an organism depends on its capacity to survive in an adverse environment condition. Maximum growth of *A. niger* and *A. flavus* was obtained when exposed to alternated 12 hours light and 12 hours darkness. Morphological variability in colour of colony, colony growth and sporulation was observed of *A. niger* and *A. flavus*

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