STUDY OF MYCOFLORA ASSOCIATED WITH CITRUS *SINENSIS* L.

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A B S T R A C T

Fruits play a vital role in human nutrition, it contains vitamins and essential minerals that can help keep a good and normal health. They can play an important role in health and prevention of heart disease and cancer. Vitamins and minerals are used as nutritional food for many patients suffering from different ailments such as diabetes, constipations and stroke [11]. But such valuable fruits are attacked by various pathogens that influence the fruit economic value. Throughout the world about 20–25% of the harvested fruits are decayed by pathogens during post–harvest handling [3]. Microorganisms are associated, in a variety of ways with all the food we eat. At the time of transporting and handling there was chance to contamination of microorganism [9]. These microorganism alter the quality and quantity of our food materials such as fruits and vegetables. Particularly fungi play important role in this regard, so there has been a need to identify and isolate these associated fungi with fruit spoilage. Therefore there has been an increasing need to identify and isolate the fungi associated with the fruit spoilage. Hence, there is need to isolate and identify these microorganisms responsible for spoilage particularly fungi. The samples were surfaced sterilized with ethanol and the small pieces were cultured on Potato Dextrose Agar and incubated at room temperature for 7days at 28°C. The pure cultures were maintained, fungi were identified morphologically and microscopically. The investigation showed that the samples were infected with several fungal species. Some fungi are most pathogenic, some are saprophytic. The most predominant fungi isolated from sweet orange was *Aspergillus, Penicillium, Cladosporium* spp (40%), *Fusarium* spp (30%), *Alternaria* spp (20%) , *Rhizopus* and *Mucor* ..

***Keywords*: Isolation, identification, fungi, fruits spoilage**

INTRODUCTION:

Sweet orange (*Citrus sinensis*) is a evergreen small tree in 7.5m height Origin of Citrus sinensis is southern China, but is today grown commercially worldwide in tropical, semi–tropical and some warm temperate regions. Orange having evergreen leaves of different shapes, size from elliptical to oblong to oval, and wings on the petioles. White flowers either singly or in whorls, having fragrant smell. The fruit is sweet and juicy, in color from yellow to orange to red. Juice of fruit contains vitamins and having nutritional value. All cultivated citrus species grow very well in both tropical and subtropical parts of the world provided there is sufficient moisture and the temperature.. Citrus grows best under 1100 to 1500mm of rain per annum, distributed over nine months of the year. The optimum at 28°C temperature range plant showed good growth and requires a period of dry but cool weather for final ripening of the fruits.

Sweet orange is one of the important and major fruit crop. It contain vitamin C therefore it consumed both as fresh fruit or juice. Unfortunately it is known to be fruit attacked by several microorganisms; these microbes alter the fruit quality. Microorganism can be contaminated to fruit at any stage i.e. seedling, crop growth in the field during harvesting post harvest stage, storage and transporting. When fruit goes to spoil it is hazard to human and animals. Because the associated pathogenic fungi were produce secondary metabolites which referred as mycotoxins [6], [10]. Different fungi produce different toxins at different toxicity level [8],[13].

The objective of this study was to isolate and identify fungi associated with postharvest deteriorated fruits of sweet orange.

MATERIALS AND METHODS

The material and reagents used in the laboratory for this investigation includes; Potato dextrose agar (PDA), ethanol, Petri plates, conical flasks, cotton wool, lacto phenol cotton blue, sterilized knife, glass rod, test tubes, streptomycin, filter paper, microscope, Cork borer, slide and cover slip.

Collection of samples

Sweet orange fruits both fresh and spoilt were purchased from Jalna market. A total of ten fruits were purchased from three different points and transported to the laboratory in an ethanol sterile polythene bag for analysis.

Isolation of fungi

Isolation of the fungi was carried out as described by Baiyewu [1] . Small pieces (3–5cm) of tissues from the spoilt fruits was cut with sterile scalpel and placed on potato dextrose agar containing streptomycin (to prevent growth of bacteria) in petri plates and incubated at room temperature (28^0C) for 5 days. Isolated fungi were maintained in pure culture form for further study..

Identification of fungi

Identification of the fungi was done according to Fawole & Oso [5]. A drop of lacto phenol cotton blue was used for staining placed on a slide and then with help of needle; a small portion of the mycelium from the pure cultures of fungi was removed and placed in the drop of the stain. The mycelium was spread on the slide and a cover slip was put on it. The slide was obsered under the microscope. Morphological characteristics of the fungi such as type of hyphae and structure of conidia or spore were observed which was helpful for identification of fungi.

RESULT AND DISCUSSION

Table No. 01 Isolation of Mycoflora from *Citrus sinensis* on PDA and CZA

Name of Fungi	**Percentage incidence of fungi on PDA**	**Percentage incidence of fungi on CZA**
Alternaria Spp.	**20**	**10**
Aspergillus flavus	**50**	**40**
Aspergillus niger	**40**	**30**
Cladosporium spp	**40**	**20**
Fusarium oxysporum	**30**	**25**
Mucor spp	**30**	**25**
Penicillium chrysogenum	**40**	**20**
Rhizopus stolonifer	**30**	**30**

Table No. 02 Cultural and Morphological Characteristics of Fungi

Sr.No.	Name of fungi	Cultural and Morphological Characteristics of Fungi
1	*Alternaria Spp.*	Colonies olvaceous, black in colour.Conidia in short chains, ellipsoidal, tapering to abeak,8-12 transverse and 2-6 logitudinal septa.
2	*Aspergillus flavus*	Yellowish green colour colonies, powdery, septate and branched erect conidiophores, conidial heads radiating conidia globose to subglobose.
3	*Aspergillus niger*	Colonies black on development of conidia, powdery, septate and branched hyphae .spherical vesicle, swollen at the apex ,dark brown, conidia heads are biseriate, conidia catenatr, rough walled, dry, globose, covering entire vesicle.
4	*Cladosporium spp*	Play grey or grayish brown colour colonies growth pattern cottony, conidiophores micronematous, with swellings, distinctly nodose, plae brown in colour, smooth conidia, cylindrical or ellipsoidal, rounded at the tip.
5	*Fusarium oxysporum*	Colonies initially white, cottony and fast growing. Conidiophores branched bearing a whorl of phialids, microconidia hyaline, sickle shaped, multiseptate, and fusiform.
6	*Mucor spp*	Colonies white, aseptate hyphae with right angle branching, columellate sporangiophore singly from the mycelium. Without rhizoids, spherical sporangia, sporangiophore smooth walled, globose to ellipsoidal
7	*Penicillium chrysogenum*	Bluish green colour colony with white mycelium at center, fluffy. Velvety, Hyphae non septate, hyaline branched conidiophores, brush like ending in phialids, conidia smooth and basipetal.
8	*Rhizopus stolonifer*	Grey or dusty white colonies rapidly growing, fluffy, dense cottony mycelium, aseptate smooth sporangiophore in groups from stolons opposite to the rhizoids, sporangium colourless and turn black at maturity.


Photo plate No. 1
Alternaria alternata
Aspergillus flavus
Aspergillus niger
Fusarium oxysporum
Penicillium chrysogenium
Rhizopus stolenifer

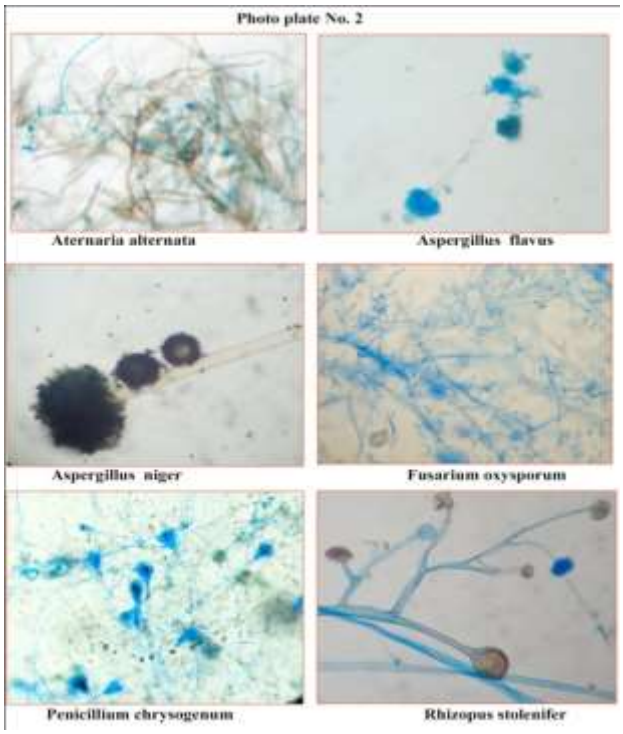
Photo plate No. 2
Aternaria alternata
Aspergillus flavus
Aspergillus niger
Fusarium oxysporum
Penicillium chrysogenum
Rhizopus stolenifer

This study shows (Table No.01 and Photoplate No. 1 and 2) that *Aspergillus, Penicillium, Fusarium* spp*, Cladosporium* spp*, Mucor* spp , *Rhizopus* spp and *Alternaria* spp were found in the spoilt sweet orange. Out of the fungi isolated from sweet orange, *Aspergillus Cladosporium* spp, and *Penicillium* spp have the highest frequency of 50% and 40% followed by *Fusarium* spp, *Rhizopus, Mucor* and *Alternaria* spp with 30% and 20% frequency of occurrence on PDA, somewhat similar observation was also seen on CZA. The characteristics symptoms originally observed were also noticed. All the organisms were successfully taking part in the decay and are thus confirmed as the causal organism of fruit decay [2], [12] .Thus, these fungi were also found to be associated with the deterioration of sweet orange. All the organisms isolated were confirmed to cause spoilage on the sweet orange in varying degrees. Of all the isolated fungi from sweet orange, *Aspergillus ,Cladosporium* spp and *Fusarium* spp were the most pathogenic (virulent) with rapid disintegration of the fruits in 7days having a rot .While the least pathogenic fungi was *Alternaria* spp and *Rhizopus* spp [7],[14]. Generally, fungi that cause spoilage are considered toxigenic or pathogenic. Some fungi may produce mycotoxins.[8] the fungi isolated in this study have been reported to produce secondary metabolites in plant tissues. These secondary metabolites are potentially harmful to humans and animals [4],[2]. A good example is aflatoxin which produced by *Aspergillus* has been implicated in cancer of the liver (heplatoma), aflatoxicosis.

CONCLUSION

In this study, *Aspergillus, Cladosporium* spp, *Fusarium* spp, *Penicillium* spp, Alternaria spp *Mucor spp* and *Rhizopu*s were detected in spoilt sweet orange. The presence of these fungi on sweet orange poses a serious threat to the health of consumers because these organisms could produce mycotoxins, which are harmful when consumed. Most of the fungi isolated were observed to be able to infect healthy orange fruits within a short time, which make a serious economic loss to sellers of these fruits. These fungi alter the quality of fruits. Result was also recorded from CZA medium but there was less frequency as compared to PDA. Therefore there is need to prevent the infection of such harmful fungi to minimize the loss [15].

ACKNOWLEDGEMENT

Authors are thankful to Principal of Lalbahadur Shastri Senior College Partur for providing all necessary facilities.

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