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FUNGAL ISOLATION AND CHARACTERIZATION FROM SPOILED VEGETABLES *LYCOPERSICON ESCULENTUM, BRASSICA OLERACEA, SPINACIA OLERACEA*

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Abstract:

This study investigated the micro organisms associated with the spoilage of vegetables of Tomato (Lycopersicon esculentum), Spinach (Spinacea oleracea), Cabbage (Brassica oleracea) obtained from local market of Haridwar. Five vegetable spoilage fungi were isolate & identified as follows Aspergilus, Fusarium, Penicilium, Trichoderma. In antifungal sensitivity test different antifungal drugs were used to test the susceptibility behavior as the isolated organism. Antifungal activity was determined by agar well diffusion technique. Penicillium show resistance to both antifungal resistance to griseofluvin & sensitive to fluconazole & inhibition zone was 30mm against fluconazole, gave highest inhibition zone against fluconazole that was 32nm. Trichoderma shows sensitivity to fluconazole & resistance to fluconazole, inhibition zone is 31nm. The need for microbial assessment of vegetables for production of salads and other use is also emphasized to reduce possible contamination.

Key words: *fungal, Lycopersicon esculentum , Brassica oleracea, Spinacea oleracea,*

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INTRODUCTION:

Vegetable nutrition has widely drawn the attention of fitness conscious as well as food scientists alike for their proven health benefits. Majority of day to day used vegetables are very low in calories per 100g. And again here is the long list of vegetables whose calorie is less them 20 per 10g such as bottle gourd , bitter melon, cabbage, Chinese cabbage, bok-choy, spinach, summer squash etc. Scientific studies have shown that low-calories but nutrient rich foods help the human body stay fit and diseases free. Vegetable, like fruits, are low in fat but contain good amounts of vitamins and minerals. All the Green, Yellow, Orange vegetables are rich source of calcium, magnesium, potassium, iron, beta-carotene, vitamin B complex, Vitamin C, vitamin A, and vitamin K. As in vegetables are home for many antioxidants that, firstly help protect the human body from oxidant stress, diseases and cancer, and secondly, help the body develop the capacity to fight against threes by boosting immunity. Additionally, vegetables are packed with soluble as well as insoluble dietary fibre known as non-starch polysaccharides (NSP) such as cellulose, mucilage, hemicellulose, gums, pectin etc.

It has been estimated that one-fourth of all produce harvested is spoiled before consumption Fungal spoilage of vegetable often result in water soaked, mushy areas while fungal rots of fleshy fruits such as apples and peaches frequently show brown or cream colored areas in which mold mycelia are growing in the tissue below the skin and aerial as “dry rot”. Vegetables are vital source of nutrient of human beings [1]. They give the body the necessary vitamin, fats, minerals and oil in the right proportion for human growth and development. Micro-organism are associated, in a variety of ways with all the food we eat. They may influence the quality availability, and quantity of our food. Naturally occurring food such as fruits and vegetables normally contain some micro-organism and may be contaminated with additional organism during handling. it is therefore necessary and important that both the farmer who harvest the fruits into bag for transportation, The marketers and consumers take necessary and appropriate precautions in preventing contamination and eating of contaminated fruits [2].

Food can serve as a medium for the growth of micro-organisms and as a result, transmit diseases. Numerous microbial defects [Signs and Symptoms] of agriculture crops are characterized by the types of micro-organism responsible for the deterioration; the process of infection in the case at fungal invasion follows the development of fungal penetrating structure. Spoilage microorganism exploits the host using extra cellular lytic enzyme that degrade these polymers to release water and plant’s other intracellular constituents for use as nutrients for their growth [3]. Some spoilage microbes are capable of colonizing and creating lessons on healthy,undamaged plant tissues[4].

The colonization process involves the ability of micro-organism (Fungi) establish itself with in the produce (host). This is initiated when fungi (following adhesion and release of enzymes) depolymerize certain specific cell wall polymers of the produce [5]. Susceptibility of vegetables is largely due to differential chemical composition such as pH and moisture contents. The higher pH (near neutrality) and moisture contents are associated with higher greater predisposition to fungal spoilage. The occurrence of fungal spoilage of vegetables is also recognized as a source of potential health hazard to man and animals [6]. The contamination of vegetables by fungi could also be as a result of poor handling practices in food supply chain storage condition, distribution (Effiuvwevewere BJO, 2000). Fungi affecting Tomatoes (*Lycopersicon esculentum*) include *Fusarium oxysporium* ,*Fusarium moniliforms*, *Aspergillus niger* and *Rhizopus stolonifer*. They are responsible for tomato soft rot . Result shows that *Rhizopus stolonifer* caused the greatest rot on tomato fruit. *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus nigrican*, *Curvularia lunata*, *Rhizopus oryzae*, *Fusarium equiseti* and *Fusarium moniliforme* were responsible for post harvested losses in Pawpaw (*Caricapaya*) in Southern Nigeria reported by Gupta. The principle of spread of fungal infection in fruits supports that a single infected orange can be the source of infection to other oranges during storage and on transit. Soil-infesting fungi that cause loss of fleshy tissue typically infect plants at the time of or just before harvesting. Infection may occur, however, during post harvested handling or storage. Common air molds such as *Penicillium* species may gain entry into the susceptible tissue and cause loss during packaging [7].

MATERIAL AND METHODS:

Sample collection: The sample of spoiled vegetable like tomato, spinach and cabbage were isolated from the local market of singdwar from haridwar. Isolation of fungi Carried out by following 2 methods.

1. **Serial dilution**

Labled sterile water blanks test tube with 1gm of spoilage in 10ml sterile distilled water. It was used as stock solution & then plating out low dilution 10^{-2} to 10^{-4} of sample on to SDA medium. The inoculated plates were then incubated at 28^{0}C for 5 days.

2. Leaf impression method

In impression method, firstly the spoiled tissue of samples were cut off and wash with the sterile distilled water, Then the spoiled tissue was put over the SDA medium with the help of forcep and incubated the plates at 28^0C for 5-6 days.

Characterization and identification of the fungi

The identification of fungi was done on the basis of morphology, colour shape, lactophenol cotton blue staining.

Antifungal sensitivity test

Agar well diffusion method used for the antifungal sensitivity test. Agar plates were prepared by dissolving Sabouraud's dextrose agar medium in sterilized water. 0.1ml of inoculum suspension was spreaded on the Sabouraud's agar medium. Using flamed sterile borer the medium was bored & the prepared antifungal solution of fluconazole & griseolfluvin 3mg/ml were taken. 0.5ml antifungal was added in each bore. The plates were left for diffusion for one hour in refrigerator & then incubated at 28^0C for inhibition zone.

RESULTS AND DISCUSSION:

In present study *Alternaria, Aspergillus, Fusarium, Penicillium and Trichoderma* were isolated from spoiled vegetables like tomato, spinach. Total fungal colonies were enumerated in the vegetable sample (Table1). In case of fungi, observation was taken after 5 days of incubation. The fungal isolates from the spoiled vegetables are shown in table 2. They were identified as *Fusarium, Penicillium, Trichoderma, Alternaria and Aspergillus* by fungal characteristics (Table 3).Colonies appeared after 5 days of incubation at 28^0C in Sabouraud's agar medium, were cottony,woolly, greenish black, whitish rey, whitish pink growth (Table 2).During the study of following results were obtained *Penicillium* resistance to fluconazole & griseofluvin. *Aspergillus* shows resistance to griseofluvin and sensitive to fluconazole. *Alternaria* shows sensitivity to fluconazole and resistance to griseofluvin. *Fusarium* also shows resistance to griseofluvin & sensitive to fluconazole. *Trichoderma* shows sensitivity to fluconazole and resistance to griseofluvin (Table4 and Fig 1: a,b,c,d,e,)

Table 1: Enumeration of Fungi in vegetable sample (Average of 3 replicates)

S.No.	Sample	Average No. of Colonics ± S.E.
1	Tomato	19± 1.73
2	Spinach	62±4.36
3	Cabbage	-

Table 2: Cultural Characteristic of isolated fungal species

S.No	Characteristic	*Alternaria*	*Aspergillus*	*Fusarium*	*Penicillium*	*Trichoderma*
1	Colour	Grayish Green	Greenish Blue, Black or Green colonies	White to Pink	Greenish or bluish colonies	White to green colonies
2	Shape		Conidiophore arising from a foot cell	Wooly sicked shaped	A brush like head	Phalides shape
3	Mycellium (septate or non septate)	Seeptate	Saptate mycelium	Septate micro Conidia	Septate	Non Septate
4	Fruiting body shape	Conidia are produced singly or chains	Basipetal-conidia on phailedsl or 2 series on vesicles	Sickle shaped conidia	Conidia in chains	Conidia in Balls

Table 3: Identified fungal isolates from Tomato, Spinach and Cabbage

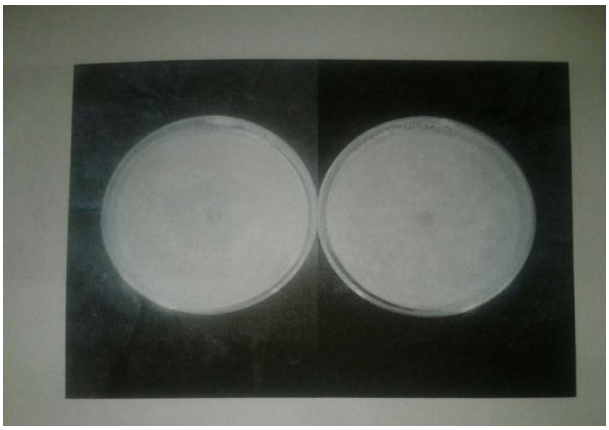
S.No	Fungi	Tomato	Spinach	Cabbage
1	*Alternaria*	+	+	-
2	*Aspergillus*	-	+	-
3	*Fusarium*	+	-	-
4	*Penicillium*	+	-	-
5	*Trichoderma*	+	+	-

Table 4: Identified fungal isolates from Tomato, Spinach and Cabbage

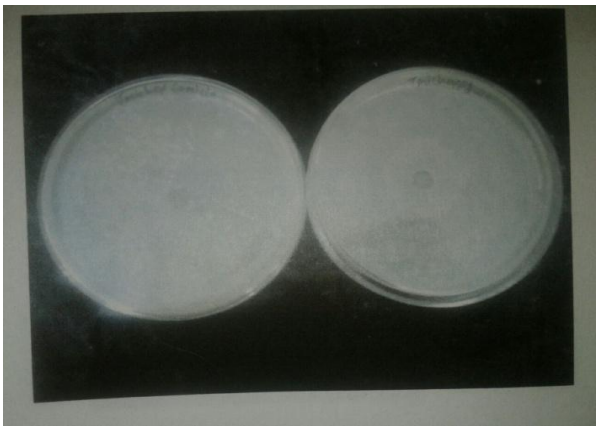
S.No.	Fungi	Zone inhibition	
		Griseofluvin	Fluconazole
1	*Alternaria*	-	30mm
2	*Aspergillus*	-	11mm
3	*Fusarium*	-	32mm
4	*Penicillium*	-	-
5	*Trichoderma*	-	31mm

The principal fungi fruit rots reported all over the world with carrying intensities on tomato includes *Alternaria* rot caused by *A. Solani* and *A. tenuis*, *Phytophthora* rot caused by *Phytophthora infestans*, *Phytophthora nicotianae* var, parasitica, Anthracnose ripe rot caused by *Colletotrichum, phomoides*, *Phoma* rot *Phoma destructiva* and *Fusarium* rot caused by *Fusarium spp.* [8-11]. Tomato crop and yield is suffered every year due to number of pathogenic diseases. Such diseases are caused by fungi, due to the presence of fungi, spoilage of vegetables occur. Baiyewn et al reported the similar results, which is the same genus with those isolated from spoiled vegetables.

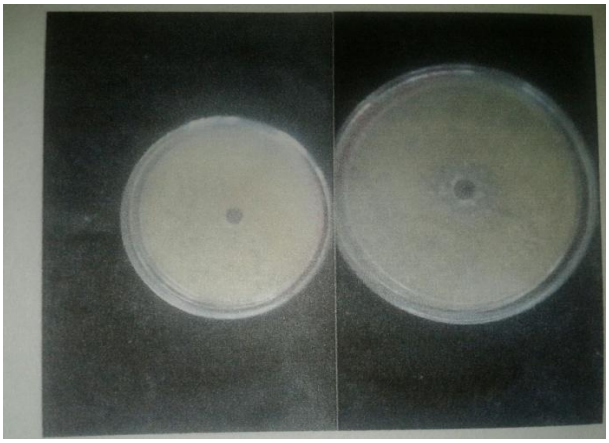
Alternaria spp. causes spoilage in spinach, symptoms of spoilage like rotting and wiltingblight, leaf spot, powdery mildew, downy mildew, rust and anthracnose. The fungal isolates were identified by the lacto phenol. In this study include *Aspergillus, Alternaria, Fusarium, Penicillium, Trichoderma*. In present study, antifungal sensitivity tested by the different drugs and *Penicillium* showed resistance to antifungal drugs. To escape from contamination subsequent handling, packaging and storage is necessary. All the vegetables should therefore be adequately washed and processed before consumption either by consumer processor.



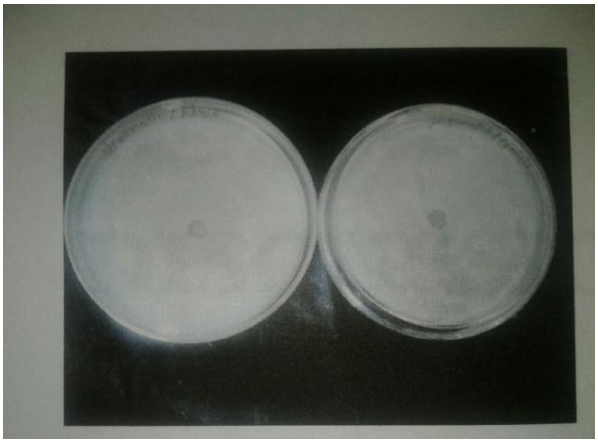
(a) Inhibition Zone of *Fusarium*



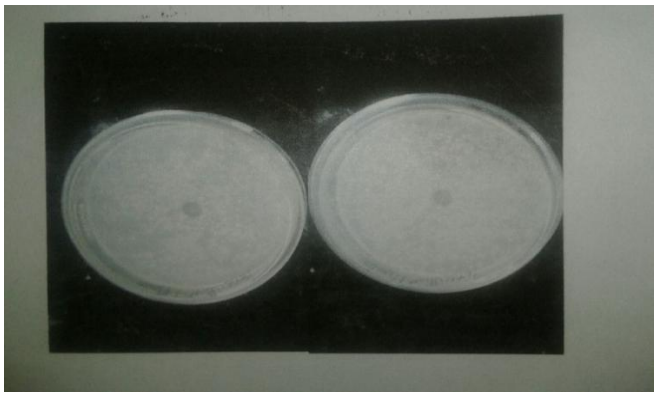
(b) Inhibition Zone of *Trichoderma*



(c) Inhibition zone of *Aspergillus*



(d) Inhibition Zone of *Alternaria*



(e) Inhibition Zone of *Penicillium*
Fig 1: Showing Inhibition Zone of isolated fungi

CONCLUSION:

Fruits and vegetables are important and have dietary nutritional qualities.Fresh vegetables fruits of local market are harboring many microbial Contaminants and pathogens.Tamatoes are eaten raw or cooked. Vegetables contains Pathogenic micro-organism from the local market, To escape from contamination subsequently handling, packaging and storage is necessary. Fresh vegetables should be microbiologically safe. In India majority of the people prefer to buy the local fresh vegetables and quality of local market's fresh vegetables are low. However, the quality of the fresh vegetable of the local market must be maintained in hygienic condition and proper handling, transport, storage must be controlled so that the risk of contamination decreases and changes of food borne outbreaks can be minimized. The high prevalence of some fungi demand demand that appropriate control measures produce against infection should be employed if former expect good performance of their produce. These can be done by pretreatment of fresh vegetables by various antifungal. Adequate microbiological knowledge and handling practices of these produces would therefore help minimize waste due to deterioration.

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