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"Isolation And Identification Of Mycoflora Of Fruit And Vegetable Market Of Davanagere

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Introduction

Fruits and vegetables are very important and have dietary and nutritional qualities. It has been known that fruits and vegetables plays a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health. In India the loss of fruits and vegetables due to storage fungi is very high. In the present study, main emphasis has been laid on the isolation and identification of fungi directly responsible for storage rots in markets. Towards a better understanding of the ecological relationships of the pathogenic mycoflora causing market diseases, it was thought necessary to study the general surface mycoflora of fruits and vegetables. Contamination of fruits and vegetables takes place at all stages of processing if proper sanitary and hygiene conditions are not maintained (Beuchat, 1995). The present investigations were carried to study the isolation, identification and characterization of fungi associated with the fruits and vegetables of Davanagere market. Literature survey reveals that the merge report in diversity of fungi present in fruits and vegetables of Davanagere market.

Key words: Isolation, Mycoflora, Fruit, Vegetable, Davanagere

Materials & Methods

Study Area:

The area of investigation of Davanagere, approximately lies between 14°28′00″N 75°55′27″E, but the most commonly cited coordinates are around 14.46°N and 75.92°E. Davanagere is one of the central district of Karnataka. The district is generally dry with temperatures ranging from 17^0c in winter to 41^0c in summer the normal rainfall is 487 mm, with a record rainfall of 459.30 mm. The soil types commonly composed of red sandy, deep black, red loamy, mixed red and black, medium black.

Sampling Site:

The sampling site is the fruit and vegetable market of Davanagere, which is located beside the private bus stand. It is the major market of Davanagere. The vegetables and fruits were mainly brought to the market from different areas such as Challakere, Hosadurga, Holalkere, Molakalmuru, Hiriyur Shimogga, Davangere, and coming from surrounding villages.

Sample Collection:

A total twenty three different infected fruits and vegetables were collected from the market of Davanagere. For the purpose of isolation of pathogen. They are listed as follows

1. **Fruits:** *Malus domestica(*Apple), *Vitis vinifera***(**Grapes), *Punica granatum*(Pomegranate), *Mangifera indica(*Mango*), Achras sapota*(Sapota), *Citrus sinensis*(Orange), *Musa paradisiaca*(Banana), *Citrus limon*(Lemon), *Citrus aurantiifolia(*Lime*).*
2. **Vegetables:** *Solanum tuberosum*(Potato), *Solanum lycopersicum*(Tomato), *Solanum melagena* (Brinjal), *Cucurbita maxima*(Pumpkin), *Momordica charantia*(Bitter gourd), *Allium cepa*(Onion), *Cucumis sativus*(Cucumber), Abelmoschus esculentus L.(Ladies finger), Daucus carota(Carrot), *Phaseolus vulgaris L.*(Beans), *Capsicum annum*(Chilli), *Raphanus sativus*(Raddish), *Luffa acuntangula*(Ridge gourd), *Capsicum sps.*(*Capsicum*)

Media and media composition:

The media used for the isolation & culturing of fungi include different media such as Potato dextrose agar medium (PDA), Sabouraud's dextrose agar (SDA), Czapek's agar media etc are used for the growth of fungi. The medium used here is Potato dextrose agar medium (PDA).

Isolation of fungi:

Small portions of disease tissue (3-5cm) were cut with sterile scalpel from which samples were taken of these regions for culture. The samples were plated out directly onto the media after cutting out small portions of infected parts using sterile Forceps. These cut out portions were aseptically placed either onto or into the previously prepared solidified PDA media plates for isolation of fungi in aseptic condition. The plates were incubated in incubator at 28 ± 3^0c and examined daily for the growth of pathogen, after 5-6days growths of fungal colonies were observed **(**Amusa et.al, 2002 and Baiyewu et.al, 2007).

Identification of fungal isolates:

The fungal growths that appeared were primarily identified using cultural and morphological features. The mould isolates were identified by staining with Lacto phenol cotton blue (Mc Lean and Lvimey 1965). It allows for the identification of various fungal structures such as presence or absence or rhizoids, hyphae, spores as well as other additional structures.(Barnett and Hunter,1987).

Results

In the present study different fungi associated with the deterioration of fruits and vegetables were isolated from the collected samples. A total eight fungal species belonging to six different genera were isolated and identified on the basis of cultural and morphological characteristics as presented in (Table 1).

A total eight fungal species isolated from twenty three different samples of fruits and vegetables collected from Davanagere market (Plate 1).The fungal isolates were identified to the species level based on the morphological identification of fungal culture colony or hyphae, the Characteristics of the spores and reproductive structures (Barnett and Hunter, 1987, illustrated genera of imperfect fungi 4th edition). (Table 2 & 3).

A total six species of fungi were isolated from nine samples of decaying fruits were identified as, *Aspergillus niger* from grapes, lime, apple, pomegranate, sapota, mango, orange. *Aspergillus flavus* from orange, *Rhizopus sp*. from grapes, banana, orange, *Mucor sp.* from apple and sapota, *Alternaria sp*. from lime, *Penicillium sp.* from lemon. (Table 3 & Plate 2).

Among these fungal isolates *Aspergillus* came in the first position with high incidence as highest fungal genera represented by the two species namely, *Aspergillus niger, Aspergillus flavus,* were more prevalent than any other fungal species isolated in this study. The *Aspergillus spp.* was recovered from grapes, lime, apple, pomegranate, sapota, banana, and mango, orange. The eight *Aspergillus spp.* Was isolated from eight fruits. Hence the genus *Aspergillus spp.* were the predominating fungi in the fruits. *Rhizopus sp.* was the second highest disease causing fungi in fruits, the *Rhizopus sp.* was recovered from grapes, banana, and orange. The three *Rhizopus sp.* isolated from three fruits. *Mucor sp*. was the third highest

disease causing fungi in fruits, and it is recovered from apple and sapota. The two *Mucor sp.* was isolated from two fruits. The remaining two fungal species like *Alternaria sp.* and *Penicillium sp*., the *Alternaria sp.* was isolated from lime, *Penicillium sp*. was isolated from lemon (Table 4).

In vegetables, the fungi associated with the spoilage were isolated from fourteen different samples of vegetables (Plate 5). A total eight species of fungi were isolated from fourteen samples of decaying vegetables were identified as *Aspergillus niger* from tomato, capsicum, ladies finger, onion, ridge gourd, *Aspergillus* flavus from pumpkin, *Aspergillus fumigatus* from onion, *Mucor sp.* from brinjal, chilli, potato, cucumber, *Rhizopus sp.* from brinjal, capsicum, Bitter gourd, *Penicillium sp.* from carrot. *Fusarium sp.* from beans. *Alternaria sp*. from potato(Table 5 & Plate5).

Among these fungal isolates *Aspergillus* was the highest disease causing fungi in vegetables and the fungal genera represented by *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* were more prevalent than any other fungal species isolated in this study. The *Aspergillus spp.* was recovered from tomato, capsicum, ladies finger, onion, pumpkin, ridge gourd. The eight *Aspergillus spp.* isolated from six vegetables. Hence *Aspergillus spp* .was the predominating fungi in vegetables. *Mucor sp.* was the second highest disease causing in vegetables, the Mucor sp. recovered from brinjal, chilli, potato, cucumber. The four *Mucor sp.* isolated from four vegetables. This is followed by *Rhizopus sp.* was also disease causing fungi in vegetables, the *Rhizopus sp.* was recovered from brinjal, capsicum, Bitter gourd, raddish. The four *Rhizopus sp.*isolated from four vegetables. The remaining three fungal species like *Alternaria sp., Fusarium sp. and Penicillium sp.,* these are the single species obtained from vegetables. *Alternaria sp.* was isolated from potato, *Fusarium sp.* was isolated from beans, *Penicillium sp.* was from carrot (Table 5 & fig 2).

Similar results on post harvest fungi on storage fruits and vegetables were reported by many investigators. (Sharma, et.,al. 1998, Ghurde and pachkhede 2010, Cherian 2005)

Table 1: Cultural, Morphological Characteristics and Identification of Fungi

Isolate	Cultural Characteristics	Morphological Characteristics
Rhizopus sp.	Large fluffy white milky colonies which later turns black as Culture ages.	Non-septate hyphal with upright sporangiophores connected by stolon and rhizoids, dark pear-shaped Sporangium on hemispherical columella.
Mucor sp.	Cream white/large fluffy white colonies almost covering the whole surface	Sporangium comes out directly from the hyphal without stolon or rhizoids collumella.
Penicillium sp.	Large fluffy white colonies almost covering the whole surface	Non–septate branched hyphal enlarge at the apex to form conidiophores they produce brownish black Conidia in chains.
Aspergillus sp.	Very common colors of colony (black and white).	Conidia borne in 360 arrangements covering the upper 2/3 of the conidiophores.
Aspergillus niger	Black color fluffy growth with white edges	Large conidial, head dark brown becoming radiate and split to columns
Aspergillus flavus	Lemon green powdery growth	Radiate conidial head, coarsely roughened, vesicle conidia and flask shaped phayalides chains of rough.
Aspergillus fumigatus	Grey, green fluffy growth or bluish green.	Vesicle pyriform, uniseriate, Clavate,vesicle, thickwalled, Smooth, conidia globose to sub globose, echinulate
Alternaria sp.	Flat white growth	Erect conidiophores, septate hyphae with cylindrical conidia.
Fusarium sp.	Rapidly growing wooly to cottony lemon and yellow.	Multicellular distinctive sickle shaped macro conidia.

Table 2: The Fungal species isolated from infected Fruit samples

Fruits	Botanical Name	Fungus
Grapes	*Vitis vinifera*	*Aspergillus niger, Rhizopus sp.*
Lemon	*Citrus limon*	*Pencillium sp.*
Lime	*Citrus aurantiifolia*	*Aspergillus niger, Alternaria sp.*
Apple	*Malus domestica*	*Aspergillus niger,Mucor sp.*
Pomegranate	*Punica granatum*	*Aspergillus niger.*
Sapota	*Achras sapota*	*Aspergillus niger, Mucor sp.*
Banana	*Musa paradisiaca*	*Aspergillus flavus, Rhizopus sp.*
Mango	*Mangifera indica*	*Aspergillus niger.*
Orange	*Citrus sinensis*	*Aspergillus niger, Aspergillus flavus, Rhizopus sp.*

Table 3: The Fungal species isolated from infected Vegetable samples

Vegetables	Botanical Name	Fungus
Tomato	*Solanum lycopersicum*	*Aspergillus niger, Aspergillus fumigatus*
Brinjal	*Solanum melagena*	*Rhizopus sp, Mucor sp.*
Capsicum	*Capsicum sps.*	*Rhizopus sp, Aspergillus niger.*
Bitter gourd	*Momordica charantia*	*Rhizopus sp.*
Chilli	*Capsicum annum*	*Mucor sp.*
Ladies finger	*Abelmoschus esculentus L.*	*Aspergillus niger*
Potato	*Solanum tuberosum*	*Mucor sp, Alternaria sp.*
Cucumber	*Cucumis sativus*	*Mucor sp.*
Onion	*Allium cepa*	*Aspergillus niger, Aspergillus fumigatus*
Beans	*Phaseolus vulgaris L.*	*Fusarium sp.*
Radish	*Raphanus sativus*	*Rhizopus sp.*
Pumpkin	*Cucurbita maxima*	*Aspergillus flavus*
Ridge gourd	*Luffa acuntangula*	*Aspergillus niger*
Carrot	*Daucus carota*	*Pencillium sp.*

Discussion

The present study was confined to diseases occurring in fruits and vegetables under the conditions of storage and marketing at Davanagere.

The isolated fungi were identified by their cultural and morphological Characteristics as presented in (Table 1).In some cases the infected tissues were stained by Lactophenol cotton blue (Mc Lean and Ivimey, 1965) and observed under compound microscope, the morphological identification of fungal culture colony or hyphae, the Characteristics of the spores and reproductive structures (Barnett and Hunter, 1987).The findings of this study showed that *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Rhizopus sp., Mucor sp., penicillium sp., Alternaria sp., Fusarium sp.,* were found in fruits and vegetables.

In fruits, the fungi associated with the spoilage of grapes were *Aspergillus niger* and *Rhizopus sp*., which is the same genus with those isolated from grapes, *Penicillium sp.,*were found associated with deterioration of lemon. The isolation of *Aspergillus niger, Alternaria sp.,* from lime, *Aspergillus niger,Mucor sp.,* were found associated with deterioration of apple, In pomegranate *Aspergillus niger* is responsible for the deterioration of fruit, *Aspergillus niger* and *mucor sp.* Were found associated with deterioration of sapota, In banana *Aspergillus flavus,Rhizopus sp.,* are responsible for the spoilage of fruit, the isolation of *Aspergillus niger* from mango, In this study three fungal species *Aspergillus niger, Aspergillus flavus,Rhizopus sp.,* were found associated with deteriorating of orange.

Among these fruits, *Aspergillus spp*. is the highest disease causing fungi in fruits like grapes, lime, apple, pomegranate, sapota, banana, and mango, orange are affected by *Aspergillus spp.,* were the predominating fungi in many fruits. Similar results on post harvest fungal pathogens on market storage of fruits were reported by earlier workers (Bhale, 2011). *Rhizopus sp.* is the second highest disease causing fungi in fruits like grapes, banana, and orange. *Mucor spp.,* is the third highest disease causing fungi in fruits like apple, sapota. *Alternaria sp.* Is isolated from lime alone, this is followed by *penicillium sp.* isolated from lemon.

In vegetables, the fungi associated with the spoilage of tomato were *Aspergillus niger, Aspergillus fumigatus,* in brinjal *Rhizopus sp., Mucor sp.,* are responsible for their spoilage, *Rhizopus sp., Aspergillus niger* were found associated with the deterioration of capsicum, the isolation of *Rhizopus sp.,* from bitter gourd, *Mucor sp.,* is isolated from the chilli, in Ladies finger Aspergillus niger is responsible for their spoilage, *Mucor spp.,*and *Alternaria sp.,* were found associated with the deterioration of potato, in cucumber *Mucor sp.,* is responsible for the spoilage, the isolation of *Aspergillus niger* and *Aspergillus fumigatus* from onion, the *fusarium sp.,* is isolated from the beans, in raddish *Rhizopus sp.,* are responsible for their spoilage, the fungi associated with the spoilage of pumpkin is *Aspergillus flavus,Aspergillus niger* is found association with the ridge gourd, the *penicillium spp.* is found in the carrot responsible for the spoilage.

Among these vegetables *Aspergillus sp*. is the highest disease causing fungi in vegetables like tomato, capsicum, ladies finger, onion, pumpkin, Ridge gourd, this reveals that species of *Aspergillus sp*. were predominating fungi in vegetables, *Mucor sp*. is the second highest disease causing fungi in vegetables like brinjal, chilli, potato, cucumber, *Rhizopus sp.* Is isolated from brinjal, capsicum, bitter gourd, raddish, hence *Rhizopus sp*. is the third highest disease fungi in vegetables, the *penicillium sp*. is isolated from carrot only, this is followed by *fusarium sp*. isolated from beans and *Alternaria sp*. isolated from potato.

Conclusion

Fruits and vegetables are the essential requirement of human diet, and these are producing the chief source of vitamin C, minerals and salts. During the survey it is observed that the high number of contaminants and pathogens which cause the spoilage of fruits and vegetables during the storage. Exposure on consumption of these spoiled fruits and vegetables may be responsible for serious health hazards. In present investigation, among fruits *Aspergillus niger, Aspergillus flavus, Rhizopus, Mucor, Penicillium,* and among vegetables *Aspergillus niger,Aspergillus flavus,Aspergillus fumigatus and Rhizopus Alternaria, Fusarium ,Penicillium,* were found to be major disease causing organisms. The major dominant fungi like *Aspergillus, Mucor, Rhizopus, Fusarium, Alternaria and penicillium* species were found on fruits and vegetables which may causes allergic effects on human health.

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Plate 1: The Fruits Infected By Fungal Pathogens

Vitis vinifera **(**Grapes)

Citrus limon (Lemon)

Citrus aurantifolia (Lime)

*Malus domestica (*Apple)

Punica granatum (Pomegranate)

Achras sapota (Sapota)

Musa paradisiaca (Banana)

*Mangifera indica (*Mango)

Citrus sinensis (Orange)

Plate 2: Culture Plates of Fruits

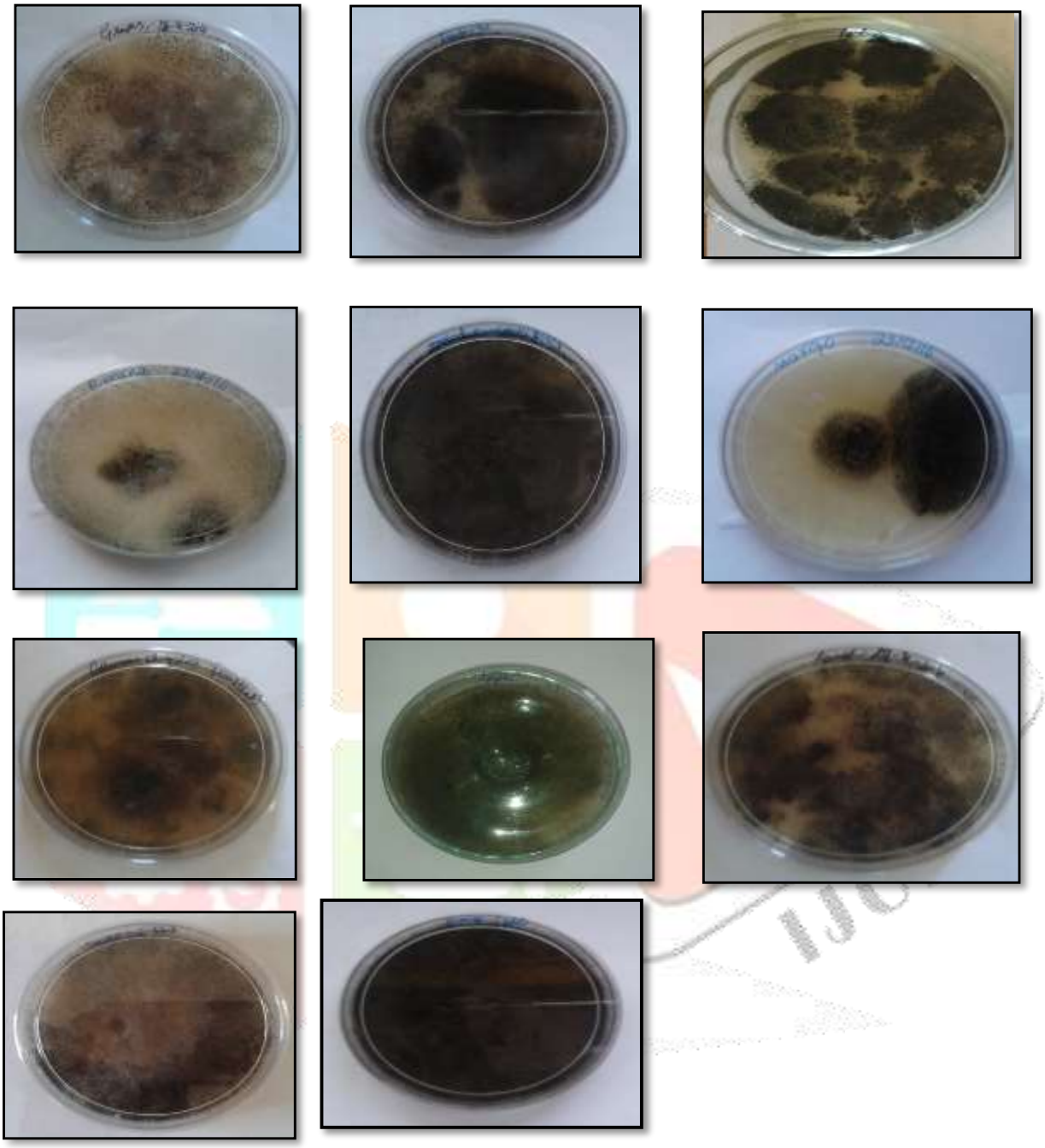
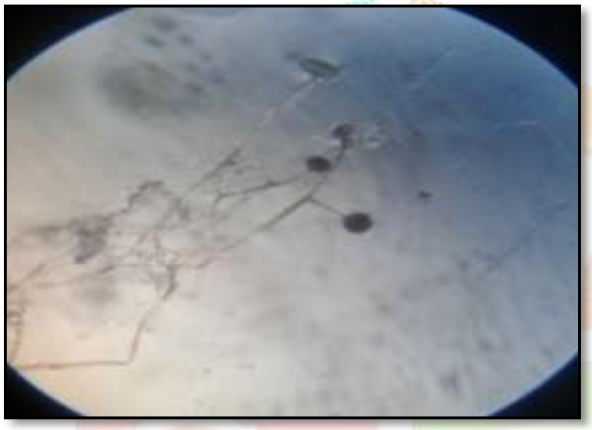


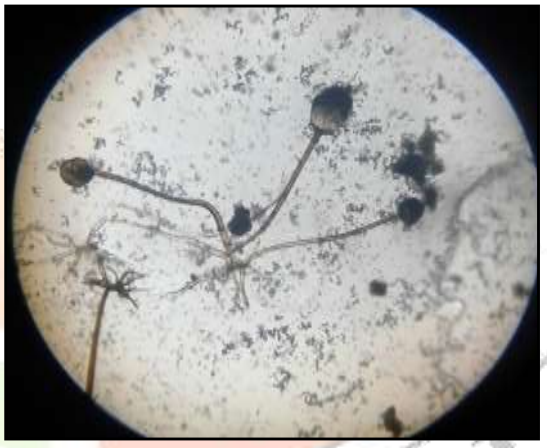
Plate 3: Fungal Isolates from Fruits

Aspergillus niger

Aspergillus flavus

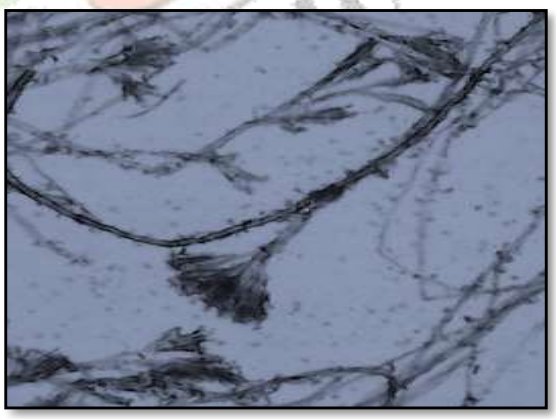


Mucor sp.



Rhizopus sp.

Alternaria sp.



Penicillium sp.

Plate 4: The Vegetables Infected By Fungal Pathogens

Solanum lycopersicum (Tomato)

Solanum melagena (Brinjal)

Capsicum sps.(Capsicum)

Momordica charantia (Bitter gourd)

Capsicum annumn (Chilli)

Abelmoschus esculentus L.

Solanum tuberosum (Potato)

Cucumis sativus (Cucumber)

Allium cepa (Onion)

Phaseolus vulgaris L. (Beans)

Daucus carota (Carrot)

Cucurbita maxima (Pumpkin)

Plate 5: Culture Plates of Vegetables

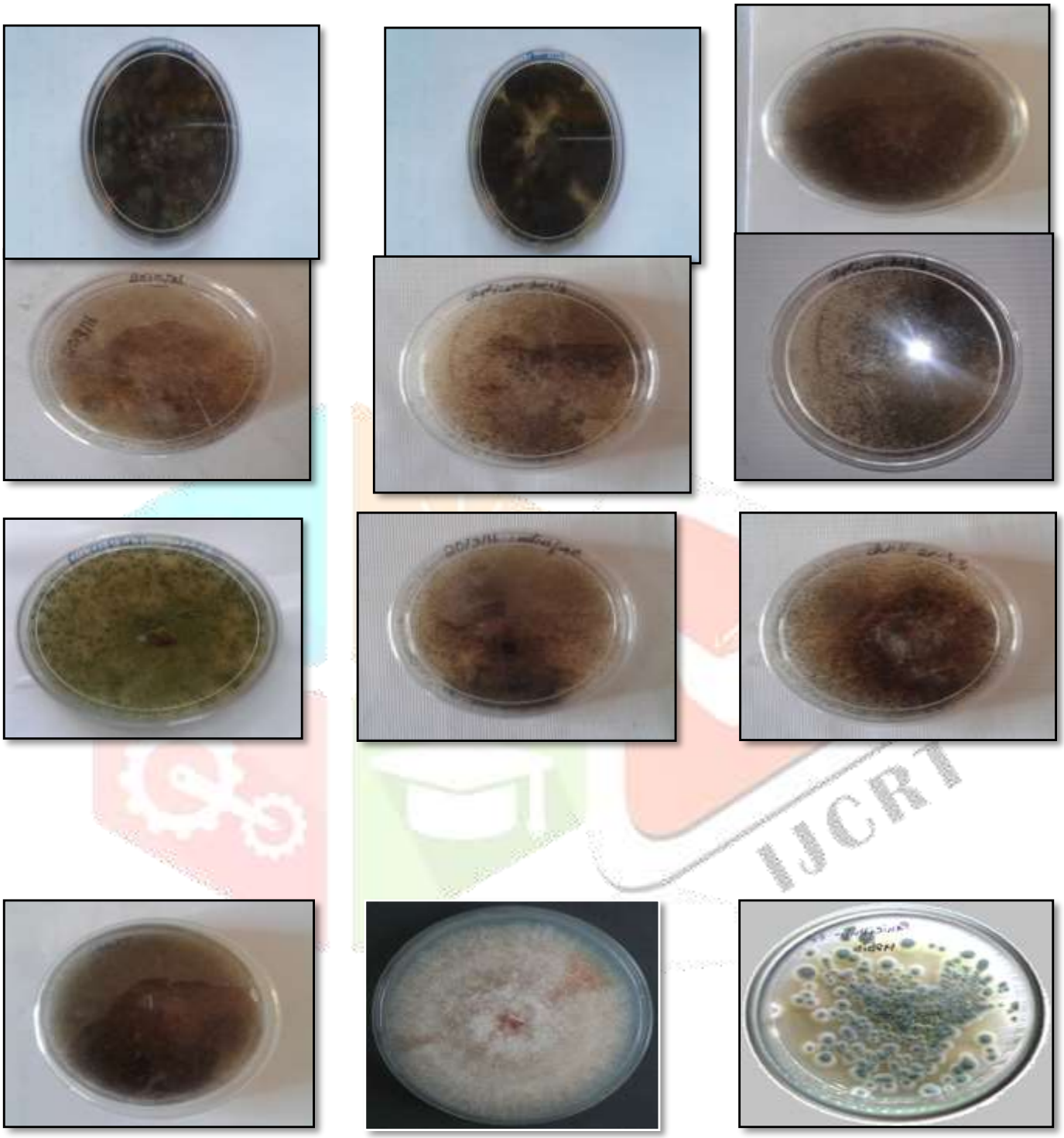
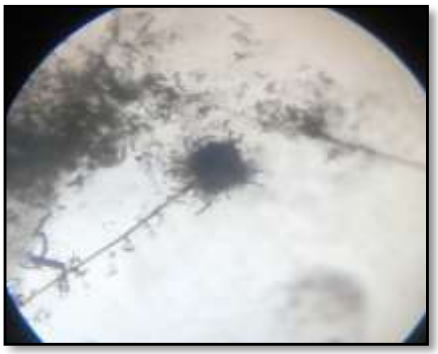


Plate 6: Fungal Isolates of Vegetables



Aspergillus niger

Aspergillus fumigatus

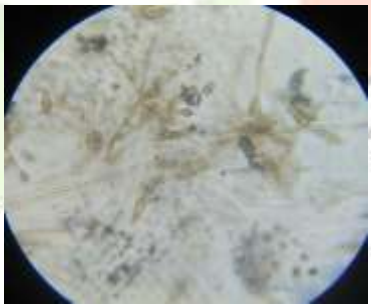
Aspergillus flavus

Mucor sp.

Rhizopus sp.

Penicillium sp.

Fusarium sp.



Alternaria sp.