

Alternaria: Isolation and Identification from Different Plant Parts

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Abstract:- *Alternaria* is a genus of ascomycete fungi. *Alternaria* species are known as major plant pathogens. There are 299 species in the genus they are ubiquitous in the environment and are a natural portion of fungal flora almost everywhere. The club shaped spores are single or form long chains. So Main objective of this investigation is to isolate different *Alternaria* species from different portions of plant and diagnosed their species. They are standard agents of decay and decomposition. At least 20% of agricultural spoilage is triggered by *Alternaria* species not all *Alternaria* species are pests and pathogens; some have listed promise as biocontrol agents against invasive plant species. The genus is now known to be polyphyletic. *Alternaria* alternate generates early blight of potato, leaf spot disease in *Withania somnifera* and can infest several alternative plants. *Alternaria abroscens* generates stem cancer of tomato, *Alternaria arbsti* causes Leaf lesions on Asian pear, *Alternaria blumeae* generates cessions on *Blumea aurita*, *Alternaria brassicae* infests numerous vegetables and roses. *Alternaria carotiinculata* generates leaf blight on corrot, *Alternaria citri* generates black rot on citrus plants. *Alternaria cucumerina* grows on multiple cucurbits, *Alternaria infectoria* infests Wheat, *Alternaria solani* generates early blight in potatoes and tomatoes. *Alternaria* toxins are mycotoxins fabricated by *Alternaria* species. The genus *Alternaria* currently encompasses around 50 species. At the cellular level. Toxins are fabricated by AAL that are necessary for pathogenicity on tomato. The fungus lives in seeds and seedlings and is also spread by spores. The disease flourishes in dead plants that have been left in dead plants that have been left in gardens over winter. There are no insect vectors for this disease.

I. INTRODUCTION

Alternaria mold is commonly identified in the outdoors and usually grows on plants and other surfaces such as wood *Alternaria alternate* often grows on materials such as wallpaper, canvas, plaster bricks and tiles. *Alternaria* blight triggered by the fungus *Alternaria cucumerina* is a common disease of the most cucurbits in warm rainy weather *Alternaria* leaf spot or *Alternaria* Leaf blight are a cohort of fungal infections in plants, that have a range of hosts. *Alternaria* reproduces only by conidia which are generated at the tips of conidiophores. The detection of *Alternaria* species necessitates a mixture of morphological features and molecular methods. Morphological features such as conidial shape, size quantity of primary conidium, conidial branching arrangement and primary conidiophore length were utilized in the identification by Simmons and Roberts 1993 Indian mustard (*Brassica juncea* (Linn.) Czern. and

cross.) is an important oil seed crop, grown both in tropical and sub-tropical regions of the world. It yields important edible oil, which cannot be conveniently replaced. The major limitations in growing mustard are diseases, aphid pest frost injury, non-availability of high yielding varieties suitable for high input conditions and in variations in weather conditions (Kumar, 1999.) among all these infections such as white rust (*Albino candida* (pers.) Kuntze), Downy mildew (*Peronospora parasita* (pers.) deBary) and *Alternaria* blight (*Alternaria brassicicola* (Schw.) Wiltshire play a salient role in reducing the productivity of mustard. *Alternaria* blight reduced 1000 seed weight generating loss of 35.38 percent (Kolte et al., 1987). *Alternaria alternata* has previously been stated in Pakistan as a saprophytic pathogen of tomato generating post-harvest losses at high frequency (Akthar et al., 1994). Papaya (*Carica papaya* L.) is cultivated in tropical and sub-tropical regions of all over the world (Baiyewu et al., 2007). After harvesting during storage and transportation these fruits are subjected to numerous biotic stresses (Bhale, 2011), which plays a pivotal role in collaboration of different pathogens and formation of diseases over papaya fruit for instance *Rhizopus* rot, *Asprgillus* rot, *Penicillium* rot (Sharma, 2015). *Fusarium* rot, *Alternaria* rot and Anthracnose (Singh et al., 2012).

Leaf spot disease of brinjal triggered by *Alternaria alternata* in Jaipur district of Rajasthan, Premila, 2014 recorded 9.5 to 29.5 percent frequency of Leaf spot disease on brinjal in Manipur. Similar conclusions about the occurrence of the disease have also been reported by Balai et al., 2010. Worldwide, different *Alternaria* species have been linked with *Alternaria* Leaf blotch and Fruit spot on apple. The most quoted causal agent of *Alternaria* leaf blotch on apple is *A. mali* (Filajdic & Sutton, 1991; Bulajic et al., 1996). other *Alternaria* species such as *A. alternata* sensu lato have been implicated for *Alternaria* leaf blotch on apple (Kusaba & Tsuge, 1994). Although the conidia of the genus *Alternaria* are distinct and easy to recognize and conveniently separated into large-spored and small-spored species groups, it is complicated to elucidate the multiple species within the genus owing to high scope of similarity in their morphological characteristics (Simmons, 1992). Small pored *Alternaria alternate* intricate causing multiple human and plant infections (Anissie et al., 1989; Thomas, 2003). *Alternaria* species cause four infections of citrus, including *Alternaria* brown spot of tangerine (citrus reticulate Blanco) and their hybrids, *Alternaria* leaf spot of rough lemon *Alternaria* is a cosmopolitan genus that consists of different saprophytic and pathogenic species *Alternaria* was divided into 26 sections by Woudenberg et al. (2013). Fruit rot of chilli (*Capsicum annum* L.) caused by *Colletotrichum capsici* (Syd.) Butler and Bibby and *Alternaria alternata* (Fr.) Keissler causes

severe losses both in yield and quality of the product. The disease is prevailing in almost all major chilly growing areas and it is reported to cause 25-48% deficiency in different parts of india (Muthu Lakshmi 1990; Dater 1995; Ekbote2001) *Alternaria* leaf spot or *Alternaria* blight is common foliar disease of cotton occurring in most regions of the world (Hillocks, 1992; Steinberg, 1993)

Cotton seedlings became less susceptible to *Alternaria* leaf spot in response to cotyledonary treatment not simply with INA formulated in a wettable powder as illustrated *Alternaria* leaf spot disease triggered by *Alternaria cucumerina* (E11-& EV) Elliot was reported to infect cucumbers during the growing in numerous countries (7,12,14,) Yacoub Batta 2003.

II. MATERIAL AND MAETHODS

Samples were collected different fruit and leaves from Aurangabad city, Gavtala, Kalash seed Jalan Bajra Research Center, University campus and market .Infected Fruit and leaves samples were collected in pre-sterilized polythene bags. From the market to examine postharvest fungi. Different type of fungal pathogens was collected from the fruits and leaves identification are based largely on the morphological characters of spores and spore bearing structure by using direct microscopy. Identification of fungi was also based on the color of mycelia and microscopic examination of vegetative structure.

A. Collection of samples:

Infected Fruits and leaves samples are collected in sterile sample bags.

The sample of infected fruits and leaves are used for isolation of pathogenic fungi

- Culture Technique:
- Direct inoculation:

The samples were plated out directly onto the media after cutting out small portion of infected part using sterile scalpel. These cut out portions were aseptically placed either onto or into the media for isolation.

B. Processing Of sampled for culture:

- The samples of Fruits and leaves were washed with water.
- Cut the infected portion of Fruit and leaves with the help of blade or scalpel.
- Cut into small pieces 2-3mm.
- The infected pieces of fruits and leaves were inoculated on media for isolation of fungi

a) Sterilization of Glassware's:

Dry heat was the method employed with the hot air oven being the equipment used. . The glassware include Petri plates, conical flask, beaker, test tube, glass rod, measuring cylinder etc. they were sterilized in hot air over. Beaker measuring cylinder and conical flasks were plugged with cotton wool for sterilization. Test-tube, glass rod scalpel was wrapped in Aluminum foil.

C. Preparation of media

- Selection of media for isolation
During investigation usually Potato Dextrose Agar (PDA) and Czapek Dox Agar (CZA) medium were used for the isolation and maintenance of pure cultures. The constituents of the medium are as follows.

PDA	1000 ml	500 ml	250 ml
Peeled Potato	200 gm.	100 gm.	50gm
Dextrose	20gm.	10 gm.	5 gm.
Agar	20 gm.	10 gm.	5 gm.
Distilled Water	1000 ml	500 ml	250 ml
PH	5.6	5.6	5.6

Table 1: Composition of media used in isolation Potato dextrose agar (PDA) (Rangaswami.1996)

- Procedure:

Take above measure constituents, Peeled Potatoes were boiled until soft and passed through muslin cloth. Then dextrose was added in it and make a final volume of solution agar was added PH was adjusted.

Czapek Agar	1000ml	500ml	250 ml
Sucrose	30gm	15gm	7.5gm
NaNO3	2gm	1gm	0.5gm
KH2PO4	1 gm	0.5 gm	0.25gm
MgSO4	0.5gm	0.25 gm	0.25gm
KCI	0.5gm	0.25gm	0.125 gm
GeSO4	0.01gm	0.005gm	0.0025gm
Agar	20gm	10gm	5 gm
D.W.	100ml	500ml	250ml
pH	5.6	5.6	5.6

Table 2: Czapek Box Agar (CZA):

- Procedure:
Take above measurable constituents respectively, mixed in distilled water and make a countable final volume. Then adjust PH.

D. ISOLATION OF MICROORGANISMS

Potato Dextrose Agar (PDA) was for isolating fungi. The infected parts of fruits and leaves were inoculated with the help of forceps or scalpel. PDA Plates were labelled properly and incubated appropriately PDA plates were incubated on the room temperature (25-28°C) For 5-7 day

- **Procedure for Sub culturing:** Pure isolates were obtained by selecting discrete colonies and having them subculture on petri-dishes containing freshly prepared PDA media
- **Preservation for Isolates:** Pure isolates were inoculated onto freshly prepared PDA Slant in for fungi Preservation

respectively. Cultures were incubated for 24hrs. in an incubator at 37°C and then stored in refrigerator at 4°C. PDA cultures were incubated for 24hrs. On the Workbench (28°C) and stored in the refrigerator at 4°C.

III. IDENTIFICATION OF FUNGI

PDA medium was used to maintain stock cultures

The micro slides were prepared in cotton blue and lactose phenol small tuft of the fungus usually with spore and spore bearing structures were transferred into the spore with the help of a Flamed. Cool needle the fungal material was teased using two mounted needle's structure mixed gently in the stain. The coverslip was placed over the fungal materials without air bubbles in the stain the microphotograph's were taken for every isolated fungal from leaves and fruits.

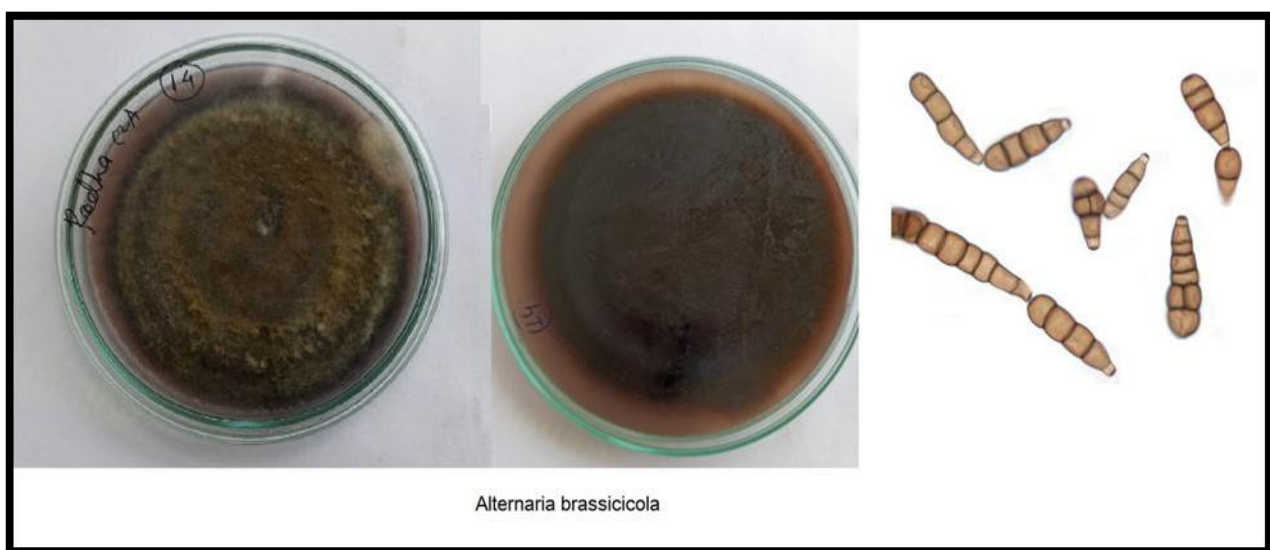


Fig. 1: *Alternaria brassicicola*

Colonies amphigenous effuse, dark olivaceous brown to dark blackish brown, velvety. Mycelium immersed; hyphae branched, septate, hyaline at first, later brown or olivaceous brown, inter and intracellular, smooth, 1.5-7.5 thick. Conidiophores arising singly or in sets of 2-12 or more, emerging through stomata, generally simple, erect, or ascending. Straight or curved, sometimes geniculate, cylindrical but often marginally swollen at the base, septate, pale to mid olivaceous brown, smooth, up to 70 long 5-8m thick. conidia mostly in chains of up to 20 or more, occasionally branched, acropleurogenous, arising through tiny pores in the conidiophores wall straight, approximately cylindrical, generally tapering marginally towards

the apex or obclavate, the basal cell rounded, the beak generally almost non-existent, the apical cell being more or less rectangular assembling a truncated cone, sometimes better evolved but then always short and thick with 1-11 mostly less than 6 transverse septa and generally few but up to 6 longitudinal septa, often marginally constricted at the septa. Pale to dark olivaceous, Brown, smooth or becoming marginally wrinkled with age, 18-30m long, and 8-20m thick in with the beak 1/6 the length of the conidium and 6-8µ thick. On leaves of Cruciferae forming dark brown to almost black, circular zone spots 1-10mm diam. More common and causing more severe disease than *A. brassicae* in seed crops.

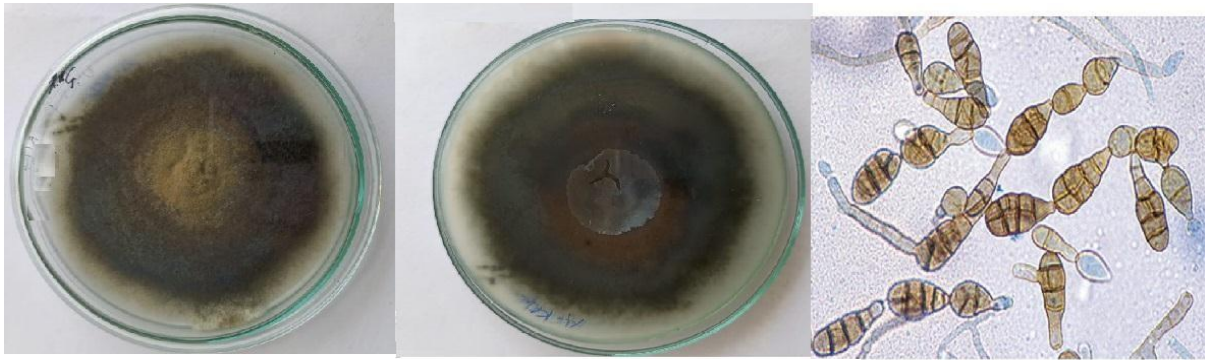


Fig. 2: *Alternaria alternata*

Colonies usually black or olivaceous black, sometimes grey. Conidiophores arising singly or in small groups, simple or branched. Straight or flexuous, sometimes geniculate, pale to olivaceous or golden brown, smooth, up to 50 μ long, 3-6 μ thick with 1 or several conidial scars. Conidia formed in long often branched chains. Obclavate, obpyriform ovoid or ellipsoidal often with a short conical or cylindrical beak, sometimes up to but not more than one third the length of the conidium, pale to mid golden brown, smooth or verrucose, with up to 8 transverse and usually several longitudinal or oblique septa, overall length 20-63 (37) μ , 9-18 (13) μ thick in the broadest part; beak pale, 2-5 μ thick.

An extremely common saprophyte found on many kinds of plants and other substrates including food stuffs, soil and textiles; cosmopolitan Colonies amphigenous conidiophores arising singly or in tiny groups, erect, straight or flexuous, sometimes geniculate, cylindrical, septate, pale to mid brown up to 100 long, 6-10 thick, generally with several well established conidial scars. Conidia solitary

or sometimes in chains of 2, obclavate rostrate, the beak longer, often much longer than the body of the spore, pale to mid golden brown, smooth to verrucose, overall 130-220(180) long. 15-24(20) thick longitudinal and oblique septa; beak pale brown, septate, not branched, 4-5 thick at the base quickly narrowing generating leaf blight of cucurbits, often economic significance in U.S.A. spots at first small, circular, water soaked, whitish or tan. Later expanding and often zonate, with a clear brown margin Conidiophores arising singly or in tiny groups, straight or flexuous, septate somewhat pale brown out 110 long, 6-10 thick. conidia generally solitary, straight or marginally flexuous, obclavate or with the body of the conidium along or ellipsoidal tapering to beak which is commonly the same length as or rather longer than the body, pale or mid pale golden or olivaceous brown, smooth, overall length usually 150-300 μ , 15-19 μ thick in the broadest part, with 9-11 transverse & 0 or few longitudinal or oblique septa; beak flexuous, pale, sometimes branched, 2.5-5 μ thick tapering gradually

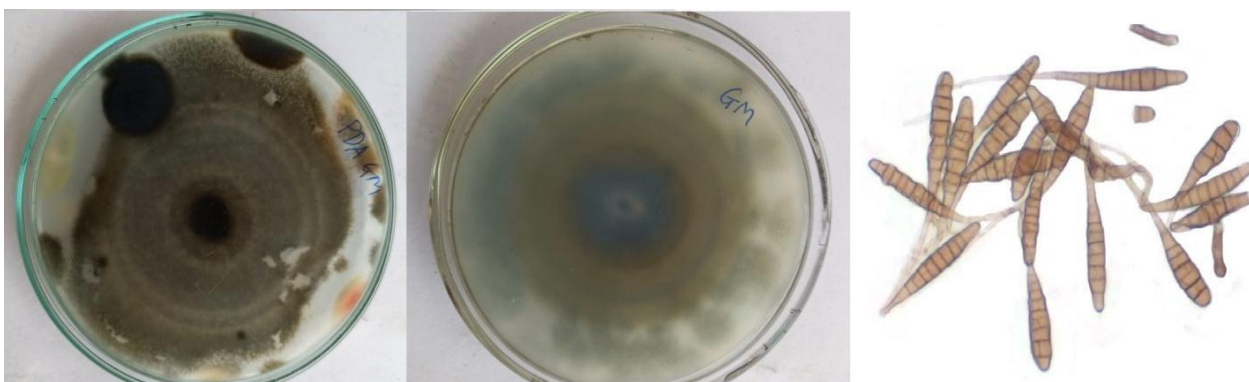


Fig. 3: *Alternaria solani*

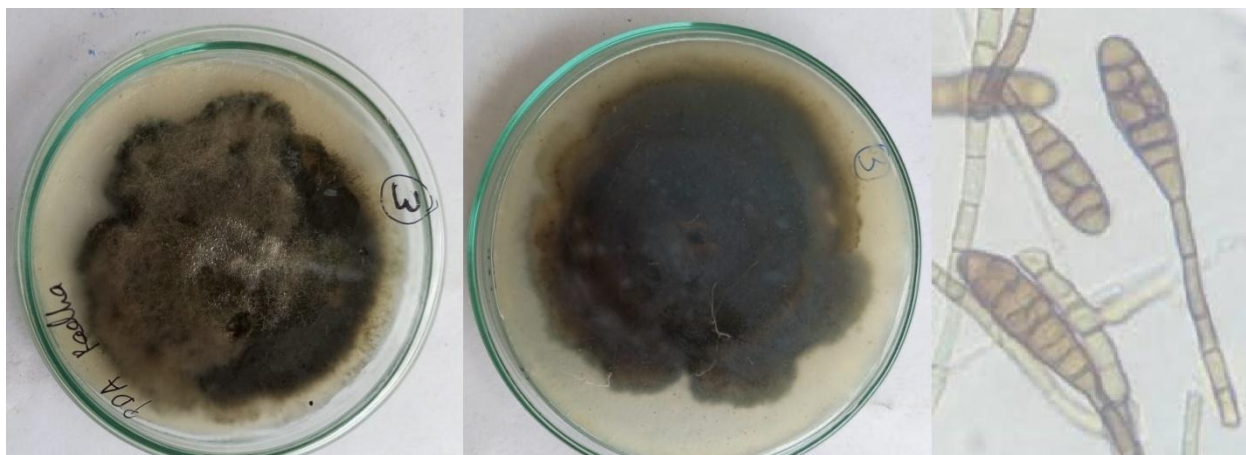


Fig. 4: *Alternaria cucumerina*

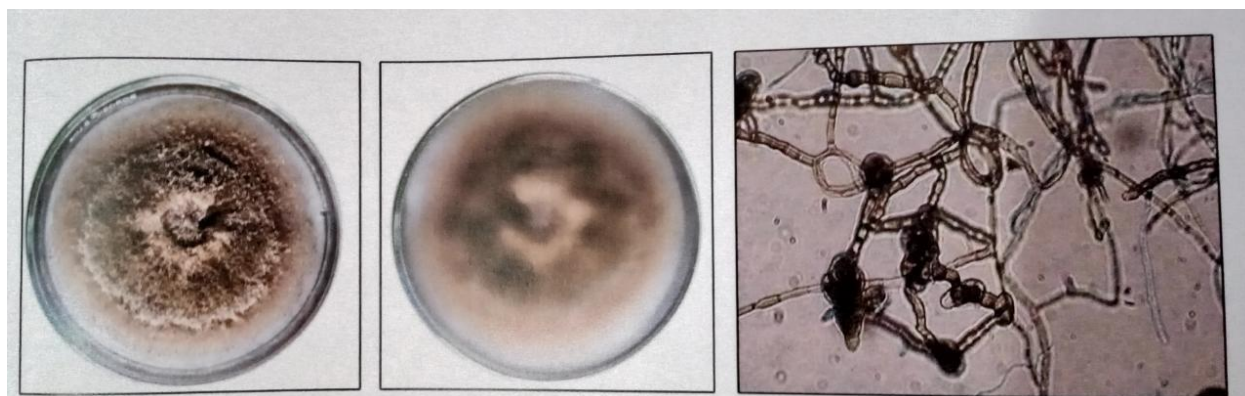


Fig. 5: *Alternaria citri*

On tomato, potato eggplant and other plants belonging to the Solanaceae and has been reported on other hosts. on the upper side of leaf Colonies effuse, olivaceous to black; in culture grey, olivaceous brown or black sometimes zonate. Conidiophores single or branched, straight or flexuous, septate, pale to mid brown or olivaceous brown up to 300 μ long, 3-5 μ thick, with a terminal scar and sometimes one or two lateral ones conidia solitary or in simple, or branched chains of 2-7, straight or slightly curved, variously shaped but commonly obclavate or oval, often rostrate, pale to mid or

sometimes dark brown or olivaceous brown, smooth to verruculose with 8 transverse and numerous longitudinal or oblique septa, constricted at the septa, 8-60(42) μ long including beak when present 6-24(17) μ thick in the broadest part; beaks mostly 8 μ or less long 2.5-4 μ thick, colourless or rather pale brown. Isolates of *A. citri* often become sterile after sub-culturing. Responsible for various type of injury to fruits and leaves of citrus sp .including black rot of oranges, stem end and rot of lemons and a leaf spot of rough lemon.

Name of fungi	Name of infected leaves				
	<i>Brassica juncea L.</i>	<i>Spinacia oleracea L.</i>	<i>Gossypium hirsutum L.</i>	<i>Cucumis Sativus</i>	<i>Solanum melongea</i>
<i>Alternaria brassicicola</i>	+	-	-	-	-
<i>Alternaria Alternate</i>	-	+	+	-	+
<i>Alternaria cucumerina</i>	-	-	-	+	-
<i>Alternaria Solani</i>	-	-	-	-	+
<i>Alternaria Citri</i>	-	-	-	-	-

Table 3: Isolation of *Alternaria* from Leaves

Name of fungi	Name of infected fruits				
	<i>Solanum lycopersicum L.</i>	<i>Capsicum Annum</i>	<i>Psidium guajava</i>	<i>Carica papaya</i>	<i>Citrus sinensis</i>
<i>Alternaria Solani</i>	+	-	-	-	-
<i>Alternaria Alternate</i>	+	+	-	+	-
<i>Alternaria Citri</i>	-	-	-	-	+
<i>Alternaria Raphani</i>	-	-	+	-	-
<i>Alternaria arboresecens</i>	+	-	-	-	-

Table 4: Isolation of *Alternaria* from Fruits

The given table in which I have selected 5 samples of infected fruits and 5 samples of infected leaves samples of infected leaves. The infected fruit sample in which different type of *Alternaria* present. The infected fruits of tomato, chilli, guava, papaya, and orange were collected from Aurangapura market, Jadhav mandi market, Parkhead market. The tomato chilly & papaya in which *A. alternata* present, orange in which *A.citri* is present and guava fruit in which *A. raphani* is present. Infected laves samples of *brassico juncea L. Spinacia oleraceaL., Gossypium hirsutumL., Cucumis sativus , Solanum melongea were collected from Bajra Research Center, Aurangapura market, Kalash seeds Jalna, farms.*

A. Alternata mostly fund in infected samples, Brassica juncea L. in which A. brassicicola is present, spinacia oleraceaL., Gossypium hirsultum L. solanum melongea in which A. alternnata present. Cucumis sativus in which A. Cucumerina present Brassicaceae, citrus family solanaceae family in which specific species are present that is Alternarna brassiocola, A. citri, A. Solani,.

IV. RESULT AND DISCUSSION

For investigation of isolation of fungi species from different sources like infected leaves, fruit, stem, etc. for the study of genus *Alternaria* I have selected infected fruits and leaves samples. During the study isolates of different sources among the 10 isolates were identified taken for morphological and microscopic characters

It was clear from the table infected fruits and infected leaves collected from different localities and it gives result of genus *Alternaria* like *A.solani, A.alternata, A. citri, A. raphani, A. brassicicola, A. cucumerina*.etc.I have collectd samples of infected fruits and they were chilli, tomato, guava, papaya, orange and infected leaves sample of *Brassica juncea L. Spinacia oleracea L. Gossypium hirsutum L., Cucumis Sativus, Solanum Melongea..*Mostly, observed *A. alternata* for eg. *A.alternata* were observed in infected fruit of tomato, chilli , papy and infected leaves of *Spinacia Oleracea L. Gossypium hirsutum L., Solanum Meleongea.*The isolation of fungi used different type of media. I have used Potato Dextrose Agar and Czapek Dox Agar medium. PDA media in which growth of fungi within 4-5 days but in CZA media growth of fungi observed slowly than PDA. Avoid bacterial contamination of PDA Streptomycin were used most of plant in which *Alternaria* are endemic.

V. CONCLUSION

Alternaria is genus of Ascomycete fungi at least 20% if agricultural spoilage is caused by *Alternaria* species; most severe losses may reach up to 80% of yield. *Alternaria* spot is yellow, dark brown to black circular with concentric rings. Disease caused by *Alternaria* occurring in different plant. Parts. *Alternaria* causes Foliar damage. *Alternaria* causes serious damage to brassicas: *A. Brassicicola, A.raphani, A.brassica.* *Alternaria* causes infection to garden plants and fruits.

It is saprophytic and found on decaying plant tissue. A wide variety of fungal pathogens causes post-harvest disease in fruit and vegetable. Dark brown to black circular spot present on leaves, fruit, vegetables in which most of cases *Alternaria* is present. Severe infection of plants, is controlled by copper fungicides or Captan, fungicide sprayed directly on infected plants. Sanitation and crop rotation are also control the *Alternaria* infection. The distribution of *Alternaria* all over it found in leaves, fruit, seed, grasses, vegetables. Solanaceae family plant in which *A.solani*, Brassicaceae family in which *A.brassicicola* and Citrus family in which *A.citri* present.

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