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Mycoflora Associated with Stored Onion and their Cultural Characteristics

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A B S T R A C T

Onion being important vegetable crop grown in India is preferred mainly because of its green leaves, immature and mature bulbs which are either eaten raw or cooked as a vegetable. Post-harvest losses due to desiccation, decay and sprouting possess a great threat for effective economic use of onion. Infected large and small sized onions (495 numbers) were collected from different market places of Bhubaneswar, Odisha for association of fungal mycoflora. Out of these *Aspergillus niger, Fusarium oxysporum* and *Botrytis sp* were found to be associated with 214,169 and 112 number of rotted samples of both large and small sized onion samples respectively. Whitish black rotten areas with blackish powdery masses and water soaked patches on the skin were found on different size of onion. Whitish fungal growth of *F.oxysporum* was found in Potato dextrose agar (PDA) which turned pink colour after seven days of inoculation and its conidia measured to 18.39-25.63 × 3.58-4.93µm. Green white mycelium was developed on the petridish by *Botrytis sp.* and measured 7.77 ×4.58 µm. Mycelium of *A.niger* was submerged, hyaline, septate and complete black colouration on Potato Dextrose Agar (PDA). Vesicle measured 55-85 µm. Potato Dextrose Agar (PDA) medium supported highest growth of *F.oxysporum* (80.13mm) followed by Richard's agar (76.63mm). Potato Rose Bengal Agar supported least growth 38.23mm. PDA also supported highest radial growth of *A.niger* (81mm). The growth of *F.oxysporum* increased from 15°C reaching maximum at 25°C (82.25mm) and declined thereafter with minimum in 35°C (64.75mm).

Introduction

Onion (*Allium cepa L.*) is one of the oldest bulb crops, known to mankind. It is one of the most important vegetable crops grown in India and believed to be originated in Central Asia. Onion is preferred mainly because of its green leaves, immature and mature bulbs which are either eaten raw or cooked as a vegetable. It is valued because of its distinct pungent flavour and is an essential ingredient for the cuisine of many regions (Kukanor, 2005). Despite of availability of good varieties of onion and achievement in production technology, the post-harvest losses during storage is still an ailing cause which leads to significant quantitative and qualitative losses during storage upto 25-30 %.

Onion is susceptible to numerous foliar, bulb and root pathogen that ultimately reduce yield and quality. It is attacked by several fungal, bacterial, and viral diseases among which

Fusarium oxysporumf. sp. cepae, *Botrytis cinerea* and *Aspergillus niger* are important. Onion is known to get infected with *Fusarium oxysporumf. sp. cepae* causing basal bulb rot of onion, which caused considerable damage to onion bulb(Patil, 2012). The disease is characterized by wilting and rapid dying back of leaves from the tips of the plants near maturity.

Infected plants can be pulled out easily because they have a retarded root system (Ilhe *et al.,* 2013).Several Allium crop diseases are also affected by *Botrytis spp*, which results in yield losses in different parts of the world. *Botrytis cinerea* causes leaf fleck, blight and is the causal agent of neck rot (Droby *et al.*, 2007, Wright *et al.*, 2001). *Aspergillus niger* is one of the important storage infectant of onion bulb. It has highest percentage frequency of occurrence in onion. The percentage of their frequency is 50.3 (Khatoon *et al.*, 2017). In view of this an investigation was conducted for association of any fungal flora among stored onion in Bhubaneswar township. The detailed study of decay fungi associated with stored onion were conducted in Plant Pathology Department, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha.

Materials and Methods

Collection of infected samples

Rot affected onion bulbs exhibiting black, sunken and water soaked lesions were collected from different market places of Bhubaneswar. Infected samples were brought to the laboratory and microscopically observed for associated pathogen. These were surface sterilized and kept in a moist chamber aseptically for about 72 hours so as to develop the fungal colonies. Microscopic examination of fungal growth were conducted.

Isolation of pathogen

The infected onion sample having both healthy and diseased portion were sterilized using 1% sodium hypochlorite for 3-4 minute. These were washed 3 times with sterilized distilled water. These portions were transferred to plate containing solidified potato dextrose agar(PDA) medium.

sThe observations were taken periodically after incubation at 27±1°C. Colony of fungal mycelium was found on plates after seven days of plating. Hyphal tip method was followed for preparing the pure culture of all associated fungal mycoflora.

Pathogenicity test

Healthy onion samples were collected from different markets of Bhubaneswar, Odisha. Healthy onions were washed thoroughly with tap water followed by distilled water. These were surface disinfected with 95% ethyl alcohol. Fungal discs of each isolated pure culture were inserted in the holes made with the help of cork borer in healthy onion and kept on laboratory table for initiation of symptoms.

Cultural characteristics

The cultural characteristic of the causal pathogen were studied using different media. Media like Czapek's dox agar medium,Potato rose Bengal agar medium**,** Richards agar medium**,** Malt extract agar medium, Sabouraud's agar medium, Brown's agar medium and Potato dextrose agar medium were prepared using standard protocol and was sterilized in the autoclave at 15psi pressure for 20 minute.

The radial growth of causal pathogen was carried out in temperature ranges like15^0C, 20^0C, 25^0C, 30^0C, 35^0C using incubator.

Results and Discussion

Collection of infected onion sample

The infected onion bulbs showing symptoms of rotting were collected from different places of Bhubaneswar. A total no of 495 infected samples (316 number large sized and 179 number of small sized) exhibiting black, sunken, water soaked lesions were brought to the laboratory and microscopically observed for associated pathogen (Table 1).

Symptomatology

Fusarium oxysporum

Whitish black rotten areas were found at the tip to downward with foul smell. The tips of the large onion were found to be depressed. Faint black rotting areas were found on the skin of the small onion. The extents of rotting on the large onion were more than that of small onion. Sumner (1995 a, b) reported brown colouration with watery appearance in *Fusarium oxysporum* infected bulb. Soft rot and semi water symptoms on onion bulb infected with *Fusarium oxysporum* were also reported by Fageria *et al.,* (2003).

Aspergillus niger

Healthy samples were completely free from diseases with red colour skin but in infected bulb, red colour of skin was completely absent due to black spore coverage. Blackish powdery masses were found on the surface of large onion which could be rubbed off easily.

Small sized onions were also found to be infected by blackish spore mass from the tip downward. Walker (1952) also noted that in case of black the black powdery masses of spores were borne on exterior of the scales and can be rubbed off readily. Tiwari *et al.*, (1984), Singh, (1995) also reported that invaded tissue becomes water soaked at first and a white mold develops between the scales, soon produced black spores.

Botrytis species

Large onions were found to be rotting from the tip with black coloration and water soaked patches on the skin of the onion. Very small whitish fungal growths were observed on the blackish rotten area at the tip of the onion.

Faint whitish fungal growths were also found on small onion without any rotting area. Elad *et al.,* (2007) reported *Botrytiscinerea* causing neck rot in onion. Fillinger *et al.,* (2016) reported neck rot caused by *Botrytis cinerea* was destructive pathogen of stored onion.

Pathogenicity test

Fusarium oxysporum

Pure culture of *F. oxysporum* were grown for 7 days and inoculated with cork borer method to both large and small sized onion. Onion bulbs were completely damaged in 10 days after inoculation. The internal tissues were rotted, putrified by the fungus and upon re isolation, the same fungus was isolated.

Christopher (2000) and Behrani *et al., (*2015) proved pathogenicity by inoculating the seedling of onion and by soil inoculation method.

Aspergillus niger

Inoculated onions of both large and small sized were completely rotted within 10 days of inoculation. On splitting large onion after 10 days, rotted putrifying tissues with blackish colouration was found. Upon isolation the same fungus was isolated. Ara *et al., (2008)* proved pathogenicity by inoculating the seedling of onion.

Botrytis species

Inoculated onions of both large and small sized were completely rotted within 10 days of inoculation. On splitting large onion after 10 days, rotten faint whitish colouration was found. Upon isolation the same fungus was isolated.

Mycoflora associated with stored onion

Infected samples were observed under microscope for the association of microorganism. The detailed association of microorganism with the samples collected from different market were described below. A total number of 316 large sized samples were evaluated and out of that 141 samples were associated with *A. niger* followed by *F. oxysporum*(107) and *Botrytis sp.*(68). In small onion also maximum samples were associated with *A. niger* (73), *F. oxysporum*(62), *B. cineria*(44)(Table 2).

Fusarium oxysporum

Whitish fungal growths present on the infected onion were observed under microscope. Mycelial beads were taken to potato dextrose agar (PDA) aseptically grown for 7 days and again purified by fungal tip method. Cottony whitish growth was observed on the PDA plate turning to slightly pink colour after 7 days of inoculation. The growth of the fungus also became completely white and turned to pink colour. Sporodochia consisted of branched conidiophore. The aerial mycelium appeared white and changed to pink colour afterwards.

The mycelium was extensive intra and inter cellular. Macro conidia are grown in sporodichia. They are mostly long, slender, pointed at both the ends, dorso ventrally curved, sickle cell, septate. The conidia was measured and varied from 18.39-25.63×3.58-4.93µm. The fungus was identified as *F.oxysporum.* Micro conidia were also observed in the culture. They are usually one celled, oval shaped. Chlamydospores were also observed in the culture which were characterized by the appearance of thick rounded double layer dark spots found in chains and measured 5.16 µm as shownin Fig 1(a,b).

Brayford (1996), Kawade *et al.,* (2012) and other workers reported presence of macro, micro conidia and chlamydospore in *F. oxysporum* culture. Patra and Biswas (2016) also reported conidial size ranging from 13-15 x 2-3 µm to 15-19 x 3-4 µm in culture of *Fusarium oxysporumf. sp. cicero.* In the current study thick round double layer dark chlamydospores also observed in chains measuring 5.16µm in old culture of *Fusarium oxysporum.* Cramer (2000) also reported the existence of chlamydospores in old mycelia as half round cell with thick cell wall which confirmed the finding of the current study.

Botrytis species

Infected onion bulbs developed semi watery starting from neck region, grey white mycelia were developed on the petridish. The fungus covered in entire plate within 8 days of inoculation. Abundant short conidiophores about 1mm long were found. Conidia were round to oval, single celled and measured as 7.77×4.58µm as shown in Fig 2 (c,d). The fungus was identified as *Botrytis sp.* as per the available literature. This was also reported by Nielsen *et al.,* (2001) who has found white fluffy mycelium and sparse production of conidia by *Botrytis sp.* Shirane *et al., (*1989) reported two groups of spore size of *Botrytis allii* of onion i.e 10- 11 µm x 5-6 µm, and the other 8-9 µm x 4-5 µm. The current measurement was confirmed by the above one.

Aspergillus niger

The blackish sporulation found on the infected onion was transferred to PDA and pure culture was prepared. Colonies of PDA were found to be faint white and gradually changed to black colour due to aerial sporulation by the fungus. Mycelium was submerged hyaline and septate. Conidiophores arised on the mycelium were yellow brown near the head, thick walled, mostly non septate. Conidial heads were brown to black, globose forming a vesicle and measured 55-85 µm in diameter. Phialedes were born directly and conidia were found in chains.

The conidia were round, smooth at first and later turned to serrated impacting rough appearance to the outer peripheral wall as shown in Fig 3 (e,f). The fungus was identified as *A. niger* according to the available literature. Alexopoulous (1952) and Walker (1952) described *A.niger* associated with onion having continuous dark walled spherical conidia 2-5 µm in diameter born in chains.

In the current study, conidia are present in chains on the black globose vesicle measuring 55-85 µm. The conidia were found as round and serrated with rough appearance with outer peripheral wall.

Table.1 Collection of infected onion sample from different market places

Sl. No	Name of market places of Bhubaneswar	No of infected samples	
		Large size onion	Small size onion
1	No 1 market	82	75
2	Delta square market	58	26
3	Siripur market	80	15
4	Ruchika market	96	63
Total		316	179
Grand total		495	

Table.2 Microscopic observation of different infected samples collected

No of infected samples collected									
Sl No	**Name of the market place of Bhubaneswar**	**Large size onion**				**Small size onion**			
		Total no of samples	***Botrytis sp.***	***Fusarium oxysporum***	***Aspergillus niger***	**Total no of samples**	***Botrytis sp.***	***Fusarium oxysporum***	***Aspergillus niger***
01	Unit 1	82	11	23	48	75	15	18	42
02	Delta square	58	7	21	20	26	06	11	9
03	Siripur	80	16	26	38	15	4	6	5
04	Ruchika	96	27	34	35	63	19	27	17
	Total	316	68	107	141	179	44	62	73

Table.3 Growth behaviour of *Fusarium oxysporum* in different media 7 days after inoculation

Treatment	Different solid media	Mean colony diameter (mm)	Percent inhibition in comparison to PDA
T1	Potato dextrose agar	80.13	0.0
T2	Brown's agar	70.20	12.39
T3	Czapek'sdox agar	68.20	14.8
T4	Sabouraud's agar	74.00	7.65
T5	Richard's agar	76.63	4.36
T6	Potato rose Bengal agar	38.23	52.29
T7	Malt extract agar	54.80	31.61
	SE(m)±	1.713	
	CD(5%)	5.247	

Table.4 Growth behaviour of *Aspergillus niger* in different media 5 days after inoculation

Treatment	Different solid media	Mean colony diameter (mm)	Percent inhibition as compared to PDA
T1	Czapekdox agar	56.66	30.05
T2	Malt extract agar	55.00	32.10
T3	Brown's agar	52.01	35.79
T4	Richard's agar	71.66	11.53
T5	Sabouraud's agar	61.00	24.69
T6	Potato rose Bengal agar	61.00	24.69
T7	Potato dextrose agar	81.00	0.00
	SE(m)±	2.031	
	CD(5%)	6.22	

Table.5 Radial growth of *Fusarium oxysporum* in different temperature ranges

Sl. No	Temperature (°C)	Mean radial growth(mm)
1	15	62.0
2	20	62.0
3	25	82.25
4	30	74.0
5	35	64.75
	SE (m)	1.77
	CD 5%	5.41

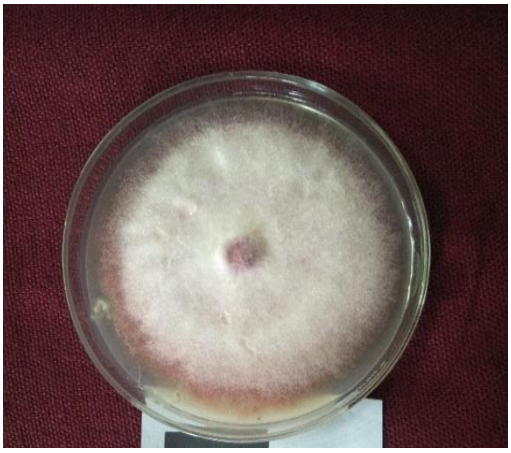

Fig.a

Fig.b

Fig.1 (a) Pure culture of *Fusarium oxysporum* plate and (b) Microscopic View of *Fusarium oxysporum*

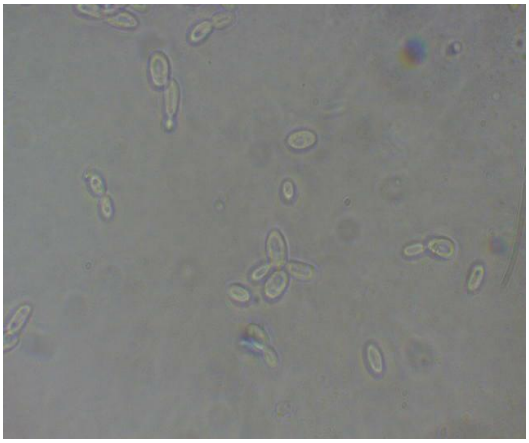

Fig.c (40x)

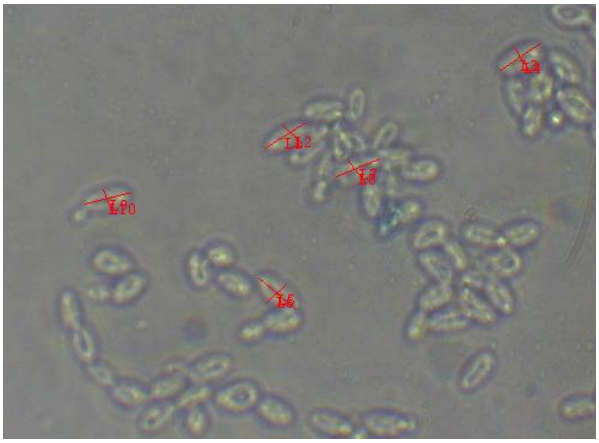

Fig.d (100x)

Fig.2 Microscopic View of *Botrytis species* (Fig c, d)

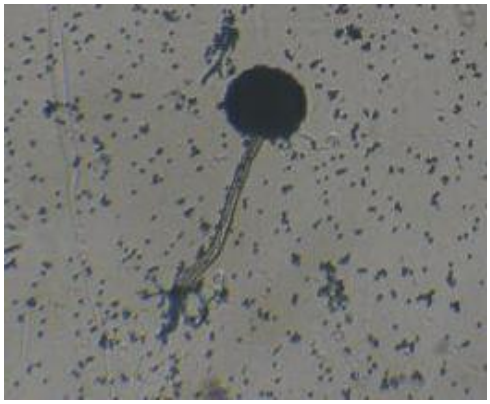

Fig.e (10x)

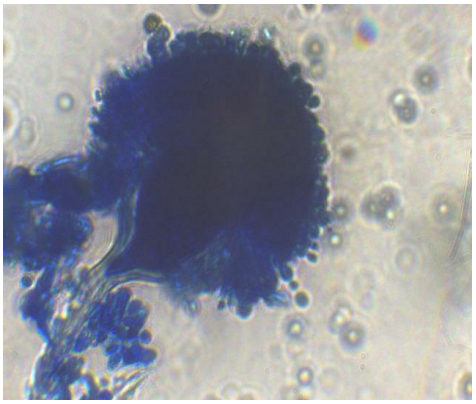

Fig.f (100x)

Fig.3 Microscopic view of *Aspergillus niger* (Fig e,f)

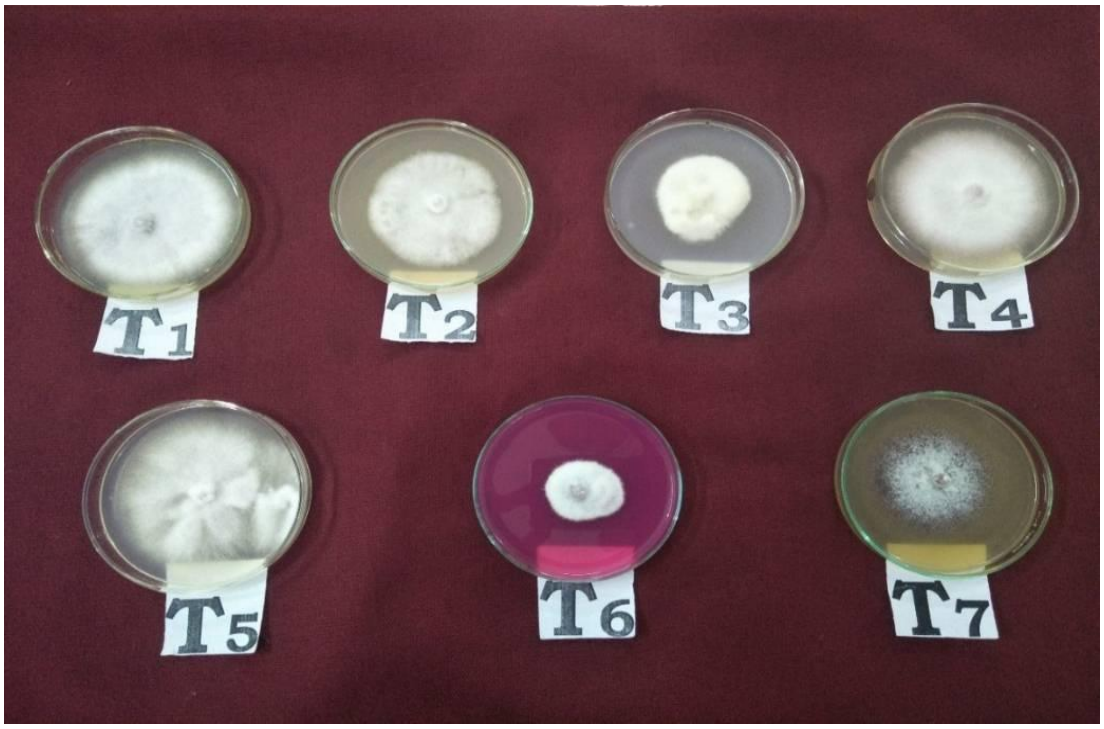

Fig.4a Growth behaviour of *Fusarium oxysporum* in different media
T1: PDA, T2: Brown's Agar, T3: Czapek's Agar, T4: Sabouraud's Agar,
T5: Richard's agar, T6: Rose Bengal agar, T7: Malt extract agar

Fig.4b Growth behaviour of *Aspergillus niger* in different media
T1: Czapek's Agar, T2: Malt Extract Agar, T3: Brown's Agar, T4: Richard's Agar,
T5: Sabouraud's Agar, T6: Potato Rose bengal Agar, T7: PDA

Cultural study

Growth behaviour of test pathogen in different media

F.oxysporum

A total number of seven media were evaluated for the growth of test pathogen *F.oxysporum.* Significant radial growth was observed among all the media. Highest growth was observed in PDA (80.13mm) followed by Richard's agar (76.63mm) which was at par of PDA.

Sabouraud's agar (74.00mm) also behaved similarly for the growth of *F.oxysporum* whereas potato rose Bengal supported the least growth (38.23mm) (Table 3).Ilhe *et al.,* (2013) found excellent growth of *F.oxysporum f.sp. cepae* in PDA followed by host leaf extract.

Other workers like Pokhar and Thakore (2003), Kulkarni,(2005) and Sharma *et al.,* (2012) also found PDA as best growth medium for *F. oxysporumf.sp. gladioli* & *F.oxysporum f. sp. Lycopersici* respectively.

Aspergillus niger

Significant difference were also observed in all the growth media by *A.niger* with PDA showing highest radial growth (81mm) followed by Richard's Agar (71.66 mm). Sabouraud's agar and Potato rose Bengal were found to be same (61mm) for the growth of *A.niger* (Table 4).

Prakash and Siradhana (1978) reported maximum growth in PDA followed by Richard's agar media. The current study of Richard's agar media was 71.66mm radial growth which was next to PDA. Pathak (1993) reported better growth of *A.niger*in Richard's solution followed by PDA.

Growth behaviour of *F.oxysporum* in different temperature ranges

The test pathogen showed highest radial growth of 82.25 mm in 25°C followed by in 30°C (74.00 mm). The radial growth of the fungus increased from 15°C upto 25°C and then declined when temperature increased (Table 5).Similar observations were reported by Sharma *et al., (*2012), Chaturvedi *et al.,* (2003) which confirmed the findings of current study. Gupta *et al.,* (2010) and Mishra *et al.,* (2010) reported maximum growth of *F.oxysporum*at 28° C followed by 34°C.

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