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Study of fungi responsible for the post-harvest deterioration of onion (*Allium cepa* L.)

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Abstract
Onion (*Allium cepa L*) is extremely important vegetable crop not only for internal consumption but also as highest foreign exchange earner among the fruits and vegetables. Maharashtra is India's top onion producing state accounting for one-third of the total annual onion production of 16 million tonnes. Onions (*Allium cepa* L.) are commercially valuable and important seasonal crop of Maharashtra (India), Onion (*Allium cepa* L.) is one of the important vegetable and consumed daily as a flouring agents. The characteristics aroma and odor of onion is due to allicin (diallylthiosulfate), which accounts for its use as medicine, food, salad, spice, and condiment in the Indian foods. Many fungi are responsible for the deterioration of onion bubs due to improper handling and storage practices. The aim of the present investigation is to find out the fungi responsible for the deteriorating of onion in post harvest conditions.
The onion bulbs were collected from different markets of Mumbai (India) and screened aiming to record the incidence of fungi responsible for the deterioration of Onion bulbs during storage in different seasons. The investigation revealed that four major fungal species were prevalent on Onion bulbs. *viz. Aspergillus niger, A. flavus, Penicillium* sp. and *Rhizopous stolonifer. A. niger* and *Penicillium sp.* were the most dominated and frequently isolated fungi. Considering abundant host range and worldwide distribution of these fungi, common management strategy may not be effective. There is no statistical relationship established between the samples collected from different markets and the different climatic conditions (seasons). In all seasons the dominant fungi were present in the samples collected from different markets. This article will be helpful for providing information about integrated disease management of associated fungal disease of onion during post harvest condition and in various aeromycological investigations.

Keywords: deterioration; *Aspergillus sp.;* fungi; onion; post-harvest; allicin; seasonal crop; disease management; vegetables; climatic conditions

Introduction
India is the second largest onion producer in the world after China. India has great diverse climatic conditions which results in variety of vegetables production. In spite, of this India is far behind in vegetable production than other developed global markets. Major reason behind it is post harvest decaying of vegetables and infectious microorganisms present in environment. *Allium cepa* commonly called as pyaj in Hindi and Kanda in local language is one of the most popularly grown crops in India. It adds different flavors in cooking hence known as King of the kitchen. Onions contain phenolics and flavonoides which are potential anti-inflammatory, anti- cholesterol, anticancer and antioxidant properties. Dropsy, kidney, heart, liver, diabetes, bulbculosis are some of the important diseases which are cured by regular consumption of onion. Most onion cultivars contains about 89% water, 4% sugar, 1% protein, 2% fiber and 0.1% fat, vitamin C, vitamin B6, folic acid and numerous other nutrients in small amounts (Anonymous, 2013) [6]. It shows that the importance of onion for human use and encourages for increasing its production and productivity.
The Major Onion producing states area Maharashtra, Karnataka, Madhya Pradesh, Gujarat, Bihar, Andhra Pradesh, Rajasthan, Haryana and Telangana. Throughout India, Maharashtra ranks first in Onion production with a share of 28.32%. Nasik, Ahmednagar, Pune, Sholapur are the Concentrated Pockets of Onion growing regions of Maharashtra. More than 100,000 hectares is under onion production in Nashik alone. Lasalgaon in Nashik is the biggest onion market in Asia (APEDA, 2020).
The major varieties found in India are Agrifound Dark Red, Agrifound Light Red, NHRDF Red, Agrifound White, Agrifound Rose and Agrifound Red, Pusa Ratnar, Pusa Red, Pusa White Round. There are certain varieties in yellow onion which are suitable for export in European countries Tana F1, Arad-H, Suprex, Granex 55, HA 60 and Granex 429.There is a lot of demand of Indian Onion in the world, the country has exported 11,49,896.85 MT of fresh onion to the world for the worth of Rs. 2,320.70 crores/ 324.20 USD Millions during the year 2019-20 (APEDA, 2020).
Several field and storage diseases affecting productivity and storage capabilities of onion have been reported, among which black mould rot of onion is an important post harvest disease. Post harvest diseases are the diseases caused after harvesting the crop. About 35-40 % fruits and vegetables including onion is lost due to damage caused by different diseases all over the world and incurred great economic losses (Gupta and Verma, 2002, Kakde *et al*., 2012) [14, 21]. Inadequate and improper field processing during and after harvest, infection by different pathogen, sprouting and also poor storage methods being practiced by the farmers are the main reasons of prevailing losses.
The fungus, *Aspergillus niger* is wide spread in distribution and is found in almost all kind of soils (Domich *et al*., 1980) [10] and is capable tolerate a wide range of pH, salinity, alkalinity (Kis-Papo *et al*., 2003) [22]. This fungal pathogen

causing several important post- harvest rot diseases including onions. Onion black mould rot disease is the maximum destructive disease of storages condition. The fungus causing black mold disease is the main member of *Aspergillus* and is predominantly a plant pathogen responsible for post harvest deterioration of many stored food materials (Baytak *et al*., 2005; Ahmad *et al*., 2006, Kakde, 2012) [9, 2, 21]. Being saprophytic and filamentous in morphology *Aspergillus niger* resides and perpetuates in soil, forage, organic debris and food products causing black mold disease during post-harvest stage of onion bulbs, McDonald *et al*. (2004) [25]. A gray mold develops between the onion scales, later producing small to large black bodies (sclerotia) which develop as a solid layer around the neck. Due to the infection of *Botrytis* sp. (Botrytis Neck Rot) (Anonymous, 2009) [4]. Blue mold of onion is caused by several *Penicillium* species. Smudge disease caused by *Colletotrichum sp.* appears on dried outer scales and lower portions of the bulb as dark green dots which turn black. The symptoms may be scattered but often appear in distinct circular, concentric rings. The fungus produces enzymes that break down cell walls and allow mycelium to proliferate throughout the bulb.

All these above fungi attack a wide range of fruits, vegetables, bulbs, and seeds and hence they are common in the soil and market yards where they grow saprophytically on dead tissue on infected plant debris and produce spores profusely. Spores are also common in the air, which will settles on fresh commodities and deteriorate the vegetable and fruits. The spores of these fungi are a common inhabitant of the soil and air. The deterioration of onion bulbs occurs due to fungal disease favored by humidity and warm temperatures in transit storage conditions. Some bulbs will show no external symptoms, but when the bulb is cut open, central portions may be gray to black.

The aim of the investigation was to analyze the variations occurred in fungus in different seasons on different selected market places. The results will be helpful in understanding the relationship between the market sites and infectious fungus variations. This evaluation may provide a different aspect to study aeromycological microorganism present in various market places in accordance with integrated post harvest management.

Materials and Methods

a. Study site and Sample collection:

The Onion bulbs were collected from 4 selected markets of Mumbai city i.e. Fort, Dadar, Ghatkopar. The healthy as well as spoiled and discarded Onions including mechanically injured wounded etc. were collected in pre-sterilized polythene bag and brought back into the laboratory for further investigation. Fruit surface showing symptoms of fungal infections were cultured to identify the associated pathogens. The unwashed samples were kept in pre-sterilized moist blotter chambers separately. The samples were incubated at room temperature (28˚ C) for 3 to 5 days to allow the growth of fungi associated with it. The diseased or decayed samples were examined for the fungi by scrapping the surface issues from the infected part or margins. The disease incidence was recorded by counting number of fruits showing fungal infection against total number of fruits taken and per cent d*isease Incidence* was calculated using formula:

$$\%\,Disease\ Incidence = \frac{Number\ of\ infected\ fruits \times 100}{Total\ number\ of\ fruits\ observed}$$

b. Identification of fungi

Small sections of infected fruit were cut and surface sterilized individually in 2% sodium hypochlorite for 1 min and rinsed twice in sterile distilled water to remove the tresses of $HgCl_2$. The tissues were transferred aseptically to Petri dish containing Potato dextrose agar (PDA) and CZ. The plates were incubated for the growth till the formation of spores. Fungi were carefully isolated on the slides and mounted using lactophenol cotton blue stain. The microscopic slides were examined under the microscope for morphological examination. The culture thus obtained was observed under the microscope for various cultural and morphological characters *viz*., mycelia growth, shape, size, colour and microscopical characters of the fungus so as to identify the pathogen upto species level. On the basis of cultural and morphological characteristics the fungal pathogens were identified with the help of descriptions given in standard literatures (Ainsworth, *et al*., 1972; Ellis, 1971; Ingold, 1974; Gilman, 1957; Smith, 1969) [3, 11, 19, 12, 30].

Result and Discussion

Onion (*Allium cepa*) is one of the oldest cultivated plants in the world and is rich in flavonols and natural organosulfur compounds (OSCs). Many pathogenic and saprophytic fungi are responsible for the deterioration of food many commodities including vegetable and fruits. In the present investigation total nine major fungal species were found associated with the onion bulbs throughout the year. The list of dominant fungi associated with onion bulbs collected from different markets in different months during the period of investigation is given in Table 1.

Maximum incidences of fungi were registered in Monsoon than winter and summer season. The most favorable temperature conditions for the growth of the fungus is 28° - 34°C followed by the warm and moist conditions eliciting infection (Tysoni *et al*., 2004) [32]. The optimum temperature for growth of *Aspergillus niger* which ranges from 28 to 34 °C, and it is inhibited below 17 and above 47 °C (Sumner, 1995) [31]. Sporulation can also be take place in 24 h after infection (Salvestrin and Letham, 1994) [28]. Similar situation also prevailing in Mumbai, as weather conditions is hot and humid during summer. Due to climatic factors and variations in temperature and RH, fungal species also showed differential prevalence (Fig.2). In the present investigation the maximum fungal attack was found in the month of July and August.

Aspergillus niger infected bulbs shoed masses of black powdery spores on both exterior and between the scales of the bulbs. *Aspergillus niger* was isolated throughout the year almost every time and found dominant in all seasons than other fungi. In summer it contributed 20.14, in Monsoon it is 36.11 while in winter is 26.39 percent. Black mold rot are the most destructive diseases of onion in storage and in the field and Aspergillus niger Van Tieghem was found to be associated with black mold rot of onion (Wani *et al*., 2011) [33]. Qadri *et al*., (1982) [26] reported spoilage caused by *A. niger* was as high as 80 per cent. *Aspergillus niger* is soil saprophyte being ubiquitous in occurrence attacks onion by producing various enzymes and toxins and establishes itself in bulb and other tissues.

Hot and humid climate is favorable for the growth of these saprophytic fungi. No much difference was recorded in the sample collected from different markets. Infections spread

from bulb to bulb by direct contact, through bruises or wounds, by mechanical means or by air-borne spores. Rajam (1992) [27] reported that among the post-harvest diseases of onion, black mould rot caused by Aspergillus niger was the predominant one. In the present investigation also A. niger is most prevalent fungi thought the year from the onion samples collected from different markets. Commonly occurring fungi such as *Aspergius spp.*, *Penicillium*, *Fusarium* etc. reported as mycotoxigenic and can be controlled with clove oil (eugenol) hence regulate the production of aflatoxins (Juglal *et al*., 2002) [20].

Penicillium species is on second position in terms of association with onion bulbs collected from different markets. Maximum infestation of *Penicillium* sp. was observed during monsoon (25.0%), winter (21.5%) and minimum in summer (11.8%). The blue moulds are frequently isolated from stored diseased bulbs of local cultivars of onion by Hussain *et al*., (1977). List of dominant fungi associated with Onion in different seasons during storage has been depicted in Figure 1.

Identification of pathogens which causes several diseases in onion is essential for effective inhibition of target pathogens. Different species of *Aspergillus* pathogens are reported to cause black and blue mould disease on onion bulb during storage. The fungal infestation stats at germination or seedling stage and continue throughout the storage (Hayden and Maude, 1992; Hayden *et al*., 1994 a & b; Koycu and Ozer, 1997; Sirois *et al*., 1998) [15, 16, 17, 23, 29]. The fungus causing black mold is the main member of *Aspergillus* and is predominantly a plant pathogen responsible for post harvest deterioration of stored food materials (Marziyeh Tolouee *et al*., 2010) [24]. Chemical treatment can be found best to inhibit black mold and other fungal pathogens disease in the onion bulbs (Grinstein *et al*., 1992) [13].

Statistical analysis

The statistical analysis i.e. Chi square test was performed to check the seasonal variations between fungal species at different selected market sites. It was observed that all the fungal species not showed variations depending on the area and climatic fluctuations. Most of the variation was recorded for the period of winter and monsoon. *Aspergillus spp, Penicillium spp, Rhizopus stolonifer and* shown highest growth monsoon season (Table 3).

Table 1: Fungi associated with onion bulbs collected from different markets in different seasons/months during the period of investigation

Name of Fungi	Area/Market	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Total	Percentage
Total number of Samples		12	12	12	12	12	12	12	12	12	12	12	12	144	
Alternaria spp	Fort	2	1	0	1	2	1	3	1	0	2	1	0	14	9.7
	Dadar	0	3	2	0	3	2	2	0	2	3	0	1	18	12.5
	Ghatkopar	1	0	0	0	1	1	3	3	0	1	0	3	13	9.0
Aspergillus flavus	Fort	2	2	3	1	3	5	6	3	5	3	4	2	39	17.4
	Dadar	4	1	2	1	2	7	3	5	4	2	3	3	37	17.4
	Ghatkopar	5	3	2	3	4	4	6	4	3	4	3	2	43	11.8
Aspergillus niger	Fort	3	2	2	1	3	3	0	3	3	0	2	3	25	27.1
	Dadar	2	3	0	2	3	2	2	4	0	2	3	2	25	25.7
	Ghatkopar	1	0	0	1	0	4	2	1	3	3	0	2	17	29.9
Botrytis spp	Fort	0	1	0	0	1	2	1	2	1	0	0	1	9	6.3
	Dadar	0	0	0	0	0	3	1	0	0	0	0	0	4	2.8
	Ghatkopar	2	0	0	0	0	1	0	0	0	0	1	1	5	3.5
Colletotrichum spp	Fort	0	0	1	1	2	3	1	2	3	0	0	2	15	10.4
	Dadar	1	1	0	0	0	3	0	1	2	0	0	3	11	7.6
	Ghatkopar	2	0	1	0	1	2	1	1	0	3	1	0	12	8.3
Fusarium oxysporum	Fort	0	0	0	0	0	1	2	0	0	0	0	1	4	2.8
	Dadar	0	1	0	0	0	1	1	0	0	1	2	0	6	4.2
	Ghatkopar	0	0	0	1	0	2	2	0	1	0	1	1	8	5.6
Geotrichum candidum	Fort	0	0	0	0	0	1	1	1	0	0	1	0	4	2.8
	Dadar	0	0	0	0	1	0	0	0	0	0	0	0	1	0.7
	Ghatkopar	0	0	1	0	0	1	0	0	0	0	1	0	3	2.1
Penicillium spp	Fort	1	3	0	1	3	3	2	3	3	2	3	2	26	18.1
	Dadar	2	3	1	1	4	2	3	4	5	4	2	2	33	22.9
	Ghatkopar	3	1	1	0	2	3	4	3	1	3	3	1	25	17.4
Rhizopus stolonifer	Fort	1	0	2	1	2	2	2	2	2	0	2	0	16	11.1
	Dadar	0	0	1	0	1	1	1	0	3	2	1	1	11	7.6
	Ghatkopar	1	2	0	3	2	2	3	2	0	3	2	2	22	15.3

Table 2: Prevalence of dominant fungi during different seasons and its percent contribution

S N	Name of Fungi	Summer	Monsoon	Winter
		(%)	(%)	(%)
1.	*Alternaria spp*	6.94	15.28	9.03
2.	*Aspergillus flavus*	11.81	18.75	15.97
3.	*Aspergillus niger*	20.14	36.11	26.39
4.	*Botrytis spp*	2.08	7.64	2.78
5.	*Colletotrichum spp*	4.86	11.81	9.72

6.	*Fusarium oxysporum*	1.39	6.25	4.86
7.	*Geotrichum candidum*	0.69	3.47	1.39
8.	*Penicillium spp*	11.81	25.00	21.53
9.	*Rhizopus stolonifer*	7.64	13.89	12.50

Table 3: Chi square test for relationship between seasonal variations and incidence of postharvest fungal diseases

Observed	Expected	(Oi-Ei)^2/Ei
32	33.05829596	0.03387925
31	31.75336323	0.01787389
34	32.18834081	0.10196577
72	67.8206278	0.25754925
62	65.14349776	0.1516894
65	66.03587444	0.01624929
48	51.12107623	0.19054992
53	49.10313901	0.30925773
49	49.77578475	0.01209106
	Calculated value=	1.09110557
Chi Square Table Value=		9.487729

*Calculated Value < Table Value, Accept H_o (There is no association between Area and Season)

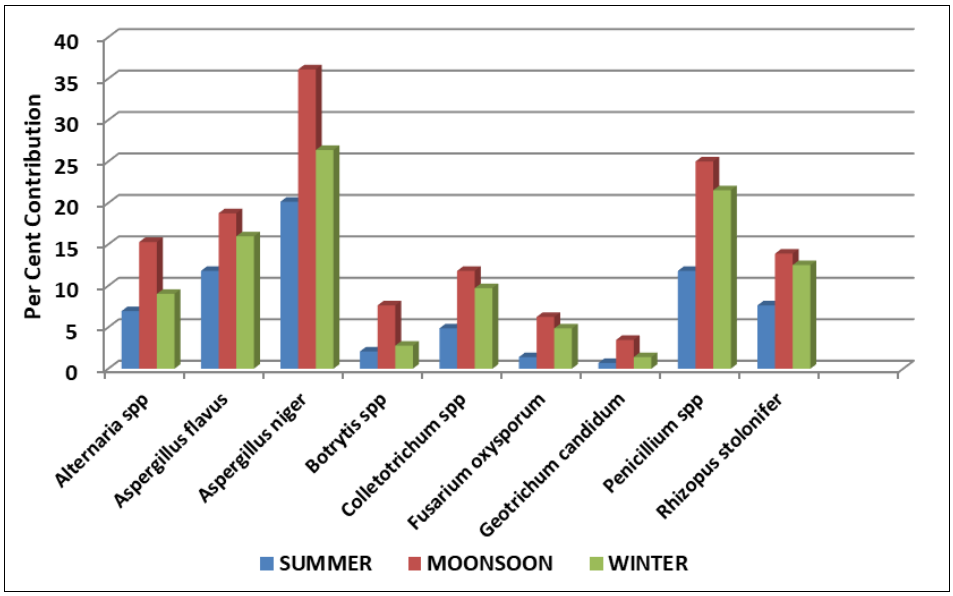

Fig 1: Dominant fungi associated with Onion bulbs during storage

Conclusions

The most common and dominant fugal species were recorded in the sample was *Aspergillus spp.*, *Penicillium sp. and Rhizopous stolonifer*. Onion black mould rot disease vaused by *Aspergillus niger* is the maximum destructive disease of storages condition in the onion bulbs collected from different markets. The fluctuations in the fungal incidences are non-significant with respect to location and seasonal variations. Maximum fungal incidences were recorded during monsoon than winter and summer. Certain pre-harvest treatments and post-harvest processing like curing and moisture reduction in onion bulbs may reduce the post-harvest losses. The study provides useful insight that according to various climatic conditions the fungal infections may vary. The study will be helpful in various aeromycological studies and for more strategies to prevent post-harvest fungal infections on *Allium cepa*.

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