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Influence of Fertilisers, Host Factors and Storage Conditions in Relation to Disease Severity: A Case Study of Black Mould Rot of Onion

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Aspergillus niger was found to be the predominant pathogen associated with black mould rot of onion during storage. Market survey for the assessment of spoilage caused by the fungus recorded a loss of 2.9% to 12.09% during the period from June 1998 to February 1999. Application of higher doses of calcium in the form of gypsum (400 kg/ha) and lower dose of nitrogen in the form of urea (50 kg/ha) to the field and advancing the harvest of onion bulbs by fifteen days significantly reduced the spoilage of bulbs during storage. An inverse relationship existed between neck length of the bulbs and spoilage at storage. Bulb rotting was noticed when the storage temperature was between 30 °C and 40 °C and the relative humidity was above 80%. Further, *A. niger* infection caused reduction in pungency of onion bulbs which was more pronounced at grade 4 than grade 1. The culture filtrates of *Aspergillus niger* and *Aspergillus flavus* isolated from onion as well as the extracts from onion bulbs infected with the above fungi were free from aflatoxin contamination.

Keywords: Onion, black mould, *A. niger,* fertilisers, host, storage, pungency, aflatoxin.

Onion (*Allium cepa* L.) an important spice crop widely grown in India is seasonal, despite the demand being constant and hence there is preharvest scarcity and postharvest glut for this commodity. In order to regulate the supply and enable the farmer to get a remunerative price for the produce long-term storage of onion is a prerequisite. Losses of onion during storage are considerable mainly due to sprouting and contamination by several microorganisms. A general estimate of storage losses of onion in developing countries was given as 16 to 35% (NAS, 1978). Nearly 40% of the production is lost during postharvest handling and sprouting, of which microbial spoilage alone contributed 15–20% of the total loss (Pantastico and Bantista, 1976; Bhagchandani et al., 1980). In India, Venkatarayan and Delvi (1951) and Dang and Thakur (1972) reported 15% loss of onion bulbs due to fungal spoilage. Quadri and Srivastava (1985) observed that the spoilage caused by *A. niger* was as high as 80%.

A. niger, a soil saprophyte being ubiquitous in occurrence by virtue of its ability to contaminate and produce various enzymes and mycotoxins, attacks onion and establishes itself in bulb and other tissues. A perusals of literature revealed that little work have been done on the various factors influencing the development of black mould rot of onion. Hence the present study was undertaken to assess the extent of loss caused by *Aspergillus* rot, to study the influence of fertilisers, host factors such as age and length of topping and environmental factors during storage on postharvest spoilage of onion bulbs as well as to study the quality deterioration of contaminated bulbs.

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Materials and Methods

Assessment of A. niger *contamination in onion bulbs*

Fortnightly market surveys were made during June 1998 to February 1999 to identify the fungi associated with contaminated onion bulbs with special reference to *A. niger.* Hundred onion bulbs and godown sweepings showing distinct symptoms of rotting were collected at random from storage centers of Tiruppur Kumaran Market, Coimbatore, Tamil Nadu (India) and also from farmers holdings in and around Coimbatore. The disease was scored on a 0–5 scale formulated as given below, with grade specifications given based on the area of scale involved in spoilage. The Percent Disease Index (PDI) was worked out by using the formula of Rose (1974).

Isolation

The dried outer scales, fleshy inner scales and rootlets from contaminated bulbs were cut into small pieces and surface sterilized with 0.1% mercuric chloride for 2 min. They were then washed in three changes of sterile distilled water and plated on potato dextrose agar (PDA), Czapecks agar (CZA) and oats agar (OA) media in sterile Petri dishes and incubated at room temperature (28 ± 2 °C). The different fungi associated were isolated and purified by single spore isolation technique (Riker and Riker, 1936) and maintained on PDA, CZA, and OA slants.

Pathogenicity

The pathogenicity test for *A. niger* isolated from contaminated bulbs was done as per the method described by Venkatarayan and Delvi (1951).

Effect of fertilizer application on black mould rot

CALCIUM

A field experiment was conducted at the Agricultural College and Research Institute, Coimbatore during May – October 1999. Five levels of calcium (Ca) 0, 100, 200, 300, 400 kg/ha were tried and the nutrient was applied in the form of gypsum ($Ca\ So_4$) twice, one before transplanting and another fifteen days after transplanting of seedlings in the field. Matured bulbs were harvested and stored at room temperature (28 ± 2 °C) and *A. niger* contamination of bulbs was recorded at weekly intervals and expressed as PDI. The available Ca present in the soil as well as plant sample was estimated as per the procedure prescribed by Jackson (1973).

NITROGEN

The experiment was laid out in randomized block design with four levels of nitrogen 50, 100, 150, 200 kg/ha replicated five times at Agricultural College and Research Institute, Coimbatore during the same period. Phosphorus (150 kg/ha) and potash (75 kg/ha) were applied for all the treatments. Nitrogen (N) was applied in the form of urea.

Half of N and full dose of phosphorus and potassium were applied before transplanting. The remaining half of N was applied 30 days after transplanting. Matured bulbs were harvested and yield data as well as fungal contamination in storage were recorded. Alkaline KMNo4 mineralizable N was estimated by the method described by Subbiah and Asija (1956). Total N from the plant sample was estimated based on micro Kjeldahl method of Jackson (1973).

Host factors influencing black mould rot of stored onions

AGE AND SUSCEPTIBILITY OF ONION BULBS TO BLACK MOULD ROT

Bulbs used for the study were harvested at three stages of maturity viz. tops up and green (fifteen days before normal harvest period), tops fallen over but still green (normal harvest period) and tops fallen over and dry (fifteen days after actual harvest period) and were stored at room temperature (28 ± 2 °C).

EFFECT OF LENGTH OF TOPPING ON THE SUSCEPTIBILITY OF BULBS TO BLACK MOULD ROT

Harvested onion bulbs were topped at 0, 2, 4 and 6 cm height and stored at room temperature (28 ± 2 °C).

Environmental factors at storage influencing black mould rot of stored onions

TEMPERATURE

The effect of different temperatures on black mould rot of onion bulbs in storage, was studied in BOD incubators and at room temperatures (28 ± 2 °C). *A. niger* inoculated bulbs were incubated at 0 °C, 10 °C, 20 °C and 40 °C and stored at room temperature (28 ± 2 °C).

RELATIVE HUMIDITY

The effect of relative humidities on spoilage of onion bulbs in storage was studied in desiccators having relative humidities of 100%, 95%, 75%, 50%, 35%. The relative humidities were obtained by the method described by Solomon (1951). In all the above four experiments, fifty bulbs were used per sample and they were replicated three times. Observations on the extent of rotting of bulbs at storage were taken at weekly intervals.

Quality deterioration due to A. niger

ESTIMATION OF PUNGENCY

Onion bulbs were analysed for their pungencies by the method suggested by Schwimmer and Weston (1961). Five different samples based on the area of infection as given below were taken for the analysis of pyruvic acid.

1. Samples having 25% infection – grade 1
2. Samples with 25–50% infection – grade 2
3. Samples with 50–75 infection – grade 3
4. Samples with 75–100 infection – grade 4
5. Samples with no infection – grade 0

Aflatoxin in onion bulbs

FROM CULTURE FILTRATE

Onion isolates of *A. flavus* and *A. niger* were tested for their ability to produce aflatoxin in culture media as per the method of Diener and Davis (1966) in a thin layer chromatography (TLC).

AFLATOXIN IN ONION BULBS

Fifty g of bulbs infected with *A. niger* was ground in a pestle and mortar and taken in a 250 ml conical flask and moistened by adding 10–15 ml of distilled water and 200 ml chloroform was added. The mouth of the flask was stoppered with a cotton plug in aluminium foil and shaken mechanically for one h. After shaking, the slurry was filtered through a Buckner funnel under mild suction. The flask and the slurry were thoroughly washed with additional chloroform (25 ml) and the filtrate was collected. The filtrate was transferred quantitatively to a separating funnel and shaken with water and one half volume of chloroform. After the separation of the phases, the bottom (chloroform) layer was drained into a flask containing about 10 g sodium sulphate (anhydrous) to absorb water. The clear chloroform extract was concentrated *in vacuo* over warm water bath using quick fit distillation set. The concentrate was made up to known volume with chloroform and stored in amber coloured vials under refrigeration until analysis.

With the help of micropipette 50 µg of test material was spotted on TLC plates, coated with silica gel HR 25. Three µg of standard aflatoxin B_1, was spotted on the plate. The plates were placed in the solvent chamber containing chloroform: acetone (9 : 1) for about 50 min till the solvent reached 3/4 height of the plate. The plates were then air dried and visualized under UV light (320–360 nm), in a dark room. The same procedure was followed for the infected samples of maize and groundnut which were used as checks.

Statistical analyses

Statistical analyses of the experiments were performed using the IRRISTAT modules of the International Rice Research Institute, Manila.

Results

Market survey

The results of the fortnightly surveys to assess the loss in onion bulbs due to *Aspergillus* sp. in onion mundies in and around Coimbatore indicated that spoilage of onion due to *A. niger* and *A. flavus* were 6.78% and 5.07%, respectively. The decay due to *A. niger* ranged from 2.90% to 12.09%, while it was 1.49% due to *A. flavus. A. flavus* was conspicious by its absence during the month of December.

The mean spoilage of onion bulbs by black mould rot was more during the month of June (11.82%), followed by July (10.09%). The spoilage was minimum during the month of December (1.45%). A positive correlation was observed between temperature

and spoilage of onion bulbs as the spoilage was high during June when the temperature was also higher (34.5 °C) followed by July (32.3 °C) and it was lower during December which recorded a lower temperature of 24.5 °C *(Table 1).*

Table 1

Market survey of black mould rot of onion bulbs (1998–99)

Month	Mean temperature		RH %	Extent of spoilage (PDI)			
	Max	Min		*A. niger*	Mean	*A. flavus*	Mean
June	34.5	24.5	76.0	12.65 (20.88)		11.83 (20.05)	
	33.4	24.4	75.0	11.52 (19.82)	12.09 (20.36)	11.24 (19.55)	11.54 (19.82)
July	32.3	22.0	72.0	11.22 (19.55)		8.05 (16.54)	
	32.5	23.0	74.0	11.56 (19.91)	11.39 (19.73)	9.53 (17.95)	8.79 (17.26)
Aug	31.6	21.3	74.0	8.38 (16.85)		8.53 (16.95)	
	30.5	22.5	76.0	7.91 (16.32)	8.15 (16.64)	6.38 (14.65)	7.46 (15.89)
Sep	31.3	22.0	78.0	8.25 (16.74)		4.53 (12.25)	
	31.2	23.5	80.0	7.38 (15.79)	7.82 (16.22)	5.42 (13.44)	4.98 (12.92)
Oct	29.0	21.2	89.0	5.05 (13.05)		5.23 (13.18)	
	31.0	22.0	88.0	5.41 (13.44)	5.23 (13.18)	4.68 (12.52)	4.96 (12.92)
Nov	28.0	21.5	86.0	3.23 (10.30)		2.98 (9.97)	
	30.0	22.0	88.0	3.53 (10.78)	3.38 (10.62)	–	1.49 (7.03)
Dec	24.5	20.3	91.0	2.83 (9.63)		–	
	26.3	21.0	91.0	2.97 (9.97)	2.90 (9.80)	–	–
Jan	30.0	22.4	92.0	4.34 (11.97)		3.29 (10.47)	
	32.5	21.0	91.0	4.15 (11.83)	4.25 (11.97)	3.14 (10.14)	3.22 (10.30)
Feb	32.5	23.3	89.0	5.65 (13.69)		3.13 (10.14)	
	33.0	22.5	89.0	5.93 (14.06)	5.79 (13.94)	3.27 (10.47)	3.20 (10.30)
Mean	30.8	22.2	83.0	6.78 (15.12)	6.78 (15.12)	5.07 (13.05)	5.07 (13.05)

Figures in parentheses are arcsine transformed values

Isolation of postharvest microflora in onion

Five fungi were identified from the diseased onion bulbs collected from various store houses, either individually or in combinations. They were *A. niger*, *A. flavus, Fusarium oxysporum* f. sp. *cepae, Penicillium notatum, Rhizopus stolonifer. A. niger* was the predominant pathogen (73.33%) followed by *P. notatum* (15%) in dried outer scales of onion. *A. flavus* and *F. oxysporum* f. sp. *cepae* were not present in the dried outer scales. *A. niger* was abundantly present (60%) in fleshy scales of onion bulbs followed by *F. oxysporum* f. sp. *cepae* (30%). These two fungi could be isolated in all the three media. However, *P. notatum* was isolated only on oats medium and that too to a lesser extent of 3.33% and *R. stolonifer* was absent in fleshy scales of onion. *A. niger* was more frequently isolated (65%) from the soil adhering to onion bulbs followed by *F. oxysporum* f. sp. *cepae* (21.67%). *R. stolonifer* was not present in soil plated in all the three media. Even in the rootlets of onion bulbs, *A. niger* was found to be the most predominant fungi (48.33%) followed by *F. oxysporum* f. sp. *cepae* (28.33%), *R. stolonifer* (15%) and *A. flavus* (8.33%) *(Table 2).*

Table 2

Occurrence of postharvest pathogens in onion

Fungi isolated	Dried outer scales			Mean	Fleshy scales			Mean	Rhizosphere soil			Mean	Rootlets			Mean
	OA	CZA	PDA		OA	CZA	PDA		OA	CZA	PDA		OA	CZA	PDA	
A. niger	60	85	75	73.33	55	65	60	60.00	60	75	60	65.00	55	55	35	48.33
A. flavus	–	–	–	–	5	15	–	6.67	–	–	–	–	–	25	–	8.33
F. o. f. sp. *cepae*	–	–	–	–	30	20	40	30.00	25	15	20	21.67	30	10	45	28.33
P. notatum	15	15	15	15.00	10	–	–	3.33	15	5	10	10.00	–	–	–	–
R. stolonifer	25	–	10	11.67	–	–	–	–	–	–	–	–	15	10	20	15.00

Table 3

Effect of calcium application on the black mould rot of onion bulbs

Calcium dose kg/ha	Spoilage (PDI)/days after storage													Mean	Yield t/ha	Percent red. over control
	7	14	21	28	35	42	49	56	63	70	77	84	91			
100	8.63	13.50	20.00	24.5	28.25	32.63	36.00	42.00	45.50	48.50	51.88	55.75	59.63	35.91	3.73	5.50
	(17.05)	(21.56)	(26.57)	(29.67)	(32.14)	(34.82)	(36.87)	(40.40)	(42.42)	(44.14)	(46.03)	(48.33)	(50.53)	(36.81)		
200	7.50	11.88	17.38	21.88	26.25	29.13	32.88	37.25	40.50	43.88	48.13	51.50	55.00	32.35	3.81	14.34
	(15.89)	(20.18)	(24.65)	(27.90)	(30.85)	(32.65)	(35.00)	(37.64)	(39.52)	(41.50)	(43.91)	(45.86)	(47.87)	(34.82)		
300	7.50	10.50	15.38	19.33)	22.13	25.50	29.00	33.00	35.75	38.88	42.63	45.63	49.13	28.82	3.88	34.16
	(15.89)	(18.91)	(23.11)	(26.28	(28.04)	(30.33)	(32.58)	(35.06)	(36.75)	(38.59)	(40.74)	(42.48)	(44.48)	(32.46)		
400	6.88	9.38	13.63	16.25	18.75	21.50	24.00	27.00	30.38	33.00	36.63	38.50	41.50	24.42	3.90	35.74
	(15.23)	(17.85)	(21.64)	(23.73)	(25.70)	(27.62)	(29.33)	(31.31)	(33.46)	(35.06)	(37.23)	(38.35)	(40.11)	(29.60)		
Control	9.75	14.75	21.13	26.50	30.00	34.38	38.50	43.00	47.50	51.00	55.13	59.38	63.00	38.00	3.50	–
	(18.24)	(22.63)	(27.35)	(30.98)	(33.21)	(35.91)	(38.35)	(40.98)	(43.57)	(45.57)	(47.93)	(50.42)	(52.54)	(38.06)		
Mean	8.05	12.00	17.50	21.75	25.11	28.63	32.08	36.45	39.93	43.05	46.88	50.15	53.65	31.94	–	–
	(16.54)	(20.27)	(24.73)	(27.76)	(30.07)	(32.33)	(34.51)	(37.71)	(39.17)	(41.03)	(43.22)	(45.11)	(47.12)	(34.39)		

Figures in parentheses are arcsine transformed values

	CD (5%)	
Treatments	0.1067	CD (P = 0.05) 0.022
Days	0.1924	
Interaction	0.3849	

Nutritional factors influencing the disease development

CA APPLICATION ON THE BLACK MOULD ROT OF ONION BULBS

Field data's in *Table 3* revealed that increased dose of Ca application (400 kg/ha), besides reducing storage rot (24.42%), increased the yield (3.9 t/ha). Where there was no application of Ca, a higher rotting (38%) was noted with a lesser yield (3.5 t/ha). Analysis of Ca content in soil and bulb samples showed that the Ca content was found to be more in treatment receiving more Ca (400 kg/ha), when compared to untreated control (5440 kg/ha) with a bulb Ca level of 1.28% *(Table 4).*

Table 4

Calcium content in soil and bulb samples

Calcium level (kg/ha)	Soil (kg/ha)	Bulb (%)
100	5680	1.68
200	6160	1.84
300	8080	2.00
400	9200	2.72
Control	5440	1.28

N APPLICATION ON THE BLACK MOULD ROT OF ONION BULBS

The spoilage percentage was higher in the onion samples from plots applied with higher dosage of N (200 kg/ha) as compared to those receiving lesser amount. Reduced levels of rotting (26.69%) was observed in samples obtained from plots receiving N dose of 50 kg/ha in the field. However, the yield from these plots were lower (1.92 t/ha). Samples from plots receiving higher dose of N (200 kg/ha) recorded not only higher incidence of rotting (43.2%), but also higher yield (3.8 t/ha) *(Table 5).*

The available N from the soil and plant samples were also higher in plots which received 200 kg/ha of N, the quantity being 225 kg/ha and 1.44%, respectively. Soil and plant N in plots which received only 50 kg/ha recorded 192 kg/ha and 1.29%, respectively *(Table 6).*

Table 6

Nitrogen content in soil and bulb samples

Nitrogen level (kg/ha)	Soil (available N) (kg/ha)	Bulb (%)
50	192	1.29
100	202	1.36
150	215	1.42
200	225	1.44

Table 5

Effect of nitrogen application on the black mould rot of onion bulbs

Nitrogen dose kg/ha	Spoilage (PDI)/days after storage													Mean	Yield t/ha	Percent red. over control
	7	14	21	28	35	42	49	56	63	70	77	84	91			
50	7.50	10.50	13.50	17.00	20.50	23.10	27.00	30.00	33.50	36.20	39.70	43.00	45.50	26.69	1.92	29.58
	(15.89)	(18.91)	(21.56)	(24.35)	(26.92)	(28.73)	(31.31)	(33.21)	(35.37)	(36.99)	(39.06)	(40.98)	(42.42)	(31.11)		
100	8.00	12.00	16.20	20.50	26.00	29.60	33.00	36.70	39.50	42.60	46.50	49.10	51.90	31.66	2.50	16.46
	(16.43)	(20.27)	(23.73)	(26.92)	(30.66)	(32.96)	(35.06)	(37.29)	(38.94)	(40.16)	(42.99)	(44.48)	(46.09)	(34.27)		
150	9.50	13.06	20.60	26.60	30.00	34.40	38.50	43.00	47.50	51.40	55.10	59.50	63.00	37.90	3.40	–
	(17.95)	(21.64)	(26.99)	(31.05)	(33.21)	(35.91)	(38.35)	(40.98)	(43.57)	(45.80)	(47.93)	(50.48)	(52.54)	(38.00)		
200	9.30	14.90	23.50	30.00	35.60	39.50	44..40	49.70	55.00	59.00	63.30	67.00	70.70	43.20	3.80	–13.98
	(17.76)	(22.71)	(29.00)	(33.21)	(36.63)	(38.94)	(41.78)	(44.83)	(47.87)	(50.18)	(52.71)	(54.94)	(57.23)	(41.09)		
Mean	8.58	12.75	18.45	23.53	28.03	31.65	35.73	39.85	43.88	47.30	51.15	54.66	57.78	34.86	–	–
	(17.05)	(20.96)	(25.47)	(29.00)	(31.95)	(34.27)	(36.69)	(39.17)	(41.50)	(43.45)	(45.69)	(47.64)	(49.94)	(36.21)		

Figures in parentheses are arcsine transformed values

	CD (5%)	
Treatments	0.1436	CD (P = 0.05) 0.022
Days	0.2589	
Interaction	0.5177	

Host factors influencing the disease development

AGE OF ONION BULBS AT HARVEST AND SUSCEPTIBILITY TO BLACK MOULD ROT

It was observed that the bulbs harvested fifteen days before normal harvest (145 days) recorded a lesser spoilage (31.20%) due to *A. niger* as compared to the increased spoilage (41.95%) in bulbs lifted 15 days after normal harvest. At three months after storage, the percentage of fungal spoilage was 64.93% in bulbs harvested fifteen days after normal harvest as against 51.14% in bulbs harvest fifteen days earlier *(Table 7).*

NECK HEIGHT AND DISEASE INCIDENCE

An inverse relationship was noticed between neck heights at harvest and spoilage due to *A. niger* in onion bulbs. Bulbs harvested with 6 cm neck height recorded less spoilage (22.93%), followed by 26.30% spoilage in bulbs with a neck height of 4 cm. Bulbs left without any neck showed more spoilage (35.41%) *(Table 8).*

Environmental factors influencing the disease development

EFFECT OF TEMPERATURE ON BLACK MOULD ROT OF ONION BULB

Bulb rotting was more with increase in storage temperature. The bulbs stored at 0 °C and 10 °C recorded no spoilage even after 35 days. Whereas those stored at 40 °C, recorded the maximum spoilage of 54.40% followed by bulbs at 30 °C (40.98%). At 20 °C, the percentage of spoilage was least (24.40%). At 20 °C, 30 °C and 40 °C, the spoilage rate increased with increase in storage period. At thirty-five days after storage, 95.5% of the bulbs stored at 40 °C were spoiled due to *A. niger (Table 9).*

EFFECT OF RELATIVE HUMIDITY ON BLACK MOULD ROT OF ONION BULB

Maximum rotting of 60.53% was observed in bulbs stored at a relative humidity of 100% followed by 50.25% in those stored at 90%. Bulbs stored at 100% relative humidity also recorded higher sprouting losses. Those stored at 35% relative humidity recorded a rotting of 26.43% and it was on par with those stored at 50% relative humidity (26.53%). Shrivelling and desiccation were noted in bulbs stored at 35% and 50% relative humidities. At 35 days after storage, cent % rotting was observed in bulbs stored at 100% relative humidity, as against 46.5% rotting in bulbs stored at 35% relative humidity. The increase in spoilage was higher at 100% relative humidity, as compared to those stored at 35% relative humidity *(Table 10).*

Quality deterioration

EFFECT OF *A. NIGER* ON PUNGENCY OF ONION BULBS

Reduction in pyruvic acid content was observed in infected bulbs which was pronounced with increasing grades. The pyruvic acid content was lowest (3.38 µg/g) in onion bulbs with grade 4 and highest in onion bulbs with grade 1 (6.78 µg/g) compared to healthy bulbs (7.1 µg/g) *(Table 11).*

Table 7

Age of bulbs in relation to susceptibility

Days of harvest	Spoilage (PDI)/days after storage)													Mean
	7	14	21	28	35	42	49	56	63	70	77	84	91	
145 days	7.36	10.43	18.21	21.50	25.43	29.00	32.50	36.00	38.50	42.00	45.07	48.50	51.14	31.20
	(15.79)	(18.81)	(25.25)	(27.62)	(30.26)	(32.58)	(34.76)	(36.87)	(38.35)	(40.40)	(42.19)	(44.14)	(45.63)	(33.89)
160 days	8.36	10.71	18.57	26.50	31.50	35.00	39.50	43.21	47.50	50.07	52.50	55.07	57.79	36.64
	(16.85)	(19.09)	(25.55)	(30.98)	(34.14)	(36.27)	(38.94)	(41.09)	(43.57)	(45.06)	(46.43)	(47.93)	(49.49)	(37.23)
175 days	9.50	14.43	23.50	32.50	36.50	39.50	43.50	49.00	53.50	56.50	59.50	62.50	64.93	41.95
	(17.95)	(22.30)	(29.00)	(34.76)	(37.17)	(38.94)	(41.27)	(44.43)	(47.01)	(48.73)	(50.48)	(52.24)	(53.67)	(40.40)
Mean	8.41	11.86	20.09	26.83	31.14	34.50	38.50	42.74	46.50	49.52	52.36	55.36	57.95	36.60
	(16.85)	(20.18)	(26.64)	(31.18)	(33.89)	(35.97)	(38.35)	(40.87)	(42.99)	(44.71)	(46.38)	(48.10)	(49.60)	(37.23)

Figures in parentheses are arcsine transformed values

	CD (P = 0.05)
Treatments	0.0942
Days	0.1961
Interaction	0.3398

Table 8

Neck height and its influence on black mould rot of onion bulbs

Neck height	Spoilage (°PDI)/days after storage)													Mean
(cm)	7	14	21	28	35	42	49	56	63	70	77	84	91	
0	9.40	13.50	20.50	26.40	29.90	34.00	37.30	40.60	44.50	47.40	50.90	54.40	58.80	35.41
	(17.85)	(21.56)	(26.92)	(30.92)	(33.15)	(35.67)	(37.64)	(39.58)	(41.84)	(43.51)	(45.52)	(47.42)	(50.07)	(36.51)
2	9.40	12.00	18.90	25.60	22.40	36.50	33.60	35.90	39.40	41.90	44.90	48.50	52.10	32.39
	(17.85)	(20.27)	(25.77)	(30.40)	(28.25)	(37.17)	(35.43)	(36.81)	(38.88)	(40.34)	(42.07)	(44.14)	(46.20)	(34.70)
4	6.40	9.10	13.40	17.70	21.00	23.50	26.90	29.90	32.60	35.10	38.60	42.10	45.60	26.30
	(14.65)	(17.56)	(21.47)	(24.88)	(27.27)	(29.00)	(31.24)	(33.15)	(34.82)	(36.33)	(38.41)	(40.45)	(42.48)	(30.85)
6	6.10	8.90	12.40	15.20	18.60	20.00	23.40	25.40	27.70	30.10	33.70	37.00	38.60	22.93
	(14.30)	(17.36)	(20.62)	(22.95)	(25.55)	(26.57)	(28.93)	(30.26)	(31.76)	(33.27)	(35.49)	(37.46)	(39.00)	(29.59)
Mean	7.82	10.87	16.30	21.22	22.97	28.50	30.30	32.95	35.97	38.65	42.02	45.50	49.02	29.25
	(16.22)	(19.28)	(23.81)	(27.42)	(28.66)	(32.27)	(33.40)	(35.06)	(36.87)	(38.47)	(40.40)	(42.42)	(44.43)	(32.77)

Figures in parentheses are square root transformed values

	CD (0.05)
Treatments	0.1067
Days	0.1924
Interaction	0.3849

Table 9

Effect of temperature on black mould rot of onion bulbs

Temperature	Spoilage (PDI)/days after storage)					Mean
	7	14	21	28	35	
0	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)
10	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)	0.00 (4.10)
20	9.38 (17.95)	15.38 (23.11)	23.13 (28.73)	32.63 (34.82)	41.50 (40.11)	24.40 (29.60)
30	14.50 (22.38)	25.00 (30.00)	41.00 (39.82)	55.38 (48.10)	69.00 (56.17)	40.98 (39.82)
40	18.63 (25.55)	32.38 (34.70)	52.00 (46.14)	73.50 (59.02)	95.50 (77.75)	54.40 (47.52)
Mean	8.50 (16.95)	14.55 (22.38)	23.23 (28.79)	32.30 (34.63)	41.20 (39.93)	23.96 (29.33)

Figures in parentheses are arcsine transformed values

	CD (0.05)
Treatments	0.5124
Days	0.5124
Interaction	0.9073

Table 10

Effect of relative humidity on black mould rot of onion bulbs

Relative humidity (%)	Spoilage (PDI)/days after storage)					Mean
	7	14	21	28	35	
100	20.38 (26.85)	38.50 (38.35)	59.88 (50.71)	83.38 (65.96)	100.00 (90.00)	60.53 (51.06)
90	15.88 (23.42)	30.50 (33.52)	48.38 (44.08)	71.00 (57.42)	85.50 (67.62)	50.25 (45.17)
75	10.63 (19.00)	19.38 (26.13)	30.63 (33.58)	41.88 (40.34)	53.50 (47.01)	31.20 (33.96)
50	8.50 (16.95)	15.38 (23.11)	26.38 (30.92)	35.88 (36.81)	46.50 (42.99)	26.53 (30.98)
35	8.00 (16.43)	15.25 (23.03)	26.38 (30.92)	36.00 (36.87)	46.50 (42.99)	26.43 (30.92)
Mean	12.68 (20.88)	23.80 (29.20)	38.33 (38.23)	53.63 (47.06)	66.40 (54.57)	38.97 (38.65)

Figures in parentheses are arcsine transformed values

	CD (0.05)
Treatments	0.6299
Days	0.6299
Interaction	1.4085

Table 11

Effect of *A. niger* infection on pungency of onion

Area of bulb infected	Pyruvic acid content u/g	Percent reduction over control
Grade 1	6.78	4.51
Grade 2	5.48	22.82
Grade 3	5.13	27.75
Grade 4	3.38	52.39
Grade 0	7.1	–
CD (5%)	0.1823	–

PRODUCTION OF AFLATOXIN BY *A. NIGER* IN ONION BULBS

Characteristic blue fluorescent spots was absent under UV-light (320–360 nm) in samples extracted from infected onion bulbs while the spots were observed in maize and groundnut samples *(Table 12).* Further, the isolates of *A. niger* and *A. flavus* from onion did not elaborate aflatoxin even under *in vitro* conditions.

Table 12

Occurrence of aflatoxin in *A. niger* contaminated commodities

Commodity	Culture filtrate	Sample
Maize	++	++
Groundnut	++	++
Onion	–	–

++ (present)
– (absent)

Discussion

Onion (*Allium cepae* L.) is an important vegetable and finds a place in all food preparations of Indian household, in both vegetarian and nonvegetarian diet. Several weeks lapse before the commodity reaches the kitchen. The onion bulbs have to be necessarily stored atleast for few months to avoid preharvest scarcity and postharvest glut. Since onion bulbs are lifted from soil, they are vulnerable to contamination with many soil fungi, which results in lowering of its production and utilization. Among the several diseases that affect onion bulbs after harvest, black mould rot caused by *A. niger* is observed to be the major one. Earlier, Rajam (1992) reported that among the postharvest diseases of onion, black mould rot caused by *A. niger* was the predominant one. The present investigation was therefore undertaken to assess the various factors influencing black mould rot development

in storage and consequent quality deterioration in contaminated onion bulbs so that suitable disease management practices can be formulated to contain the spoilage.

Onion bulb being a perishable commodity contains about 86.8% of moisture and forms an ideal medium for the proliferation of many storage fungi. The survey to assess the extent of spoilage of onion bulbs due to black mould rot, for a period of nine months from June 1998 through February 1999, showed that contamination by *A. niger* and *A. flavus* was up to 11.85 percent. The occurrence of *A. niger* was observed to be higher (2.9–12%) than *A. flavus* (1.49–11.54%). Going through the incidence of spoilage, higher spoilage noticed during June with a temperature range of 33.95 °C to 24.45 °C indicated that high temperature favours disease development as low spoilage was noticed during December which experienced a low temperature range of 30.78 °C to 22.24 °C. Tiwari and Pandule (1984) found that the actual and net marketable loss due to black mould was up to 12.8% in a survey conducted with the traders and farmers of Maharashtra.

Among the microorganisms responsible for storage decay of onion bulbs, fungi play an important role. The predominant fungal flora isolated from various parts of onion bulbs in storage using different media showed that *A. niger* was the predominant fungus as its occurrence ranged from 48.33 to 73.33%, in dried outer scales, fleshy scales, soil particles and rootlets of bulbs. The loss due to *A. niger* in stored onions in an ill ventilated and humid conditions was to the extent of 5–30% (Venkatarayan and Delvi, 1951). Musa et al. (1973) and Qadri and Srivastava (1985) stated that *A. niger* was the dominant fungi infecting more than 80% of the bulbs in onions during postharvest handling.

Various factors including cultural practices as well as postharvest handling methods influence development of black mould rot of onion bulbs during storage (Tanaka and Nonaka, 1981). In the present study, the influence of Ca and N fertilisers towards black mould rot development in storage indicated that increased Ca application, decreased the spoilage. Further the soil as well as plant Ca levels have increased with increase in field application of Ca. Certain physiological disorders and diseases of storage organs, such as fruits and vegetables, were related to the Ca content of their tissues (Huber, 1981). Sharples and Johnson (1977) and Conway (1982) stated that decay caused by fungal pathogens in apple was effectively reduced with application of Ca. The reduction in spoilage of onion bulbs in the present study may be due to the increased uptake of Ca by onion bulbs in the respective plots. Higher Ca content of apples and potatoes have reduced the decay, by strengthening the cell wall and membrane structure by forming Ca pectate and making them more resistant to fungal enzyme activity and slowing the rate of decay as reported by Conway et al. (1992).

Contrary to the above result, increased application of N increased the fungal spoilage. However, lower N dose besides reducing the disease incidence also reduced the bulb yield. Increased N application, increased the available soil and plant N. The present findings corroborate the earlier views of Vaughan (1960) and Jones and Mann (1963). Bedlan (1985) recommended avoidance of excessive N fertilizer to control *Botrytis* storage rot of onion. The rotting of bulbs stored under room conditions, increased with increased application of N fertilizer (Kato et al., 1987). Pronounced spoilage due to higher N supply, produced bulbs with thick necks and since these necks did not shrink easily on drying such

bulbs did not keep well in storage (Jones and Mann, 1963). Further, increased N might delay bulb maturity or increase bulb susceptibility by reducing alkaloids and phenols in bulb leading to more spoilage during storage (Boyd, 1972).

The present studies on the influence of age at harvest towards susceptibility of onion bulbs to black mould rot showed that advancing the harvest by 15 days caused a reduction in spoilage when compared to normal harvest. However, bulbs lifted 15 days after the normal harvest, increased the rotting. Earlier, Boswell (1923) stated that the storage losses from decay were less in the early maturing onions than in the late maturing ones. In a season when high incidence of neck rot was expected, it might be advantageous to harvest the crop early (Thompson et al., 1972).

Increase in neck length caused decrease in black mould rot at storage. With 6 cm neck length, the spoilage was less, whereas with full cutting of tops (0 cm), the loss was high. Jones et al. (1944) recommended, against too close topping since a large open wound did not dry and allow decaying organism to enter. Onions stored with tops on, had higher percentage of health bulbs, than those stored with the tops removed.

Storage losses of onion bulbs due to postharvest diseases are governed by environmental conditions. In the present study, the effect of temperature and relative humidity on the deterioration of onion bulbs due to *A. niger* was studied. It was observed that *A. niger* caused more spoilage at a temperature range of 30 °C to 40 °C. Maude et al. (1985) found that *Aspergillus* spp. were predominant in onion bulbs at 30 °C to 40 °C.

Relative humidity in the storage environment is the critical factor in the development of black mould rot. The effect of relative humidities at different levels were studied on the storage rot of onion and the results revealed that at 100% relative humidity, the spoilage was cent%, followed by 90%. The incidence and severity of disease increased with increase in relative humidity and vice versa. The increase in spoilage due to rise in ambient relative humidity might be due to adsorption of water vapour by the outer scales, rendering them susceptible to *A. niger* decay (Thamizharasi and Narasimham, 1991). Free water essential for spore germination is provided at high humidity condition (Scott, 1952).

The pungency of onion is expressed from the quality of pyruvic acid present. Increase in disease intensity causes reduction in pungency of onion bulb. To find out the effect of *A. niger* infection on the pungency of onion, the quantity of pyruvic acid present in infected onion showing different grades of the disease was estimated. The bulbs with grade 4 showed pronounced reduction in pungency followed by those with grade 3. Bedlan (1985) observed that pungent and coloured cultivars were less susceptible to the storage rot caused by *B. cinerea* and *B. aclada.* However, Owen et al. (1950) found that there was no indication of an influence of pungency in relation to black mould.

The elaboration of aflatoxin by *A. niger* in onion bulbs was studied along with contaminated maize and groundnut kernels for comparison. It was found that the culture filtrates of *A. niger* and *A. flavus* isolates from onion and also the extracts from onion bulbs were free from aflatoxin even though they were severely infected by *A. niger*. Earlier, the presence of inhibitory compounds and antifungal substances in onion might have checked the population of aflatoxin by *A. niger* in onion bulbs. Ibrahim et al. (1990) observed that only two samples among 20 gave positive results for aflatoxin B_1, in poorly

stored onion bulb sample. Thus, the present studies on the various factors influencing black mould rot development in onion during storage enable us to develop integrated disease management practices to contain the disease.

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