

## Management of black mold rot of onion

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### Abstract

Black mold rot are the most destructive diseases of onion in storage and in the field. The present study was carried out to isolate the fungus associated with black mold rot of onion and to control disease with seven different systemic and non-systemic fungicides. On the basis of morphological characteristics, *Aspergillus niger* Van Tieghem was found to be associated with black mold rot of onion. It was observed that all the fungicides caused significant reduction in the mycelial growth, lesion diameter, spore germination and rot severity. Amongst systemic fungicides, carbendazim brought about highest reduction followed by hexaconazole, bitertanol and myclobutanil, respectively. Amongst non-systemic fungicides, the mancozeb was found the most effective followed by captan and zineb, respectively. The higher concentrations (1000 ppm and 2000 ppm) of all the fungicides proved more effective than lower concentrations (125 ppm and 500 ppm). The current study showed effective utilization of hexaconazole and mancozeb for the control of black rot of onion caused by *A. niger*.

**Keywords:** *Aspergillus niger*, black mold, fungicides, onion, rot severity.

### Introduction

Onion is native to south west Asia or Mediterranean, considered important commercial vegetable crop in the world as well as in India. It is grown during kharif and rabi seasons as a essential part of food, while utilized as salad and also cooked as vegetable (Singh *et al.*, 1994). Though India ranks first in the world in area under onion cultivation and second in its production, but the productivity is still quite low (Shrivastva *et al.*, 1996; Anonymous, 2001). The production in India spread over an area of about 0.33 million hectares with an average annual production of 4.70 million tones (Anonymous, 2001).

In Jammu and Kashmir onions are grown extensively as main season crops and have become highly remunerative enterprise for farmers. However, like other crops these are attacked by variety of pathogens in the field and as well as in storage, which degrade its quality and yield (Anonymous, 2001). The production of onion is limited to specific period and storage of this vegetable is important to make it available to consumers throughout all seasons of the year. Massive storage loss has occurred due to rotting fungal pathogens (Taskeen-un-Nisa, 2010). Little attention has been attributed towards the menace caused by fungal rot of onion in Kashmir. The fungal pathogen, *Aspergillus niger* is known to cause decaying in storage onion bulbs. This fungus cause a disease known as black mold on several vegetables including onions. However, no detailed studies have been carried out to identify the fungus

which causes rotting of onion bulbs in Kashmir valley. Application of fungicides is one of the most effective control methods for the management of fungal pathogens. Therefore necessity has arisen to study the post harvest fungicidal treatments on the control of black mold rot of storage onion bulbs. Therefore present study entitled, "Management of black mold rot of onion in Kashmir valley," was carried out with the main objective of isolating and identifying the fungal pathogen causing black mold rot of onion in the field as well in storage from different areas or localities of Kashmir valley and to work out the management strategies for the control of fungal rot of onion with some fungicides.

### Materials and Methods

The diseased onion bulbs were collected in polythene bags from fields, markets, godowns and at storage places from different areas of Kashmir valley. These samples were either used immediately or stored at 10 °C in the laboratory for different pathological studies. The aseptically isolated portions from onion bulb were purified and maintained on Potato Dextrose Agar (PDA) medium. The isolated pathogen was identified on the basis of morphological, reproductive and cultural characteristics.

For pathogenicity and mode of infection, fungal pathogen was re-inoculated onto the healthy onion bulbs by different methods as adopted by Muimba *et al.* (1983). One set of onion bulbs (surface) was sprayed with spore suspension without injury. Another set of onion bulbs was

inoculated by introducing the drop of suspension after pricking them with some needles. Un-inoculated served as control. All the onion bulbs were kept in clean polythene bags, incubated at 23+ 1°C for ten days. Thereafter, disease symptoms were studied and identification of pathogen was ascertained by comparing with original culture following Koch's postulates.

### Chemical control

Seven fungicides, both systemic and non-systemic, viz. carbendazim, myclobutanil, bitertanol, hexaconazole, mancozeb, captan and zineb were used in different concentrations to control the black mold (Table 1) through food poisoning technique (Falck, 1907; Grower and Moore, 1962). Appropriate quantity of each fungicide was separately dispensed in molten sterilized PDA medium to make desired concentrations for each fungicide. The mycelial discs of 5 mm diameter, taken from 10 days old culture of the fungal pathogens were aseptically placed in the center of solidified poisoned PDA. Five replications were maintained for each concentration. The Petri-plates were incubated at 24 ± 2°C and observations on the mycelial growth of test fungus were recorded after seven days of incubation. The growth of test fungus on non-poisoned PDA served as a control. The percent inhibition in growth due to various fungicidal treatments at different concentrations was computed.

Mycelial growth inhibition (%) =  $[(dc-dt)/dc] \times 100(\%)$   
 dc = average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group.

**Table 1.** Different concentration of fungicides used for the management of Black mold rot of onion.

Fungicides	Concentrations (ppm)			
Carbendazim50WP	125	250	500	1000
Bitertanol10WP	125	250	500	1000
Myclobutanil25W	125	250	500	1000
Hexaconazole5Ec	125	250	500	1000
Mancozeb75WP	500	1000	1500	2000
Captan50WP	500	1000	1500	2000
Zineb75WP	500	1000	1500	2000

### Effect of fungicides on lesion diameter

Fresh samples of onion bulbs were dipped separately in different fungicidal concentrations for 5 minutes before inoculation of the pathogen by pin prick method as above. Onion bulbs were then dried under shade and kept in card board trays and incubated at 24 ± 2°C for different duration.

The observations were recorded after 4days to 10 days of incubation by measuring the average diameter of the resultant lesion.

### Effect of fungicides on rot severity

Different concentrations of fungicides were evaluated for their efficacy on rot severity under storage conditions. Rot severity was recorded as per the grade scale (Table 2) and formula adopted by Mckinney (1923).

$$\text{Rot severity} = \frac{\text{Sum of all numerical rotting}}{\text{No. of vegetables examined} \times \text{Maximum grade value}} \times 100$$

**Table 2.** Grade scale used for evaluation of rot severity.

Grade	Extent of rotting	Numerical score (%)
0	No rotting	0
1	Pin head to 10mm	10
2	Upto 1/4 <sup>th</sup> of the onion bulb	25
3	Upto 1/2 of onion bulb	50
4	Upto 3/4 <sup>th</sup> of the onion bulb	75
5	More than 3/4 <sup>th</sup> of onion bulb	100

## Results

*A. niger* was identified as a causal organism of black rot of onion. The primary symptom was a black discoloration of tissue on onion bulbs. Infected bulbs showed blackening at the neck; streaks or spots of black colour appeared on or beneath the outer scales. In advanced stages, the entire bulb appeared black and become shriveled (Plate 1 A&B).

Colonies on Potato dextrose agar (PDA) at 24 ± 2°C were initially white, quickly turns black with conidial production. Reversely pale yellow growth may produce radial fissures in the agar (Plate 1C). The mycelium was septate and hyaline. Conidiophore arise from the mycelium. Conidial heads are (Delete 'are') radiate initially, and then split into columns at maturity. The conidiophores is biseriate (vesicles produces sterile cells known as metulae that support the conidiogenous phialides). Conidiophores are long (400-3000 µm), smooth, and hyaline becoming darker at the apex and terminating in a globose vesicle (30-75 µm in diameter). Metulae and phialides cover the entire vesicle. Conidia are brown to black, very rough, globose and measure 4-5 µm in diameter (Plate 1D).

The different concentrations of systemic and non-systemic fungicides evaluated for their effect on the inhibition of the mycelial growth, lesion

diameter and disease severity showed significant reduction in fungal rot particularly at greater concentration (Table 1). However, the carbendazim at highest concentration brought about highest reduction in colony diameter (0 mm) followed by hexaconazole, (17mm), bitertanol (24.8mm) and myclobutanil (27mm), respectively in comparison to control. Other concentrations of systemic fungicides also showed reduction in colony diameter but to lesser extent. Amongst non-systemic fungicides, the mancozeb was found highly effective in reducing the colony diameter (0mm) of *A. niger* followed by Zineb (6.00mm) and Captan (15mm), respectively over control.

Lesion diameter was also significantly reduced due to applications of systemic and non-systemic fungicides (Table 2). Amongst the systemic fungicides, the hexaconazole at highest concentration (1000 ppm) exhibited the highest reduction in lesion diameter on onion (100%) followed by carbendazim (100%), myclobutanil (33.34%) and bitertanol (31.82%), respectively. Amongst non-systemic fungicide the mancozeb at highest concentration (2000 ppm) was found most effective (100%) in reducing the lesion diameter followed by zineb (37.88%) and captan (25.76%), respectively.

All the systemic fungicides at different concentrations brought about significant inhibition in the spore germination. Spore germination was significantly suppressed due to application of hexaconazole and mancozeb results (Table 3 & 4).

It was observed from the results (Table. 5) that the severity of black mold rots of onion reduced significantly after dip treatment in all the concentration of fungicides for different duration. The maximum reduction in the severity of black mold rot was found at highest concentration (1000 ppm) followed by lower concentrations i.e. 500 ppm and 250 ppm, respectively. Amongst systemic the (Delete "the") fungicides, the carbendazim and mancozeb in reducing the severity of onion rot caused by *A. niger*.

## Discussion

The causal agent of black mold rot of onion showed similar symptoms as described earlier by different workers on onion bulbs in storage (Dang and Singh, 1982; Quadri *et al.*, 1982; JoonTaek *et al.*, 2001). The results showed that infection occurred on the bulbs of onion as indicated by death of the foliage at maturity. The neck and shoulders of infection bulbs develop black spore and infected scale shrivels. As the disease progress, the fungus may infect the freshly inner

scale and entire outer surface may become black and may cause decay. Such findings are in agreement to those of Frisvad *et al.* (2005) and Valdaz *et al.* (2006) on onion bulbs. Bedlan (1985) reported that storage disease of onion bulbs is caused by *Botrytis* sp. Incidence of fungal rot on onion caused by different pathogens have also been reported by some workers under storage conditions (Gupta *et al.*, 1984; Giridhar and Reddy, 1989; Kamlesh *et al.*, 2002)

Pathogenicity test of fungi studied under present investigation showed that the injury prior to infection was pre-requisite for the diseases. Our findings are in conformity with those of Harja and Batra (1978) who considered *Phoma destructiva*, *Fusarium oxysporum* and other species of fungi more virulent on injured than on non-injured tomato fruits. Taskeen-un Nisa (2010), while testing different methods of inoculation for *Fusarium* spp. and other vegetable rot fungi found pin-prick methods as the best methods for quick infection.

Antigungal activity of seven fungicides acquired in present study is in conformity with several previous findings (Singh *et al.*, 1997; Srinivasan and Shanmugam, 2006) on different rot fungi. Khan *et al.* (1995) and Patel *et al.* (2005) evaluated five different fungicides such as mancozeb, carbendazim, copper-oxychloride and potassium permanganate *in-vitro* for their efficacy mycelial growth of *Alternaria* sp. and observed that all tested fungicides at different concentration resulted in significant reduction in the colony diameter as compared to control.

Presently, hexaconazole and mancozeb proved effective in reducing the lesion diameter and spore germination. The reduction in the lesion diameter may be due to effect of fungicides on the mycelial growth of fungal pathogens responsible for rotting of these vegetables in storage. Similar findings were observed by Patel *et al.* (2005). They observed that fungicides such as mancozeb, carbendazim, copper-oxychloride, potassium permanganate caused reduced mycelial growth of *Alternaria* rot of tomato. Suryavanshi and Deokar (2001) noticed maximum inhibition of mycelial growth of *A. niger* by captan. Several fungicides have also been found effective against pathogenic fungi responsible for fungal rots in storage and in field conditions on onion (Shahnaz *et al.*, 2007).

Severity of black mold rot of onion reduced significantly after dip treatment in all the concentration of fungicides for different duration. The present findings are in agreement with those of Nagaraju and Urs (1998). Gambhir and Khainar (1986) found *Penicillium brefeldianum* was unable

to tolerate even 500 ppm of coperoxychloride (Bilitox.). Onion seeds, when tested with benomyl+thiram and mancozeb at two doses were effective in controlling *Pencillium* spp.

(Tylkowska *et al.*, 1998). The current study concludes effective utilization of hexaconazole and mancozeb for the management of black rot of onion.

**Table 1:** Effect of different concentration of fungicides on the colony diameter of *Aspergillus niger*.

Concentration Treatment	Colony diameter (mm)				
	125ppm	250ppm	500ppm	1000ppm	Control
<b>Systemic fungicide</b>					
Hexaconazole	47.00	32.40	24.00	17.00	85.60
Myclobutanil	60.20	43.80	32.00	27.00	80.60
Carbendazim	11.80	7.80	0.0	0.0	90.00
Bitertanol	56.00	42.80	30.00	24.80	90.00
<b>Non-Systemic</b>	<b>500ppm</b>	<b>1000ppm</b>	<b>1500ppm</b>	<b>2000ppm</b>	<b>Control</b>
Mancozeb	0.00	0.00	0.00	0.00	90.00
Zineb	37.80	28.00	17.20	6.00	90.00
Captan	45.60	31.20	23.40	15.00	90.00

	SE. diff	C.D (P=0.05)	C.D (P= 0.01)
Fungicide	0.89	2.42	
Concentration.	1.32	2.36	3.41
Fungicide x Conc.	2.71	3.17	5.32

**Table 2:** Effect of different concentrations of fungicides on the lesion diameter of *Aspergillus niger*.

Treatment	Concentration	Lesion diameter (cm) after incubation				Mean	% diameter of rot with respect to control	% age reduction over control
		4days	8days	12days	15days			
Bitertanol	1000ppm	2.4	3.6	4.9	6.9	4.5	68.19	31.82
Carbendazim	1000ppm	0.0	0.0	0.0	0.0	0.0	0.0	100
Hexaconazole	1000ppm	0.0	0.0	0.0	0.0	0.0	0.0	100
Myclobutanil	1000ppm	1.9	3.1	5.5	7.3	4.4	66.66	33.34
Mancozeb	2000ppm	0.0	0.0	0.0	0.0	0.0	0.0	100
Zineb	2000ppm	2.4	4.0	5.7	7.5	4.9	74.24	25.76
Captan	2000ppm	1.9	3.1	4.8	6.7	4.1	62.12	37.88
Control		3.7	5.7	7.0	10.0	6.6		

	SE. diff.	C.D (P = 0.05)	C.D (P = 0.01)
Fungicides	0.56	0.49	1.94
Concentration	1.83	1.96	2.17
Fungicide x conc.	2.80	4.33	5.35

Mean of five replicates; \*\* Figures in parentheses are arc Sin $\sqrt{\%$ age transformed value and are statistically identical.

**Table 3:** Effect of systemic fungicides on spore germination.

Concentration Treatment	Spore germination (%)				
	0.0	125ppm	250ppm	500ppm	1000ppm
Carbendazim	95.20 (77.35)	58.64 (49.98)	47.20 (43.40)	32.53 (34.78)	24.47 (29.65)
Bitertanol	93.10(74.78)	52.52 (46.45)	39.84 (39.14)	31.30 (34.02)	21.85 (27.87)
Myclobutanil	91.71 (73.26)	59.98 (50.76)	51.97 (46.13)	44.54 (41.87)	30.54 (33.55)
Hexaconazole	91.40 (72.95)	45.22 (42.26)	32.60 (34.82)	20.99 (27.27)	9.86 (18.31)
	SE. diff.	C.D (P = 0.05)	C.D (P = 0.01)		
Fungicides	0.56	1.49	1.94		
Concentration	0.83	1.66	2.17		
Fungicide x conc.	2.80	3.33	4.35		

Mean of five replicates; \*\* Figures in parentheses are arc Sin $\sqrt{\%$ age transformed value and are statistically identical.

**Table 4:** Effect of non-systemic fungicides on the spore germination.

Concentration Treatment	Spore germination (%)				
	0.0	500ppm	1000ppm	1500ppm	2000ppm
Mancozeb	89.42 (71.02)	22.57 (28.37)	16.55 (24.01)	9.10 (17.56)	(0.00) (0.00)
Captan	90.53 (72.08)	42.52 (40.70)	38.53 (38.37)	31.30 (34.02)	21.91 (27.91)
Zineb	91.53 (73.9)	51.44 (45.83)	43.12 (41.05)	35.91 (36.82)	21.78 (27.82)
	<b>SE. diff.</b>	<b>CD (P = 0.05)</b>	<b>CD (P = 0.01)</b>		
Fungicides	0.78	1.57	2.04		
Concentration	0.90	1.81	2.36		
Fungicide x conc.	1.57	3.14	4.09		

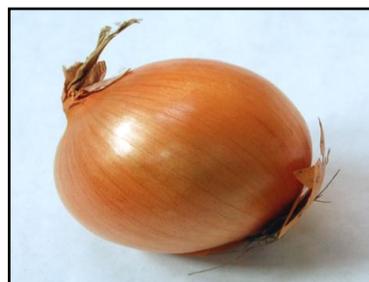
Mean of five replicates; \*\* Figures in parentheses are arc Sin<sup>1/2</sup>%age transformed value and are statistically identical.

**Table 5:** Effect of fungicidal dips applied as pre-inoculation treatments on the severity of *Aspergillus* rot.

Treatment	Concentration	Rot severity (%) after incubation			
		4 <sup>th</sup> days	8 <sup>th</sup> days	12 <sup>th</sup> days	16 <sup>th</sup> days
Carbendazim	1000ppm	0.00	1.07 (5.95)	4.50 (12.26)	13.69 (21.72)
	500ppm	2.05 (8.25)	7.25 (15.63)	11.38 (19.72)	20.96 (27.25)
	250ppm	4.21 (11.85)	9.80 (18.25)	12.99 (21.13)	25.95 (30.63)
Hexaconazole	1000ppm	0.00	0.00	4.46 (12.20)	10.77 (19.16)
	500ppm	2.51 (9.12)	6.92 (15.26)	12.56 (20.76)	21.40 (27.56)
	250ppm	9.36 (17.82)	15.70 (23.35)	23.72 (29.15)	28.49 (32.26)
Myclobutanil	1000ppm	10.72 (19.12)	16.86 (24.25)	25.00 (30.00)	35.58 (36.62)
	500ppm	13.26 (21.36)	21.53 (27.65)	31.69 (34.26)	41.59 (40.16)
	250ppm	17.31 (24.59)	26.86 (31.22)	46.80 (43.17)	61.62 (51.72)
Bitertanol	1000ppm	13.23 (21.33)	20.82 (27.15)	29.13 (32.67)	55.50 (48.16)
	500ppm	18.14 (25.21)	27.33 (31.52)	52.98 (46.71)	63.09 (52.59)
	250ppm	24.87 (29.92)	33.63 (35.45)	60.90 (51.30)	74.30 (59.54)
Zineb	2000ppm	5.60 (13.70)	10.80 (19.26)	17.98 (25.09)	27.22 (31.45)
	1500ppm	8.01 (16.45)	13.47 (21.54)	24.56 (29.71)	42.12 (40.47)
	1000ppm	12.40 (20.62)	24.50 (29.67)	34.13 (35.75)	54.68 (47.69)
Mancozeb	2000ppm	0.00	0.00	0.00	0.00
	1500ppm	0.0	0.0	0.0	2.52 (9.140)
	1000ppm	0.0	4.42 (12.15)	8.53 (16.99)	12.55 (20.75)
Captan	2000ppm	4.61 (12.41)	10.88 (19.26)	20.96 (27.25)	37.32 (37.66)

	1500ppm	9.13 17.59	13.97 21.95	25.94 (30.62)	43.50 (41.27)
	1000ppm	9.19 17.65	19.91 (22.72)	28.96 32.56	45.27 42.29
Control		32.81 (34.95)	49.37 (44.64)	73.35 (58.92)	96.22 (78.79)
	<b>SE. diff.</b>	<b>C.D (P = 0.05)</b>	<b>C.D (P = 0.01)</b>		
Fungicides	1.80	1.61	2.09		
Concentration	0.93	1.86	2.42		
Fungicide x conc.	1.61	3.22	4.19		

Mean of five replicates; \*\* Figures in parentheses are arc Sin<sup>1/2</sup>age transformed value and are statistically identical.



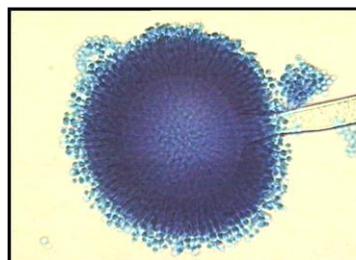
A. Healthy onion



B. Infected onion



C. Pure Culture of *A. niger* on PDA



D. Conidiophores with conidia (400x)

**Plate 1(A-D):** Black mold rot of onion caused by *Aspergillus niger*.

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