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In vitro evaluation of fungicides against the pathogens associated with post-harvest bulb rot of onion

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Abstract
The present study was carried out to evaluate the efficacy of seven fungicides against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *cepae* which are associated with post-harvest bulb rot of onion using poisoned food technique. The fungicides, evaluated *in vitro* were found fungistatic/antifungal against test pathogens. Propiconazole was found most fungistatic and recorded cent per cent mycelial growth inhibition. The next best fungicides were SAAF (93.70%) and Tebuconazole (92.59%). Copper oxychloride was found less effective with 28.89 per cent mycelial growth inhibition against *A. niger*. Fungicides, Tebuconazole, Propiconazole and SAAF were found most fungistatic and recorded significantly the cent per cent mycelial growth inhibition and azoxystrobin was found less effective with 50.76 per cent inhibition of mycelial growth against *A. flavus*. Cent per cent inhibition of mycelial growth was observed in fungicides Tebuconazole and Propiconazole were found most fungistatic. Fungicides, Copper oxychloride was found less effective with minimum 65.54 per cent mycelial growth inhibition against against *F. oxysporum* f. sp. *cepae*.

Keywords: Onion, *A. niger*, *A. flavus*, *F. oxysporum* f. sp. *cepae*, fungicide

Introduction
Onion *(Allium cepa* L.) is a bulbous, it is a biennial herb and one of the most important vegetable crop grown in India. It originated in the region of central Asia. It is a commercially grown underground bulbous vegetable crop with an extended range of adaptations and a relatively high production potentiality. It belongs to the family Amaryllidaceae and genus *Allium* is an important vegetable. The reddish color of the outer peel of the onion is due to catechuic acid, protocatechuic acid and phenolic factors which are present in red onions and they have antifungal properties also. Thus, it is known as Queen of the kitchen.

About 35-40% of post-harvest loss onion is lost due to damage caused by storage diseases. The bulb rot of onion imparts about 15-30% losses during the storage. There are diverse fungal pathogen species like *Aspergillus spp*, *Botrytis spp*, *Fusarium spp*, *Colletotrichum spp*, *Penicillium spp*, *Rhizopus spp*, *Erwinia spp*, *Pseudomonas spp*, *Lactobacillus spp* and *Alternaria spp*, which attacks onion bulb during the post-harvest storage period, where *Aspergillus niger* is the most virulent pathogen in the field condition and storage (Kumar *et al*., 2015) [3]. Post-harvest diseases of the onion are caused by latent infection from field conditions and if these infections are reduced before harvest, post-harvest losses can be minimized. The fungicide molecules are to be evaluated from time to time to find out superior fungicides to recommend an economical spray schedule with which the disease can be controlled. Hence, the screening of the fungicides to manage post-harvest diseases of onion both at pre and post-harvest stages is essential. Keeping this in view the present

Research work was undertaken to find out the fungi associated with bulb rot of onion in stored condition. This paper also deals with the *in vitro* management of pathogens associated with post- harvest bulb rot of onion.

Material and Methods
Experimental site
All the experiments (*In vitro*) were conducted at the Department of Plant Pathology, College of Agriculture, Pune.

Collection of disease samples
Onion bulbs showing symptoms of rot, black, brown and discolouration were randomly collected in the bags from the various markets and fields in Pune district.

These collected bulbs were brought to the Plant Pathology, Laboratory, College of Agriculture, Pune and subjected for further studies.

Culture media

Potato dextrose agar (PDA), the common laboratory culture medium was used as basal medium for isolation, purification, multiplication and maintenance of the pure culture of diseases.

Glass-wares

The common glass-wares (Borosil and corning make) *viz*., Petri dishes, test tubes, conical flasks, volumetric flasks, measuring cyclinder, glass rods, beakers, funnel, pipettes etc. were obtained from the Department of Plant Pathology, College of Agriculture, Pune.

Equipments

The laboratory equipments *viz.,* Autoclave, Hot air oven, Laminar-airflow Cabinet, BOD incubator, Refrigerator, Binocular Research Microscope, Electronic balance, pH meter, Mixer-cum-grinder etc. available at the Department of Plant Pathology, College of Agriculture, Pune were utilized, as and when required.

Disease management Strategies

***In vitro* evaluation of fungicides**

Efficacy of Seven fungicides *viz.*, Propineb (70 WP), Propiconazole (25 EC), SAAF (75 WP), Tebuconazole (25.9 EC), Azoxystrobin (23 SC), Copper oxychloride (50 WP) and Difenoconazole (25 EC) were evaluated *in vitro* against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *cepae* by applying poisoned food technique (Nene and Thapliyal, 1993) [6]. The requisite quantity of each fungicide based on active ingredient was calculated and mixed thoroughly with autoclaved and cooled (40^0 C) Potato dextrose agar medium (PDA) in conical flasks to obtain desired concentrations. Untreated PDA medium without fungicide served as control. Fungicide amended PDA medium was then poured in Petri plates (90 mm dia.).

After solidification of the medium, all the plates were inoculated aseptically with 5 mm culture disc of the test fungus obtained from a week old culture of *A. niger*, *A. flavus* and *Fusarium oxysporum f. sp. cepae*. The disc was placed on PDA in the centre of the Petri plate and plates were incubated at 27 ±1 ^{0}C in inverted position. Suitable numbers of replication were maintained for each treatment. When medium in the untreated control plates was fully covered with mycelial growth of the test fungus, radial mycelial growth was measured in all the treatment plates. The diameter of the colony was measured in two directions and average was recorded. Per cent inhibition of mycelial growth in treated plates was calculated by applying the formula given by Vincent (1947) [8].

$$I = \frac{100(C - T)}{C}$$

Where

I = Per cent inhibition of fungal growth.

C = Growth (mm) of the test fungus in control plate.

T = Growth (mm) of the test fungus in treated plate.

Table 1: *In vitro* evaluation of fungicides treatment details is given below,

Tr. No.	Common name	Concentration
T_1	Difenoconazole	0.05%
T_2	Tebuconazole	0.1%
T_3	Copper oxychloride	0.25%
T_4	Azoxystrobin	0.1%
T_5	Propineb	0.25%
T_6	Propiconazole	0.1%
T_7	Carbendazim 16% + Mancozeb 63%	0.2%
T_8	Control	-

Results and Discussion

Present studies on the post-harvest and storage diseases of onion were undertaken during *Rabi*-2020 on the aspects of *in vitro* evaluation of fungicides. The results obtained on these aspects are presented in the following paragraphs.

In vitro* evaluation of fungicides against *A. niger

Radial mycelial growth

The highest mean radial mycelial growth was recorded in copper oxychloride (64.00 mm) which was followed by Propineb (54.67 mm), Azoxystrobin (40.67 mm), Difenoconazole (11.67 mm), Tebuconazole (6.67 mm) and SAAF (5.67 mm). Significantly the least mean radial mycelial growth was recorded with Propiconazole (0.00 mm).

Mycelial growth inhibition

The fungicide Propiconazole was found fungistatic which recorded the highest mycelial growth inhibition of cent over untreated control (00.00%). The second and third best fungicides found were SAAF (93.70%) and Tebuconazole (92.59%). This was followed by Difenoconazole (87.04%), Azoxystrobin (54.81%) and Propineb (39.26%) However, Copper oxychloride (28.89%) was found less effective with minimum mycelial inhibition (Table 2 and Plate 1).

Thus, all the fungicides tested were found fungistatic / antifungal against *A. niger* and significantly inhibited its mycelial growth. These results are similar to the findings of several scientists. Nandeesha *et al*., (2013) [5], Futane *et al*., (2018) [1] and Raju and Naik (2006) [7].

Table 2: *In vitro* effect of fungicides on mycelial growth and growth inhibition of *A. niger*

Sr. No.	Fungicides	Concentration	Mean colony Diameter (mm)	Growth Inhibition%
1	Propineb	0.25%	54.67	39.26 (38.80)
2	Tebuconazole	0.1%	6.67	92.59 (74.24)
3	Propiconazole	0.1%	0.00	100.00

				(90.00)
4	Difenoconazole	0.05%	11.67	87.04 (68.97)
5	Carbendazim+Mancozeb	0.2%	5.67	93.70 (75.48)
6	COC	0.25%	64.00	28.89 (32.48)
7	Azoxystrobin	0.1%	40.67	54.81 (47.76)
8	Control	-	90.00	0.00 (0.00)
SE (m) ±		1.01		0.88
CD at 1%		4.19		3.70
CV (%)		5.14		2.49

In vitro* evaluation of fungicides against *Aspergillus flavus

Radial mycelial growth

The highest mean radial mycelial growth was recorded in Azoxystrobin (43.33 mm), which was followed by Propineb (35.33 mm), Difenoconazole (13.67 mm) and Copper oxychloride (8.33 mm). Significantly the least mean radial mycelial growth was recorded in Tebuconazole, Propiconazole and SAAF (0.00 mm) compared to maximum radial growth (88.00 mm) in untreated control plates.

Mycelial growth inhibition

The fungicides such as Tebuconazole, Propiconazole and SAAF were found most fungistatic which recorded significantly the highest mycelial growth inhibition of 100 per cent over untreated control (00.00%). This was followed by copper oxychloride (90.53%), Difenoconazole (84.47%) and Propineb (59.58%). However, Azoxystrobin (50.76%) was found less effective with minimum mycelial growth inhibition (Table 3 and Plate 2).

Thus, all the fungicides tested were found fungistatic / antifungal against *A. flavus* and significantly inhibited its mycelial growth. These results are similar to the finding of Nagpurne Vinay *et al*., (2020) [4] investigated the efficiency of non-systemic fungicide and systemic fungicide *viz*. Mancozeb (Indofil M-45), copper oxychloride (Blitox 50 WP), carbendazim (Bavistin 50 WP) and hexaconazole (Contaf 5 EC) at 0.2 percent of concentration evaluated against *A. flavus*. The complete mycelial growth inhibition was found in carbendazim followed by copper oxychloride (90.2%).

Table 3: *In vitro* effect of fungicides on mycelial growth and growth inhibition of *A. flavus*

Sr. No.	Fungicides	Concentration	Mean colony Diameter (mm)	Growth Inhibition%
1	Propineb	0.25%	35.33	59.58 (50.69)
2	Tebuconazole	0.1%	0.00	100.00 (90.00)
3	Propiconazole	0.1%	0.00	100.00 (90.00)
4	Difenoconazole	0.05%	13.67	84.47 (66.80)
5	Carbendazim+Mancozeb	0.2%	0.00	100.00 (90.00)
6	COC	0.25%	8.33	90.53 (72.12)
7	Azoxystrobin	0.1%	43.33	50.76 (45.43)
8	Control	-	88.00	0.00 (0.00)
SE (m) ±		0.94		0.73
CD at 1%		3.89		3.06
CV (%)		6.92		1.74

In vitro* evaluation of fungicides against *F. oxysporum* f. sp. *cepae

Radial mycelial growth

The highest mean radial mycelial growth was recorded in Azoxystrobin (33.00 mm) which was followed by Propineb (32.00 mm), Copper oxychloride (30.67 mm), SAAF (20.33 mm) and Difenoconazole (12.00 mm). Significantly the least mean radial mycelial growth was recorded with Tebuconazole (0.00 mm) and Propiconazole (0.00 mm) compared to maximum radial growth (89.00 mm) in untreated control plates.

Mycelial growth inhibition: The fungicides such as Tebuconazole and Propiconazole were found most fungistatic which recorded significantly the highest mycelial growth inhibition of 100 per cent over untreated control (00.00%). This was followed by Difenoconazole (86.52%), SAAF (77.15%), Copper oxychloride (65.54%) and Propineb (64.04%). However, Azoxystrobin (62.92%) was found less effective with minimum mycelial growth inhibition (Table 4 and Plate 3).

Thus, all the fungicides tested were found fungistatic / antifungal against *F. oxysporum* f. sp. *cepae* and significantly inhibited its mycelial growth. These results are similar to the findings of several scientists. Futane *et al*., (2018) [1] and Kavitha *et al*., (2017) [2].

Table 4: *In vitro* effect of fungicides on mycelial growth and growth inhibition of *F. oxysporum* f. sp. *Cepae*

Sr. No.	Fungicides	Concentration	Mean colony Diameter (mm)	Growth Inhibition%
1	Propineb	0.25%	32.00	64.04 (53.16)
2	Tebuconazole	0.1%	0.00	100.00 (90.00)
3	Propiconazole	0.1%	0.00	100.00 (90.00)
4	Difenoconazole	0.05%	12.00	86.52 (68.50)
5	Carbendazim+Mancozeb	0.2%	20.33	77.15 (61.47)
6	COC	0.25%	30.67	65.54 (54.07)
7	Azoxystrobin	0.1%	33.00	62.92 (52.50)
8	Control	-	89.00	0.00 (0.00)
SE (m) ±		1.07		0.84
CD at 1%		4.43		3.55
CV (%)		6.86		2.17

Plate 1: Evaluation of different fungicides against *A. niger*

T_1- Propineb
T_2- Tebuconazole
T_3- Propiconazole
T_4- Difenoconazole
T_5- Carbendazim+Mancozeb
T_6- Copper oxychloride
T_7- Azoxystrobin
T_8- Control

Plate 2: Evaluation of different fungicides against *A. flavus*

T_1- Azoxystrobin
T_2- Tebuconazole
T_3- Propineb
T_4- Difenoconazole
T_5- Propiconazole
T_6- Copper oxychloride
T_7- Carbendazim+Mancozeb
T_8- Control

Plate 3: Evaluation of different fungicides against *F. oxysporum* f. sp. *cepae*

T_1- Propineb
T_2- Tebuconazole
T_3- Propiconazole
T_4- Difenoconazole
T_5- Carbendazim+Mancozeb
T_6- Copper oxychloride
T_7- Azoxystrobin
T_8- Control

Conclusion

Thus from the results obtained on various aspects during investigation on postharvest and storage diseases of onion, following conclusions are being drawn.

All the test fungicides, evaluated *in vitro* found fungistatic / antifungal to the test pathogens. However, fungicides such as Propiconazole and Tebuconazole were found most effective against *A. niger*, *A. flavus* and *F. oxysporum* f. sp. *cepae*.

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