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

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Abstract
A laboratory experiment was conducted to study the efficacy of seven botanicals 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 *in vitro* (each @ 10%). Among the botanicals garlic (71.11%) showed the highest mycelial growth inhibition. This was followed by ghaneri (70.37%) and tulsi (8.52%) was found less effective with minimum mycelial growth inhibition against *A. niger*. However, Garlic (69.26%) showed the highest mycelial growth inhibition. This was followed by Neem (61.85%) and tulsi (9.63%) was found less effective with minimum mycelial growth inhibition against *A. flavus*. Among the botanicals evaluated aginst *F. oxysporum* f. sp. *cepae*, Onion (60.37%) showed the highest mycelial growth inhibition. This was followed by Garlic (51.11%), Eucalyptus (47.04%) and Ginger (32.59%) was found less effective with minimum mycelial growth inhibition respectively.

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

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) [4]. 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. However, Fungicides would be harmful to human health because of residual effects and increased pathogen resistance, they are not safe due to public concerns about food and environmental safety (Samuel and Ifeanyi, 2015) [6]. As a result, it's important to develop strategies to reduce storage losses using various methods like the effective application of plant-derived compounds (Kumar *et al*., 2015) [4] and (Samuel and Ifeanyi, 2015) [6].

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.

Plant extracts / botanicals
Seven plant species / botanicals which were locally available were used for *in vitro* studies. Botanicals were locally available and collected from the farms of College of Agriculture, Pune.

Disease management Strategies
***In vitro* evaluation of botanicals**
A total of seven botanicals *viz.*, tulsi (*Oscimum sanctum*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), ghaneri / lantana (*Lantana camera*), neem (*Azadirachta indica*), eucalyptus (*Eucalyptus globulus*) and onion (*Allium cepa*) were evaluated at 10% *in vitro* against *A. niger* by applying Poisoned food technique (Nene and Thapliyal, 1993) [5]. The radial mycelial growth and per cent inhibition of the test pathogen was recorded after seven days.
Aqueous leaf extracts of the test botanicals were prepared by grinding with mixture-cum grinder the mixture was filtered through double layered muslin cloth. Each of filtrates obtained were further filtered through Whatman No.1 filter paper using funnel and volumetric flasks (100 ml) the final clear extracts formed the standard plant extracts of 100 per cent concentration. These were evaluated (@10%) *in vitro* using Potato Dextrose Agar (PDA) as basal culture medium. An appropriate quantity of each plant extract (100%) was separately mixed thoroughly with PDA medium in conical flasks (250 ml) to obtain desired concentration of 10 per cent and autoclaved at 15 lbs/inch pressure for 15 to 20 minutes. Sterilized and cooled PDA medium mixed separately with plant extract was then poured (15 to 20 ml/plate) into sterile glass Petri plates (90 mm) and allowed to solidify at room temperature. Each plant extract and its respective concentration were replicated three times.
The plates containing PDA without any plant extract were maintained as untreated control. After solidification of PDA, all the treatment and control plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a week old actively growing pure culture of *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *cepae*. Plates containing without plant extract PDA and inoculated with mycelial disc of the test fungus served as untreated control. All these plates were then incubated at 26 ± 2 °C temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus. 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) [10].

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

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.

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 botanicals. The results obtained on these aspects are presented in the following paragraphs.

In vitro* efficacy of botanicals against *A. niger
Radial mycelial growth
The radial mycelial growth of the test pathogen ranged from 26.00 (garlic) to 82.33 mm (tulsi). However, it was maximum in tulsi (82.33 mm). This was followed by neem (78.67 mm), eucalyptus (42.00 mm), onion (38.67 mm), ginger (28.33 mm) and lantana / ghaneri (26.67 mm). The least radial mycelial growth was recorded with garlic (26.00 mm) over untreated control (90.00 mm).

Mycelial growth inhibition
The radial mycelial growth inhibition at 10 per cent of the test pathogen ranged from 8.52% (tulsi) to 71.11% (garlic). However, significantly highest mycelial growth inhibition was recorded with garlic (71.11%). This was followed by lantana (70.37%), ginger (68.52%), onion (57.04%), eucalyptus (53.33%) and neem (12.59%). However, Tulsi (8.52%) was found less effective with minimum mycelial growth inhibition (Table 1 and Plate 1).
These results are in agreement with the findings of scientists Shricharan *et al*., (2020) [8] and Saranya *et al*., (2018) [7].

Table 1: *In vitro* effect of different botanicals on growth and inhibition of *A. niger*

Sr. No.	Botanicals	Concentration	Mean colony Diameter (mm)	Growth Inhibition%
1	Neem	10%	78.67	12.59 (20.57)
2	Tulsi	10%	82.33	8.52 (16.88)
3	Eucalyptus	10%	42.00	53.33 (46.91)
4	onion	10%	38.67	57.04 (49.05)
5	Garlic	10%	26.00	71.11 (57.50)
6	Lantana	10%	26.67	70.37 (57.02)
7	Ginger	10%	28.33	68.52 (55.91)
8	Control	-	90.00	0.00 (0.00)
SE (m) ±		1.42		1.29
CD at 1%		5.88		5.40
CV (%)		4.78		5.12

In vitro efficacy of plant extracts against *A. flavus*

Radial mycelial growth

The radial mycelial growth of the test pathogen ranged from 27.67 mm (Garlic) to 81.33 mm (Tulsi). However, it was maximum in tulsi (81.33 mm). This was followed by onion (72.00 mm), ginger (72.00 mm), lantana / ghaneri (59.00 mm) and eucalyptus (60.67 mm) and neem (34.33 mm). The least radial mycelial growth was recorded in garlic (27.67 mm) over untreated control (90.00mm).

Mycelial growth inhibition

The mycelial growth inhibition of the test pathogen ranged from 9.63% (Tulsi) to 69.26% (Garlic). The highest mycelial growth inhibition was recorded with Garlic (69.26%). This was followed by Neem (61.85%), Eucalyptus (32.59%), Lantana (34.44%), Ginger (20.00%), Onion (20.00%), and. However, Tulsi (9.63%) was found less effective with minimum mycelial growth inhibition (Table 2 and Plate 2).

These results are in agreement with the findings of scientists. Bhosale *et al*., (2018) [2] demonstrated the effect of nine botanicals under *in vitro* conditions. However, maximum mycelial growth inhibition was recorded with neem (72.17%), garlic (71.5%). Khatun and Shamsi, (2016) [3] evaluated efficency of five plant extracts at 10 per cent against *A. flavus*. At 10 and 20 per cent concentration extracts of *A. sativum* was responsible for the complete inhibition of mycelial radial growth of *A. flavus*.

Table 2: *In vitro* effect of different botanicals on growth and inhibition of *A. flavus*

Sr. No.	Botanicals	Concentration	Mean colony Diameter (mm)	Growth Inhibition%
1	Neem	10%	34.33	61.85 (51.87)
2	Tulsi	10%	81.33	9.63 (17.86)
3	Eucalyptus	10%	60.67	32.59 (34.81)
4	Onion	10%	72.00	20.00 (26.54)
5	Garlic	10%	27.67	69.26 (56.35)
6	Lantana	10%	59.00	34.44 (35.89)
7	Ginger	10%	72.00	20.00 (26.48)
8	Control	-	90.00	0.00 (0.00)
SE (m) ±		1.80		1.51
CD at 1%		7.41		6.35
CV (%)		5.00		7.32

In vitro efficacy of plant extracts against *F. oxysporum* f. sp. *cepae*

Radial mycelial growth: The mycelial growth of the test pathogen ranged from 35.67 mm (Onion) to 60.67 mm (Ginger). The maximum growth of the pathogen with ginger (60.67 mm). This was followed by tulsi (58.00 mm), lantana / ghaneri (50.33 mm), neem (49.67 mm), eucalyptus (47.67 mm) and garlic (44.00 mm). The least radial mycelial growth was recorded with onion (35.67 mm) over untreated control (90.00mm).

Mycelial growth inhibition

The radial mycelial growth inhibition of the test pathogen ranged from 32.59% (Ginger) to 60.37% (Onion). However, significantly highest mycelial growth inhibition was recorded with onion (60.37%). This was followed by garlic (51.11%), eucalyptus (47.04%), neem (44.81%), lantana (44.07%) and tulsi (35.56%). However, ginger (32.59%) was found less effective with minimum mycelial growth inhibition (Table 3 and Plate 3).

These results are in agreement with the findings of several previous workers. Taskeen-Un-Nisa *et al*., (2011) [9] evaluated efficiency of three plant extract against *F. oxysporum*. The maximum inhibition found in *A. sativum*. Ashwini (2015) [1] evaluated nine botanicals under *in vitro* conditions. At 10 per cent concentration highest mycelial growth inhibition was recorded with *A. sativum* (50.99%).

Table 3: *In vitro* effect of different botanicals on growth and inhibition *F. oxysporum* f. sp. *Cepae*

Sr. No.	Botanicals	Concentration	Mean colony Diameter (mm)	Growth Inhibition%
1	Neem	10%	49.67	44.81 (42.01)
2	Tulsi	10%	58.00	35.56 (36.59)
3	Eucalyptus	10%	47.67	47.04 (43.30)
4	Lantana	10%	50.33	44.07 (41.59)
5	Garlic	10%	44.00	51.11 (45.64)
6	Onion	10%	35.67	60.37 (50.99)
7	Ginger	10%	60.67	32.59 (34.80)
8	Control	-	90.00	0.00 (0.00)
SE (m) ±		1.75		1.21
CD at 1%		7.24		5.11
CV (%)		5.57		4.99

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

T_1- Neem (10%)
T_2- Tulsi (10%)
T_3- Eucalyptus (10%)
T_4- Ginger (10%)
T_5- Garlic (10%)
T_6- Ghaneri (10%)
T_7- Onion (10%)
T_8- Control

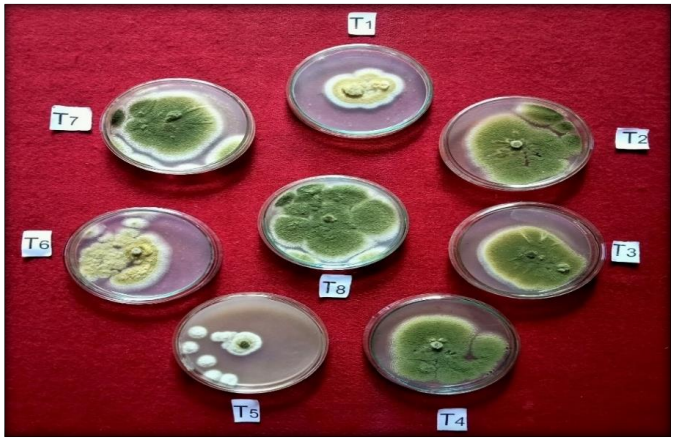

Plate 2: Evaluation of different botanicals against *A. flavus*

T_1- Neem (10%)
T_2- Tulsi (10%)
T_3- Eucalyptus (10%)
T_4- Onion (10%)
T_5- Garlic (10%)
T_6- Ghaneri (10%)
T_7- Ginger (10%)
T_8- Control

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

T_1- Neem (10%)
T_2- Tulsi (10%)
T_3- Eucalyptus (10%)
T_7- Ginger (10%)
T_4- Garlic (10%)
T_5- Ghaneri (10%)
T_6- Onion (10%)
T_8- Control

Conclusion

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

All the test botanicals, evaluated *in vitro* found fungistatic / antifungal to the test pathogens. However, garlic was found effective against *A. niger*, *A. flavus* and *F. oxysporum* f. sp. *cepae*.

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