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Effect of Botanicals and Bioagents on Growth of *Aspergillus niger* (Van Tiegh) Causing Black Mold in Onion

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ABSTRACT

For eco friendly, cost effective and non phytotoxic management eight phytoextracts were tested against black mold (Aspergillus niger) of onion under in vitro condition. Out of eight phytoextracts, maximum inhibition 71.19 per cent was found in Lawsonia inermis which followed by Ocimum sanctum leaf extract (62.97%), Zingiber officinale rhizome extract (62.26%) and Lantana camera leaf extract (61.59%). Minimum inhibition of test fungus was recorded in Jetropha curcas leaf extract (53.20%). Among the six biocontrol agents, Tricoderma viride isolate 6 significantly reduced the growth (78.63%) of test fungus followed by the isolate T. viride isolate-9 with (75.51%) growth inhibition, while least antagonism (61.27%) was obtained with biocontrol agent Basillus subtilis.

Key words: *Onion, Black mold, Aspergillus niger, Phytoextracts, Biocontrol agents.*

INTRODUCTION

Onion (*Allium cepa* L.) bulb of family *Amaryllidaceae* is an important vegetable crop widely cultivated and used throughout the world. Among the vegetables it enriches health of the people. As a foodstuff they are usually cooked or used as a vegetable, but can also be eaten raw or used to make pickles or chutneys. Most onion cultivars contains about 89% water, 4% sugar, 1% protein, 2% fiber, 0.1% fat, vitamin C, vitamin B6, folic acid and numerous other nutrients in small amounts. This shows the importance of onion for human use and thus encourages for increasing its production and productivity. Onion suffers from many post harvest diseases namely black mold, blue mold, neck rot, soft rot and smudge, among which black mold and blue mold restrict the availability of onion to domestic and international trades due to damage in storage. It is pertinent to generate information on the efficacy of available botanicals and bioagents for managing the disease. Hence, the present study was undertaken to screen various botanicals and bioagents under *in vitro* to manage black mold.

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More over plant extracts and bioagents are eco friendly, accessible to rural dwellers, cost effective and non or less phytotoxic and have been successfully used to control a number of plant diseases.

MATERIAL AND METHODS

a) *In vitro* evaluation of different phytoextracts against black mold fungi of onion

Eight locally available plants were used for their evaluation against *Aspergillus niger*. For growth inhibition of *Aspergillus niger* procedure given by Ansari[1] was followed with a slight modification. Fresh leaves, rhizomes or cloves of respective plants as shown in Table 1 were first washed with tap water and then with sterilized water. Each sample was then homogenized in sterile distilled water at the rate of 1 ml/g of tissues (1:1 V/W) with a mixer and filtered through fine muslin cloth. The filtrate was centrifuged at 5000 rpm for 20 minutes and the supernatant was filtered with fine muslin cloth, which formed the standard plant extract solution (100%). The extracts were individually incorporated into PDA medium to prepare 2, 5 and 10 per cent concentration in 250 ml conical flasks separately and sterilized at 1.036 kg/cm^2 for 15 minutes. These were poured in 90 mm sterilized Petri plates keeping three replications for each concentration of extract. PDA without extracts was maintained as control. All the Petri plates were centrally inoculated with one week old four mm mycelial disc of the test pathogen and incubated at 28 ± 2°C. Seven days after incubation, the radial growth of mycelium was recorded and per cent inhibition of fungal growth for each treatment and concentration was calculated by using the formula:

$$I = \frac{C - T}{C} \times 100$$

Where,

I = per cent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

Table 1: List of different phytoextracts tested and their concentration

Sr. No.	Plant	Plant parts used	Concentrations (%)		
1	*Allium sativum* L.(Garlic)	Cloves	2	5	10
2	*Zingiber officinale* Rosc. (Ginger)	Rhizomes	2	5	10
3	*Ocimum sanctum* L.(Tulsi)	Leaves	2	5	10
4	*Lantana camera* L. (Lantana)	Leaves	2	5	10
5	*Jatropha curcas* L.(Jatropha)	Leaves	2	5	10
6	*Lawsonia inermis* L.(Mahandi)	Leaves	2	5	10
7	*Azadirachta indica* (Neem)	Leaves	2	5	10
8	*Curcuma longa* L.(Turmeric)	Rhizomes	2	5	10
9	Control	-	-	-	-

b) Evaluvation of different bio control agents *in vitro* against black mold of onion

Different bio control agents (Table 2) were tested *in vitro* against *Aspergillus niger* by dual culture method[5]. All the fungal isolates and the pathogen were multiplied in Richards medium in which potassium nitrate substituted by ammonium sulphate for ten days, where as bacterial antagonists were multiplied in Nutrient agar (NA) media. Twenty millilitre of Richards medium was poured aseptically in

each of Petri plates and allowed to solidify. Mycelial disc of four millimetre diameter of each antagonist and test fungus were placed on opposite ends of Richards medium containing Petriplates, where as bacterial antagonists were streaked at one end of the medium with the help of the metal loop. Each treatment was replicated thrice. The plates were incubated at 28 ± 2ºC for seven days. After incubation the growth of antagonist and test fungus was measured by linear measurement. Index of antagonism was determined by following formula:

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent antagonism index
C = Area of test fungus in control (mm)
T = Area of test fungus in respective treatment (mm)

Table 2: List of different biocontrol agents tested

Sr. No.	Name of the antagonist
1	*Trichoderma viride* isolate 6
2	*T. viride* isolate 9
3	*T. gliocladium*
4	*T. hamatum*
5	*Pseudomonas fluorescens*
6	*Bacillus subtilis*
7	Control

RESULTS AND DISCUSSION

a) Effect of various phytoextracts on the growth of *A. niger*

Effects of eight different phytoextracts on the growth of test fungus was evaluated at 2, 5 and 10 per cent concentrations by poisoned food technique. The observations on growth in each treatment including control were taken and per cent inhibition was calculated on the basis of difference in growth obtained in respective treatments and control. The data regarding per cent inhibition of the growth are presented in Table 3 and Plate-1.

The results presented in Table 3 revealed that all phytoextracts like lantana, mehandhi, tulsi, jatropha, garlic, turmeric, ginger and neem at 2, 5 and 10 % concentrations were found to be effective against the test fungus *Aspergillus niger*. Among all treatments maximum inhibition was obtained in *Lawsonia inermis* (mehandi) leaf extract (71.19%) which followed by *Ocimum sanctum* (tulsi) leaf extract (62.97%). *Zingiber officinale* (Ginger) rhizome extract (62.26%) and *Lantana camera* (Lantana) leaf extract (61.59%) were at par with *Zingiber officinale* (Ginger) rhizome extract (62.26%). Minimum inhibition of test fungus was recorded in *Jatropha curcas* (Jatropha) leaf extract (53.20%).

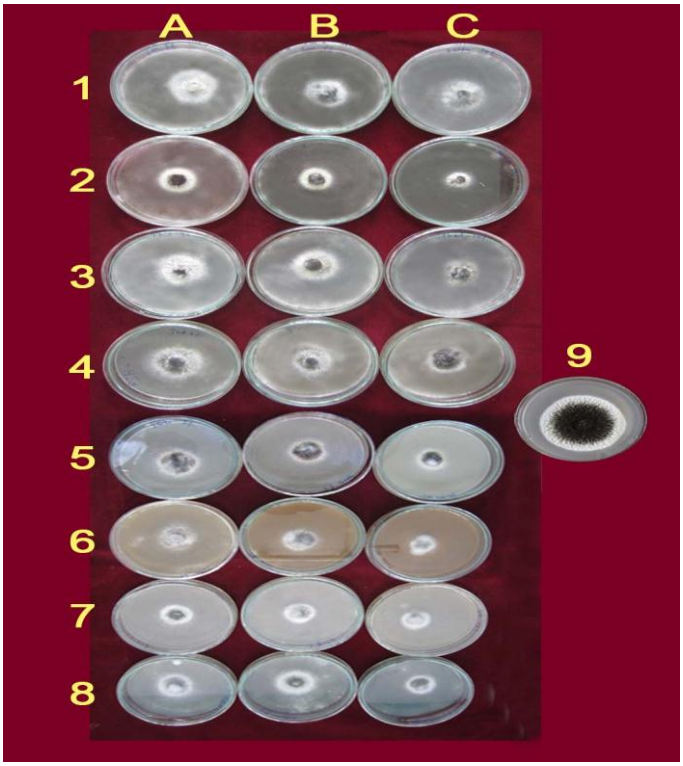
Within the phytoextracts, all three levels of phytoextracts significantly differed from each other. Higher concentration of all the phytoextracts gave significantly higher inhibition as compared to their lower level.

Irkin and Korukluoglu[4] reported that *Allium* plants have antifungal effects to *Aspergillus niger*. Girase *et al*.[3] suggested that solvent extracts of the *Lantana camara* leaves can be used to control *A. niger* as an alternative of chemical pesticides. Leaf extracts like turmeric, onion, tulsi and coleus were found to be effective against spoilage organisms *Aspergillus* spp. reported by Roopa and Suvarna[6].

Table 3: Effect of phytoextracts on growth inhibition of *A. niger in vitro*

Sr. No.	Phytoextract	Concentration in (%)/ per cent inhibition			Mean
		A 2	B 5	C 10	
1	*Lantana camera* L.(Lantana)	53.68	62.76	68.10	61.59
2	*Lawsonia inermis* L.(Mehandi)	68.54	70.36	74.56	71.19
3	*Ocimum sanctum* L.(Tulsi)	58.10	61.62	69.00	62.97
4	*Jatropha curcas* L.(Jatropha)	47.89	54.70	56.97	53.20
5	*Allium sativum* L.(Garlic)	51.52	52.43	69.81	58.07
6	*Curcuma longa* L.(Turmeric)	53.15	56.97	69.11	59.86
7	*Zingiber officinale* Rosc. (Ginger)	57.89	62.42	66.39	62.26
8	*Azadirachta indica* (Neem)	53.68	58.34	66.74	59.65
9	Control	-	-	-	-
	Mean	55.56	59.95	67.59	-
		Phytoextract (P)	Concentration (C)	P×C	
	S.Em.±	0.49	0.16	0.85	
	C.D.at 5%	1.39	0.46	2.41	
	C.V.%	3.21			

Plate 1: *In vitro* evaluation of different plant extracts against the mycelial growth of *Aspergillus niger*



1.*Lantana camera* L.(Lantana)
2.*Lowsonia inermis* L.(Mehandi)
3. *Ocimum sanctum* L.(Tulsi)
4. *Jatropha curcas* L.(Jatropha)
5. *Allium sativum* L.(Garlic)
6. *Curcuma longa* L.(Turmeric)
7. *Zingiber officinale* Rosc. (Ginger)
8. *Azadirachta indica* (Neem)
9. *Control*

a) Effect of different bio control agents on the growth of *A. niger*

Six different fungal/ bacterial bio control agents were used against *A. niger* under *in vitro* condition *i.e.* dual culture test. The observations on growth of test fungus and antagonism index were recorded and are presented in Table 4, Plate-2 and Fig.2.

Data presented in Table 4.3 revealed that all the isolates show impressive results with above 61 per cent growth of test fungus. Among the six bio control agents, *Tricoderma viride* isolate 6 significantly reduced the growth (78.63%) of target pathogen and showed severe antagonism followed by the isolate *T. viride* isolate 9 with (75.51%)

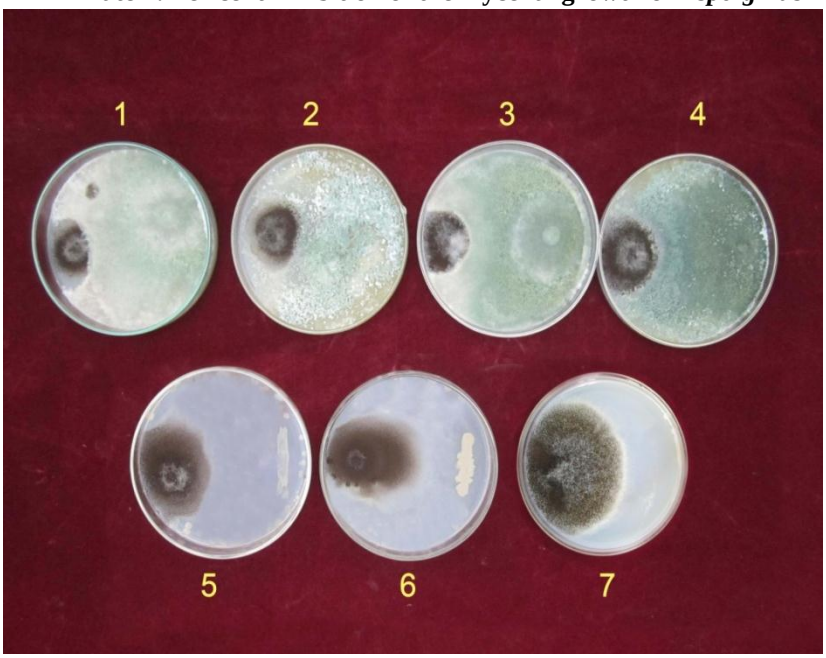
growth, which was statistically at par with *T. viride* inhibition isolate 6. However, *T. gliocladium* and *T. hamatum* were also showing severe antagonism as they reduced the growth of *A. niger*, by 73.01 and 69.26 per cent, respectively. While least antagonism (65.02%) and (61.27%) were obtained with bio control agent *Basillus subtilis* and *Pseudomonas fluorescens*, respectively.

Gajera and Vakharia[2] observed 86.2 per cent inhibition of *T. viride* 60 followed by 80.4 per cent inhibition of *T. harzianum* 2J.

Table 4: Effect of different bio control agents on the growth inhibition of *A. niger*

Sr. No.	Name of the antagonist	Growth reduction (%)
1	*Trichoderma viride* isolate-6	78.63
2	*T. viride* isolate-9	75.51
3	*T. gliocladium*	73.01
4	*T. hamatum*	69.26
5	*Pseudomonas fluorescens*	65.02
6	*Bacillus subtilis*	61.27
7	Control	0.00
S.Em. ±		1.52
C.D. at 5%		4.61
C.V.%		4.36

Plate 2: Per cent inhibition of the mycelial growth of *Aspergillus niger* by different bioagents



1. *Trichoderma viride* isolate-6
2. *T. viride* isolate-9
3. *T. gliocladium*
4. *T. hamatum*
5. *Pseudomonas fluorescens*
6. *Bacillus subtilis*
7. Control

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