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Eco-friendly management of black mould rot of onion (*Aspergillus niger* Van Tieghem)

B. K. PRAJAPATI[1]* AND R. K. PATIL[2]

Directorate of Research,
S. D. Agricultural University, Sardarkrushinagar, Banaskantha-385 506, Gujarat

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ABSTRACT

Black mould rot of onion incited by *Aspergillus niger* is an economically important post-harvest disease of onion under storage conditions and cause extensive losses. Application of fungicides to manage the disease cause residue problems, as it is directly consumed. Therefore, the study was undertaken to manage the black mould rot disease through application of phytoextracts and salts *in vitro* and *in vivo*. Among all phytoextracts screened, custard apple, neem, ghaneri and amaranthus extracts at 10 per cent concentration completely inhibited the mycelial growth of *A. niger* followed by moringa (97.30 %) over control. The results of *in vivo* study revealed that custard apple, neem, ghaneri and amaranthus leaf extracts (10 %) found most effective in controlling the black mould rot over control on 7th and 14th day after inoculation in pre- and post-inoculation treatments. Among the salts screened, aluminum acetate, sodium benzoate, sodium carbonate, sodium bicarbonate and ammonium phosphate at 0.2 M concentration completely inhibited the mycelial growth of *A. niger* over control on 7th day after inoculation *in vitro*. In pre-inoculation treatment, severity of black mould rot disease was negligible in bulbs treated with aluminum acetate (0.00 & 0.67 %) at 7th and 14th day after inoculation. Similar trend of results was noted in post-inoculation treatments.

Key words : *Aspergillus niger*, black mould rot, onion, phytoextracts, salts

Onion (*Allium cepa* L.) is an important vegetable crop widely cultivated and used in the world. After harvest of the bulbs when they are preserved for longer period, many fungal and bacterial pathogens cause deterioration of bulbs. Black mould rot is the most destructive disease of onion in storage as well as under field conditions (Wani and Nisa, 2011). Quadri *et al.*, (1982) reported that the spoilage caused by *A. niger* was as high as 80 per cent. Onion black mould rot disease can occasionally be seen in the field at harvest; it is primarily a post-harvest disease and can cause extensive losses in storage under tropical conditions (Thamizharasi and Narasimham, 1992). Rajam (1992) reported that among the post-harvest diseases of onion, black mould rot caused by *A. niger* was the predominant one. So far, no any effective management practices have been found suitable to manage black mould rot of onion during storage. The present study was undertaken to manage the black mould rot disease of onion by employing phytoextracts and salts.

[1]Assistant Research Scientist, Directorate of Research, S. D. Agricultural University, Sardarkrushinagar, Banaskantha-385 506, Gujarat [2]Ex. Professor, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand
*Email : bindesh_prajapati@yahoo.in

MATERIALS AND METHODS

Phytoextracts study:

In vitro: Bio-efficacy of different phytoextracts of plant species were tested *in vitro* by Poisoned Food Technique (Nene and Thapliyal, 1979) against black mould rot pathogen. The list of plant species used for phytoextracts study are given in Table 1. The phytoextracts were tested at 10 per cent concentration. Fresh and healthy plant parts of each species were thoroughly washed with tap water and then with distilled sterilized water. They were macerated separately in grinder by adding 100 ml ethanol. The mixture was filtered through two fold sterilized muslin cloth and the filtrate was centrifuged at 5,000 rpm for 10 min. and the clear supernatant extract was collected in sterilized conical flasks. After evaporating the ethanol from extract, the clear extract was collected and diluted

with 100 ml distilled sterile water to make volume 1:1 (W/V). This was considered as 100 per cent concentration for the study to test the efficacy of phytoextracts (Sinha and Saxena, 1989). Control was maintained for each set where fungal disc were placed on PDA medium without phytoextracts. Each treatment was repeated thrice. The inoculated plates were then incubated at 25 ± 1°C in BOD incubator. The observations on mycelial growth (mm) and per cent growth inhibition of test fungi were recorded after 7 days of incubation. The per cent growth inhibition (PGI) of pathogen in each treatment was calculated by following formula (Asalmol *et al.*, 1990).

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

Where,

I = Inhibition per cent

C = Colony diameter (mm) in control plate

T = Colony diameter (mm) in treated plate

In vivo: The phytoextracts which found promising *in vitro*, were further tested to study their efficacy in controlling black mould rot of onion.

Pre- inoculation: Healthy, mature uniform size onion bulbs of cultivar Nasik red variety were collected from Sardar Patel vegetable market, Anand. The bulbs were thoroughly washed in tap water, then surface sterilized by dipping in 1 per cent NaOCl solution for one minute followed by three successive washings with distilled sterile water and then were first dipped in 10 per cent phytoextracts for five minutes separately, air dried and after 12 hrs. treated bulbs were inoculated with black mould rot pathogen (10^6 spores/ml) and then inoculated bulbs were bagged in sterilized polythene bags with sterilized moist absorbent cotton swab. The mouth of the bag was loosely tied with rubber bands and inoculated bulbs were incubated at room temperature.

Table 1 List of phytoextracts used for the study

Sr. No	Botanical Name	Common Name	Family	Plant Part used
1.	*Annona squamosa* L.	Custard apple	Annonaceae	Leaf
2.	*Ocimum sanctum* L.	Tulsi	Labiatae	Leaf
3.	*Aloe barbadensis* Mill.	Aloe vera	Asphodeloideae	Leaf
4.	*Azadirachta indica* A. Juss	Neem	Meliaceae	Leaf
5.	*Lantana camara* L.	Ghaneri	Verbenaceae	Leaf
6.	*Andrographis paniculata* (Burm.f.) wall. Nees	Kalmegh	Acanthaceae	Leaf
7.	*Centelly asiatica* L.	Mandukaparni	Apiaceae	Leaf
8.	*Amaranthus viridis* L.	Amaranthus	Amaranthaceae	Leaf
9.	*Moringa oleifera* Lam.	Moringa	Moringaceae	Leaf

Post- inoculation: Healthy mature onion bulbs were inoculated with test pathogen (10^6 spores/ml) and after 12 hrs., the inoculated bulbs were dipped in phytoextracts separately for five minutes. Observations on percent disease severity were recorded after 7th and 14th days of inoculation with the help of standard assessment key.

Standard key for disease severity assessment: (Wani and Nisa, 2011)

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

$$\text{Severity (\%)} = \frac{\text{Area of infected bulbs}}{\text{Total area of blub tissue}} \times 100$$

Salts study:

In vitro: Efficacy of different organic and inorganic salts was tested *in vitro* by "Poisoned food technique" against *A. niger*. The list of salts used for the study is given in Table 2. .

Table 2 List of organic and inorganic salts used for the study

Sr. No.	Salts	Concentration (Molarity)
1.	Aluminum acetate ($C_2H_5O_4Al$)	0.2 M
2.	Aluminum chloride ($AlCl_3 \cdot 6H_2O$)	0.2 M
3.	Calcium chloride ($CaCl_2.H_2O$)	0.2 M
4.	Potassium chloride (KCl)	0.2 M
5.	Sodium benzoate ($C_7H_5O_2Na$)	0.2 M
6.	Sodium bicarbonate ($NaHCO_3$)	0.2 M
7.	Sodium carbonate (Na_2CO_3)	0.2 M
8.	Sodium chloride (NaCl)	0.2 M
9.	Ammonium phosphate {$(NH_4)_3PO4$}	0.2 M

Observations on per cent growth inhibition were recorded after 7 days of incubation.

In vivo: The salts which found promising *in vitro*, were further tested to study their efficacy in controlling black mould rot of onion.

Pre-inoculation: Similar methodology was followed as described in pre-inoculation of phytoextracts.

Post-inoculation: Similar methodology was followed as described in pre-inoculation of phytoextracts. Observations on percent disease severity were recorded after 7th and 14th day of inoculation.

RESULTS AND DISCUSSION

Phytoextracts study:

In vitro: Among the phytoextracts screened, five were found significantly superior in inhibiting the mycelium growth of *A. niger* over control. Custard apple, neem, ghaneri and amaranthus extracts completely inhibited the mycelial growth of *A. niger*. The next best treatment in order of merit was moringa recording 97.30 per cent mycelial growth inhibition over control. Mandukaparni (0.74 %) and Tulsi leaf extract (1.22 %) found least effective in suppressing the mycelial growth of the *A. niger* (Table 3). The results of present study are in agreement with the results obtained by Mushtaq *et al.*, (2012). They reported that chloroform leaves extract of *Amaranthus viridis* and *Malvestrum coromendalianum* (0.2 g/ml) gave effective inhibition of *A. niger* through poisoned food technique *in vitro*. Leaf extract of *Annona squamosa* gave good control over sporulation of *A. niger in vitro* isolated from cow pea, ground nut, maize and melon seeds through poisoned food technique (Onyeani *et al.*, 2012). Gupta *et al.*, (2012) reported that leaf extracts of *Datura stramonium*, *Ocimum sanctum*, *Azadirecta indica*, *Eucalyptus* spp. (5 ml/ 10g seeds) were found most effective against *A. niger* isolated from onion *in vitro* by seed soaking and germination method. Girase *et al.*, (2012) found that petroleum ether extract of *Lantana camara* at 125 and 250 ppm gave 100 per cent mycelial growth inhibition of *A. niger* through agar well diffusion assay method *in vitro*.

Table 3 Evaluation of phytoextracts (10 %) against *A. niger in vitro*

Sr. No.	Treatments	Mycelial growth (mm) 7 DAI*	Per cent growth inhibition (PGI)
1.	Custard apple (*A. squamosa* L.)	00.00	100.00
2.	Tulsi (*O. sanctum* L)	88.90	01.22
3.	Aloevera (*A. barbadensis* Mill)	75.67	15.92
4.	Neem (*A. indica* A. Juss)	00.00	100.00
5.	Ghaneri (*Lantana camara* L.)	00.00	100.00
6.	Kalmegh (*A. paniculata* (Burm.f.) wall. Nees)	66.33	26.30
7.	Mandukaparni (*C. asiatica* L.)	89.33	00.74
8.	Amaranthus (*A. viridis* L.)	00.00	100.00
9.	Moringa (*M. oleifera* Lam.)	02.43	97.30
10.	Control	90.00	-
	S.Em.±	0.708	-
	C.D. at 5%	2.104	-
	C.V.%	2.972	-

*DAI= Days after inoculation

In vivo: Among five phytoextracts screened *in vivo*, all were found significantly superior in reducing the black mould rot severity as compared to control on 7th and 14th day after inoculation both in pre- and post-inoculation treatments at 10 per cent concentration (Table 4).

Pre-inoculation: The results revealed that no black mould rot severity was recorded in bulbs treated with ghaneri, kalmegh, amaranthus, custard apple and neem leaves extracts on 7th and 14th day after inoculation as compared to control (13.67 & 25.33 %).

Post-inoculation: Similar trend of results were also noted in post-inoculation treatment. Leaf extracts of ghaneri, kalmegh, amaranthus, custard apple and neem at 10 per cent concentration completely controlled black mould rot severity on 7th and 14th day after inoculation as compared to control (14.67 & 26.33 %). The results of present findings are quite confirmative with the results reported by Kaur and Verma (2002). They noted that dry neem leaf extracts (5×10^4 µg/ ml) found most effective in pre- (94.44 %) and post inoculation treatments (50.0 %) in controlling green mould rot (*Penicillium digitatum*) of kinnow fruits. Singh and Sumbali (2003) noted that leaf extract of *Azadirachta indica* (10 %) found most effective in controlling the Penicillium rot (*Penicillium expansum*) infecting apple fruits. Damaram (2013) reported that Fusarium fruit rot severity in tomato fruits treated with neem leaf extracts in both pre- and post-inoculation treatments showed lower fruit rot severity (18.33 & 19.33 %) as compared to control (47.33 %) after 8 days of inoculation. Reddy (2012) reported that neem leaf extract gave lower Penicillium fruit rot severity (18.33 & 17.50 %) both in pre- and post-inoculation treatments after 8 days of inoculation in citrus fruits.

Salts study:

In vitro: Among the organic and inorganic salts screened, five salts were found significantly superior revealing complete mycelial growth inhibition of *A. niger* over control. Aluminum acetate, sodium benzoate, sodium carbonate, sodium bicarbonate and ammonium phosphate completely inhibited the mycelial growth of *A. niger* over control on 7th day after inoculation. Calcium chloride (90.00 mm, PGI-00.00), potassium chloride (87.33 mm, PGI- 2.97) and sodium chloride (86.33 mm, PGI- 3.70) found least effective at 0.2 M concentration in restricting the mycelial growth of the *A. niger* (Table 5). The results of present investigation corroborate with the results reported by Nwafor and Ikenebomeh (2009). They reported that sodium benzoate at 0.05 per cent (w/v) conc. showed complete inhibition of *A. niger*.

Table 4 Evaluation of phytoextracts against black mould rot of onion (*A. niger*) *in vivo*

Sr. No.	Treatments	Per cent rot severity			
		Pre-inoculation		Post-inoculation	
		7th DAI*	14th DAI	7th DAI	14th DAI
1.	Ghaneri	0.00	0.00	0.00	0.00
		(0.71)**	(0.71)	(0.71)	(0.71)
2.	Kalmegh	0.00	0.00	0.00	0.00
		(0.71)	(0.71)	(0.71)	(0.71)
3.	Amaranthus	0.00	0.00	0.00	0.00
		(0.71)	(0.71)	(0.71)	(0.71)
4.	Custard apple	0.00	0.00	0.00	0.00
		(0.71)	(0.71)	(0.71)	(0.71)
5.	Neem	0.00	0.00	0.00	0.00
		(0.71)	(0.71)	(0.71)	(0.71)
6.	Control	13.67	25.33	14.67	26.33
		(3.76)	(5.08)	(3.88)	(5.18)
	S.Em.±	0.048	0.058	0.062	0.013
	C.D. at 5%	0.149	0.179	0.192	0.040
	C.V.%	6.881	7.015	8.708	1.562

* DAI= Days after inoculation

**Figures in parentheses are square root transformed ($\sqrt{x + 0.5}$) values

Table 5 Efficacy of organic and inorganic salts against mycelial growth of *A. niger in vitro*

Sr. No.	Treatments	Mycelial growth (mm) 7 DAI*	Per cent growth inhibition (PGI)
1.	Aluminum acetate	00.00	100.00
2.	Aluminum chloride	56.33	37.41
3.	Calcium chloride	90.00	00.00
4.	Potassium chloride	87.33	02.97
5.	Sodium benzoate	00.00	100.00
6.	Sodium bicarbonate	00.00	100.00
7.	Sodium carbonate	00.00	100.00
8.	Sodium chloride	86.33	03.70
9.	Ammonium phosphate	00.00	100.00
10.	Control	90.00	00.00
	S.Em.±	1.633	-
	C.D. at 5%	4.817	-
	C.V.%	6.893	-

*DAI= Days after inoculation

Heydaryinia *et al.*, (2011) reported that sodium benzoate at 0.1 per cent concentration found most inhibitory to mycelial growth of *A. niger* followed by potassium sorbate (0.1 %). Ghadiri *et al.*, (2013) showed that ammonium phosphate gave highest inhibition of the mycelial growth of *F. solani* (92.29%) on PDA medium followed by potassium carbonate (54.92%), potassium bicarbonate (46.14%), sodium carbonate (42.60%) and sodium bicarbonate (42.33%). Pandya (2015) found that aluminum acetate and aluminum chloride salts at 0.2 M concentration completely inhibited the mycelial growth of *Fusarium solani in vitro*.

In vivo: Five salts which were found most effective *in vitro* were further screened to test their efficacy in controlling black mould rot of onion at 0.2 M concentration by following both pre- and post-inoculation methods.

Pre-inoculation: The results presented in Table 6 revealed that the severity of black mould rot disease was negligible in onion bulbs treated with sodium benzoate (0.00 & 0.33 %) and sodium bicarbonate (0.00 & 0.33 %) followed by aluminum acetate (0.00 & 0.67 %) after 7th and 14th day of inoculation. Ammonium phosphate (0.33 & 1.00 %) and sodium carbonate (0.33 & 1.93 %) showed mediocre effect over control (16.67 & 27.67 %).

Post-inoculation: Similar trend of results was noted in post-inoculation treatment. Significantly very low black mould rot severity was recorded in bulbs treated with sodium benzoate (0.00 & 1.06 %) and sodium bicarbonate (0.73 & 1.33 %) followed by aluminum acetate (0.67 & 1.50 %) after 7th and 14th days of inoculation. The next best treatment in order of merit was sodium carbonate (1.10 & 2.47 %) followed by ammonium phosphate (1.77 & 4.37 %). The results of present study are in agreement with the results obtained by Mecteau *et al.*, (2008). They showed that application of aluminum chloride gave 40 per cent decrease in dry rot severity of potato infected with *Fusarium solani* var. *coeruleum* compared with the control. Pandya (2015) reported that severity of Fusarium dry rot was not observed in potato tubers treated with aluminum acetate and aluminum chloride on 4th and 8th day after inoculation in pre- and post-inoculation treatments.

Table 6 Evaluation of organic and inorganic salts in management of black mould rot (*A. niger*) of onion *in vivo*

Sr. No.	Treatments	Per cent rot severity			
		Pre-inoculation		Post-inoculation	
		7th DAI*	14th DAI	7th DAI	14th DAI
1.	Aluminum acetate	0.00	0.67	0.67	1.50
		(0.71)**	(1.05)	(1.05)	(1.41)
2.	Sodium benzoate	0.00	0.33	0.00	1.06
		(0.71)	(0.88)	(0.71)	(1.25)
3.	Sodium bicarbonate	0.00	0.33	0.73	1.33
		(0.71)	(0.88)	(1.08)	(1.35)
4.	Sodium carbonate	0.33	1.93	1.10	2.47
		(0.88)	(1.56)	(1.26)	(1.72)
5.	Ammonium phosphate	0.33	1.00	1.77	4.37
		(0.88)	(1.22)	(1.50)	(2.20)
6.	Control	16.67	27.67	22.33	41.33
		(4.33)	(5.30)	(4.77)	(6.46)
	S.Em.±	0.128	0.135	0.124	0.069
	C.D. at 5%	0.394	0.415	0.381	0.212
	C.V.%	16.200	12.835	12.372	4.957

* DAI= Days after inoculation

**Figures in parentheses are square root transformed ($\sqrt{x + 0.5}$) values

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