

## Isolation and identification of fungal pathogens causing fruit rot of Bottle gourd in some villages of Kosi division

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### ABSTRACT

In the present study, fungal pathogens were isolated from fruit rot of bottle gourd collected from seven different villages of Saharsa district. Disease incidence was calculated in each village which varied from 10% to 60%. Maximum disease incidence was observed in Jahmhra village. Altogether three isolates *Fusarium oxysporum*, *Alternaria alternata* and *Colletotrichum lagenarium* were isolated. Growth was observed in three different media. Maximum growth occurred in PDA medium. Effect of temperature on growth was observed. Maximum growth was observed at a temperature of 28°C. Pathogenicity of *Fusarium oxysporum*, *Alternaria alternata* and *Colletotrichum lagenarium* was observed as 80%, 100% and 60% respectively.

**Key Words** - Isolates, Disease incidence, Pathogenicity, Fruit rot, Bottle gourd

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### INTRODUCTION

Bottle gourd is a climber plant belonging to cucurbitaceae. It is a tropical vegetable grown in India for its fruits. Leaves and twigs of bottle gourd are also used as leafy vegetable. Woody exocarps of dry fruits are used in the manufacture of containers, music instruments and fishing floats (Heisser-1979). Bottle gourd is rich in minerals like potassium and calcium as well as contains vitamin A and vitamin B. In addition to minerals and vitamins bottle gourd contains biologically active constituents such as saponin, triterpenes and flavonoids. Therefore bottle gourd is an important source of pharmaceuticals. Its use in diet is advised for diabetes, Jaundice, ulcer, cardiac disorders and blood pressure control (Prajapati *et al.*, 2010). Plant of bottle gourd is attacked by several fungal diseases like downy mildew, powdery mildew, root rot, fruit rot during various stages of growth and even at post-harvest stage which causes low productivity of fruits (Neeraj and Verma-2010). *Alternaria alternata* causes black fruit rot of bottle

gourd at blossom and end stage. This disease causes huge economic loss to bottle gourd grower. Fruit rot disease of bottle gourd was first reported by Singh and Chouhan (1980) from North India.

In Saharsa district, Baghwa, Amahi, Sihoul, Jamhra, Balutola, Barsam and Lagma villages are important bottle gourd grower area where fruit rot is common causing huge economic loss to bottle gourd grower. The objective of the present study is to isolate and identify fungal pathogens causing fruit rot of bottle gourd.

### MATERIAL & METHODS

A survey was conducted in bottle gourd fruits in villages, Baghwa, Amahi, Sihoul, Jamhra, Balutola, Barsam and Lagma of Saharsa district. In each field total number of fruits and infected fruits were counted and disease incident was calculated by the formula:

$$\text{Disease incidence} = \frac{\text{No. of rotten fruits}}{\text{Total no. of fruits}} \times 100$$

Infected fruits were collected in polythene bags and brought to the laboratory.

#### Isolation of pathogens:

Infected parts of fruits were cut into small pieces using a sharp sterile blade and surface sterilized with 0.5% sodium hypochlorite. Rotten pieces of fruits were washed 3-4 times with sterilized distilled water.

#### Culture media used:

Three different media: PDA medium, Oat meal agar medium (OMA) and Richard's medium were used for the culture of fungal pathogens.

#### Isolation of fungal pathogens:

For the isolation of fungal pathogens PDA medium was selected. Sterilized rotten pieces of fruits were inoculated on PDA plates and incubated at  $28\pm 2^{\circ}\text{C}$  for 3 days. Colonies and mycelial mats were observed and sub-cultured to get pure culture.

#### Growth and sporulation of isolates on different media:

To observe the growth and sporulation rate of isolates, pure culture of isolates were inoculated in PDA medium, OMA medium and Richard's medium and incubated for six days. After six days, radial growth of mycelium was measured and growth of spores was observed in each medium.

#### Effect of temperature on growth of isolates:

To observe the effect of temperature on the growth of isolates mycelial mat (immediately) of each isolate from pure culture was inoculated on moist suitable medium and incubated at temperature  $15^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$ ,  $28^{\circ}\text{C}$ ,  $35^{\circ}\text{C}$  and  $40^{\circ}\text{C}$  for 6 days. Growth of each isolate at different temperature was measured. Isolated fungal pathogens were identified on the morphological characters of mycelium and spores with the help of standard monographs (Barnett and Hunter-1972, Botton *et al.*, 1990, Elliot-1971). Verification of Koch's postulates (pathogenicity test). This test was performed in vivo for each isolate. Five healthy fruits of cultivated area were selected for the test. Each healthy fruit was surface sterilized with 70% ethanol. 4-5 scratch were made on each fruit and inoculated with spore suspension of isolate. For

control one healthy fruit was mounted with sterilized distilled water after scratching. All inoculated and control fruits were covered with sterilized polythene bags. Small holes were made in polythene bags for aeration. After 6 days all treated fruits were observed for rot development.

#### RESULT & DISCUSSION

A survey was conducted in seven villages namely Baghwa, Amahi, Sihoul, Jamhra, Balutola, Barsam and Lagma of district Saharsa to observe fungal disease of Bottle gourd. Disease incidences in bottle gourd field of selected villages were calculated.

Disease incidence varied in between 10% to 60% in different fields. Maximum disease incidence was recorded from Bottle gourd field of Jamhra (60%) and lowers in Bottle gourd field of Barsam (10%) (Fig. 1). Altogether, three fungal pathogens *Fusarium oxysporum*, *Alternaria alternata* and *Colletotrichum lagenarium* were isolated from fruit rot of Bottle gourd in different localities. *Fusarium oxysporum* was isolated from fruit rot of Bottle gourd collected from Baghwa, Balutola and Sihoul, *Alternaria alternata* from Amahi, Jamhra, Sihoul and Barsam, *Colletotrichum lagenarium* from Jamhra, Balutola and Lagma. Difference in occurrence of fungal pathogens in different localities may be due to composition of vegetation in surrounding area as well as to the association of Bottle gourd with other crops (Anahosur, 1992).

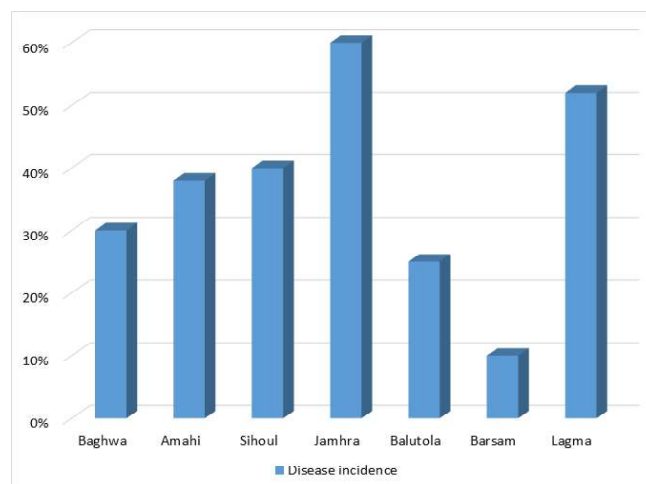


Fig. 1- Disease incidence in Bottle gourd at different villages

Occurrence of a fungal pathogen in a particular area may be due to its presence on host plant in another sink of same locality and due to favorable environment (Banvart, 1998) (Table 1).

**Table 1: Fungal pathogen isolated from Bottle gourd**

Villages	<i>Fusarium</i>	<i>Alternaria</i>	<i>Colletotrichum</i>
Baghwa	+ve	-ve	-ve
Amahi	-ve	+ve	-ve
Sihoul	+ve	+ve	-ve
Jamhra	-ve	+ve	+ve
Balutola	+ve	-ve	+ve
Barsam	-ve	+ve	-ve
Lagma	-ve	-ve	+ve

Effect of different media on growth of isolates was tested in PDA medium, OMA medium and Richard's medium after incubation for 2 days, 4 days and 6 days. Maximum growth of all isolates was observed in PDA medium followed by OMA medium (Table 2). Prasad and Kulshrestha (1999) reported to excellent growth of *Alternaria helianthi* in PDA medium supplemented with calcium carbonate.

**Table 2: Mycelial growth and sporulation of isolates in different medium.**

Medium	Days	Isolate	Mycelium	Spore
PDA	2	<i>Fusarium</i>	4.94	-
	2	<i>Alternaria</i>	4.73	-
	2	<i>Colletotrichum</i>	5.31	-
	4	<i>Fusarium</i>	6.54	+
	4	<i>Alternaria</i>	6.34	+
	4	<i>Colletotrichum</i>	7.85	+
	6	<i>Fusarium</i>	8.95	++
	6	<i>Colletotrichum</i>	9.45	++
OMA	2	<i>Fusarium</i>	3.75	-
	2	<i>Alternaria</i>	4.72	-
	2	<i>Colletotrichum</i>	5.43	-
	4	<i>Fusarium</i>	5.85	+
	4	<i>Alternaria</i>	6.45	+
	4	<i>Colletotrichum</i>	7.46	+
	6	<i>Fusarium</i>	7.83	++
	6	<i>Colletotrichum</i>	9.21	++
Richard's	2	<i>Fusarium</i>	4.73	-
	2	<i>Alternaria</i>	4.42	-
	2	<i>Colletotrichum</i>	5.21	-
	4	<i>Fusarium</i>	5.98	+
	4	<i>Alternaria</i>	6.21	++
	4	<i>Colletotrichum</i>	7.53	+
	6	<i>Fusarium</i>	8.43	+++
	6	<i>Colletotrichum</i>	9.17	++

Effect of temperature was tested on the growth of isolates at a temperature range of 15°C to 40°C. It was observed that excellent growth of all isolates occurred at a temperature of 28°C followed by 35°C. At low temperature and above 35°C temperature growth reduced.

**Table 3: Effect of different temperature on isolates in PDA medium**

Days	Isolate	15°C	20°C	28°C	35°C	40°C
2	<i>Fusarium</i>	1.64	2.43	4.94	4.75	1.78
2	<i>Alternaria</i>	1.53	2.87	4.73	4.68	1.79
2	<i>Colletotrichum</i>	1.48	2.74	5.31	5.23	1.41
4	<i>Fusarium</i>	2.73	5.96	6.54	6.43	1.85
4	<i>Alternaria</i>	2.78	5.23	6.34	6.14	2.12
4	<i>Colletotrichum</i>	2.75	6.42	7.85	7.68	1.95
6	<i>Fusarium</i>	2.83	7.13	8.95	8.78	2.13
6	<i>Alternaria</i>	2.56	7.21	8.72	8.46	1.98
6	<i>Colletotrichum</i>	2.14	8.13	9.45	9.26	2.49

Pathogenicity test (verification of Koch's postulate) was performed on fresh fruits. *Alternaria alternata* showed 100% pathogenicity, *Fusarium oxysporum* showed 80% and *Colletotrichum lagenarium* showed 60% pathogenicity.

**Table 4: Pathogenicity test of isolates**

Pathogen	Healthy fruit	Rot developed	Percentage
<i>Fusarium</i>	5	4	80%
<i>Alternaria</i>	5	5	100%
<i>Colletotrichum</i>	5	3	60%

## CONCLUSION

A survey was conducted in Bottle gourd fields of seven villages of Saharsa district. District incidence was calculated in each locality. Disease incidence varied from 10% to 60%. Maximum disease incidence was observed in Jamhra village. Fungal pathogens were isolated from fruit rot of Bottle gourd. Altogether, three isolates *Fusarium oxysporum*, *Alternaria alternata* and *Colletotrichum lagenarium* were isolated from fruit rot of different localities. *Fusarium oxysporum* was isolated from fruits of Baghwa, Sihoul and Balutola. *Alternaria alternata* was isolated from fruit rot of Amahi, Sihoul, Jamhra and Barsam. *Colletotrichum lagenarium* was isolated from fruit rot of Balutola and Lagma.

Effect of different media and temperature was tested on growth and sporulation of isolates.

Maximum growth of all isolates was observed in PDA medium at 28°C temperature.

Pathogenicity test for each isolate was performed by infecting fresh fruit with isolate. Pathogenicity of *Fusarium oxysporum*, *Alternaria alternata* and *Colletotrichum lagenarium* were observed as 80%, 100% and 60% respectively.

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