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## Studies on physiological parameters of *Choanephora cucurbitarum*, the incitant of wet rot of cucumber

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### Abstract

A newly documented disease, namely *Choanephora* wet rot of cucumber (*Cucumis sativus* L.) caused by *Choanephora cucurbitarum* was found at SKN COA, Jobner, in Zayad 2014. The colonies of the causal fungus grown on potato dextrose agar at 25°C are white at the early stage of growth with abundant aerial mycelium, but at later growth stages turn pale yellow. Sporangia and sporangiola are readily observed on the tip of upright sporangiophores, which are numerous on aerial mycelium. Effect of temperature, RH and media on mycelia growth of *Choanephora cucurbitarum* was studied in vitro. Maximum mycelia growth was found at 25 C and 30 C. 100% relative humidity favours the disease also. A significantly decrease in mycelia growth was observed at 70% relative humidity. Among five different media PDA was found superior. This was followed by martin medium. Mycelial growth was not recorded on Oat meal agar.

**Keywords:** *Choanephora cucurbitarum*, choanephora wet rot

### Introduction

Cucumber (*Cucumis sativus* L.) is known as “*Kheera*” in Hindi. It is a popular and widely cultivated summer vegetable in India which belongs to family *Cucurbitaceae*. In India, cucumber is commonly grown in Andhra Pradesh, Karnataka, Assam and Rajasthan, generally towards the riversides. Rajasthan state provides the maximum potential for the production of cucumber because of its agro-climatic conditions that are best suited for their growth and yield. “*Kheera*” is eaten raw with salt and pepper or as salad with onion and tomato. Cucumber is rich in vitamin B and C as well as in minerals such as calcium, phosphorus, iron and potassium. Cucumber is a warm season crop but it is also grown in summer and rainy season. It requires 18 °C minimum temperature for seed germination and 20-30 °C for growth and development of plant. However, a number of fungal diseases which have been reported to cause heavy losses to the crop. *Choanephora* wet of cucumber is a major disease of cucumber and cause severe losses in mainly green house and poly house condition.

### Material Method

#### Physio-pathological studies

All the glasswares were thoroughly cleaned and rinsed with distilled water. Chemicals of analar grade were used. Five different synthetic and semi-synthetic media were prepared by weighing the different constituents of each medium and autoclaved at 1.045 kg/cm<sup>2</sup> for 20 minutes.

In all three physiopathological experiments, inoculation was done with 5 mm diameter bit taken from 7 days old fungal culture and incubated at 25±1°C (except for temperature study) for 3 days. The each experiment, under physio-pathological study was arranged in completely randomized design (CRD) with four replications.

#### Effect of temperature on mycelial growth

Effect of temperature on mycelial growth of *Choanephora cucurbitarum* was studied *in vitro*. Twenty ml of sterilized PDA was poured in each sterilized Petriplate. Inoculation was made with 5 mm disc of 7 days old culture of *Choanephora cucurbitarum* with the help of sterilized cork borer and incubated at different levels of temperature viz. 20, 25, 30, 35 and 40 °C for 3 days. Observations on mycelial growth was recorded after 3<sup>rd</sup> day of incubation.

#### Effect of relative humidity on mycelial growth

To study the effect of relative humidity on mycelial growth of *Choanephora cucurbitarum*,

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five different levels of relative humidity i.e. 60, 70, 80, 90 and 100 per cent were maintained by using the concentrate sulphuric acid and sterilized distilled water in different proportion in glass desiccators according to the method suggested by Buxton and Mellanby (1934) [4]. The composition of the acid solution used was as follows.

RH (%)	Stock solution (ml)	Distilled water (ml)
60	374.0	396.0
70	348.0	510.3
80	294.0	640.0
90	161.0	712.0
100	0.00	Only distilled water

Petriplates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of *Choanephora cucurbitarum* with the help of sterilized cork borer. Inoculated Petriplates were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportion and incubated at  $25 \pm 1^\circ\text{C}$  for 3 days. Observations on mycelial growth was recorded after 3<sup>rd</sup> day of incubation.

#### Effect of media on mycelial growth

Growth on solid media was determined by measuring the colony diameter along with the two diagonals passing through the center of colony by excluding initial diameter (5 mm) of bit. Five solid media whose composition is given below were taken for *in vitro* studies. Petriplates having sterilized medium were inoculated with 5 mm disc of mycelial growth with the help of sterilized cork borer and incubated at  $25 \pm 1^\circ\text{C}$  in incubator for 3 days. Observations on mycelial growth (radial growth) was taken after 3<sup>rd</sup> day of incubation. Medium giving best radial growth of mycelium used for further studies.

#### Constituents of different media

S. No.	Medium	Constituents	Quantity
1.	PDA	Agar-agar Dextrose Peeled-potato Distilled water	20.00 g 20.00 g 250.00 g 1000 ml
2.	Czapeck's Dox Agar medium	Agar-agar Sucrose Distilled-water Dipotassium-phosphate- Magnesium Potassium chloride Sodium nitrate Ferrus sulphate	15.00 g 30.00 g 1000 ml 1.00 g 0.50 g 0.50 g 2.00 g 0.01 g
3.	Oat meal medium	Agar-agar Glucose Oat meal Distilled water	20.00 g 20.00 g 20.00 g 1000 ml
4.	Corn meal medium	Agar-agar Corn meal Glucose Distilled water	20.00 g 20.00 g 20.00 g 1000 ml
5.	Martin's medium	Agar-agar Dextrose Peptone Distilled water Rose Bengal Potassium-dihydrogen phosphate Magnesium sulphate Streptomycin	15.00 g 10.00 g 5.00 g 1000 ml 1 part in 3000 part of media 1.00 g 0.50 g 30.00 mg

## Results

### Effect of temperature on mycelial growth

The entire microorganisms grow under certain range of temperature within which a minimum, optimum and maximum temperature could be located. It is evident from the data that the fungus grew at all the temperature range of  $20^\circ\text{C}$  to  $40^\circ\text{C}$ . Maximum mycelial growth of the fungus was observed at  $25^\circ\text{C}$  (90 mm) and  $30^\circ\text{C}$  (90 mm) at 3<sup>rd</sup> day of incubation and found at par with  $35^\circ\text{C}$  (89.25 mm). A gradual decrease in mycelial growth was observed at  $20^\circ\text{C}$  (75.25 mm). Minimum mycelial growth (35.56 mm) of the fungus was observed at  $40^\circ\text{C}$ .

**Table 1:** Effect of temperature on mycelial growth of *Choanephora cucurbitarum*

S. No.	Temperature ( $^\circ\text{C}$ )	Mycelial growth (mm)*
1	20	75.25
2	25	90.00
3	30	90.00
4	35	89.25
5	40	35.56
	SEm $\pm$	1.26
	CD (p = 0.05)	3.89

\* Average of four replications

### Effect of relative humidity on mycelial growth

To evaluate the effect of atmospheric moisture, the fungus was exposed directly to different levels of relative humidity viz. 60, 70, 80, 90, 100 per cent and incubated at  $25 \pm 1^\circ\text{C}$  for 3 days. It was observed that all the five humidity levels include the growth of *Choanephora cucurbitarum*.

Perusal of data showed that maximum mycelial growth (90 mm) of *Choanephora cucurbitarum* was observed at 100 and 90 per cent relative humidity and found at par with 80 per cent (89.00 mm) relative humidity. A significantly decrease in mycelial growth was observed at 70 per cent (81.25 mm) relative humidity. Minimum mycelial growth (76.00 mm) was observed at 60 per cent relative humidity.

**Table 2:** Effect of relative humidity on mycelial growth of *Choanephora cucurbitarum* at  $25 \pm 1^\circ\text{C}$

S. No.	Relative humidity (%)	Mycelial growth (mm)*
1	60	76.00
2	70	81.25
3	80	89.00
4	90	90.00
5	100	90.00
	SEm $\pm$	1.31
	CD (p = 0.05)	4.03

\* Average of four replications

### Effect of media on mycelial growth

To find out a suitable medium for mycelial growth of *Choanephora cucurbitarum*, five different media were tested. Perusal of data revealed that potato dextrose agar medium was significantly superior in supporting maximum mycelial growth (90.00 mm). This was followed by Martin's medium (36.66 mm), corn meal agar (9.5 mm) and Czapeck's Dox agar (8.45 mm). Mycelial growth of the fungus was not recorded on oat meal agar.

**Table 3:** Effect of different media on mycelial growth of *Choanephora cucurbitarum* at 25 ± 1°C

S. No.	Medium	Mycelial growth (mm)*
1	PDA	90.00
2	Corn meal agar	9.50
3	Oat meal agar	0.00
4	Czapeck's dox agar	8.45
5	Martin's medium	36.66
	SEm±	0.54
	CD (p = 0.05)	1.68

\* Average of four replications

### Discussion

Nutrition plays an important role in growth and sporulation of the fungus. In order to determine basal medium for growth and sporulation of *Choanephora cucurbitarum*, five different solid media were tested *in vitro*. The potato dextrose agar medium was supported maximum mycelial growth (90.00 mm) and it was followed by Martin's medium (36.66 mm). Minimum mycelial growth (0.00 mm) was found on oat meal agar. Similarly, Kuo *et al.* (1999)<sup>[7]</sup> and Mishra and Mishra (2012)<sup>[9]</sup> found good growth on PDA.

Temperature is one of the important factor for the growth and sporulation of an organism which also influences the occurrence and development of disease and most of the organisms grow between 0 to 42 °C (Wolf and Wolf, 1947). Results of temperature studies showed the maximum growth (90.00 mm) at 25 °C and 30 °C, at par with 35 °C (89.25 mm) and least mycelial growth (35.56 mm) was found at 40 °C temperature. Optimum temperature 25 °C and 30 °C for mycelial growth of *Choanephora cucurbitarum* was also observed by Kuo *et al.*, (1999)<sup>[7]</sup> and Abdel-Motaal *et al.*, (2010)<sup>[1]</sup>.

In the present investigation it was observed that pathogen *Choanephora cucurbitarum* grew and sporulated efficiently at 80 to 100 per cent relative humidity, whereas, decline was observed at lower humidity levels. Maximum growth and sporulation of *Choanephora cucurbitarum* was also observed best at 90 to 100 per cent relative humidity by earlier workers Kwon and Hyeong, (2005)<sup>[8]</sup> and Hussein and Ziedan, (2013)<sup>[6]</sup>.

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