Biological Forum – An International Journal **14(1): 158-163(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

First Report of *Choanephora* Fruit Rot (*Choanephora cucurbitarum*) on cucumber and their Incidence and Occurrence under Protected Cultivation in India

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(Received 11 October 2021, Accepted 08 December, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: *Cucumis sativam* (L.) is creeping vine grown as an off-season vegetable crop under protected poly houses. During survey of major cucumber growing districts of Rajasthan, a new disease "*Choanephora* fruit rot of cucumber" was first time observed and recorded during in 2019-2020. The survey provided baseline information on the distribution, occurrence and incidence of *Choanephora* fruit rot in the major cucumber growing area of Rajasthan state. During the survey, discussions were held with the farmers concerned, regarding occurrence, incidence and yield losses due to this disease. *Choanephora cucurbitarum* is the new emerging pathogen as well as serious threat to cucumber and other vegetables in India. The disease incidence was recorded ranged from 45.61 % to 64.22 %. During the rainy season (July-November), the temperature was 28-30°C with high humidity (70-90%) that favored disease development. During survey typical symptoms of *Choanephora* fruit rot were observed under polyhouse condition as water-soaked lesions on immature & young fruits, leaf and flowers that progressed to rapidly soft rotting of the infected tissues, the fruit surface was covered by a dense whitish cottony mycelial growth of fungal hyphae beared black to brown fructifications and on the surface of diseased fruit, a cushiony mat resembling developing sporangiola emerged. Cotton-like white mycelium and black spore masses were formed by the fungus isolated from diseased tissues on PDA. Pathogenicity tests were performed by spore suspension method and single mycelial disc inoculation technique. Re-isolation of pathogenic fungus from diseased inoculated fruit completed Koch's postulates. The causative fungus was identified as *Choanephora cucurbitarum* (Berk & Ravenel) Thaxt. based on cultural and morphological characteristics. According to the available literature, the current studies confirm the first occurrence of cucumber fruit rot caused by *Choanephora cucurbitarum* in India.

Keywords. Cucumber, *Choanephora* fruit rot, *Cucumis sativam Choanephora cucurbitarum*, Survey, Disease incidence.

INTRODUCTION

Cucumis sativam (L.) is a creeping vine vegetable crop originated from Southern Asia (Renner *et al.*, 2007) that bears cucumiform fruit used for culinary purpose and belongs to the family *Cucurbitaceae*. "Kheera" is eaten raw with salt and pepper or as salad with onion and tomato. Its fruits also used as pickle in India. Cucumber is grown either in the open field or under protected poly houses but, in the recent years, has become important and gaining popularity in polyhouse cultivation grown as an off-season vegetable crop during summer and rainy season in India.

Although *Zygomycetes* are known to be weak plant pathogens (Agrios, 2005), some *Zygomycetous* fungi have evolved their behaviour in such a way that their extensive adaptability favours new host range as an alternative for their trend line. The genus *Choanephora*, a *Zygomycetes* member is known to attack decaying floral portions of various plants after fertilization and invades the fruits, causing soft rot of summer squash or pumpkin, pepper, and okra (Agrios, 2005). However, plant diseases caused by *Zygomycetous* fungus, on the other hand, have received limited attention on cucumber in India.

Choanephora cucurbitarum is causing fruit rots, flower rot and leaf blights on a variety of plants including squash, pumpkin, pepper, pea and bean. The fungus also causes pod blight known as wet rot, blossom blight and whisker rot (Kacharek *et al.*, 2003). Wet rot of flower and fruit of Egusi melon (*Citrullus lanatus*) caused by *Choanephora cucurbitarum* and symptoms appeared on a vine, flowers, blossoms and fruit as whitish fungal growth resembling numerous fruiting

bodies consisted of white stalks with white to brown heads developed on the infected surface. The affected plant tissue became translucent, water-soaked and rotten. The fungus was usually confined to the end of the fruit, but during wetness, the entire fruit decayed. Fruits also rotted backwards from the blossom end. Affected fruits dropped off prematurely (Kehinde, 2013). *Choanephora* flower and fruit rot, caused by *Choanephora cucurbitarum*, is a common disease of many vegetable crops including beans, cantaloupe, eggplant, okra, peas, pumpkin and squash. Squash fruit that are near or on the ground are more likely to become diseased and found 50-60 per cent of blossoms or fruit with *Choanephora* fruit rot symptoms (Lewis Ivey, 2015).

Various diseases caused by *Choanephora cucurbitarum* have recently been reported, including Leaf rot of cauliflower (Gogoi *et al.*, 2016), leaf blight of papaya, twig blight of green pea, blossom blight of teasle gourd, blossom and leaf blight of aubergine (Das *et al.*, 2017), Inflorescence blight and pod rot of dolichos bean & yard long bean (Kurian *et al.*, 2018), Twig blight of Lalshak (Das *et al.*, 2018) and Twig Blight of Chilli (Chandrakala and Vidyasagar, 2018) in India. *Choanephora* fruit rot of summer squash, cucumber and pumpkin caused by *Choanephora cucurbitarum* appeared as wilting of blossoms suddenly collapse and decay. A profuse, fuzzy fungal growth with large masses of black spores formed on infected blossoms, fruit and soft, watery rot at the blossom end (Emily Pfeufer, 2021).

Survey and incidence of *Choanephora* fruit rot disease. During mid-September – November, a disease survey was conducted in major cucumber growing districts of Rajasthan, and a new disease "*Choanephora* fruit rot of cucumber" was observed and recorded during in 2019-2020. The first occurrence was noted from Jaipur, Tonk, Dausa, Alwar, Ajmer and Jhunjhunu districts of Rajasthan, India. The goal of this research was to isolate and identify the causal agent of fruit rot disease in cucumber-growing areas in Rajasthan, India. Our current study discovered the new an emerging pathogen *Choanephora cucurbitarum*, which causes severe fruit rot symptoms.

Disease incidence of *Choanephora* fruit rot of cucumber was recorded as per formula given by Al-Sadi *et al.*, (2011) as given below.

Percent disease incidence =

$$\frac{\text{No. of fruits infected}}{\text{Total no. of fruits examined}} \times 100$$

SYMPTOMS

Typical symptoms of fruit rot appeared as water-soaked and dark green lesions on young fruits and flowers that progressed to a soft rot. After infection the diseased young fruits were rotten rapidly under favourable environmental conditions (Fig. 1 A-D). Usually whitish mycelial growth and monosporous sporangiola were produced on the lesions. The suspect pathogen had black to brown fructifications in mature lesions. On the surface of diseased fruit, black pinheads resembling developing sporangiola created a cushiony mat. The pathogen was most susceptible to infection in immature tender fruits. The fruit surface was frequently coated with fungal hyphae and profuse sporangia and sporangiospores in the fields. Soft rot caused by *Rhizopus* spp. or *Mucor* spp. had similar symptoms. A total of 24 fungal isolates were recovered from infected cucumber fruit, with isolate CcAl-1 being the most virulent; although, no much more mycological differences were observed between the isolates. The pathogen preferred monsoon or rainy season with high moisture (70-90%) and high temperature (30-35°C) favours the outbreak of the disease (Kwon and Hyeong, 2005).

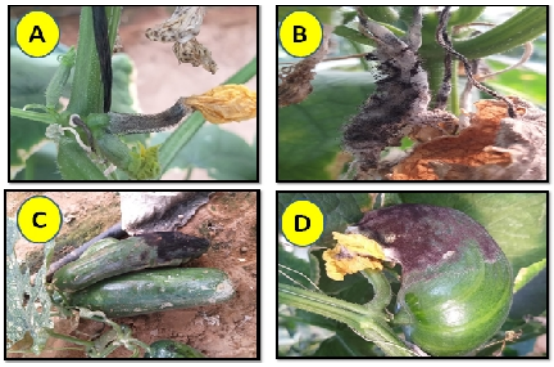

Fig. 1. Syndromes of soft rot under natural field infestation showing whitish mycelium growth with sporangiola (A&B) A symptom on immature fruit with abundant sporulation. (C&D) A typical symptom of the soft rot of cucumber on young fruits.

MATERIALS AND METHODS

A. Isolation, Purification and Identification of the causative pathogen

Pathogen was isolated on Potato dextrose agar medium under the optimum temperature range of 28±2ºC. Pathogen isolation was done by three ways: (i) from the infected part, (ii) by picking single sporangiola and (iii) by single spore dilution method. In 2019-20, cucumber fruits with soft rot symptoms were sampled from polyhouses in the surveyed location. To isolate the causative pathogen, the diseased tissues of fruits were cut into small pieces. The small pieces, approximately 5 × 5 mm in size, were sterilized with a 1 per cent NaOCI solution for 1 minute before being placed on Potato Dextrose Agar (PDA) and left at 28±2ºC for 24 hours. For further examination, the fungal tip coming out of the tissues was transferred to potato dextrose agar (PDA), and the culture was purified using the single spore technique. The morphology and colony features of the associated fungus were used to identify it. A total of 24 fungal isolates were obtained from infected cucumber fruit during survey.

Cultural morphology of the pathogen was studied on PDA from 1- 3 days after inoculation at 28±2ºC. A fungus that formed white aerial mycelia that eventually turned pale yellow and tentatively designated as a *Choanephora* sp. was consistently isolated from infected plant tissues. Colony margins were then transferred onto (PDA) as part of the culture purification process by single spore culture technique.

B. Cultural and morphometric characteristics of the causative pathogen

During the survey, 24 isolates of the fungus were obtained from diseased cucumber fruit. The most virulent isolate was found CcAl-1; however there were no much more mycological differences found between the isolates. Fungal culture was maintained in PDA (Fig. 2A-C). On PDA, the fungal colonies varied in colour from white to pale yellowish. The fungus growth temperature ranged from 15 to 35°C, with the optimum temperature being at 28±2°C on PDA. The growth rate of the isolate CcAl-1 was very first and encroached whole Petri dish (9 cm diam.) within 30 hours and remaining isolates covered within 31-42 hours. Abundant monosporous sporangiola were rapidly formed on the medium. There was not much more cultural differentiation found among all the isolates collected from surveyed area. Mycelia were hyaline and non-septate in nature. Due to β-carotene synthesis, a creamish white culture develops with yellow pigmentation.

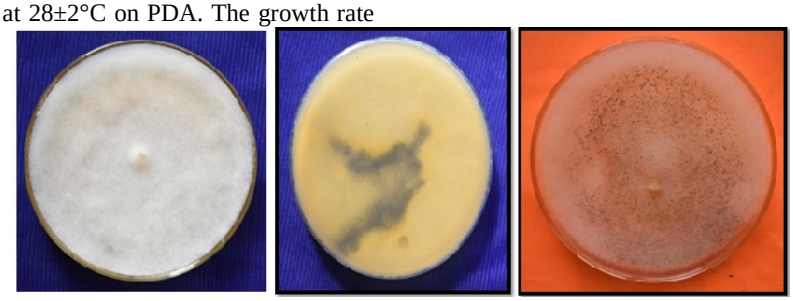

Fig. 2. A cultural pattern of *Choanephora cucubitarum* on PDA. A. Mycelial growth (upper surface), B. Mycelial growth (Lower surface), D. Sporulation.

C. Pathogenicity test

Pathogenicity test of the isolate CcAl-1 was confirmed by cross-inoculation of 23 different isolates by single mycelial disc inoculation technique. Among 24 different isolates, isolate CcAl-1 showed virulence. The same experiment was also conducted by spraying of spore suspension method (1×10^6 spores/ml). Among different isolates, isolate CcAl-1 showed virulence, which consecutively triggered symptom development. To determine the fungal pathogenicity to fruits of cucumber, an incision was made with sterilized knife on healthy young cucumber fruits with length of 2 cm, width 1 cm and 1 cm depths. Wounded fruits were inoculated by white fungal mycelial bits taken from Potato Dextrose Agar medium inserted into the incision and surface of the incision was covered by moist cotton. As a control, a sterile plug (PDA) free from fungal growth was used. After inoculation, fruits were covered with plastic bags (90-95 per cent RH) kept in growth chambers with temperatures set at 28±2ºC for five days (Fig. 3A-D). In another way, spore suspension method used for pathogenicity to fruits of cucumber, plants were grown in sterilized soil in earthen pots for 60 days. Conidial suspension of an isolate (CcAl-1) was prepared from 7 day old culture. Concentration of spore suspension was adjusted to 1×10^6 /ml by using a Haemocytometer and sprayed artificially wounded cucumber fruits (50ml/fruit). The inoculated plants were kept in a humid chamber with 80-90 per cent relative humidity at 28±2 ºC for 24 hours and returned to the greenhouse until end of experiment. Wounded fruits were completely rotten with in five to seven days after inoculation, with the fruits typical greenish color turning brown, and the infected tissues fully covered with mycelia. From infected tissues, a fungus that produced cottony, white mycelium and black to brown spore. In both the cases results were found to be the same for pathogenicity test.

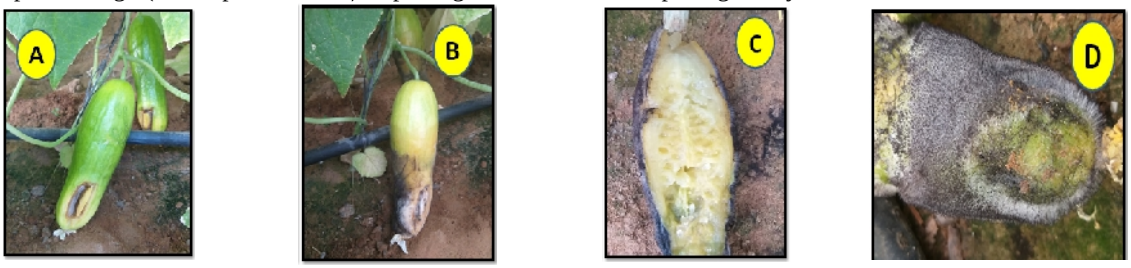

Fig. 3. (A&B) Symptoms induced by artificial inoculation. (C) Inner soft rot of the infected fruit (D) Abundant sporulation.

The percentage of diseased fruit was recorded and the progression of disease severity was observed and noted (Fig. 4) as according to the following scale given by Ziedan, (2012).

0 = no lesion observed, healthy ; 1 = 25% softening of the fruit area; 2 = 50% softening of the fruit area; 3 = 75% softening of the fruit area; 4 = 100% softening of the fruit area.

The pathogen was re-isolated from the infected fruits when symptoms appeared and the characteristics were compared with those of the original isolate thus confirmed the pathogenicity of the fungus. Five days after inoculation, typical fruit rot disease symptoms were observed, which were identical to those observed in natural conditions.

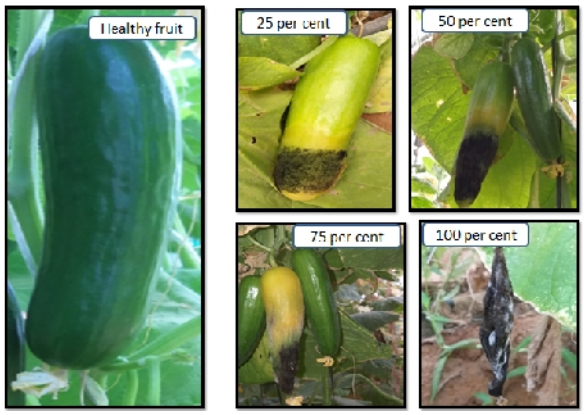

Fig. 4. Different degree of fruit soft rot severity of *Choanephora cucubitarum* on cucumber fruits from left and right (healthy), 25% soft rot of fruit, 50% soft rot of fruit, 75% soft rot of fruit, 100% soft rot of whole fruit.

RESULTS

A. Fruit rot disease syndrome on cucumber

The surveyed results proved the first time at first hand, baseline information on the distribution, prevalence of *Choanephora* fruit rot disease, incidence and severity in the major cucumber growing area of Rajasthan state. In mid-September – November 2019 through 2020, fruit rot of cucumber caused by *Choanephora cucurbitarum* was observed and recorded for the first time in major cucumber growing area of Rajasthan in India with a 45.61 % – 64.22 % disease incidence level under protected cultivation.

Symptoms primarily appeared on immature and young fruits, flowers leaf, twinges with water soaked lesions and soften of tissues on affected parts. Infected chlorophyllous tissue turned brownish, and whitish mycelial growth and monosporous sporangiola were produced on the lesions. Black pin head like emerging sporangiola formed a cushiony mat appearance on surface of infected part (Fig. 1A-D). Immature fruit may become infected and covered with a dense white mycelium. On fruits, varied degrees of disease symptoms were observed during survey. Typical symptoms of the soft fruit rot appeared at 5-7 days after inoculation on cucumber fruit. Symptoms developed on the inoculated fruit were similar to those observed in the fields (Fig. 1A-D). The fungus was re-isolated from the diseased cucumber fruits. However, the disease has never been reported in India previously.

B. Cultural and morphometric characterization of the causal pathogen

The causal fungus of cucumber fruit soft rot was identified as *Choanephora cucurbitarum* (Berk. & Ravenel) Thaxter based on cultural as well as morphological characteristics and description in the monograph by Kirk (1984) and confirmed by ITCC (Indian Type Culture Collection, ITCC No.-11,258.19 dated.16/12/2019) at ICAR-Indian Agricultural Research Institute, New Delhi, India. All isolates were able to causing soft fruit rot of wounded cucumber fruits compare the control (wounded fruits free from fungal infestation).

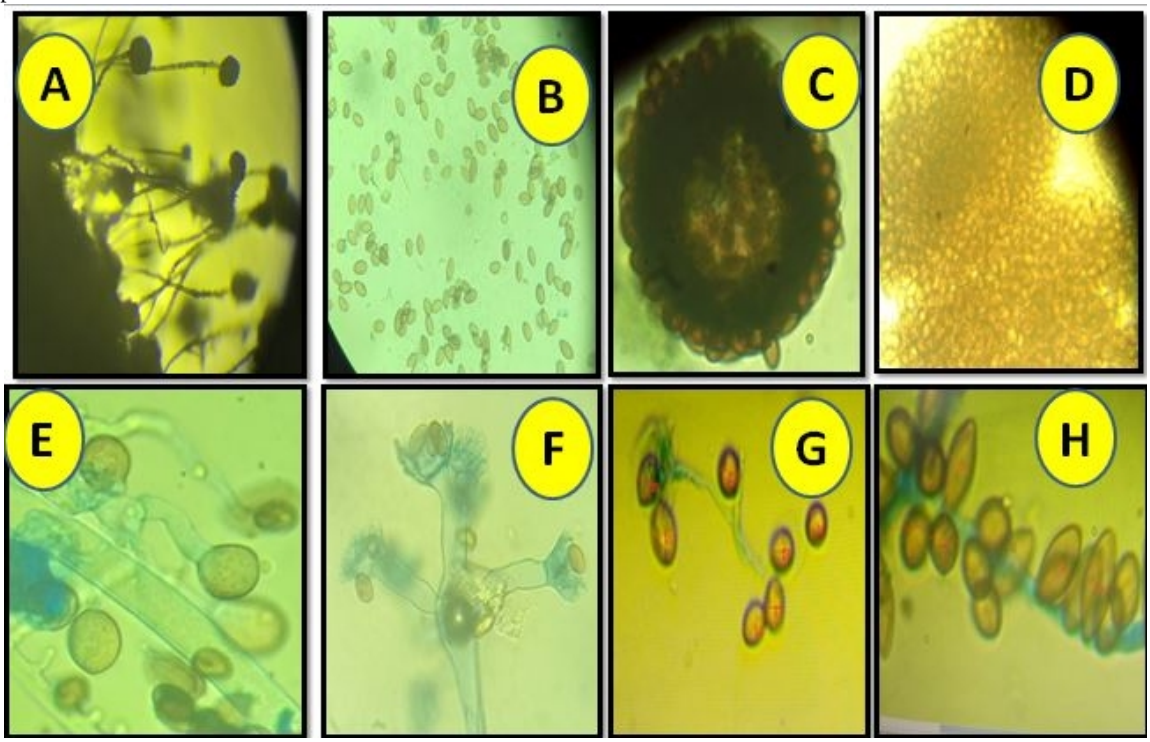

Fig. 5. (A-H) Morphological characters of *C. cucurbitarum* (A) emerging sporangiola on host surface, (B) Monosporus sporangiospores(20X), (C) sporangiola (at 20X), (D) Drooping sporangia(40X), (E) Germinating spore, (F) Secondary vesicle, (G&H) Micro-metric measurement of spores.

The morphological and cultural studies of the present isolate CcAl-1 were recorded and compared with published descriptions of soft rot-fungi (*Choanephora cucurbitarum)* described previously (Kwon and Hyeong 2005). Morphological study revealed that sporangia were sub-globose in shape, white to yellow when young, pale brown to dark brown when mature, and were 40.5 to 115.8 µm in size. Monosporus and multisporus sporangiola are the two different types of sporangiola observed under observation. Monosporous sporangiola were long, slender, elliptic, fusiform, or oval in shape, with a size range of 9.30-23.95 × 7.80 - 19.75 µm in size. At both ends, sporangispores have three or more slender appendages. Sporangiospores were elliptic, fusiform, or ovoid in shape, light brown or dark brown in colour, and ranged from 7.87-11.23 to 9.30 – 24.22 µm in size (Fig. 5 A-H).

DISCUSSION

The study established the widespread distribution and occurrence of *Choanephora* fruit rot infection on cucumber at various locations in the surveyed area of Rajasthan state and causes huge economical fruit yield losses to cucumber. The results obtained in this study demonstrated that there was a substantial occurrence of *Choanephora* fruit rot during *Kharif* 2019-2020 cropping season. The mucoraceous fungi, *Choanephora cucurbitarum* is the newly emerging pathogen as well as serious threat to the cucumber under protected cultivation. Previously *Choanephora* was considered as a weak pathogen but day by day it improved infectious behavior, wide adaptability as well as increased host range (including different plant families) spreads vigorously, turned out to be a major pathogen under changing climatic situation in India. These finding supports the fact that the *Choanephora* fruit rot disease is extremely devastating when it is appeared in rainy season. Similar finding on yield losses and incidence in different vegetable crops, fruit crops and legume crops impacted by *Choanephora cucurbitarum* infection was reported by various research workers (Gogoi *et al.*, 2016; Das *et al.*, 2017; Kurian *et al.*, 2018; Das *et al.*, 2018; Chandrakala and Vidyasagar, 2018) in India. Fruit rot of Butternut squash (*Cucurbita moschata* Duch.) caused by *Choanephora cucurbitarum* appeared as soft rot symptoms on flowers and young fruits (Emmanuel *et al.*, 2021).

This is the first report and incidence of *Choanephora* fruit rot disease of cucumber caused by *Choanephora cucurbitarum* in India. According to our knowledge, finding and based on the available literature, this is a new pathogen of cucumber fruit and the first time the pathogenicity of this fungus to cucumber fruit has been demonstrated in India, as far as the authors are aware.

FUTURE SCOPE

The present research investigation has opened up new information and given rise to new ideas on *Choanephora* fruit rot of cucumber. Hence the future lines of work are needed with there is a need to undertake an intensive survey for *Choanephora* fruit rot disease of cucumber in all agro-climatic zones of Rajasthan and quantify the loss caused by this pathogen. To identify the molecular variability in *Choanephora* fruit rot pathogen that prevalent in different agro-climatic zone of Rajasthan.

Acknowledgments. The authors are grateful to Head, Department of Plant Pathology and Dean, SKN College of Agriculture, Sri Karan Narendra Agriculture University, Jobner, Jaipur, Rajasthan-303329, India for valuable guidance, support and facilities provided to conduct the present investigation related to this research experiment successfully.

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How to cite this article: Sunil Kumar, R.R. Ahir, Mahabeer Singh, G.S. Rathore, Shivam Maurya and Poonam Kumari (2022). First Report of *Choanephora* Fruit Rot (*Choanephora cucurbitarum*) on cucumber and their Incidence and Occurrence under Protected Cultivation in India. *Biological Forum – An International Journal*, *14*(1): 158-163.