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Characterization of Cucurbit Powdery Mildews by Morphological and Microscopic Studies

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A B S T R A C T

Powdery mildew of cucurbits is caused by two causal agents, *Sphaerotheca fuliginea (Syn. Podosphaera xanthii) and Erysiphe cichoracearum (Syn. Golovinomyces cichoracearum).* To assess the distribution of powdery mildew in major cucurbit crops and also to identify peculiar characteristics of these causal agents, survey was conducted in various places of Coimbatore and Dindigal districts of Tamil Nadu. The collected powdery mildew samples were examined for the presence of conidia and conidiophores. Conidial shapes, conidiophore and conidial dimensions, presence of fibrosin bodies were established by microscopic studies. Based on the conidia and conidiophore characters, two different genera were observed in the samples collected. The conidial mean length of *Podosphaera xanthii* ranges from 30.51 to 40.88 µm whereas for *Erysiphe cichoracearum* ranges from 32.08 to 46.87 µm but the conidial mean width of *Podosphaera xanthii* ranges from 21.28 to 29.65 µm and for *Erysiphe cichoracearum* it ranges from 16.52 to 29.65 µm. For better perception of host pathogen interaction, pathogenicity assay was proved by detached leaf assay in laboratory and glasshouse *in vitro*. This prelusive study would be beneficial for early disease discernment and effective management in coming times.

Introduction

Cucurbits are economically important crops that play a major role in vegetable production. Among the different production constraints, the disease namely powdery mildew is the most common and serious fungal disease that arises both in field and greenhouse conditions all over the world. The disease is caused by two obligate biotrophs such as *Sphaerotheca fuliginea (Syn. Podosphaera xanthii)* and *Erysiphe cichoracearum (Syn. Golovinomyces cichoracearum)* (Vakalounakis *et al.,* 1994; Kristkova *et al.,* 2000). The disease causes reduction in plant growth, premature defoliation and as a result, both yield and quality of fruits gets reduced that ultimately leads to major production problem (Lebeda *et al.,* 2010).

Podosphaera xanthii occurs mostly in warmer months (Rur *et al.,* 2017) whereas *Erysiphe cichoracearum* in cooler spring and in early summer months (Aguiar *et al.,* 2012).

The two obligate biotrophs produce indistinguishable symptoms but can be easily distinguished by light microscopic studies (Braun *et al.*, 2002). The difference between the two species *Podosphaera xanthii* and *Erysiphe cichoracearum* can be made according to morphology of the conidia, the presence or absence of fibrosin bodies in interior of the conidia and the morphology of the germinative tube of conidia (Garcia *et al.*, 2010).

Therefore, the objective of the study is to identify and characterize the powdery mildew isolates from various hosts of cucurbit crops which will provide explicit information about the causal agents. This study also evaluates disease severity caused by both pathogens.

Materials and Methods

Survey with GPS coordinates

During 2019-20, powdery mildew infected leaf samples from various hosts of cucurbits were collected by conducting survey in Coimbatore and Dindigal districts of Tamil Nadu, India. The samples were collected from various locations such as Poosaripalayam, Sattakalpudhur, PN Pudhur, Thondamuthur, Kinathukadavu, Vettaikaranpudhur, Arasampalayam of Coimbatore district and Sembatti, Paraipatti, Vellodu of Dindugal district where cucurbits are largely cultivated. During the survey, observations on disease intensity, stage and variety of the crop and GPS coordinates of the locations were recorded.

Powdery mildew and its severity

The disease intensity (percentage of leaf area infected) was assessed using disease score chart which includes 0-9 scales given by Jamadar and Desai (1997). The disease score was converted to Percent disease Index (PDI) which was described by Mc Kinney's (1923). Percent Disease Index was calculated by randomly selecting fifteen leaves and worked out using the following formula

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

Microscopic studies

The morphological characters of the causal agents such as presence or absence of conspicuous fibrosin bodies, the dimensions of conidia and conidiophore, shape of conidia, type of mycelium and appresoria were characterized by means of taking observation under light microscope.

The image was captured using image analyser. The conidial numbers (25 conidia per sample) were counted and out of these, the length and width of ten conidia was measured. To study better structural aspects, the conidia and conidiophores has been stained with cotton blue in lactophenol.

Pathogenicity study

To prove the pathogenicity of causal agents, Koch's postulates have been established. Pathogenicity assay was proved for powdery mildew of cucurbits caused by two causal agents *Podosphaera xanthii* and *Erysiphe cichoracearum* in glasshouse and laboratory conditions.

In glasshouse, cucumber (Japanese Long green) and bitter gourd (CO 1) have been raised under controlled conditions in earthen pots containing the mixture of red soil, sand and farm yard manure at the ratio of 2:1:1. Diseased samples showing typical symptoms of powdery mildew caused by *Podosphaera xanthii* in cucumber and *Erysiphe cichoracearum* in bitter gourd were collected.

The conidia of *Podosphaera xanthii* was inoculated in 30 days old cucumber plant and the conidia of *Erysiphe cichoracearum* in 30 days old bitter gourd plant by brushing the conidia onto the leaf by small camel's brush and control plant was maintained for each without inoculation of conidia.

The plants were covered with polythene covers to maintain humidity for 24 to 48 hours. In laboratory conditions, healthy detached leaves of cucumber and bitter gourd were collected and surface sterilized with 70% ethanol and then washed three times thoroughly with sterile distilled water. The leaves were placed in petri dishes with three layers of moistened sterile filter paper.

The leaves of cucumber were inoculated with sporulating colonies of *Podosphaera xanthii* onto the sites of leaf by slight brushing of conidia. Likewise, the leaves of bitter gourd inoculated with *Erysiphe cichoracearum*. The petriplates were wrapped with parafilm and incubated at 25^0C with 16 hours of photoperiod. An observation on the growth of powdery patches on leaves is recorded at regular intervals.

Statistical analysis

The microscopic study has been repeated for three times to capture the images and conidia length, breadth were measured. The glasshouse study for the pathogenicity has been carried out by using completely randomized design (CRD). Statistical analysis were done using IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984) and the percentage values of the disease index were arcsine transformed. Data were subjected to ANOVA at significant level ($P < 0.05$) and means were compared by Duncan's multiple range test (DMRT).

Results and Discussion

Survey and disease severity assessment

The survey was conducted in various locations of Coimbatore and Dindugal district during the year 2019 – 2020. The cucurbits powdery mildew showing symptoms typically on both upper and lower surface of leaves and later stage on petioles stems and fruits were observed (Fig.1).

The results showed that the severity for the powdery mildew of cucurbits ranged from 30.25 to 71.24 PDI. Maximum disease severity was noticed in ash gourd (71.24 PDI) collected from PN Pudur followed by snake gourd (69.12 PDI) collected from Arasampalayam of Coimbatore district.

The minimum disease severity was noticed in watermelon (30.25 PDI) collected from Korapalayam of Dindugal district (Table 1). Pathogenecity spectrum of disease is similar to other states in India. In a study conducted on cucurbits indicated that infection in plants in one or the other locality comprising of these districts were distinct.

Among the cucurbits, ash gourd was most severely affected. The species responsible for the disease in the state were identified as *Podoshaera xanthii* and *Golovinomyces cichoracearum* (Khan and Khan., 1996; Gupta *et al.,* 2012;). The incidence and disease severity of powdery mildews in cucumber, bhendi and pea crops varied in different locations across the state which is similar to this present investigation (Parthasarathy *et al.,* 2017; Rajalakshmi *et al.,* 2016; Parameshwar Naik *et al.,* 2018).

Morphological and microscopic studies

During the survey, twenty powdery mildew infected samples from major cucurbit crops

have been collected. The morphological examinations of the collected samples from various hosts of cucurbits were done using light microscope. With the help of image analyser, the images have been captured and the results revealed that both the causal organism *Erysiphe cichoracearum* and *Podosphaera xanthii* was responsible for cucurbit powdery mildew. The two causal organisms have been distinguished by the presence of fibrosin bodies. *Podosphaera xanthii* which causes powdery mildew in cucumber contains ovoid conidia with long refractile fibrosin bodies, with discoid fibrosin bodies in snake gourd and found to be absent in *Erysiphe cichoracearum*.

The conidial mean length of *Podosphaera xanthii* ranges from 30.51 to 40.88 µm and for *E. cichoracearum* it ranges from 32.08 to 46.87 µm whereas the conidial mean width of *Podosphaera xanthii* ranges from 21.28 to 29.65 µm and for *E. cichoracearum* it ranges from 16.52 to 29.65 µm. Mycelium is hyaline with septations and condiophore with catenate type of conidia was observed (Table 2).

Similar observation is reported in *P. xanthii* which produced tubular germ tube with indistinct appresoria whereas *Erysiphe cichoracearum* produced simple apical germ tube with lobed appresoria (Fig.2). Fibriform substances was also present in the conidiophores of *P. xanthii* whereas it is absent in *E. cichoracearum*. The two obligate biotrophic polyphagous fungi cannot be distinguished visually but can be differentiated morphologically by microscopic studies (Vakalounakis *et al.*, 1994). The difference between conidial length and width ratio of the two causal agents was described by various researchers (Frolov., 1996; Rankovic., 2003). The dimensions of conidia of chilli were measured in chilli powdery mildew which is similar to present study (Bademiyya *et al.*, 2019)

Pathogenecity study

In pathogenecity assay, *Podosphaera xanthii* took initial infection after 4 days of inoculation whereas *Erysiphe cichoracearum* after 5 days of inoculation under glasshouse conditions favoured by high relative humidity and dry conditions for the development of infection. The disease looks severe after 10 days of inoculation (Fig 3 and Fig.4). The maximum of 25 and minimum of 10 conidia were observed by taking sections of artificially inoculated leaves and conidia of both causal agents were noticed similar to the conidia inoculated respectively.

Under laboratory coniditions, half of the leaf area have been covered with mildew colonies after four to seven days of inoculation and the condial characters were observed same as above for both causal agents in detached leaf assay. In accordance with Koch's postulates, healthy cucurbit plants showed typical disease symptoms whereas control plant showed no disease symptoms (Cohen *et al.*, 1996).

Rajalakshmi *et al.*, (2016) described about the pathogenecity test of bhendi and pea powdery mildew under glasshouse conditions and Parthasarathy *et al.*, (2018) described about the detached leaf assay of pea powdery mildew under glasshouse conditions. Similarly, Lovepreet Kaur (2019) also proved the pathogenicity test in case of mango powdery mildew.

A. Conidiophore of *Podosphaera xanthii* with fibriform substances B. Conidia of *Podosphaera xanthii* C. Conidia of *P.xanthii* with long, thin fibrosin bodies D. Conidia of *P. xanthii* with discoid fibrosin bodies E. Conidiophore of *E.cichoracearum* with catenate conidia. F. Erect conidiophore and conidia of Erysiphe cichoracearum stained with lactophenol G. Conidia of *E. cichoracearum* without fibrosin bodies H.

Table.1 Survey and assessment of powdery mildew in major cucurbit crops

S.No	Host Plant	Cultivar	Stage of the crop	Season	Location		GPS Coordinates		Mean PDI of powdery mildew*
					District	Village	Latitide	Longitude	
1.	Snake Gourd	CO 2	Flowering	Kharif	Coimbatore	Poosaripalyam	11.062 ºN	76.933 ºE	59.12 ^{e} (50.25)
2.	Snake Gourd	CSgH 1	Fruit Formation	Kharif	Dindugal	Sembatti	10.282 ºN	77.872 ºE	35.63 ^{o} (36.65)
3.	Snake Gourd	Kaumudi	Fruit Formation	Kharif	Dindugal	Paraipatti	10.321 ºN	77.856 ºE	38.16 ^{m} (38.15)
4.	Bitter Gourd	Palee	Fruit Formation	Kharif	Dindugal	Vellodu	10.306 ºN	77.954 ºE	32.28 ^{p} (34.62)
5.	Bitter Gourd	Arya	Flowering	Rabi	Coimbatore	Sattakal Pudhur	10.819 ºN	76.926 ºE	31.64 ^{pq} (34.23)
6.	Water melon	Local	Maturity	Kharif	Dindugal	Korapalayam	10.948 ºN	77.325 ºE	30.25 ^{q} (33.37)
7.	Ridge gourd	PKM 1	Fruit Formation	Kharif	Dindugal	Paraipatti	10.321 ºN	77.856 ºE	38.04 ^{mn} (38.08)
8.	Ash Gourd	CO 2	Fruit Formation	Rabi	Coimbatore	PN Pudhur	11.006 ºN	76.930 ºE	71.24^{a} (57.56)
9.	Cucumber	Japanese Long green	Maturity	Rabi	Coimbatore	PN Pudhur	11.006 ºN	76.930 ºE	55.32 ^{gh} (48.05)
10.	Pumpkin	Arjuna	Maturity	Kharif	Coimbatore	Thondamuthur	11.005 ºN	76.823 ºE	54.13 ^{hi} (47.37)
11.	Snake Gourd	CO 2	Maturity	Rabi	Coimbatore	Kinathukadavu	10.817 ºN	77.018 ºE	58.01 ^{ef} (49.60)
12.	Pumpkin	CO 2	Maturity	Rabi	Coimbatore	Kinathukadavu	10.817 ºN	77.018 ºE	51.63 ^{j} (45.93)
13.	Bottle Gourd	PKM 1	Fruit Formation	Rabi	Coimbatore	Poosaripalayam	11.062 ºN	76.933 ºE	58.04 ^{ef} (49.62)
14.	Snake Gourd	Local	Fruit Formation	Rabi	Coimbatore	Vettaikaranpudhur	10.603 ºN	76.902 ºE	49.64 ^{k} (44.79)
15.	Pumpkin	Local	Maturity	Rabi	Coimbatore	Vettaikaranpudhur	10.603 ºN	76.902 ºE	56.51 ^{fg} (48.74)
16.	Snake gourd	Local	Maturity	Rabi	Coimbatore	Arasampalayam	10.657 ºN	77.010 ºE	69.12^{b} (56.24)
17.	Ridge Gourd	Local	Vegetative	Rabi	Coimbatore	Arasampalayam	11.564 ºN	79.902 ºE	59.57 ^{d} (50.52)
18.	Pumpkin	Local	Fruit formation	Rabi	Coimbatore	Arasampalayam	10.654 ºN	77.010 ºE	46.31 ^{l} (42.88)
19.	Bitter Gourd	CO 1	Vegetative Stage	Rabi	Coimbatore	PN Pudhur	11.006 ºN	76.930 ºE	61.21 ^{c} (51.48)

PDI – Percent Disease Index, *Values are mean of three replicates

Values in parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table.2 Microscopic studies of causal agents of various hosts of cucurbits powdery mildew

Host	Host organism	Conidia*		Conidial shape	Mycelium	Types of Appresoria
		Length(µm)	Width(µm)			
Snake gourd	*Podosphaera xanthii*	30.514	21.283	Ovoid with discoid fibrosin bodies	Hyaline, Septate	Indistinct
Bitter gourd	*Erysiphe cichoracearum*	44.053	21.012	Cylindrical to ovoid	Hyaline, Septate	Lobed
Ridge gourd	*Erysiphe cichoracearum*	44.418	20.596	Cylindrical to ovoid	Hyaline, Septate	Lobed
Ash gourd	*Erysiphe cichoracearum*	46.871	16.525	Cylindrical	Hyaline, Septate	Lobed
Bottle gourd	*Erysiphe cichoracearum*	36.277	26.007	Cylindrical to ovoid	Hyaline, Septate	Lobed
Pumpkin	*Erysiphe cichoracearum*	32.080	23.686	Cylindrical to ovoid	Hyaline, Septate	Lobed
Cucumber	*Podosphaera xanthii*	40.887	29.656	Ovoid with long refractile fibrosin bodies	Hyaline, Septate	Forked
Water melon	*Erysiphe cichoracearum*	30.818	28.218	Ellipsoid to ovoid	Hyaline, Septate	Lobed

*Values are mean of ten conidia

Figure.1 Symptoms of powdery mildew on cucurbits observed under field conditions
A. Abundant powdery patches at severe stage of infection
B. Powdery growth on rear side of the leaf
C. Powdery growth on petiole
D. Powdery growth on fruit (ash gourd)

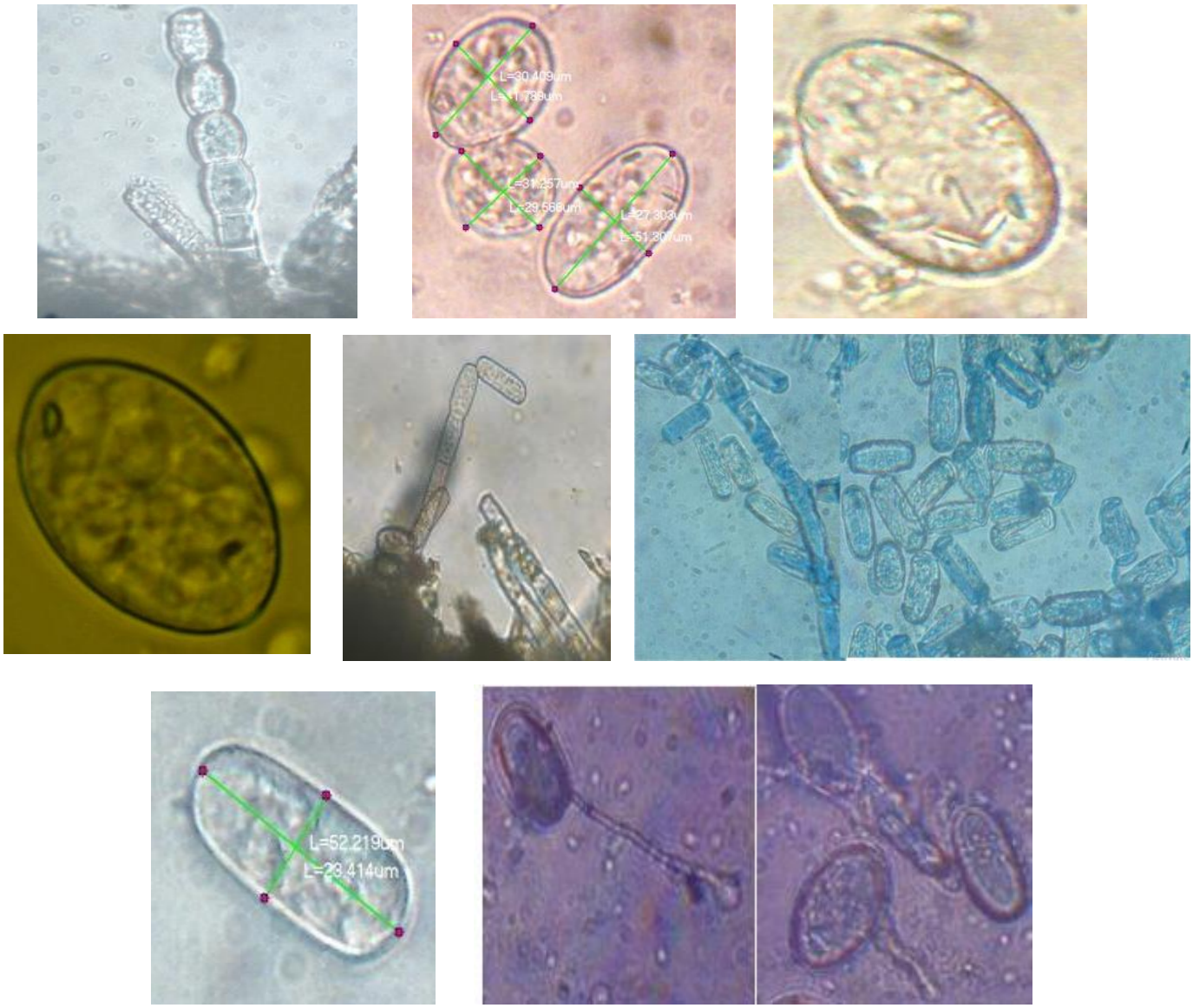

Fig.2 Microscopic studies of powdery mildew causal agents

Fig.3 Pathogenecity assay for *Podosphaera xanthii*

Fig.4 Pathogenecity assay for *Erysiphe cichoracearum*

Simple apical germ tube with lobed appresoria of *E. cichoracearum* I. Tubular germ tube of *P.xanthii*. A. Uninoculated control plant B. Inoculated plant covered with polythene covering C. Initial symptoms seen on 4th day of inoculation D. The powdery mildew symptoms on 10th day of inoculation

A. Uninoculated control plant B. Inoculated with powdery mildew conidia covered with polythene cover C. Initial symptom development after 5th day of inoculation D. The symptom at 10th day of inoculation.

This preliminary study of the morphological and microscopic characteristics of the cucurbit powdery mildew causal agents paves the way for successful disease management. Further, it has to subjected to scanning electron microscopic studies for better understanding.

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