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Morphological characterization of *Ampelomyces* spp., a hyperparasite of Bhendi (*Abelmoschus esculentus* (L.) Moench) powdery mildew

K. Athira^{1*}, N. Ragupathi² and T. Raguchander³

¹Vanavarayar Institute of Agriculture, Manakkadavu, Pollachi- 642103 (Tamil Nadu), INDIA

²Directorate of Students Welfare (DSW), Tamil Nadu Agricultural University, Coimbatore-641003(Tamil Nadu), INDIA

³Department of Plant Pathology, AC and RI, Valavachanur, Thiruvannamalai- 606753(Tamil Nadu), INDIA

*Corresponding author. E-mail: athirakk@gmail.com

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Abstract: *Ampelomyces* is a naturally occurring hyperparasite on powdery mildews. Survey was conducted in major bhendi (*Abelmoschus esculentus* (L.) Moench) growing districts of Tamil Nadu during June 2014 to assess the incidence of powdery mildew and to collect different isolates of *Ampelomyces* spp. The results of the survey revealed that the disease incidence ranged from 15.54 to 63.45 %. Ten isolates of *Ampelomyces* spp. were collected from surveyed areas of powdery mildew. Isolation of *Ampelomyces* spp. was done from powdery mildew infected bhendi leaf parasitized by *Ampelomyces* spp. using tissue segment method. All the isolates were identified by their morphological characters. The colour of the colonies in various medium was brownish black to greenish white. Most of the isolates showed radial and fluffy growth pattern with raised growth. The pycnidia of different isolates of *Ampelomyces* varied in their shape and were mostly ovoid, ellipsoid, cylindrical, pyriform to globose in shape. The size of pycnidia varied from 29.2-72.5×22.4-43.1 µm. The number of pycnidia was found to be more in isolates viz., TNAU-AQ101 and TNAU-AQ103. Pycnidiospores are hyaline, unicellular and guttulate in shape. The pycnidial production was higher in TNAU-AQ101 and TNAU-AQ103. Application of agrochemicals is one of the oldest and most effective methods to manage powdery mildew disease. However, incessant use of these agrochemicals has many demerits such as development of resistance to pathogens, residual toxicity and environmental pollution. Hence, search for an alternative means for disease management is envisaged. The genus *Ampelomyces* are the major antagonists as an alternative of Erysiphales fungi being a significant group of phytopathogens.

Keywords: *Ampelomyces*, Bhendi, Erysiphales, Hyperparasite, Pycnidia, Tissue segment method

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) belonging to the family malvaceae popularly known as Bhendi or Ladies finger is one of the most delicious and important vegetable crops grown throughout the country. It is grown for its tender green pods in tropics, subtropics and warmer parts of temperate region (Maurya *et al.*, 2013).

A number of biotic and abiotic factors are responsible for the low yield of Okra. Among the biotic factors, powdery mildew caused by *Erysiphe cichoracearum* DC is a destructive disease worldwide (Glawe, 2008) which causes an yield losses of 20 to 40 % (Agrios, 2005) or 17 to 86.6 % (Younes and Abo-Elyousr, 2014). The disease affects all stages of growth causing premature defoliation. *Erysiphe cichoracearum*, the powdery mildew pathogen of okra is causing the most widespread disease generally favoured by dry atmospheric and soil conditions (Yarwood, 1957).

Control of powdery mildews relies mainly on the use of chemical fungicides, yet intensive use of these can result in the accumulation of residual chemicals that

are potentially hazardous to humans and the environment. In order to reduce the use of chemical pesticides, alternative methods for controlling powdery mildews have been studied, including the use of microbial bio-control agents (Paulitz and Bélanger, 2001).

Ampelomyces quisqualis Ces. is a hyperparasitic fungi which can parasitize hyphae, conidiophores and chasmothecia of more than 64 different species of powdery mildew fungi, including the genera *Brasilomyces*, *Erysiphe*, *Leveillula*, *Microsphaera*, *Phyllactinia*, *Podosphaera*, *Sphaerotheca* and *Uncinula* (Kiss, 1998). The mycelial development of *A. quisqualis* suppresses both asexual and sexual sporulation of the powdery mildew pathogen (Sundheim and Krekling, 1982).

Due to its wide host range, the mycoparasite is a highly promising candidate for biological control. *A. quisqualis* best typifies the potential of mycoparasites, as it is highly specific to powdery mildews, does not produce any toxic metabolites and can easily be mass-produced *in vitro*.

With this background, the present study was carried out envisaging the following objectives i) to assess the incidence of powdery mildew in major bhendi

(*A. esculentus* (L.) Moench) growing districts of Tamil Nadu and to collect different isolates of *Ampelomyces* spp. ii) morphological characterization of *Ampelomyces* spp.

MATERIALS AND METHODS

Survey for the incidence of powdery mildew of bhendi in Tamil Nadu: Survey was conducted in major bhendi (*A. esculentus* (L.) Moench) growing districts of Tamil Nadu viz., Coimbatore, Madurai, Dharmapuri, Dindigul, Trichy, Erode, Salem, Tirupur, Theni and Cuddalore for assessing the incidence of powdery mildew of bhendi during the month of June, 2014. In each district, three villages were randomly chosen and in each village 3 farmer’s fields were selected and in each farmers field, at three places 20 plants were selected at random and Per cent Disease Index (PDI) was worked out as per the standard grade chart (Ravinder Reddy, 1982).

Disease score chart for powdery mildew

Grade	Description (% leaf area infected)
0	No sign or symptoms
1	0-10 % infection
3	11-15 % infection
5	16-25 % infection
7	26-50 % infection
9	>50 % infection

The Per cent Disease Index was worked out as described by Mc Kinney’s (1923).

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

Survey for collection of isolates of biocontrol agent *Ampelomyces* spp: The bhendi leaves showing typical

powdery mildew symptoms and hyperparasitized by *Ampelomyces* spp. were collected from farmer’s field in different districts of Tamil Nadu. The powdery mildew infected bhendi leaves hyperparasitized by *Ampelomyces* spp. were collected in polythene bags and brought to the laboratory and preserved in a refrigerator at 5°C for further studies.

Isolation of the biocontrol agent *Ampelomyces* spp: Powdery mildew infected bhendi leaves parasitized by *Ampelomyces* spp. appearing as ash to dark brown in colour collected during the surveys were examined under the stereo microscope for the presence of pycnidia of *Ampelomyces* spp. The pycnidia was transferred to the Petri plates containing potato dextrose agar medium as described by Sharma (2006).

Morphological characters of different isolates of *Ampelomyces* spp: The different isolates of *Ampelomyces* spp. were grown in potato dextrose agar medium by placing a disc of the actively growing mycelium in the centre of the petridish and incubated at room temperature. *Ampelomyces* spp. are identified based on morphological characters that is the production of pycnidia and pycnidiospores. The morphological characters of different isolates of *Ampelomyces* spp. viz., spore colour, size, shape, pigmentation, zonation, colony colour, margin of the colony and topography were studied as described by Sharma (2006).

RESULTS AND DISCUSSION

Survey for the incidence of powdery mildew disease of bhendi in Tamil Nadu: The results revealed that in general the per cent disease index (PDI) of powdery

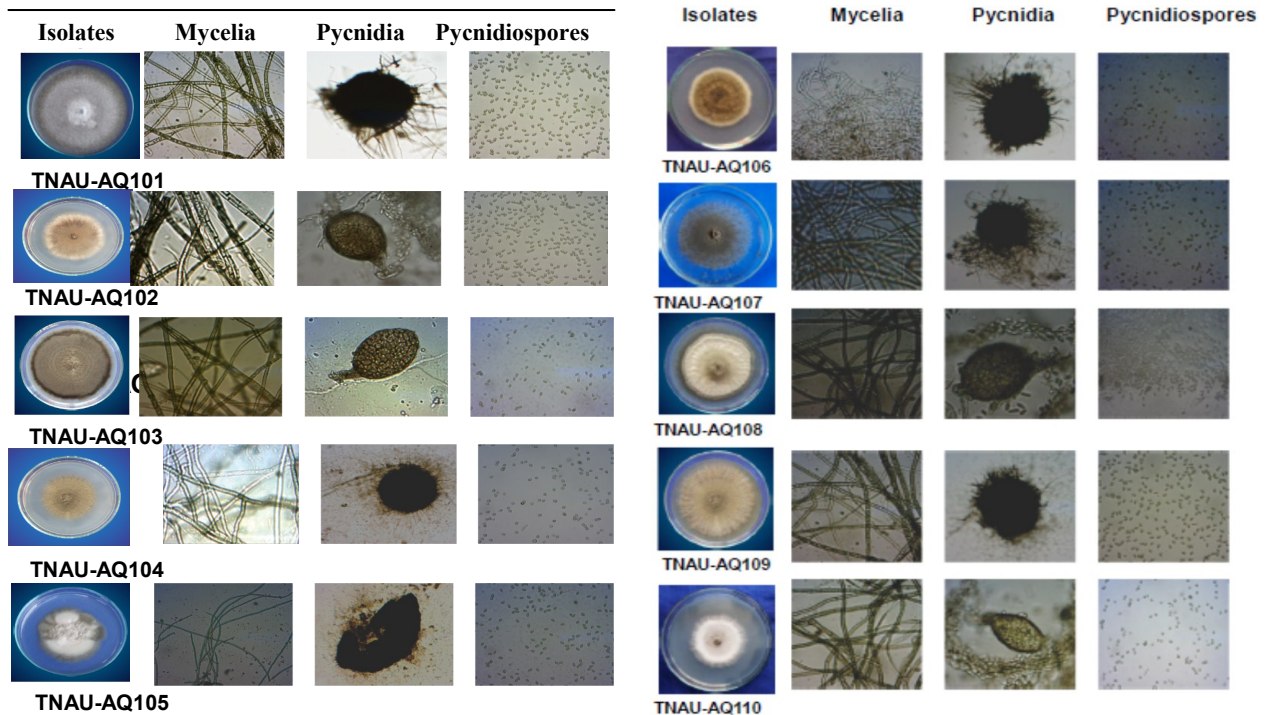


Plate 1. Morphological characters of different isolates of *Ampelomyces* spp. on potato dextrose agar medium.

Table 1. Survey for the incidence of powdery mildew and collection of isolates of *Ampelomyces* spp. in major bhendi growing districts of Tamil Nadu.

S.N.	Place of collection	Village/Taluk	Stage of the crop	Variety/ Hybrid cultivated	Hy-	Mean PDI of powdery mildew	<i>Ampelomyces</i> spp. isolates no.
1	Coimbatore	Thondamputhur	Vegetative	COBH h 1		63.45 ^a	TNAU-AQ101
2	Madurai	Devankuruchi	Pod harvest	Ganga		24.72 ^f	TNAU-AQ102
3	Dharmapuri	Papparapatty	Fruit formation	NS 801		18.53 ^g	TNAU-AQ103
4	Dindigul	Ambilikkai	Flowering	COBH h 1		54.27 ^b	TNAU-AQ104
5	Trichy	Thimampatty	Fruit formation	Sakthi		37.51 ^d	TNAU-AQ105
6	Erode	Muthampalayam	Flowering	RASI 5		43.23 ^{cd}	TNAU-AQ106
7	Salem	Idappadi	Vegetative	NS 531		15.54 ^h	TNAU-AQ107
8	Tirupur	Nallur	Fruit formation	COBH h 1		47.13 ^c	TNAU-AQ108
9	Theni	Kovilpatti	Flowering	OH-016		35.47 ^{de}	TNAU-AQ109
10	Cuddalore	Ramapuram	Fruit formation	Sakthi		30.50 ^e	TNAU-AQ110

*PDI- Per cent Disease Index, * Values are means of three replications, Means followed by a common letter are not significantly different at 5% level by DMRT

Table 2. Morphological characters of different isolates of *Ampelomyces* spp. on potato dextrose agar medium.

S.N.	Isolates	Mycelium	Topography	Colour of mature colonies	Zonation	Margin	Colony growth
1	TNAU-AQ101	Septate, hyaline	Radial	Ash	Absent	Irregular	Rapid
2	TNAU-AQ102	Septate, hyaline	Appressed	Brown	Absent	Smooth	Slow
3	TNAU-AQ103	Septate, hyaline	Flat	Brownish black	Present	Wavy	Rapid
4	TNAU-AQ104	Septate, hyaline	Fluffy	Brown	Absent	Wavy	Rapid
5	TNAU-AQ105	Septate, hyaline	Fluffy	Greenish white	Absent	Irregular	Very slow
6	TNAU-AQ106	Septate, hyaline	Fluffy	Brown	Present	Smooth	Moderate
7	TNAU-AQ107	Septate, hyaline	Floccose	Brown to black	Absent	Smooth	Moderate
8	TNAU-AQ108	Septate, hyaline	Appressed	White	Present	Smooth	Rapid
9	TNAU-AQ109	Septate, hyaline	Radial	Brown	Absent	Smooth	Rapid
10	TNAU-AQ110	Septate, hyaline	Radial	White	Absent	Smooth	Moderate

Table 3. Pycnidial characters of different isolates of *Ampelomyces* spp. on potato dextrose agar medium.

S.N.	Pycnidia	Pycnidial size (µm)	Pycnidiospore	Pycnidiospore size (µm)	No. of Pycnidia
1	Pyriiform	52.3×32.5	Unicellular, hyaline, Oval	6.6×3.3	11
2	Globose	43.5×24.7	Unicellular, hyaline, Round	6.3×3.1	5
3	Globose	72.5×43.1	Unicellular, hyaline, oval	6.8×3.5	8
4	Spherical	50.3×22.4	Unicellular, hyaline, Oval	6.6×3.2	4
5	Ovoid	30.5×23.8	Unicellular, hyaline, Oval	6.5×3.3	3
6	Spherical	65.1×35.7	Unicellular, hyaline, Round	7.0×4.1	6
7	Round	29.2×22.7	Unicellular, hyaline, Oval	6.2×3.1	7
8	Globose	32.5×24.3	Unicellular, hyaline, Oval	5.8×2.6	6
9	Spherical	30.8×23.7	Unicellular, hyaline, Oval	6.7×3.3	9
10	Oval	38.9×22.4	Unicellular, hyaline, Round	6.6×3.3	4

mildew ranged from 15.54 to 63.45%. The maximum disease incidence of 63.45 PDI was observed at Thondamuthur village in Coimbatore district followed by 54.27 PDI at Ambilikkai village in Dindigul district. Minimum incidence of 15.54 PDI was recorded at Idappadi village in Salem district (Table 1). The present study revealed that, the incidence of powdery mildew disease showed its wide spread occurrence in almost all bhendi growing areas of Tamil Nadu.

Survey for collection of biocontrol agent *Ampelomyces* spp: The hyperparasite *Ampelomyces* spp. was isolated and the isolate number was given for each isolates collected from different districts (Table 1). In the present study, the biocontrol agent *Ampelomyces* spp. was isolated from the powdery mildew infected bhendi leaves by using tissue segment method. This was in accordance with the previous study conducted

by Sharma (2006) and Angeli *et al.* (2009b) where they have isolated *Ampelomyces* spp. by transferring the conidia onto potato dextrose agar which was amended with 2 % chloramphenicol by using tissue segment method. The fungal growth was observed and purified by hyphal tip culture technique and maintained on PDA slants at 5°C. The same method was also reported by Liang *et al.* (2007). Liang *et al.* (2007) removed one or two pycnidia from the mildew mycelia and transferred to potato dextrose agar (PDA) supplemented with 0.5% chloramphenicol.

Morphological growth characters of different isolates of *Ampelomyces* spp. under in vitro conditions: The morphological characters of different isolates of *Ampelomyces* spp. viz., mycelium, topography, colour, margin of colonies, zonation, colony growth, pycnidial shape and size, pycnidiospore shape

and size were studied. In general the mycelia were septate and hyaline in nature. The topography of the each isolates varied from radial, appressed, flat, fluffy and floccose. The colour of mature colonies was also varied as ash, brown and white in nature. Zonation was observed in some isolates like TNAU-AQ103, TNAU-AQ106 and TNAU-AQ108. The pycnidia of different isolates of *Ampelomyces* spp. varied in their shape. Pycnidia were light to dark brown in colour, non-ostiolate and were mostly pyriform, globose, spherical, ovoid and round in shape. In general the pycnidia measured about $29.20-65.10 \times 22.40-43.10 \mu\text{m}$. This was in accordance with Liang et al. (2007) who reported that the pycnidia were pyriform to globose measuring about $36-123 \times 22-45 \mu\text{m}$. Similar results were reported by Kim et al. (2009) who reported that the colour of pycnidium ranged from light brown to dark brown. Angeli et al. (2009b) revealed that the pycnidia of *Ampelomyces* spp. varied in shape depending upon the fungal structure in which they were formed. They were pear-shaped, spindle-shaped or nearly spherical when they were formed inside *E. necator* conidiophores, hyphae or chasmothecia. Pycnidiospores were hyaline, one-celled, oval to round in shape measuring $5.8-7.0 \times 2.6-4.1 \mu\text{m}$. The number of pycnidia was found to be more in TNAU-AQ101 and TNAU-AQ103 (Table 3; Plate 1). The results agree with Kiss (2008) who reported that the conidia of *Ampelomyces* spp. were found to be unicellular, hyaline and guttulate in shape measuring about 11.5 to $14.5 \mu\text{m}$ in length (major axis) and 2.5 to $3.5 \mu\text{m}$ in width (minor axis). Liang et al. (2007) also reported that pycnidia of *Ampelomyces* spp. contained unicellular guttulate conidia which measured about $4.2-7.5 \times 2-3.6 \mu\text{m}$. Angeli et al. (2009b) also revealed that the conidial shape of *A. quisqualis* were fusiform to ellipsoid and their size varied from 11.5 to $14.5 \mu\text{m}$ in length and 2.5 to $3.5 \mu\text{m}$ in width.

Conclusion

The present study revealed that, the incidence of powdery mildew disease showed its wide spread occurrence in almost all bhendi growing areas of Tamil Nadu. The per cent disease index (PDI) of powdery mildew ranged from 15.54 to 63.45%. The use of *Ampelomyces* spp. is a highly elegant method of biocontrol, as the agent is specific and can expand its activity on its own and it presents no risk to the environment. The morphological characterization indicated that the mycelium is septate and hyaline in nature. The topography of different isolates varied from radial, appressed, flat, fluffy and floccose. The colour of mature colonies varied from ash to brown. Zonation was observed in some isolates. The pycnidia of different isolates of *Ampelomyces* spp. varied in their shape and colour. Pycnidia were light to dark brown in colour,

non-ostiolate and were mostly pyriform, globose, spherical, ovoid and round in shape. In general the pycnidia measured about $29.20-65.10 \times 22.40-43.10 \mu\text{m}$. Pycnidiospores were hyaline measuring $5.8-7.0 \times 2.6-4.1 \mu\text{m}$. Incessant use of agrochemicals has many demerits such as development of resistance to pathogens, residual toxicity and environmental pollution. Hence, search for an alternative means for disease management is envisaged. *Ampelomyces* are the major antagonists against powdery mildew fungi which can be used as the best alternative to fungicides.

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