

Original Research Article

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Morphological Characterization of *Ampelomyces* spp., a Hyperparasite of Grapevine [*Vitis vinifera* (L.)] Powdery Mildew

M. Banupriya¹, C. Ushamalini^{2*}, S. Nakkeeran¹ and T. Raguchander¹

¹Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India

²Coconut Research Station, TNAU, Aliyar Nagar, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India

*Corresponding author

ABSTRACT

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Survey was conducted in grapevine growing areas of Coimbatore district during October to December 2018 to assess the incidence of powdery mildew and to collect different isolates of *Ampelomyces* spp. The powdery mildew incidence was noticed from 14.65 to 63.21 PDI. Ten isolates of *Ampelomyces* spp. were collected from powdery mildew infected grapevine leaf parasitized by *Ampelomyces* spp. All the isolates were identified by their morphological characters. Most of the isolates showed flat and radial growth pattern with raised growth of mycelia brownish black to greyish white. The pycnidia of different isolates of *Ampelomyces* varied in their shape and were mostly ovoid, ellipsoid, cylindrical, spindle, pyriform to globose in shape. *Ampelomyces* spp. was studied for its morphological characters in the present work.

Introduction

Grapevine is one of the most important fruit crop and belongs to the family *Vitaceae*. It is basically a sub-tropical crop. However, in India, grapes are cultivated under tropical conditions also this crop is grown throughout the world for wine making (82% production), raisin making (10% production) and rest for table purpose (8%). In India, although it is mostly consumed as fresh fruit and only a limited quantity is utilized for the production of wine, dry fruits like raisins etc.

Grapevine cultivation is affected by various biotic and abiotic stresses, among which, Powdery mildew caused by *Erysiphe necator* (Schwein) Burrill is a destructive disease of grapes. The disease affects all stages of growth causing premature defoliation. Powdery mildew plays a major role in reducing the quality and yield of grapes since it does not need any specific humidity and temperature conditions for infection (Dry *et al.*, 2010). *Ampelomyces quisqualis* is a promising biocontrol agent for the management of powdery mildew diseases

caused by *Erysiphaceae* family. Due to its wide host range, the mycoparasite is a highly promising candidate for biological control, as it is highly specific to powdery mildews. To study its morphological characters, this study was undertaken.

Materials and Methods

Survey for the incidence of powdery mildew in grapevine cultivating areas

A roving survey was conducted in major grapes cultivating areas in Coimbatore district of Tamil Nadu viz., Theethipalayam, Madampatti, Mathipalayam, Kuppanur, Devarayapuram, Boluvampatti, Thenkarai, Alanthurai, TNAU Orchard and Deenampalayam during October-December 2018 for assessing the incidence of powdery mildew of grapes. During the survey, a Global Positioning System (GPS) was used to determine the coordinates precisely for each field visited. In each village three farmer's fields were selected and in each farmer's field, at three places 20 plants were selected at random and in each plant the per cent leaf area of the grapes affected by powdery mildew was assessed visually by following the 0-9 scale (Azmat *et al.*, 2012; Nongmaithem *et al.*, 2017). The disease score was converted to Per cent Disease Index (PDI) by following the formula given by Wheeler (1969). The Per cent Disease Index is worked out as described by Mc Kinney's (1923)

Isolation of *Ampelomyces* spp.

The grapevine leaves showing typical powdery mildew symptoms and hyperparasitized by *Ampelomyces* spp. were collected from farmer's field in different areas of Coimbatore district. The powdery mildew infected grapevine leaves hyperparasitized by *Ampelomyces* spp. appearing as ash to dark

brown in colour collected in polythene bags and brought to the laboratory and preserved in an incubator at 25°C for further studies.

Immediately after examination under image analyzer one or two pycnidia were separated with sterile needles from the powdery mildew fungal propagules and these pycnidia were placed in 2% water agarose medium (Gu and Ko, 1997) for conidial germination of *Ampelomyces* spp. After 4-5 days, the emerging colonies were quickly transferred to Potato Dextrose Agar (PDA) supplemented with 0.5 per cent chloramphenicol and 1 per cent of streptomycine sulphate (10,000 I.U. ml⁻¹) under aseptic conditions.

Pure cultures were maintained in PDA at room temperature ($\pm 25^\circ\text{C}$) and transferred at every 6-8 weeks to new Petri plates. Before isolation, the pycnidia and pycnidiospores were observed for confirmation of *Ampelomyces* spp. using stereomicroscope.

Morphological characters of the 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 Petri dish and incubated at room temperature. *Ampelomyces* spp. is identified based on morphological characters of the pycnidia and pycnidiospore.

Results and Discussion

Survey for the powdery mildew incidence of grapevine

The results revealed that the per cent disease index (PDI) of powdery mildew in Coimbatore district was ranged from 14.65 to 63.21 PDI. The maximum disease incidence of 63.21PDI was observed at Theethipalayam village followed by 52.24 PDI at Alanthurai

village and minimum incidence of 14.65 PDI was recorded at Devarayapuram village in Coimbatore district (Table 1). The present study revealed that, the incidence of powdery mildew disease showed its wide spread occurrence in almost all grape growing areas of Coimbatore district. The incidence of powdery mildew upto 32.61 to 45.55 and 14.08 to 20.84 per cent in leaves and pods was reported by Parthasarathy *et al.*, 2017.

Collection of *Ampelomyces* spp.

The hyperparasite *Ampelomyces* spp. was isolated and the isolate number was given for each isolates collected from Thondamuthur

block of Coimbatore district (Table 1). In this present study, ten isolates of *Ampelomyces* spp. was isolated from the powdery mildew infected grapevine leaves. This was in accordance with the previous study conducted by 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. 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.

Table.1 Survey for the incidence of powdery mildew and collection of isolates of *Ampelomyces* spp. in major grape growing areas of Coimbatore district

S. No	Place of collection	Stage of crop	Variety/ Hybrid cultivated	Latitude (N)	Longitude (E)	Mean PDI of powdery mildew	Isolates of <i>Ampelomyces</i> spp.
1.	Mathipalayam	Flowering	Muscat Hamburg	10.9327°	76.8415°	35.80 ^e	TNAU-AQ101
2.	Madampatti	Flowering	Muscat Hamburg	10.9698°	76.8598°	29.35 ^g	TNAU-AQ102
3.	Deenampalayam	Flowering	Muscat Hamburg	11.0044°	76.8572°	32.28 ^f	TNAU-AQ103
4.	Theethipalayam	Flowering	Muscat Hamburg	10.9523°	76.8865°	63.21 ^a	TNAU-AQ104
5.	Kuppanur	Flowering	Muscat Hamburg	10.9478°	76.8627°	46.47 ^c	TNAU-AQ105
6.	Devarayapuram	Flowering	Red globe	10.9976°	76.8159°	14.65 ^j	TNAU-AQ106
7.	Thenkarai	Flowering	Red globe	10.9366°	76.8433°	43.73 ^d	TNAU-AQ107
8.	TNAU, Orchard	Flowering	Muscat Hamburg	10.9009°	76.7856°	52.24 ^b	TNAU-AQ108
9.	Alanthurai	Flowering	Red globe	11.0098°	76.8487°	18.49 ⁱ	TNAU-AQ109
10.	Bolvampatti	Flowering	Muscat Hamburg	10.9921°	76.7221°	24.40 ^h	TNAU-AQ110

*PDI- Per cent Disease Index, * Values are means of three replications, Means followed by a common letter are significantly different at 5% level by DMRT

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

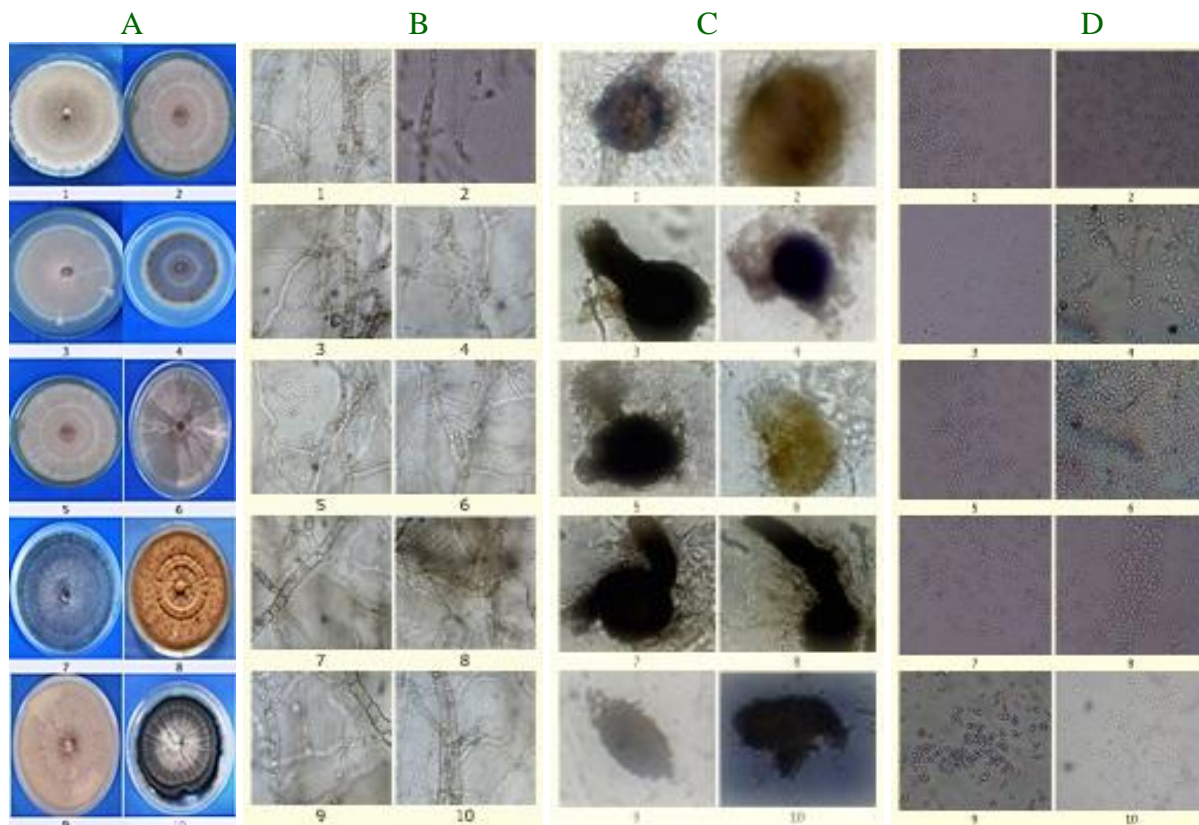
S. No	Isolates	Mycelium	Topography	Colour of mature colonies	Zonation	Margin	Colony growth
1.	TNAU-AQ101	Septate, hyaline	Radial	Greyish white	Absent	Irregular	Slow
2.	TNAU-AQ102	Septate, hyaline	Flat	Ash	Absent	Smooth	Slow
3.	TNAU-AQ103	Septate, hyaline	Flat	Brown	Present	Wavy	Moderate
4.	TNAU-AQ104	Septate, hyaline	Radial	White	Absent	Wavy	Very slow
5.	TNAU-AQ105	Septate, hyaline	Appressed	Brownish black	Absent	Smooth	Slow
6.	TNAU-AQ106	Septate, hyaline	Flat	Brownish black	Present	Irregular	Very slow
7.	TNAU-AQ107	Septate, hyaline	Radial	White	Absent	Smooth	Moderate
8.	TNAU-AQ108	Septate, hyaline	Radial	Brown	Absent	Wavy	Slow
9.	TNAU-AQ109	Septate, hyaline	Radial	Greyish white	Present	Smooth	Moderate
10.	TNAU-AQ110	Septate, hyaline	Radial	Brown	Absent	Wavy	Moderate

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

S. No	Pycnidia	Pycnidial size (µm)*	Pycnidiospore	Pycnidiospore size (µm)*
1.	Spherical	115.66×100.39	Unicellular, hyaline, Oval	3.12×1.67
2.	Pyriform	64.62×57.67	Unicellular, hyaline, Round	4.13×1.23
3.	Globose	80.73×73.7	Unicellular, hyaline, Oval	4.45×2.5
4.	Round	60.78×56.67	Unicellular, hyaline, Oval	5.87×2.36
5.	Oval	68.18×55.15	Unicellular, hyaline, Round	6.78×3.9
6.	Globose	82.18×68.18	Unicellular, hyaline, Oval	4.97×3.2
7.	Ovoid	60.46×24.5	Unicellular, hyaline, Round	3.52×2.1
8.	Spherical	66.66×58.8	Unicellular, hyaline, Oval	5.45×1.49
9.	Spindle	76.9×24.7	Unicellular, hyaline, Round	5.5×3.8
10.	Pear	89.16×24.5	Unicellular, hyaline, Round	4×3.2

* Values are means of three replications

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



A- Isolates, B- Mycelium, C- Pycnidia, D- Pycnidiospore
 1-TNAU-AQ101, 2-TNAU-AQ102, 3- TNAU-AQ103, 4-TNAU-AQ104, 5-TNAU-AQ105, 6-TNAU-AQ106, 7-
 TNAU-AQ107, 8-TNAU-AQ108, 9- TNAU-AQ109, 10-TNAU-AQ110

Morphological growth characters of isolates of *Ampelomyces spp.*

The morphological character 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 and flat. The colour of mature colonies was also varied as greyish white, brownish black, ash, brown and white in nature. Zonation was observed in some isolates like TNAU-AQ103, TNAU-AQ106 and TNAU-AQ109. 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, pear, spindle, oval and round in shape. In general the pycnidia measured about 60.46-115.66 × 24.7-100.39 μm (Table 2, plate 1). This was in accordance with Liang *et al.*, (2007) who reported that the pycnidia were pyriform to globose measuring about 36-123 × 22-45 μm. 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 and oval to round in shape measuring $3.12-6.78 \times 1.23-3.9 \mu\text{m}$. 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 μm in length (major axis) and 2.5 to 3.5 μm 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 μm in length and 2.5 to 3.5 μm in width (Table 3).

The present study revealed that, the incidence of powdery mildew disease showed its wide spread occurrence in almost all grape growing areas of Coimbatore district. The per cent disease index (PDI) of powdery mildew ranged from 14.65 to 63.21 PDI. The isolates of *Ampelomyces* spp vary in their morphological characters *i.e.*, colony colour (ash to brownish black), zonation, pycnidial shape (globose to pyriform) and pycnidiospores size ($3.12-6.78 \times 1.23-3.9 \mu\text{m}$). By studying the antibiotic nature of these isolates, effective isolate may be exploited in the biocontrol of powdery mildew pathogen in future.

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