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Natural Occurrence and Isolation of *Ampelomyces quisqualis* (mycoparasite) Associated with Rose, Other Powdery Mildew Infecting Host Plants in Himachal Pradesh, India

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Abstract

The presence of Ampelomyces spp. was quantified in naturally occurring powdery mildew infecting rose plants during the year 2015–2016. Powdery mildew infected rose samples were collected from the different rose-growing areas of Himachal Pradesh during the months of April–June and September–November. Relationships between Ampelomyces and Podosphaera pannosa were reported in four areas between September and November. Bilaspur, Mandi, Shimla, and Solan. However, there was no occurrence of Ampelomyces quisqualis in districts of Kangra and Sirmour during the present study. Nine different powdery mildew infected hosts were studied, out of which only three hosts have shown the association with A. quisqualis, which were Chotta dhatura (September–November), Zinnia and Dahlia (May–July) at Nauni. However, occurrence of A. quisqualis was reported from different powdery mildew host but it can only be isolated from Xanthium strumarium (Chota Dhatura) The effect of solid media on mycelial growth, production of pycnidium, size, shape, and color of pycnidium and conidia of A. quisqualis was investigated and maximum average mycelial growth (28.26 mm) was reported in malt extract medium followed by the Oat Meal Agar medium (22.33 mm) and Potato Dextrose Agar medium (18.50 mm).

Keywords: *Ampelomyces quisqualis*, mycoparasite, powdery mildew, rose, fungus

INTRODUCTION

Powdery mildew is a burdensome pathogen and one of the most visible plant diseases. Characterized by white and gray powdery spots on the surface of plant limbs. Although the fungus is most found on the upper surface of the leaves, but in many plants the undersides of leaves can infect other parts of the plant, such as young shoots, stems, buds, flowers, and fruits [1]. Fungi rarely kill their hosts, but they deplete nutrients, which reduces photosynthesis, increases breathing, and dehydration disturbing plant growth and can reduce yield by 20–40 percent, depending on environmental conditions favorable for the growth and reproduction of the fungus [2].

Mycoparasite *Ampelomyces quisqualis* was found to be associated with powdery mildew causing genera and establishing an interfungal parasitic relationship with mycelium, conidia; and chasmothesia of powdery mildew fungus [3]. Mycelia are spread by spraying into the phytosphere. After germination, conidia penetrate

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the hyphae of the mildew and develop internally, and host hyphae, conidiophores, and conidium initials form internal pycnidia [4]. Pycnidia also form from cleistothecia immature host [5]. Fragments of wind-borne hyphae of *Ampelomyces* are carried over long distances within infected powdery mildew conidia can cause fungal infections in other powdery mildew colonies [6–12]. Powdery mildew fungi belong to the order Erycephales and include a wide variety of genera and species, with diverse clades. These fungi infect many types of plants, such as grass, vegetables, fruits, forest trees, and agricultural crops. This causes significant annual production losses. Several strategies are being used to combat these infections. For the management of powdery mildew disease through physical, chemical, and biological methods, mycoparasite *A. quisqualis* can be successfully used as biological control agents in the management of powdery mildew diseases in various crops. The objective of present investigation was to ascertain the presence of *A. quisqualis* in powdery mildew pathogens.

MATERIALS AND METHODS

Sample Collection

Rose leaves infected with powdery mildew collected from different districts twice, that is, at the time of primary infection by powdery mildew and secondary infection by powdery mildew.

Isolations, Morphological, and Microscopic Examinations

A stereo microscope was used to detect the mycoparasite *A. quisqualis*, which are brown intercellular pycnidia in the white mycelium of powdery mildew. For the study of coparasite fungi, infected leaves are examined under a three-dimensional microscope for brown pycnidia between cells within the mycelium, white powdery mildew. Brown pycnidia were carefully collected with a dissected microscopic needle and stained with lactophenol blue cotton swab. A light microscope was used to analyze the structure of the fungus and collect images. The size of the pycnidiospores and pycnidia was measured using a micrometer. Mycoparasites were identified using identification keys provided by Belsare et al. [13]. When one or two pycnidia are identified, they were carefully scraped from the fungal mycelium with a sterile handmade glass needle [6, 14–16] and placed on Potato Dextrose Agar (PDA) pretreated with 0.5% chloramphenicol to obtain pure fungus preparation. It was quickly moved to a new plate and immediately they started growing on the media. These pure cultures were placed on PDA at room temperature and subcultured on fresh plates every 6–8 weeks.

RESULTS AND DISCUSSION

The present investigation was carried out to know the presence of natural antagonist of powdery mildew of rose, particularly *A. quisqualis* in six districts of Himachal Pradesh. Data reproduced in the Table 1 revealed that *A. quisqualis* presence was observed from September to November while absent in the months of April–June. In four districts viz. Bilaspur, Mandi, Shimla, and Solan, *A. quisqualis* was present in month of September–November. However, there was no occurrence of *A. quisqualis* in districts Kangra and Sirmour during the present study. In general, the evidence on occurrence of the mycoparasite was observed in three months, that is, September, October, and November. Pycnidia were globose to oblong in shape, light brown to dark brown color and size of *A. quisqualis* ranges from 40–45 × 32–35 µm with the biggest size of pycnidia observed in sample of Solan district of 45 × 35 µm. However, conidia of *A. quisqualis* were hyaline, ovate, to elliptical, one celled, smooth walled, and size of conidia varies from 5.28–6.98 × 2.97–3.86 µm with the largest size of conidia from district Solan of 6.98–3.8 µm.

Different powdery mildews infecting plants also were evaluated during disease progress in nearby Nauni campus areas for assessing the mycoparasites presence (Table 2). Nine different powdery mildew infected hosts were studied out of which only three hosts have shown the association with *A. quisqualis*. Association of *A. quisqualis* with different powdery mildew fungi was found in *Xanthium strumarium* during September–November, *Zinnia elegans* and *Dahlia pinnata* (May–July). Pycnidia were globose to oblong in shape, light brown to dark brown color and size of *A. quisqualis* ranges from 45–48 × 34–36 µm with the biggest size of pycnidia observed in sample of *X. strumarium* of 48 × 36 µm. However, conidia of *A. quisqualis* were hyaline, ovate to elliptical, one celled, smooth

walled, and size of conidia varies from 6.28–6.60 × 3.12–3.28 µm with the largest size of conidia from *X. strumarium* of 6.98–3.8 µm.

Table 1. Occurrence of *A. quisqualis* associated with powdery mildew of rose in Himachal Pradesh (year 2015–2016).

District (s)	*A. quisqualis* Association		Pycnidia			Conidia		
	Month (s) of Observation							
	April–June	*September–November*	*Size (µm)*	*Shape*	*Color*	*Size (µm)*	*Shape*	*Color*
Bilaspur	-	+	40.00 × 32.00	Globose to oblong	Light brown to dark brown	5.28 × 3.12	Ovate to elliptical, one celled, and smooth walled	Hyaline
Kangra	-	-	-	-	-	-	-	-
Mandi	-	+	42.00 × 34.00	Globose to oblong	Light brown to dark brown	6.18 × 2.97	Ovate to elliptical, one celled, and smooth walled	Hyaline
Shimla	-	+	43.00 × 33.00	Globose to oblong	Light brown to dark brown	6.34 × 3.46	Ovate to elliptical, one celled, and smooth walled	Hyaline
Sirmour	-	-	-	-	-	-	-	-
Solan	-	+	45.00 × 35.00	Globose to oblong	Light brown to dark brown	6.98 × 3.86	Ovate to elliptical, one celled, and smooth walled	Hyaline

**+: Present, –: Absent*

The scanning of literature revealed that several workers have reported *A. quisqualis* occurrence from different powdery mildew infected hosts [17–24] registered the presence of *A. quisqualis* in the month of November on rose powdery mildew. Previous research has shown that trees containing grapes and apples [2, 5], medicinal plants [13, 25], and boxwoods [26, 27] reported the association of *A. quisqualis* in powdery mildew of Chota Dhatura (*X. strumarium*).

Inspite of the association of *A. quisqualis* with the powdery mildew of rose, *X. strumarium*, *Z. elegans*, and *D. pinnata*, the mycoparasite was only isolated from Chota Dhatura (*X. strumarium* L.) powdery mildew infected plants and the isolated *A. quisqualis* fungus culture showed very slow growth rate [28, 29].

The effect of solid media on mycelial growth, production of pycnidium, size, shape, and color of pycnidium and conidia of *A. quisqualis* was investigated and presented in Table 3. Maximum average mycelial growth (28.26 mm) was reported in malt extract medium followed by the Oatmeal Agar medium (22.33 mm) and PDA medium (18.50 mm). In Malt Extract Agar medium, light brown to dark brown, globose to oblong pycnidia were produced with size ranged between 42.00–59.00 × 30.00–39.00 µm with average size of 48.00 × 36.00 µm while in Oat Meal Agar medium and PDA the size ranged between 40.00–56.00 × 30.00–37.00 µm (48.00 × 36.00 µm average) and 37.00–58.00 × 28.00–36.00 µm (45.00×34.00 µm average), respectively. Hyaline, ovate to elliptical, one celled, and smooth walled conidia were produced in all the solid media. Conidia of 4.00–6.54 × 3.00–4.00 µm (6.28 × 3.12 µm average) produced in PDA medium, 4.15–6.84 × 3.45–4.48 µm (6.54 × 3.45 µm average) in Oat Meal Agar medium and 4.54–7.25 × 3.65–4.82 µm (6.60 × 3.48 µm) in Malt Extract Agar medium.

Table 2. Occurrence of *A. quisqualis* associated with powdery mildew of different hosts of surrounding areas of Nauni (Solan, H.P.) in year 2015–2016.

Host (s)	**Powdery Mildew Pathogens**	***A. quisqualis* Association**		**Pycnidia**			**Conidia**		
		Month (s) of Observation		**Size (µm)**	**Shape**	**Color**	**Size (µm)**	**Shape**	**Color**
		May–July	**September – November**						
Pride of India (*Lagerstroemia indica* L.)	*Erysiphe australiana*	-	-	-	-	-	-	-	-
Kumra (*Biden pilosa* L.)	*Podosphaera xanthii*	-	-	-	-	-	-	-	-
Dahlia (*D. pinnata* Cav.)	*Erysiphe cichoracearum*	+	-	45.00 × 34.00	Globose to oblong	Light brown to dark brown	6.28×3.12	Ovate to elliptica, one celled, and smooth walled	Hyaline
Wild palak (*Beta vulgaris subsp. Maritime* L.)	*Uncinula* spp.	-	-	-	-	-	-	-	-
Khidak (*Celtis australis* L.)	*Erysiphe kusanoi*	-	-	-	-	-	-	-	-
Zennia (*Z. elegans* Jacq.)	*Erysiphe cichoracearum*	+	-	48.00 × 34.00	Globose to oblong	Light brown to dark brown	6.54×3.45	Ovate to elliptica, one celled, and smooth walled	Hyaline
Cucumber (*Cucumis sativus* L.)	*Erysiphe cichoracearum*	-	-	-	-		-	-	-
Chota Dhatura (*X. strumarium* L.)	*Podosphaera xanthii*	-	+	48.00 × 36.00	Globose to oblong	Light brown to dark brown	6.60 × 3.48	Ovate to elliptica, one celled, and smooth walled	Hyaline
Yellow woodsorrel (*Oxalis stricta* L.)	*Erysiphe cichoracearum*	-	-	-	-		-	-	-

**+: Present, –: Absent*

Czapek agar medium and potato dextrose broth was used by the Sztejnberg et al. [30] for multiplication of *A. quisqualis* [31]. Studied the effect of malt–yeast extract agar medium and found that on this medium there was production of pycnidium, but there was no conidia production [32]. Has reported maximum sporulation in PDA medium with least mycelial growth.

CONCLUSION

Ampelomyces quisqualis is found grown on the powdery mildew fungi which kill the pathogens. Ampelomyces quisqualis was found growing on the rose powdery mildew and other crops infected

with powdery mildew. Ampelomyces spp could be utilized for the management of the powdery mildew though much research work has to be done in areas like isolation and mass production. There are a lot of private sectors trying to harness possibilities but we are still very behind. Use of these mycoparasites could be used as an alternative for chemical control plant disease.

Table 3. Effect of solid media on the average mycelial growth (mm), size of pycnidia and conidia of *A. quisqualis.*

Medium	Average Mycelial Growth (mm)	Pycnidium			Conidia		
		Size (µm)	Shape	Color	Size (µm)	Shape	Color
PDA	18.50	37.00–58.00 × 28.00–36.00 (45.00 × 34.00)	Globose to oblong	Light brown to dark brown	4.00–6.54 × 3.00–4.00 (6.28 × 3.12)	Ovate to elliptical, one celled, and smooth walled	Hyaline
Oatmeal Agar	22.33	40.00–56.00 × 30.00–37.00 (48.00 × 36.00)	Globose to oblong	Light brown to dark brown	4.15–6.84 × 3.45–4.48 (6.54 × 3.45)	Ovate to elliptical, one celled, and smooth walled	Hyaline
Malt Extract Agar	28.26	42.00–59.00 × 30.00–39.00 (48.00 × 36.00)	Globose to oblong	Light brown to dark brown	4.54–7.25 × 3.65–4.82 (6.60 × 3.48)	Ovate to elliptical, one celled, and smooth walled	Hyaline
$CD_{0.05}$	3.04						

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