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## ***Ampelomyces quisqualis* - a remarkable mycoparasite on *Xanthium strumarium* powdery mildew from Himachal Pradesh India**

Ajay Kumar Gautam\* and Shubhi Avasthi\*\*

\* Faculty of Agriculture, Abhilashi University, Mandi, Himachal Pradesh, India

\*\* Department of Botany, Abhilashi Institute of Life Sciences, Mandi, Himachal Pradesh, India

\*Corresponding author: a2gautam2006@gmail.com

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### **ABSTRACT**

A powdery mildew infection was observed on *Xanthium strumarium* during the routine mycological survey in district Mandi of Himachal Pradesh, India. Morphological and microscopic examinations revealed the identity of powdery mildew as *Podosphaera xanthii*. The white powdery mildew infection was found mixed with brownish intercellular pycnidia during mycological examinations. These brown coloured pycnidia were picked up with the help of a needle under dissecting microscope and mounted in lactophenol cotton blue stain. Critical examinations identify it as a mycoparasite namely *Ampelomyces quisquali*. To best of our knowledge, this is the first report of mycoparasite *A. quisquali* on *X. strumarium* in India.

**Key Words:** *Ampelomyces quisqualis*, *Xanthium strumarium*, mycoparasitism, powdery mildew fungi.

### **INTRODUCTION**

Powdery mildew is a common fungal disease characterized by a white floury covering on aerial plant parts. It grows well in environments with high humidity and moderate temperatures. This disease affects a wide range of plants and display quite distinctive symptoms like twisting, buckle, necrosis, distortion of leaves and other infected parts which finally lead to reduced yields, shortened production times, and spoilage of infected fruits and vegetables (Otis & Inglis 1993; Jarvis et al. 2002).

Taxonomically powdery mildew belongs to order Erysiphales which contains number of genera and species distributed worldwide over diverse host range. As this disease affects many

types of plants including, grasses, vegetables, fruits, forest trees and agricultural crops, which results into huge annual yield loss every year. Therefore, considerable management methods are suggested by plant protection scientists time to time. Although, a number of methods viz. physical, chemical and biological are now in practice to control the powdery mildew diseases on various hosts. But the best method of control is prevention. There are number of biocontrol agents available in nature which help the plant to control fungal parasites. An example of such type of biocontrol association is mycoparasitism which simply an association of two fungi where one act as parasite over other. The term was introduced by Butler (1957) to elucidate the complex interrelationships between a fungal host and parasite. Number of

fungi has been reported as mycoparasites over number of fungal parasites. *Ampelomyces quisqualis* is one of the naturally occurring mycoparasites which parasitize both sexual and asexual structures of powdery mildew. This mycoparasite colonizes a large area of the target site, competes for the plant substrates and nutrients thereby causes the killing of pathogens due to starvation. It acts as a hyper parasite which penetrates the pathogen and infects it by forming pycnidia within powdery mildew hyphae, conidiophores and cleistothecia.

During the routine mycological survey in district Mandi of Himachal Pradesh, India, a powdery mildew infection was observed on leaves of *Xanthium strumarium* L. The morphological examinations of powdery mildew symptoms revealed variation in colour of fungal mycelium on leaves. Upon critical microscopic examinations, the infection was found mixed with another unknown mycoparasitic fungus. Therefore, the present study was carried out to study morphology and taxonomy of the powdery mildew disease and its mycoparasitic associate, collected the on the plant *Xanthium* sp. from Himachal Pradesh, India.

## MATERIALS AND METHODS

### Sample collection

The powdery mildew samples were collected during the phytopathological survey of district Mandi of Himachal Pradesh, India in the year 2015. These infected plant parts were dried between sheets of blotting paper and preserve for further studies. Host plants were identified and confirmed by matching the collections with herbarium and by consulting botanists. The specimens were deposited at Abhilashi University (AU), Mandi, Himachal Pradesh, India for further reference.

### Morphological and Microscopic Examinations

The infected leaves were examined primarily with a hand-lens and then with a dissecting microscope for the presence of powdery mildew symptoms. For microscopic examinations, a piece of clear adhesive tape was placed on infected leaves, stripped off and then place on a microscopic slide with one drop of clear distilled water. The microscopic observations were carried out to study the characteristics of mycelia on the host, appressoria, size and shape of conidia and conidiophores and chasmothecia. For examinations of mycoparasite, the infected leaves placed under stereomicroscope and observe d for the presence of brownish intercellular pycnidia in the white powdery mildew mycelia. These brown coloured pycnidia were picked up with the help of a needle under dissecting microscope and mounted in lactophenol cotton blue stain.

An light microscope was used to examine fungal structures, images were captured. Micrometry measurements were carried out for size of conidia, conidiophores, chasmothecia and pycnidia. Line drawings of anatomical features were drawn with the aid of Camera Lucida under oil immersion lens. All measurements were given in the form: min–max. Standard literature was consulted for identification of powdery mildew (Paul & Thakur 2006; Braun & Cook 2012) and mycoparasite (Belsare et al. 1980; Hashioka & Nakai 1980; Kiss 1998).

## RESULTS

The powdery mildew disease was observed as white powdery mass on leaves, stems and other floral parts of *P. xanthii*. The whole infected surfaces were covered fully with white powdery mass as disease progress to severity. The white powdery mildew infection was turned grayish in colour after disease severity. Morphological and microscopic analysis of diseased samples revealed the presence of a fungus *Podospaera xanthii* responsible for powdery mildew diseases. Upon further mycological examinations at later stages of infection, brownish intercellular pycnidia were found parasitic on powdery mildew. Critical examinations revealed it a mycoparasite belongs to *Ampelomyces quisquali*. Detailed description and illustrations of the specimens are given here.

*Podospaera xanthii* (Castagne) U. Braun & Shishkoff, *Schlechtendalia* 4: 31, 2000

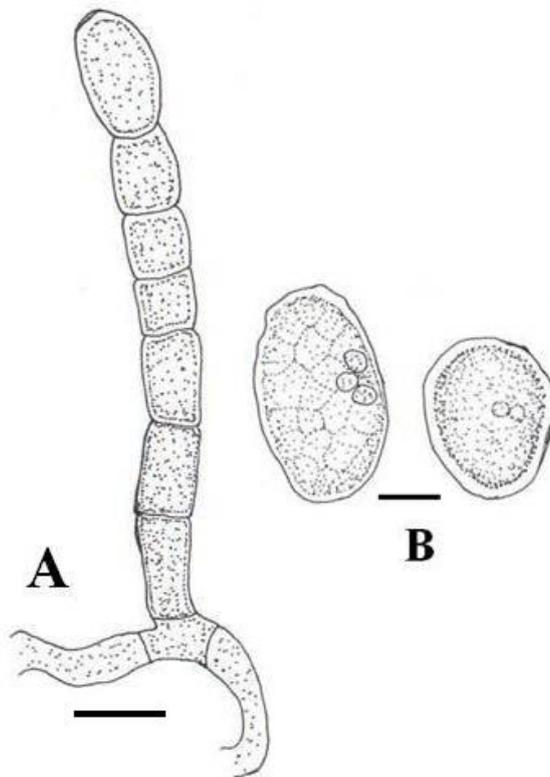
### Fig. 1 & 2

- ≡ *Erysiphe xanthii* Castagne, *Cat. pl. Marseille*: 188, 1845
- ≡ *Sphaerotheca xanthii* (Castagne) L. Junell, *Svensk Bot. Tidskr.* 60(3):382, 1966
- = *S. fusca* var. *compositarum* Y.S. Paul & V. Thakur, *Indian Erysiphaceae*: 20, 2006, *nom. inval.*; type host – *Senecio chrysanthemoides*.
- = *Euoidium leguminosarum* Y.S. Paul & J.N. Kapoor, *Indian J. Mycol. Pl. Pathol.* 17(3): 299, 1987; type host – *Phaseolus aureus*

White mycelium appeared as patches on leaves, stems and other floral parts, amphigenous, thin to dense, temporary to permanent; hyphae smooth, thin walled with simple to slightly nipple-shaped appressoria, 3.5 – 9 µm wide; conidiophores arising from the upper surface of superficial hyphae, erect, cylindrical; foot-cells short, 25 – 80 × 8 – 13 µm, following cells about as long as the foot-cell or shorter, forming catenescence conidia in chain; conidia ellipsoid-ovoid to doliiform, 23 – 44 × 14 – 21 µm, with fibrosin bodies, germ tubes lateral. No Chasmothecia were found.



**Fig. 1** – Symptoms of powdery mildew on *Xanthium strumarium*.



**Fig. 2** – *Podosphaera xanthii* anamorphic structures (A-B): **A**. Conidiophores with foot cell bearing chain of conidia; **B**. Conidia. (Scale bar: A = 20 $\mu$ m; B= 10 $\mu$ m)

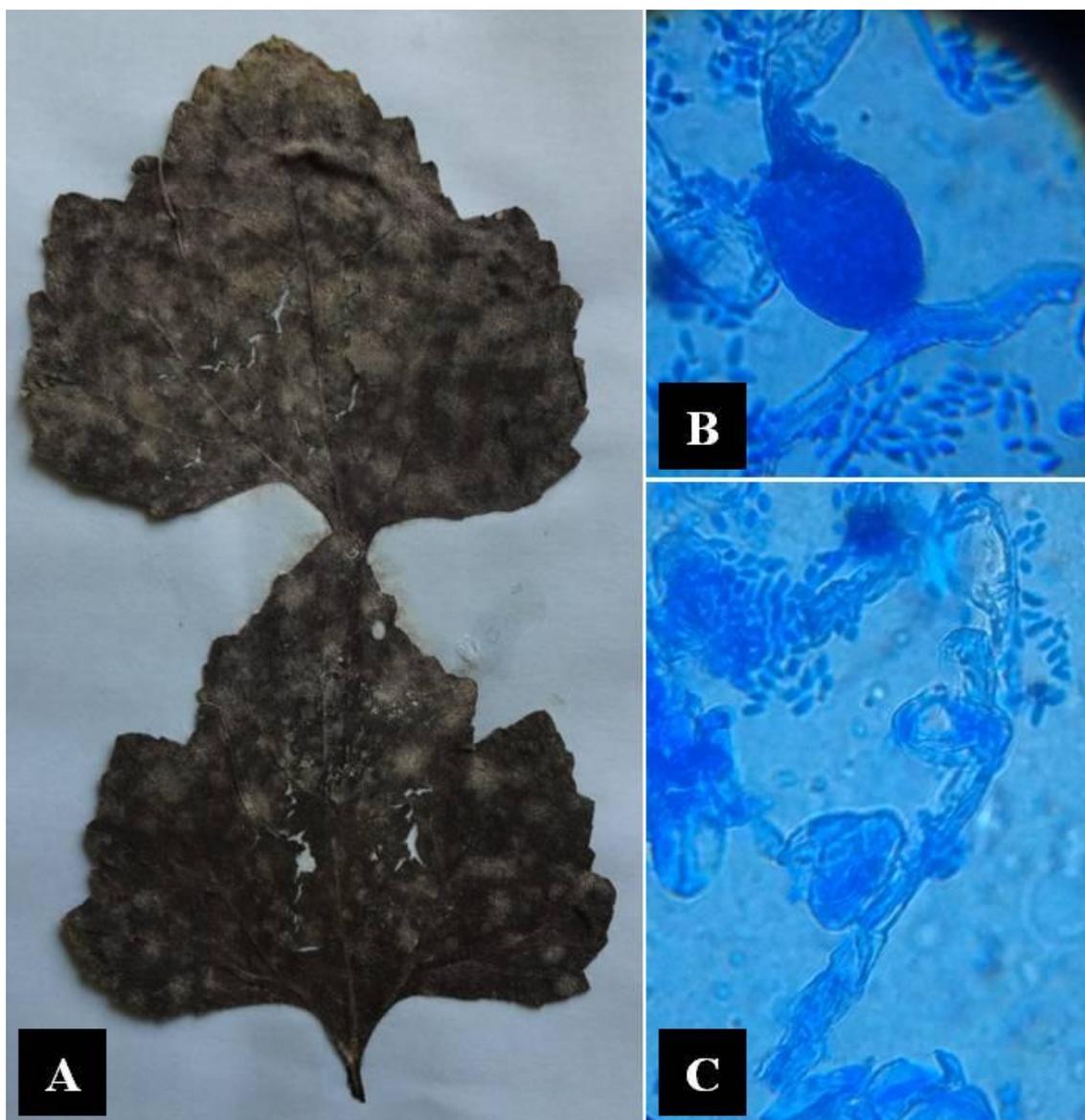
**Material examined:** on leaves of *Xanthium strumarium* L., Abhilashi University Mandi agricultural fields, India, Himachal Pradesh, at 1400 metres (4,592 ft), 12 October 2015, Ajay Kumar Gautam, (anamorph).

**Known distribution:** on numerous plant hosts belongs to family Asteraceae, Balsaminaceae, Caricaceae, Fabaceae, Malvaceae, Solanaceae Verbenaceae from America, Asia, Australia, Africa, Europe, New Zealand.

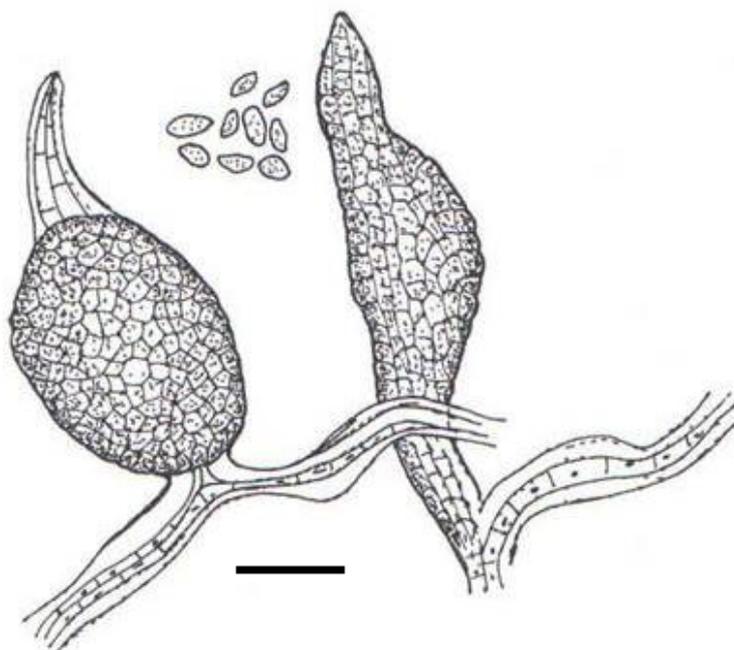
#### Identification of mycoparasite

*Ampelomyces quisqualis* Ces. Bot. Ztg. 10:301 (1852) **Figs. 3 & 4**

Hyphae of the hyper-parasite were hyaline and septate; present within the hyphae, conidiophores, and conidia of infected powdery mildews pathogen; Conidiogenous cells enteroblastic, phialidic, discrete, smooth, hyaline, 4.5 – 5.5  $\mu\text{m}$  wide, formed directly from the pycnidial wall cells. Pycnidia were light brown in transmitted light and varied in shape from sub-globose to pyriform, measured 38.5 – 79.2  $\times$  35.2 – 44 [av. ( $\pm$  SD) 62.63 ( $\pm$  12.74)  $\times$  38.97 ( $\pm$  2.53), n = 25]  $\mu\text{m}$ , with no distinct ostium; Conidia unicellular, hyaline, mostly guttulate conidia, 3.2 – 6.5  $\times$  1.1 – 3.5 [av. ( $\pm$  SD) 4.54 ( $\pm$  0.85)  $\times$  2.43 ( $\pm$  0.58.88), n = 25]  $\mu\text{m}$ , dehiscence by apical rupture of pycnidium.



**Fig. 3 – *Ampelomyces quisqualis* on *X. strumarium*.** A. Powdery mildew infection mixed with mycoparasite infection; B. Pycnidia; C. Mycelium, conidiophores and conidia of *P. xanthii* parasitized by *A. quisqualis*.



**Fig. 4** – *Ampelomyces quisqualis* anamorphic structures: **A.** Pycnidia; **B.** conidia. (Scale bar = 10µm)

## DISCUSSION

*Xanthium strumarium* is a monoecious plant species belonging to the family Asteraceae. Although, the plant probably originates in North America and has been extensively naturalized elsewhere but it is a common weed found in India. It is an annual herb with a short, hard, hairy stem. The plant has numerous medicinal properties and used in cooling, laxative, tonic, digestive, antipyretic and improves appetite, complexion and memory (Bhogaonkar & Ahmad 2012).

Like other plants, *X. strumarium* was also reported to infect with numerous plant pathogens as *Oidium xanthimi* (Paul & Thakur 2006), *Phomopsis longicolla* (Vrandecic et al. 2007), and *Curvularia lunata* & *Drechslera spicifera* (Nayla et al. 2015) from India, Croatia, and Sudan respectively. *Podosphaera xanthii* is a known pathogen for several plant hosts. It has been reported on *Momordica cochinchinensis* (Baiswar et al. 2008), Cocklebur (Lee 2013), *Bidens pilosa* (Cho et al. 2013), *Gynura bicolor* (Shen et al. 2015), red chilli pepper (Tam et al. 2015) and *Luffa cylindrical* (Cho et al. 2015).

The present study exposes mycoparasitic relationship between powdery mildew fungus (*P. xanthii*) on *X. strumarium* and *Ampelomyces quisqualis*. The powdery mildew infection on *X. strumarium* was appeared as white powdery mass but turned grayish upon mycoparasitic attack. *A. quisqualis* is most studied mycoparasites of powdery mildew disease on various crops and observed as one of the important biological control

agents (BCAs) of powdery mildew disease. There are about 18 epithets recorded worldwide till date on variety of plant hosts (www.indexfungorum.com). *A. quisqualis* is reported earlier on powdery mildew of grapes (Falk et al. 1995), apple (Vaidya & Thakur 2005); crops, weeds, medicinal plants (Belsare et al. 1980; Kiss 1998) and Buxus trees (Naseripour et al. 2014). Reports are available which shows that the mycoparasitism is not only restricted to powdery mildew; it can also parasitize *Botrytis cinerea*, *Alternaria solani*, *Colletotrichum coccodes* and *Cladosporium cucumerinum* (Jarvis & Slingsby 1977). To best of our knowledge, this is the first report of *Ampelomyces quisqualis* as mycoparasite on *Xanthium strumarium* in India.

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