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Ampelomyces quisqualis Ces. – a mycoparasite of *Euphorbia hirta* powdery mildew in Himachal Pradesh, India

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Abstract

Mycoparasitism is defined as the association of two fungi where one acts as parasite over the other. In October 2015, a powdery mildew infection was observed on *Euphorbia hirta* during the routine mycological survey in district Mandi the central region of Himachal Pradesh, India. During the course of microscopic examinations infection was found mixed with another unknown fungus. Upon morphological and microscopic examinations of infection on leaves, *Podosphaera euphorbiae-hirtae* was identified as powdery mildew fungus mixed with a mycoparasite *Ampelomyces quisqualis*. To the best of our knowledge, this is the first report of mycoparasitism of *Ampelomyces quisquali* on powdery mildew of *Euphorbia hirta* in India.

Key words: *Ampelomyces quisqualis*, *Euphorbia hirta*, mycoparasitism, powdery mildew fungi.

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Introduction

Mycoparasitism is defined as the association of two fungi where one acts as parasite over the other. This term along with mycoparasite was introduced by Butler (1957) to elucidate the complex interrelationships between a fungal host and parasite. Mycoparasites can be of balanced (biotropic) and destructive (necrotropic) type, based on the modes of host- parasite interactions (Barnett & Binder, 1973). Biotrophic mycoparasites are mainly haustorial in nature- which cause little damage to their hosts and remain active as long as the host is active. Whereas, necrotropic parasites are extremely destructive to other fungi. They often spread rapidly, completely surrounding and covering their hosts in a few hours or days (Jeffries, 1995). *Ampelomyces quisqualis* is a naturally occurring mycoparasite of powdery mildews. It is an anamorphic fungus parasitic to both sexual and asexual structures of powdery mildew pathogens. This parasite reduces growth and may eventually kill the mildew pathogen. It colonizes a large area of the target site, competes for the plant substrates and nutrients thereby causes the killing of pathogens due to starvation. Being a hyper parasite, it penetrates the pathogen and infects it by forming pycnidia within powdery mildew hyphae, conidiophores and cleistothecia (Kiss, 2008; Kiss et al., 2004). In October 2015, a powdery mildew infection was observed on *Euphorbia hirta* during the routine mycological survey in district Mandi, the central region of Himachal Pradesh, India. During the course of microscopic examinations, infection was found mixed with another unknown fungus. The detailed examination revealed the mycoparasitic nature of this unknown fungus. The present study was therefore, carried out to study morphology and taxonomy of the powdery mildew disease and its mycoparasitic associate, collected on the plant *E. hirta* from Himachal Pradesh, India.

Materials and methods

The aerial parts of *E. hirta* showing powdery mildew symptoms were collected in the course of a mycological survey from district Mandi of Himachal Pradesh. These infected leaves were dried between sheets of blotting paper and preserved for further studies. Host plants were identified and confirmed by matching the collections with herbarium and by consulting Botanists. The specimen was deposited at Abhilashi University (AU), Mandi, Himachal Pradesh, India for further reference. The infected leaves were examined primarily with a hand-lens and then with a dissecting microscope for the presence of mildew symptoms. Fungal structures present on the fresh leaves were removed using a strip of adhesive tape and examined by light microscopy with 3% potassium hydroxide as mounting medium. Microscopic observations were carried out to study the characteristics of mycelia, appressoria, size and shape of conidia, conidiophores and chasmothecia. The mycoparasite was observed with the help of a stereomicroscope and detected by 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 microspore and mounted in lactophenol cotton blue stain. Standard literature was consulted for identification of powdery mildew (Braun & Cook, 2012; Paul & Thakur, 2006) and mycoparasite (Belsare et al., 1980; Hashioka & Nakai, 1980; Kiss, 1998). An Olympus light microscope was used to examine fungal structures, and a CH2 Olympus light microscope equipped with a SONY DSC WX200 digital camera was used for microphotographs. For current name of powdery mildew fungus, MycoBank (www.mycobank.org)/Species Fungorum (www.speciesfungorum.org) websites were consulted.

Results

Identification of powdery mildew: Powdery mildew symptoms on leaves and stem of *E. hirta* were observed as white mycelium. The whole infected leaves were covered with white powdery mass as disease progress to severity. An increase in powdery mildew disease severity was observed with the decrease of temperature during winter season. Upon detailed morphological and microscopic examination it was revealed that this fungus belongs to genus *Podosphaera*. Further investigation identified it as *Podosphaera euphorbiae-hirtae*. The taxonomic descriptions and illustrations of the disease are as follows:
Podosphaera euphorbiae-hirtae (U. Braun & Somani) U. Braun & S. Takamatsu, Schlechtendalia 4: 28 (2000) = *Sphaerotheca euphorbiae-hirtae* U. Braun & Somani, Mycotaxon 25 (1): 263 (1986)= *Odium euphorbiae-hirtae* W. Y. Yen 1966.

Mycelium on leaves, amphigenous, white, persistent, forming hyaline or pale secondary hyphae, thick-walled; *conidiophores* arising from the upper surface of superficial hyphae, erect, cylindrical, *foot-cells* short, 17–27 × 7–12 μm, following cells about as long as the foot-cell or shorter, forming catenescent conidia; *conidia* ellipsoid-doliiform, 22–44 × 13–19 μm, with fibrosin bodies, *germ tubes* ± terminal, less than 30 μm long, apex with somewhat swollen (club-shaped) appressorium (Figure 1 & 3). Type: on *Euphorbia hirta*, India. Host range and distribution: on *Euphorbia pilulifera*, *Euphorbiaceae*; Asia (India).

Assessment of mycoparasitism: As powdery mildew disease attains severity, the white powdery mildew mass on infected leaves was turned to grayish at first and then brownish dots observed. Further mycological examinations under stereomicroscope, these brownish structures were identified as pycnidia within powdery mildew hyphae and conidiophores which reflected its mycoparasitic nature (Figure 1 & 2). Critical examinations revealed it a mycparasite belongs to fungus *Ampelomyces quisquali*. Detailed description and illustrations of the specimens are given here.

Ampelomyces quisqualis Ces. Botanische Zeitung 10:301(1852). Hyphae of the hyper-parasite were hyaline and septate; they were present within the hyphae, conidiophores, and conidia of infected mildews. Conidiogenous cells enteroblastic, phialidic, discrete, smooth, hyaline, 4.5-5.5 μm 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 36-123 × 22-45 µm, with no distinct ostiolum; conidia unicellular, hyaline, mostly guttulate conidia, 4.2-7.5 × 2-3.6 µm, dehiscence by apical rupture of pycnidium (Figure 2 & 4).

Figure 1: Powdery mildew on *Euphorbia hirta* (showing mycoparasitism - arrowed).

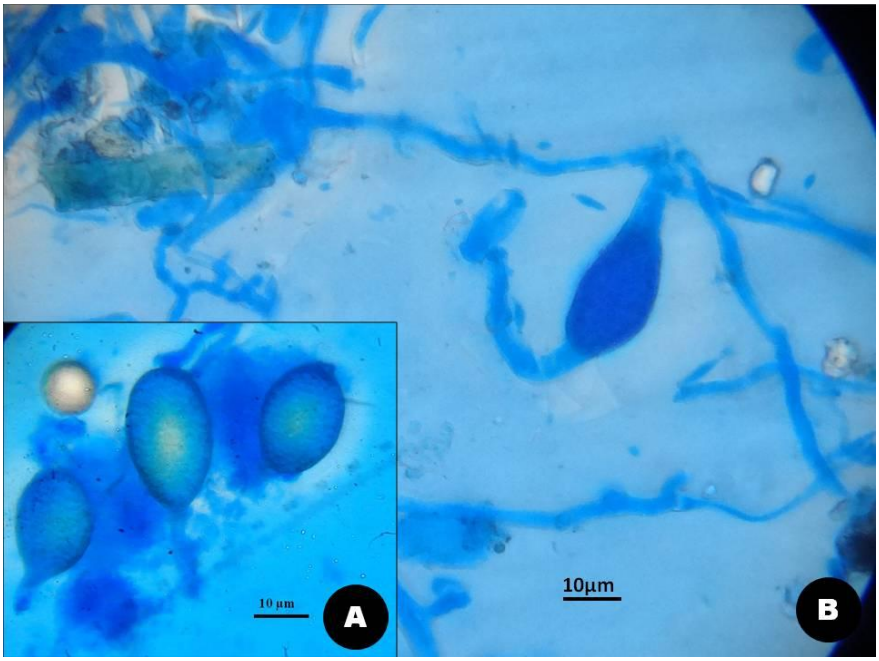

Figure 2: Mycoparasitism between *Podosphaera euphorbiae-hirtae* and *Ampelomyces quisqualis*. (A) Pycnidia of *A. quisqualis* parasitizing mycelium of *P. euphorbiae*; (B) Pycnidia of *A. quisqualis*. (Scale= 10µm)

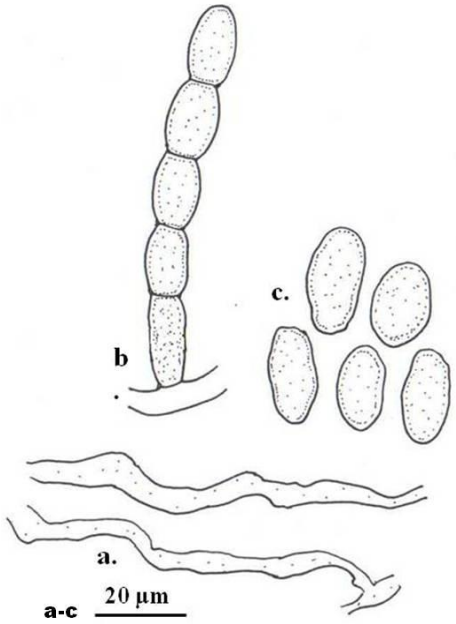

Figure 3: *Podosphaera euphorbiae-hirtae* anamorphic structures **(**a-c): a. Hyphae; b. Conidiophores with foot cell bearing chain of conidia; c. Conidia. (Scale= 20µm)

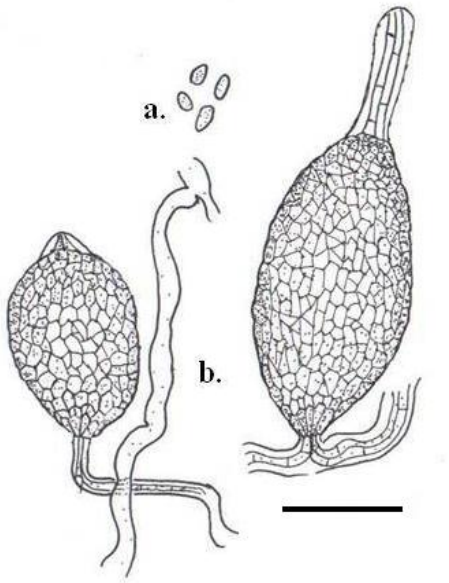

Figure 4: *Ampelomyces quisqualis* anamorphic structures (a-b): d. Pycnidia; e. conidia. (Scale= 20µm)

Discussion

Euphorbia hirta is a weed that grows in open grasslands, roadsides and pathways. It is a well-known medicinal plant and called sometime "Asthma plant" (BSBI, 2007). The stem and leaf extracts are useful in jaundice (Gautam et al., 2011). The present research shows the mycoparasitic relationship between powdery mildew fungus (*P. euphorbiae-hirtae*) on *E. hirta* and *Ampelomyces quisqualis*. The powdery mildew infection on *E. hirta* appeared as white powdery mass but turned off white upon mycoparasitic attack by *A. quisqualis*. The powdery mildew on *E. hirta* caused by various fungi is reported earlier also. The anamorphic state i.e. *Oidium* state of *Sphaerotheca fuliginea* was recorded previously by Barreto and Evans (1998) and Majumdar et al. (2007). Another powdery mildew fungus *Sphaerotheca euphorbiae* was reported from Maharashtra state also (Pawar et al., 2011). Similarly, different species of *Podosphaera* i.e. *Podosphaera euphorbiae*; *Podosphaera euphorbiae-helioscopiae*; and *Podosphaera euphorbiae-hirtae* were also reported from China, Taiwan, India, Japan, Malaysia, Singapore, Sri Lanka (Braun & Cook, 2011; Paul & Thakur, 2006). The fungal genus *Ampelomyces* is considered to be major intracellular mycoparasite of *Erysiphales* species worldwide. *Ampelomyces* mycoparasites were mostly studied for their use as biological control agents (BCAs) of powdery mildew infections of various crops (Legler et al., 2011). Review of literature provides a lot of information about *Ampelomyces* fungi on different powdery mildew fungi. *Ampelomyces quisqualis* has been reported as mycoparasitic on various powdery

mildew infections in natural conditions. The previous reports on association of *A. quisqualis* with powdery mildew of grapes (Legler et al., 2015; Caffi et al., 2012; Falk et al., 1995); apple (Vaidya & Thakur, 2005); crops, weeds, medicinal plants (Belsare et al., 1980; Kiss, 1998) and *Xanthium strumarium* (Gautam & Avasthi, 2016) revealed its wide host range and potential as biocontrol agent (Daoust & Hofstein, 1996). Genetic diversity of *Ampelomyces* mycoparasites isolated from different powdery mildew species was studied by Liang et al. (2007). *Ampelomyces quisqualis* has been found to produce certain enzymes which dissolve the host cell wall and penetrate and inactivate the host defense mechanism. Reports are available which show 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 the best of our knowledge, this is the first report of mycoparasitism of *Ampelomyces quisquali* on powdery mildew of *Euphorbia hirta* in India.

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References

Barnett HL, Binder FL, 1973. The fungal host-parasite relationship. Phytopathology **11**: 273–292.

Barreto RW, Evans HC, 1998. Fungal pathogens of *Euphorbia heterophylla* and *E. hirta* in Brazil and their potential as weed biocontrol agents**.** Mycopathologia **141**: 21–36.

Belsare SW, Moniz L, Deo VB, 1980. The hyperparasite *Ampelomyces quisqualis* Ces. from Maharashtra State, India. Biovigyanam **6**: 173–176.

Braun U, Cook RTA, 2011. Taxonomic manual of the Erysiphales (Powdery mildews). CBS Biodiversity Series No. 11, Utrecht, The Netherlands, 137–139 pp.

BSBI List, 2007. Botanical Society of Britain and Ireland.

Butler EE, 1957. *Rhizoctonia solani* as a parasite of fungi. Mycologia **49**: 354–373.

Caffi T, Legler SE, Bugiani R, Rossi V, 2013. Combining sanitation and disease modelling for control of grapevine powdery mildew. European Journal of Plant Pathology **135**(4): 817–829.

Daoust RA, Hofstein R, 1996. *Ampelomyces quisqualis*, a new biofungicide to control powdery mildew in grapes. In: Brighton Crop Protection Conference ± Pests & Diseases, vol. 1. Farnham, UK: British Crop Protection Council, 30–40 pp.

Falk SP, Gadoury DM, Cortesi P, Pearson RC, Seem RC, 1995. Parasitism of *Uncinula necator* cleistothecia by the mycoparasite *Ampelomyces quisqualis*. Phytopathology **85**: 794–800.

Gautam AK**,** Bhatia MK,Bhadauria R, 2011. Diversity and usage custom of plants of western Himachal Pradesh, India - Part I. Journal of Phytology **3**: 24–36

Gautam AK, Avasthi S, 2016. *Ampelomyces quisqualis* - a remarkable mycoparasite on *Xanthium strumarium* powdery mildew from Himachal Pradesh India. Journal on New Biological Reports **5**:1–6.

Hashioka Y, Nakai Y, 1980. Ultrastructure of pycnidial development and mycoparasitism of *Ampelomyces quisqualis* parasitic on Erysiphales. Transactions of the Mycological Society of Japan **21**: 329–338.

Jarvis WR, Slingsby K, 1977. The control of powdery mildew of greenhouse cucumber by water spray and *Ampelomyces quisqualis*. Plant Disease Reporter **61**: 728–730.

Jeffries P, 1995. Biology and ecology of mycoparasitism. Canadian Journal of Botany **73**: 1284–1290.

Kiss L, Russell JC, Szentivanyl O, Xu X, Jeffries P, 2004. Biology and biocontrol potential of Ampelomyces mycoparasites, natural antagonists of powdery mildew fungi. Biocontrol Science and Technology **14**(7): 635–651.

Kiss L, 1998. Natural occurance of *Ampelomyces* intracellular mycoparasites in mycelia of powdery mildew fungi. New Phytologist **140:** 709–714.

Kiss L, 2008. Intracellular mycoparasites in action: interactions between powdery mildew fungi and Ampelomyces. Stress in Yeasts and Filamentous Fungi **27**: 37–52.

Legler SE, Caffi T, Kiss L, Pintye A, Rossi V, 2011. Methods for screening new Ampelomyces strains to be used as biocontrol agents against grapevine powdery mildew. IOBC/WPRS Bulletin **6**: 149–154.

Legler SE, Pintye A, Caff I T, Gulyás S, Bohár G, Rossi V, Liss L, 2015. Sporulation rate in culture and mycoparasitic activity, but not mycohost specificity, are the key factors for selecting Ampelomyces strains for biocontrol of grapevine powdery mildew (*Erysiphe necator*). European Journal of Plant Pathology **144**(4): 1–14.

Liang C, Yang J, Kovács GM, Szentiványi O, Li B, Xu XM, Kiss L, 2007. Genetic diversity of *Ampelomyces* mycoparasites isolated from different powdery mildew species in China inferred from analyses of rDNA ITS sequences. Fungal Diversity **24**: 225–240.

Majumdar VL, Ahir RR, Verma OP, 2007. Anamorphic state of powdery mildew on some medicinal plants in Rajasthan. Plant Disease Research **22**(1): 95.

Paul YS, Thakur VK, 2006. Indian Erysiphaceae. Scientific Publishers: Jodhpur, India.

Pawar VP, Patil VA. 2011. Occurrence of powdery mildew on some wild plants from Khandesh region of Maharashtra state. Recent Research in Science and Technology **3**: 94–95.

Vaidya S, Thakur VS, 2005. *Ampelomyces quisqualis* Ces. – a mycoparasite of apple powdery mildew in western Himalayas. Indian Phytopathology **58**: 250–251.