



First report of *Ampelomyces* sp. as a mycoparasite of powdery mildew on *Cleome spinosa* from India

Avasthi S¹, Gautam AK², Verma RK³

¹ School of Studies in Botany, Jiwaji University, Gwalior, Madhya Pradesh- 474011, India

² Patanjali Herbal Research Department, Patanjali Research Institute, Haridwar-249405, Uttarakhand, India

³ Department of Plant Pathology, Punjab Agricultural University, Ludhiana- 141004, Punjab, India

Avasthi S, Gautam AK, Verma RK 2026 – First report of *Ampelomyces* sp. as a mycoparasite of powdery mildew on *Cleome spinosa* in India. Plant Pathology & Quarantine 16(5), 66–72, Doi 10.5943/ppq/16/1/5

Abstract

A powdery mildew infection was observed on the leaves of *Cleome spinosa* in Mandi District of Himachal Pradesh, India, during routine mycological surveys. Microscopic examination of the infected leaf samples revealed the presence of a powdery mildew pathogen and brownish intercellular pycnidia. Further critical and detailed analysis identified the fungus as *Erysiphe cruciferarum*, and the pycnidial taxon was identified as *Ampelomyces* sp., a mycoparasite. Earlier studies have reported the presence of species of *Ampelomyces* on a wide range of hosts, however, to date, there are no reports describing the mycoparasitism of *Ampelomyces* on powdery mildews on *Cleome* species. To the best of our knowledge, this is the first report of mycoparasitism of *Ampelomyces* sp. on powdery mildew of *Cleome spinosa* in India.

Keywords – *Erysiphe cruciferarum* – Hyperparasite – powdery mildew – pycnidia

Introduction

Ampelomyces is a naturally occurring fungal hyperparasite that can inhibit the growth and development of powdery mildew fungi. In this association of two fungi, one acts as a parasite over the other (also known as mycoparasitism) (Butler 1957). Here, the species of *Ampelomyces* infects the hyphae, conidiophores and fruiting bodies (cleistothecia) of a powdery mildew and produces characteristic brown pycnidia (fruiting bodies). This anamorphic fungus has the potential to infect both sexual and asexual structures of powdery mildews (Jeffries 1995). After colonizing diseased white patches of the powdery mildew that appear on the host plant, *Ampelomyces* sp. colonizes the pathogen and causes infection by producing pycnidia inside the different structures of powdery mildew. The hyperparasites spread rapidly, surrounding and covering their hosts and initiate effects of the powdery mildew in a few hours or days (Kiss et al. 2004, Kiss 2008). These biotrophic mycoparasites cause damage to their hosts and remaining active as long as the host remains active. Because of these characteristics, several *Ampelomyces* species are currently being marketed commercially as biocontrol agents to control powdery mildews on a variety of plant commodities. Some *Ampelomyces* species have recently been registered as biocontrol agents for powdery mildew, for example, the fungicide AQ10 is used commercially to control grape powdery mildew (Angeli et al. 2012, Németh et al. 2021).

The spider flower (*Cleome spinosa*) is an annual flowering plant that grows primarily in dry tropical biomes. It is native to Southern Mexico to tropical America and has been introduced to Korea, New Caledonia, the Indian Subcontinent, tropical Africa, the USA and Vietnam. This plant has applications in food, medicine, and the environment. It is used as a tall border in parks and gardens worldwide. Interestingly, this plant is pollinated by bats (Sivarajan and Sunil 1995, Bayat et al. 2018, Roy et al. 2024). During a routine mycological survey of Mandi District of Himachal Pradesh, India, a powdery mildew infection was observed on *Cleome spinosa*. Microscopic analysis revealed that the infection was combined with an unidentified fungus. Detailed examination exhibited the mycoparasitic nature of this taxon. Therefore, the goal of the present study was to examine the morphology and taxonomy of this powdery mildew and its mycoparasitic companion, which was collected from the plant *C. spinosa* in Himachal Pradesh, India.

Materials & Methods

Sample collection

The aerial parts, mostly leaves of *C. spinosa*, showing powdery mildew symptoms, were collected in labelled paper bags, dried between blotting paper sheets and preserved for further studies. Host plants were identified and confirmed by matching the collections with the herbarium.

Identification of Powdery Mildew Fungi

The infected leaf samples collected during the survey were initially assessed using a hand lens, followed by a dissecting microscope. For microscopic studies, a piece of clear adhesive tape was placed on the infected leaf exhibiting powdery mildew, stripped, and then fixed to a microscopic slide with a single drop of lactophenol cotton blue stain. Major characteristics such as mycelia on the host, appressoria, size and shape of conidia, conidiophores, and chasmothecia were analyzed through microscopic examinations. To examine mycoparasite associations, the infected leaves were inspected under a stereomicroscope for brownish intercellular pycnidia within the white powdery mildew mycelia. Under a dissecting microscope, these brown pycnidia were extracted with a needle and preserved in lactophenol cotton blue stain. Fungal structures were observed using a light microscope, photographed, and measured. Conidia, conidiophores, chasmothecia, and pycnidia sizes were quantified using micrometry. Standard literature was referenced for the identification of powdery mildew (Paul & Thakur 2006, Braun & Cook 2012) and mycoparasites (Belsare et al. 1980, Hashioka & Nakai 1980, Kiss 1998). An Olympus light microscope was employed to examine fungal structures, and a CH2 Olympus light microscope equipped with a SONY DSC WX200 digital camera was used for microphotographs. For the current name of the powdery mildew fungus, MycoBank (www.mycobank.org)/Species Fungorum (www.speciesfungorum.org) websites were consulted.

Results

The symptoms of powdery mildew disease were observed as a white powdery mass on the adaxial surface of *C. spinosa* leaves, which later turned grayish, followed by chlorotic, curled, and wilted leaves as the disease severity progressed. The examination of the powdery mildew during the onset of symptoms on the leaves revealed the presence of the fungus *Erysiphe cruciferarum*. Mycological analyses conducted in later stages of the infection revealed the presence of brownish intercellular pycnidia parasitic on the powdery mildew. After further analysis, it was determined as a mycoparasite, namely *Ampelomyces* sp. A detailed description and illustrations of both the powdery mildew pathogen and its mycoparasite are provided here.

The fungus is characterized by white or greyish white, effuse or in patches and persistent mycelium observed on leaves. Hyphae smooth, hyaline, septate, thin-walled, and had $38\text{--}72 \times 2.5\text{--}7$ μm hyphal cells, the hyphal appressoria were $3\text{--}6.5$ μm diam., single or in opposing pairs, and nearly unlobed to moderately lobed. Conidiophores erect, straight, and $45\text{--}105$ μm long. Foot cells cylindrical and relatively long, measuring roughly $15\text{--}40 \times 6\text{--}10$ μm , followed by shorter or

occasionally somewhat longer cells that form conidium singly. Conidia solitary, hyaline, cylindrical or somewhat ellipsoid-doliiform, $30\text{--}48 \times 12.5\text{--}17 \mu\text{m}$ with wrinkled, angular wall surface. Germ tubes terminated in a moderately lobed appressorium. Chasmothecia not observed.

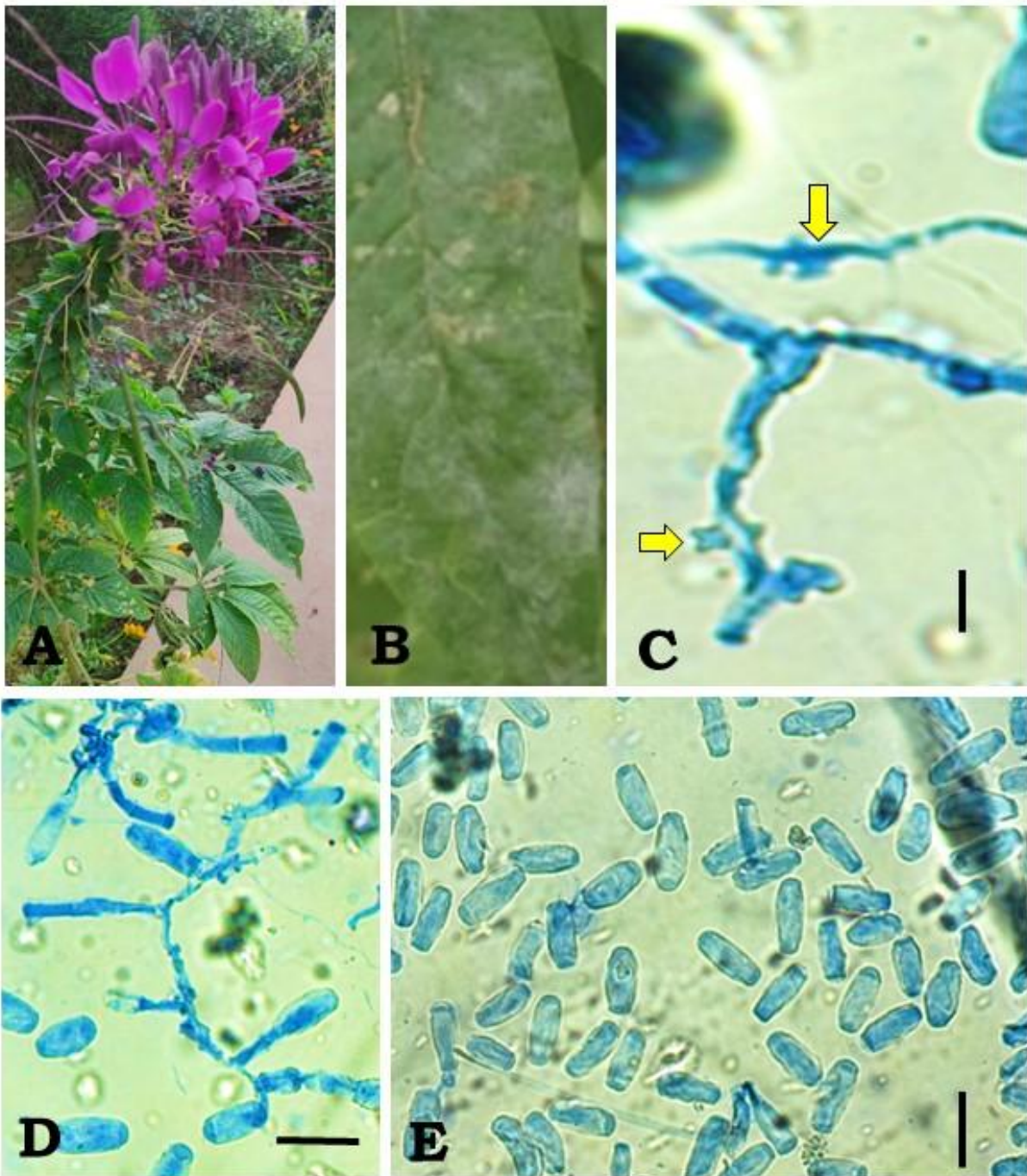


Fig. 1 – Symptoms of powdery mildew on *C. spinosa*. A-B Infection of the natural host. C hyphae with appressoria (arrow in yellow color). D Conidiophores with foot cell bearing conidia. E Conidia. Scale bars: C = $10\mu\text{m}$, D-E = $50\mu\text{m}$.

Erysiphe cruciferarum – Opiz ex L. Junell, Sv. Bot. Tidskr. 61(1): 217, 1967 (Fig. 1).
 = *E. radulescui* Docea, Lucr. Stint. Inst. Agric. Bucuresti, Ser. A, 11: 402, 1968
 = *E. cleomes* R.X. Li & D.S. Wang, Acta Mycol. Sin. 9: 266, 1990
 = *E. cruciferarum* var. *cleomes* (as “*cleomae*”) Y.S. Paul & V.K. Thakur, Indian Erysiphaceae: 31, Jodpur 2006

Mycoparasite Assessment

The white powdery mildew mass on infected leaves initially becomes gray before the appearance of brownish entities, identified as pycnidia associated with powdery mildew infection. The microscopic examination of these pycnidia revealed their mycoparasitic traits and classified this mycoparasite as the fungus *Ampelomyces* sp. A detailed description and illustrations of the specimens are provided here.

The hyphae of the hyper-parasite were septate and hyaline and were found within conidia, conidiophores and hyphae of powdery mildew pathogen. The pycnidial wall cells directly gave rise to conidiogenous cells (4–5.5 µm wide), which are enteroblastic, phialidic, distinct, smooth, and hyaline. Pycnidia were light brown in transmitted light, with a range of shapes from subglobose to pyriform, measuring 35–120 × 20–45 µm in size. The conidia aseptate, hyaline, and primarily guttulate, measuring 4.1–7.3 × 2–3.4 µm, dehiscence by apical rupture of pycnidium.

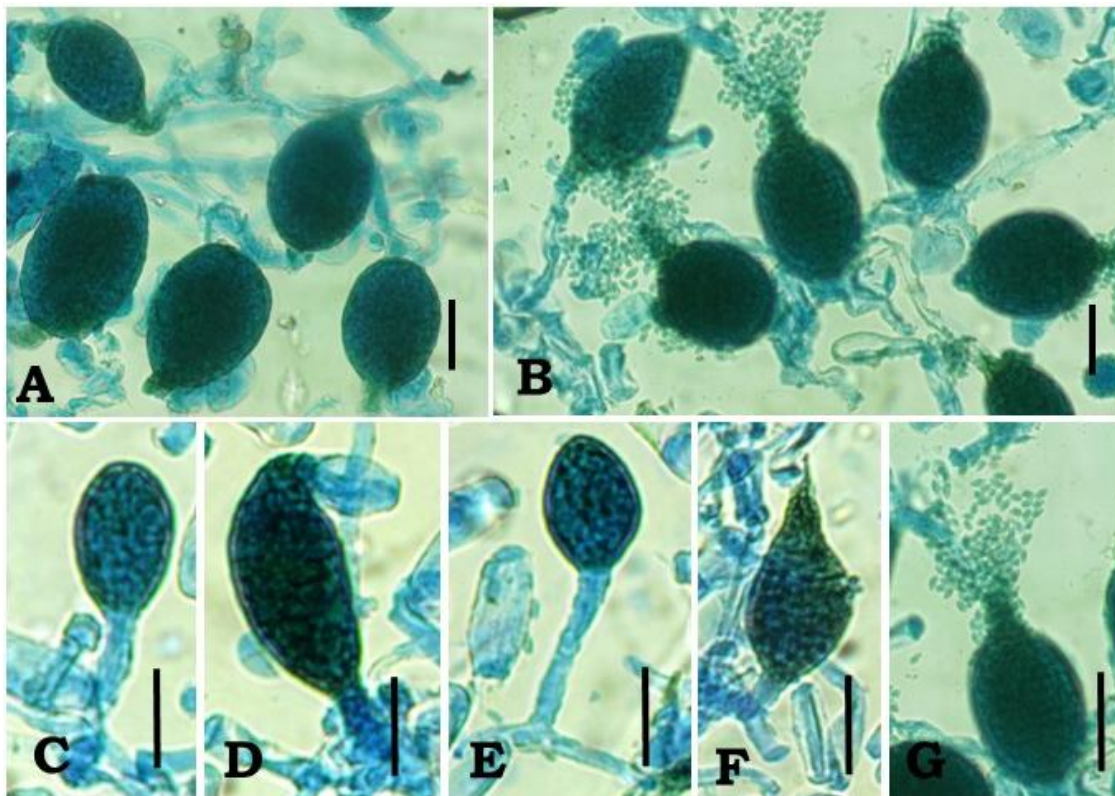


Fig. 2 – Mycoparasitism between *Erysiphe cruciferarum* and *Ampelomyces* sp. A Pycnidia of *Ampelomyces* sp. B Conidia dehiscence by apical rupture of pycnidium. C-G Different shapes and sizes of Pycnidia. Scale bar = A-G=50µm.

Ampelomyces – Ces. ex Schltdl., in Klotzsch, *Bot. Ztg.* 10: 301, 1852

(Fig. 2).

Discussion

The present study examined the *Ampelomyces* sp. and its mycoparasitic relationship with powdery mildew fungus (*E. cruciferarum*) affecting *Cleome spinosa*. The powdery mildew caused by *Erysiphe cruciferarum* has previously been reported on a broad array of hosts belonging to the families *Brassicaceae*, *Capparidaceae*, *Cleomaceae*, *Fumariaceae*, *Papaveraceae*, and *Resedaceae* (Braun & Cook 2012). However, due to its extensive host range and classification based on morphological features, *Erysiphe cruciferarum* is probably a species complex (Liu & Braun 2021). Furthermore, this fungus has been recorded on various species of *Cleome*, including *C. graveolens*, *C. hassleriana*, *C. pungens*, *C. spinosa*, and *C. viscosa* from different regions worldwide (Braun & Cook 2012). It has also been referred to as *Erysiphe cruciferarum* var. *cleomes* (Paul & Thakur

2006, Gautam & Avasthi 2018), but these names are not used according to the International Code of Nomenclature for algae, fungi, and plants under Article 40.6. Other reports of powdery mildews on *Cleome* spp. include *Erysiphe communis* (on *Cleome gigantea*, *C. pungens*, *C. spinosa* from the Czech Republic, Hungary, Switzerland, Portugal, Italy, Japan, and New Zealand) (Amano 1986), *Erysiphe cruciferarum* (on *Cleome gigantea*, *C. graveolens*, *C. hassleriana*, *C. pungens*, *C. spinosa* from the Czech Republic, Romania, Australia, Hungary, Switzerland, Italy, Portugal) (Braun 1995, Paul & Thakur 2006, Garibaldi et al. 2009), *Erysiphe polygoni* (on *Cleome gasslerana* from New Zealand and the United States) (Pennycook 1989, French 1989), *Erysiphe polyphaga* (on *Cleome pungens* from Switzerland), *Erysiphe radulescui* (*Cleome graveolens* from Romania) (Amano 1986), *Leveillula taurica* (on *Cleome glaucescens*, *C. hassleriana*, *C. spinosa* from Iran, USA, Brazil, Egypt, Italy, Romania, and South Africa) (Amano 1986, Sampangi et al. 2007, Carlos and Soares 2012), *Oidium* sp. (on *Cleome*, *Cleome* sp., and *C. spinosa* from the United States, Australia, Brazil, Zimbabwe) (Braun & Cook 2012), *Podosphaera xanthii* (on *Cleome rutidosperma* from Thailand) (Meeboon et al. 2016), and *Sphaerotheca fuliginea* (on *Cleome monophylla*, *C. spinosa*, *C. viscosa* from Thailand, India, Japan) (Amano 1986).

As a major intracellular mycoparasite of powdery mildew fungi, species of *Ampelomyces* has been extensively studied and exploited for their biological control potential (Legler et al. 2011, Prahll et al. 2023). Currently, 18 epithets are recorded worldwide on a variety of host plants (www.indexfungorum.com, last accessed on 08-06-2025), which open opportunity for researchers to explore all epithets as commercial biocontrol agents, however, some of these *Ampelomyces*-type were identified as species *Didymella* (Kiss 2004, Prahll et al. 2023). Scientific studies have also illustrated the efforts of earlier researchers to describe the mycoparasitic nature of these fungi on various powdery mildew infections (Kiss 2004). Members of *Ampelomyces* have been previously reported as a hyperparasite on powdery mildews infecting a variety of plant hosts, including grapes (Legler et al. 2015), apples (Vaidya & Thakur 2005), crops, weeds, and medicinal plants (Kiss 1998), as well as *Xanthium strumarium* (Gautam & Avasthi 2016a, Kumar & Chandel 2024), *Euphorbia hirta* (Gautam & Avasthi 2016b), and *Cucurbita maxima* (Thite et al. 2023). Its capacity to parasitise and eliminate a wide range of powdery mildew's suggests *Ampelomyces* isolates have strong potential as biocontrol agent (Daoust & Hofstein 1996). Furthermore, this genus has a broad global distribution that includes Brazil, Brunei, Bulgaria, Burma, Canada, China, Cuba, Darussalam, Fiji, India, Italy, Myanmar, New Zealand, Poland, the Russian Federation, South Africa, Spain, Sri Lanka, Ukraine, United States, and Venezuela (<https://fungi.ars.usda.gov/>). However, the taxonomic problem of *Ampelomyces* as species complex has been evaluated by Prahll et al. (2023) and Nemeth et al. (2024). Research has shown that what was once considered a single species, *Ampelomyces quisqualis*, is a complex of several distinct lineages. There is no information available that any species of *Ampelomyces* is mycoparasite on any powdery mildew infecting *Cleome* species. To the best of our knowledge, this is the first report of mycoparasitism of *Ampelomyces* sp. on powdery mildew of *Cleome spinosa*.

Acknowledgements

The authors would like to express their gratitude to their respective organizations for providing encouragement and all the assistance they needed to complete this scientific effort.

References

- Amano K. (Hirata) 1986 – Host range and geographical distribution of the powdery mildew fungi. Japan Science Society Press, Tokyo, Japan.
- Angeli D, Puopolo G, Maurhofer M, Gessler C et al. 2012 – Is the mycoparasitic activity of *Ampelomyces quisqualis* biocontrol strains related to phylogeny and hydrolytic enzyme production? *Biological Control* 63, 348–58. 9.
- Bayat S, Schranz EM, Roalson EH, Hall JC. 2018 – Lessons from Cleomaceae, the Sister of Crucifers. *Trends in Plant Science* 23(9), 808–821. Doi.10.1016/j.tplants.2018.06.010.

- Belsare SW, Moniz L, Deo VB. 1980 – The hyperparasite *Ampelomyces quisqualis* Ces. from Maharashtra State, India. *Biovigyanam* 6, 173–176.
- Braun U, Cook RTA. 2012 – Taxonomic Manual of the Erysiphales (Powdery Mildews), CBS Biodiversity Series No.11. CBS, Utrecht.
- Braun U. 1995 – The Powdery mildews (Erysiphales) of Europe. Gustav Fischer Verlag, 337.
- Butler EE. 1957 – *Rhizoctonia solani* as a parasite of fungi. *Mycologia* 49: 354–373.
- Carlos AC, Soares DJ. 2012 – The anamorphic state of *Leveillula Taurica* recorded on *Cleome spinosa* in north-eastern Brazil. *Mycosphere* 3, 289–292.
- 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.
- French AM. 1989 – California Plant Disease Host Index. Calif. Department of Food and Agriculture, Sacramento, California, U.S.A.
- Garibaldi A, Bertetti D, Gullino ML. 2009 – Outbreak of powdery mildew caused by *Erysiphe cruciferarum* on spider flower (*Cleome hassleriana*) in Italy. *Plant Disease* 93, 963.
- Gautam AK, Avasthi S. 2018 – Diversity of powdery mildew fungi from North Western Himalayan Region of Himachal Pradesh – a checklist. *Plant Pathology & Quarantine* 8(1), 78–99, Doi.10.5943/ppq/8/1/11
- Gautam AK, Avasthi S. 2016a. – *Ampelomyces quisqualis* – a remarkable mycoparasite on *Xanthium strumarium* powdery mildew from Himachal Pradesh India. *Journal on New Biological Reports* 5: 1–6.
- Gautam AK, Avasthi S. 2016b – *Ampelomyces quisqualis* Ces. – a mycoparasite of *Euphorbia hirta* powdery mildew in Himachal Pradesh, India. *Journal of Phytopathology and Pest Management* 3(2), 64–70.
- Hashioka Y, Nakai Y. 1980 – Ultrastructure of pycnidial development mycoparasitism of and *Ampelomyces quisqualis* parasitic on Erysiphales. *Transactions of the Mycological Society of Japan* 21, 329–338.
- Jeffries P. 1995 – Biology and ecology of mycoparasitism. *Canadian Journal of Botany* 73, 1284–1290.
- Kiss L. 1998 – Natural occurrence of *Ampelomyces* intracellular mycoparasites in mycelia of powdery mildew fungi. *New Phytologist* 140, 709–714.
- Kiss L. 2004 – How dangerous is the use of fungal biocontrol agents to nontarget organisms? *New Phytologist* 163, 453–455.
- Kiss L. 2008 – Intracellular mycoparasites in action: interactions between powdery mildew fungi and *Ampelomyces*. *Stress in Yeasts and Filamentous Fungi* 27, 37–52.
- Kiss L, Russell JC, Szentivanyi O, Xu X et al. 2004 – Biology and biocontrol potential of *Ampelomyces* mycoparasites, natural antagonists of powdery mildew fungi. *Biocontrol Science and Technology* 14(7), 635–651.
- Kumar V, Chandel S. 2024 – Natural occurrence and isolation of *Ampelomyces quisqualis* (mycoparasite) associated with rose, other powdery mildew infecting host plants in Himachal Pradesh, India. *International Journal of Fungi* 1(2), 23–28.
- Legler SE, Caffi T, Kiss L, Pintye A et al. 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, Caffi IT, Gulyás S et al. 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.
- Liu M, Braun U. 2021 – Powdery mildews on crops and ornamentals in Canada: a summary of the phylogeny and taxonomy from 2000 – 2019. *Canadian Journal of Plant Pathology* 44(2), 191–218. Doi.10.1080/07060661.2021.1986744

- Meeboon J, Hidayat I, Takamatsu S. 2016 – Notes on powdery mildews (Erysiphales) in Thailand I. *Podosphaera* sect. *Sphaerotheca*. Plant Pathology & Quarantine 6, 142–174.
- Németh MZ, Mizuno Y, Kobayashi H, Seress D et al. 2021 – *Ampelomyces* strains isolated from diverse powdery mildew hosts in Japan: Their phylogeny and mycoparasitic activity, including timing and quantifying mycoparasitism of *Pseudoidium neolycopersici* on tomato. PLoS One 16(5), e0251444. Doi.10.1371/journal.pone.0251444.
- Paul YS, Thakur VK. 2006 – Indian Erysiphaceae. Scientific Publishers, Jodhpur, India.
- Pennycook SR. 1989 – Plant diseases recorded in New Zealand. Pl. Dis. Div., D.S.I.R., Auckland.
- Prahl RE, Khan S, Deo RC. 2023 – *Ampelomyces* mycoparasites of powdery mildews – a review. Canadian Journal of Plant Pathology 45(4), 391–404. Doi.10.1080/07060661.2023.2206378
- Roy T, Mazumder T, Sharma P et al. 2024 – Anti-inflammatory property of ethanolic extract of *Cleome spinosa* leaves in an *in vivo* model of delayed-type hypersensitivity. Journal of Pharmacology and Pharmacotherapeutics 15(3), 306-315. Doi.10.1177/0976500X241253645
- Sampangi R, Glawe DA, Mohan SK. 2007 – First North American record of powdery mildew of *Cleome hassleriana* caused by *Leveillula taurica*. Plant Health Progress. Doi.10.1094/PHP-2007-0219-02-BR
- Sivarajan VV, Sunil CN. 1995 – *Cleome spinosa* Jacq. (Capparaceae), a new record for Peninsular India. Rheedeia 5 (2), 184–186.
- Thite SV, Pise NM, Bagal SN, Chavan CD. 2023 – *Ampelomyces quisqualis* – a remarkable mycoparasite on *Cucurbita maxima* powdery mildew from Satara (M.S.) India. Plant Pathology & Quarantine 13(1), 71–74. Doi.10.5943/ppq/13/1/7