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RESEARCH ARTICLE

Ampelomyces sp. - a mycoparasite of Powdery mildew of *Cucurbita moschata* Duch.: a report from Ziro Valley in Arunachal Pradesh

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Abstract

While conducting field studies of powdery mildew on pumpkin (*Cucurbita moschata* Duch.), in Ziro located at 27.63°N 93.83°E, 1500 meter above the sea level at Lower Subansiri district in Arunachal Pradesh, occurrence of *Ampelomyces* sp., a mycoparasite, was observed on its infected leaves during the fruiting stage. Powdery mildew is a highly destructive disease affecting wide range of host plants including members of Cucurbitaceae leading to tremendous yield loss. The study was conducted during the months of September to October, between 17.20 to 20.85 °C and relative humidity ranging from 58 to 68%. *Ampelomyces* is a mycoparasite, which parasitizes on powdery mildew and has been reported as a potential biocontrol agent (BCA) against the powdery mildew disease. It is reported to be an antagonist of powdery mildew fungal pathogen, wherein they compete for nutrition, invade the cytoplasm, inhibit sporulation of conidia leading to ultimate death of the fungal pathogen, resulting in considerable mitigation of the powdery mildew disease. In this study, sub-globose to globose brownish pycnidia of *Ampelomyces* sp. was observed parasitizing on the hyphae of powdery mildew fungal pathogen. Also, release of pycnidiospores on the conidiophore of powdery mildew pathogen was observed, representing the mycoparasitic nature of the *Ampelomyces* sp. Microscopic examination at 100x and 400x magnification revealed that the *Ampelomyces* pycnidia invades two primary targets, the hyphae and the conidiophores of the powdery mildew fungal pathogen. From the present study, the morphological examination of pycnidia has confirmed the natural occurrence of the mycoparasite, *Ampelomyces* sp., in Ziro valley, which could be explored for its further evaluation as a potential Biocontrol agent.

Key Words: Powdery Mildew; *Cucurbita moschata* Duch.; *Ampelomyces* sp.; Mycoparasite; Biocontrol agents (BCAs)

1. Introduction

Powdery mildew is a fungal foliar disease prevalent in both the green house and natural field conditions, leading to significant yield losses (Manjunath et al., 2020; Sletova, 2022). It is one of the major fungal diseases that requires extensive use of synthetic fungicides in the agricultural sector (Hewitt, 1998), which, in turn, contributes to adverse impacts on environmental health.

Pumpkin is one of the major vegetable crops cultivated worldwide due to its high nutritional values, which include: flavonoids, phenols, fibres, alpa-carotene, beta carotene, and minerals (Hussain et al., 2022). The cultivation of pumpkin in Arunachal Pradesh has long been existed as a seasonal food habit of the local ancestors and it continues to hold the same value to this day. Ziro in Arunachal Pradesh is located at 27.63°N 93.83°E, 1500 meter above the sea level, it is bestowed with favourable climate conditions, which encourages the inhabitants of the region to indulge in farming sector. Almost, every household in Ziro valley cultivate pumpkin as an important food crop in their home garden. However, the farmers also face several agricultural challenges, where powdery mildew is one of the prevalent diseases in the region, contributing to an annual production loss of 15-25%. Synergic infection of powdery mildew along with downy mildew exacerbate the damage of agricultural crops amounting to 50-70% of yield losses (Awad, 2000).

The use of chemical fungicide is the most effective control method employed against the powdery mildew disease, unfortunately it results in development of fungal resistant varieties, which leads to graduation diminution of the efficacy of existing fungicides necessitating in addition of new fungicides for the emerging resistant strains (De Miccolis Angelini et al., 2015; Pérez-García et al., 2009), creating a vicious cycle that is harmful for soil microflora and the environment. Therefore, in recent times, scientists have been focusing on researches to develop alternate safer methods as a counterpart of chemical methods, *Ampelomyces* sp. is reported as one of those methods which is regarded as a promising biocontrol agents (BCAs) against the powdery mildew disease.

The first report on parasitism of *Ampelomyces quisqualis* Ces. on powdery mildew fungus was reported by Cesati (1852), he also emphasized the culturable nature of this mycoparasite in synthetic media. In India, it has also been reported as a potent mycoparasite for successful management of powdery mildew by Kamat and Patwardhan in 1966. The first successful trial of *Ampelomyces* isolates on powdery mildew of *Cucumis sativus* has perpetuated the exploitation of *Ampelomyces* sp. that has evolved over time to be well-known as a potential biocontrol agent today. It is reported to infect the cytoplasm of the powdery mildew fungus, destroying their conidiophore and hyphae, thus collapsing the mycelium of pathogen in entirety, resulting in ultimate death of the pathogen (Kimura et al., 2023).

So, the present study was conducted to establish a report on the natural occurrence of the mycoparasite in association with powdery mildew of pumpkin (*Cucurbita moschata* Duch.) from Ziro, lower Subansiri district. Further, morphological examination was initiated to derive the identity of the mycoparasite, which will help illuminate future potential to culture, isolate and administer it as a substantial biocontrol agent against powdery mildew and several other fungal diseases that hamper the qualitative and quantitative yield of agricultural crops.

2. Material and method

2.1. Field survey

A field survey was conducted with an objective to evaluate the severity of powdery mildew of *Cucurbita mochata* Duch. in Ziro located at 27.63°N 93.83°E and about 1500 m above sea level, Lower Subansiri district during the months of September to October. During the survey, the powdery mildew infected vines constituted mixed symptoms showing white powdery patches in association with brownish pycnidia. Such infected leaves were collected in a zip-locker bag and brought to laboratory for the morphological characterization of the mycoparasite.

2.2. Morphological examination

A small segment of the collected leaves sample was excised using a scissor, visible brownish matter was scrapped onto a clean slide with a needle, mounted with cotton blue, and finally observed under compound microscope (Carl Zeiss Axio Lab. A1 in the Central Instrumentation Facility (CIF), Department of Botany, Rajiv Gandhi University) at different magnifications, 100x and 400x. Micrograph of thirty (30) matured pycnidia and pycnidiospores were carried out to analyze the shape, color, arrangement and to obtain the dimensional measurement through Axiocam ERc 5s, Zeiss camera.

3. Result

Powdery mildew infected leaves constituted white powdery patches on the surface of the leaves (Figure 1. A), while the parasitization of the mycoparasite on *Cucurbita moschata* Duch. leaves were visible as grey to dark brownish pycnidia mixed with white powdery mildew symptoms scattered all over the adaxial surface of the leaves (Figure 1.B).On average, about 20 % of the leaves of an individual plot was found to be in association with brownish pycnidia, the remaining leaves did not comprise of such pycnidial colonies though they were infected with powdery mildew disease or either the leaves were healthy and completely free from any other infections.

Microscopic examination under 100x magnification revealed mass of *Ampelomyces* pycnidia along with cylindrical and ovoidal powdery mildew conidia (Figure 1.C and Figure 1.D). At 400x magnification, the morphological features of the pycnidia were more distinct, the shape varied from sub-globose, ellipsoidal, limoniform to pyriform; surface of the pycnidia exhibits network of reticulate patterns, thick walled, light to dark brown (Figure 1.E), the measurement of size ranged between 56.18-68.86 x 36.07-47.06 µm (n=30), some of the colony of pycnidia were intermixed with mycelia of powdery mildew fungal pathogen.

The hyphae of powdery mildew pathogen were, hyaline, septate, arranged in chain on tip of conidiophore (Figure 1.F). The view of microparasitic relationship on microscope was revealed as the intracellular parasitization of *Ampelomyces* pycnidia on hyphae of powdery mildew fungal pathogen (Figure 2.G - K). Moreover, apical rupture of pycnidia released considerable amount of pycnidiosphores, morphologically it was unicellular, cylindrical, oblong, fusiform, curved, ovoid; hyaline, thin walled; aseptate; length and width measured between 6.28-9.22 x 2.99-4.36 µm (n=30) (Figure 2.L).

4. Discussion

The primary objective of the present survey was to assess the disease severity of powdery mildew of pumpkin (*Cucurbita moschata* Duch.), that has led to the observation and documentation this mycoparasite, *Ampelomyces* sp. in natural field conditions of the study site, Ziro, Lower subansiri district, Arunachal Pradesh. In this study, the occurrence of brownish pycnidia was not observed as a separate symptom, but were seen to appear conspicuously in an exclusive association over the hyphal and conidial structures of powdery mildew pathogen resulting in a composition of dual symptoms. This association reveals the natural co-existence and interactions between both the mycohost and the mycoparasite species.

The microscopic examination has revealed the morphology of the pycnidia varying from sub-globose, ellipsoidal, limoniform to pyriform; dimensional measure obtained ranged between 56.18-68.86x 36.07-47.06 µm; light to dark brown in color; and the morphology of the pycnidiophore varies from oblong to fusiform, which resembles with the morphological features of *Ampelomyces quisqualis* reported from powdery mildew of *Cucurbita maxima* by Thite et al (2023). Thus, these morphological conformations establish the identity of the examined mycoparasite as an *Ampelomyces* sp. Moreover, varied shades of pycnidial colors of this mycoparasite ranging from olive, pale green, dark brown to black has been reported from different myco-hosts with respect to the different types of plant hosts upon which they parasitize (Lee et al. 2007; Angeli et al., 2011). An experimental finding revealed that this pycnidia attains larger size when cultured on synthetic media (Hamzeh et al., 2022), whether or not, it augments the rate of pycnidiospores production is subject to contemporary research.

We have also recorded the instances of the intracellular parasitization of the *Ampelomyces* pycnidia on the hyphae of the powdery mildew pathogen, representing the mechanism of mycoparasitic relationship of the mycoparasite on the mycohost species. This interference of mycoparasite reduces the development of powdery mildew disease by impeding the life cycle of the powdery mildew pathogen, containing its development, distorting the conidia as presented in Figure (7-10), and eventually deteriorating the entire hyphae, these observations align well with the previous research publication reported by Hashioka and Nakai (1980). Release of pycnidiospores during the infection of *Ampelomyces* on the powdery mildew condiophores was also observed, these pycnidiospores germinate and grow internally by penetrating the hyphae of the mycohost, and continues its lifecycle by parasitizing upon the mycohost (Nemeth et al., 2021). As per the findings obtained from our study, we observed that the parasitism of *Ampelomyces* pycnidia often invades two primary targets, i.e. either the hyphae or the conidiophores of the powdery mildew fungal pathogen.

The average soil pH of the present study site was recorded at 6.8, it is in accordance with pH range showing maximal growth of *A. quisqualis* reported from powdery mildew of *Catha edulis* (Sztejnberg, 1979). Influence of pH for adequate sporulation and development of mycelial penetration of *Ampelomyces* at this mentioned pH has also been documented by Sharma (2006). Yuan et al (2006) had reported that the optimal pH for substantial mycelium growth of *A. quisqualis* was observed at 7.0. In this regard, it can be suggested that the ideal pH for enhanced growth and development of *Ampelomyces* sp. ranges between 6.5 to 7.0 pH.

Moreover, the detection of *Ampelomyces* sp. during the present survey infers, this mycoparasite is likely to prevail during the late harvest season of pumpkin (September-October). However, during the survey, the temperature range was recorded between 17.20 to 20.85 °C while the optimal temperature for mycelial growth of the mycoparasite is recorded to be 20-25 °C as reported by Chen et al. (2004). Kanipriya et al. (2019) had also reported that the maximum pycnidia growth of mycoparasite is observed at 25 °C. So, based on the cultivation timeline of our present study site, namely, Ziro, higher chances of its occurrence is probable during summer i.e. during the earlier stages of pumpkin crop. In Ziro, occurrence of powdery mildew is observed to be more severe during harvesting period than its earlier growth phases, and it falls during winter season where temperature drops below the optimal growth of the *Ampelomyces,* so it may be one of the reasons why occurrence of powdery mildew is intensive during the late harvest season (September to November). Conversely, in summer (July to August) the mean temperature of Ziro ranges around 20-25°C, much more ideal for growth and development of *Ampelomyces* enabling it to parasitize the fungal pathogen and thus mitigating the spread of the disease to some extent and it might be one of the reasons why spread of powdery mildew disease is lower during summer.

As the morphological examination conforms well with the characteristics of *Ampelomyces* sp., it is well established that it is a mycoparasite that parasitizes the powdery mildew fungal pathogen. At present, the primary remedial measure adopted to control powdery mildew disease is fungicides, however, prolonged application of this synthetic chemical pose serious environmental

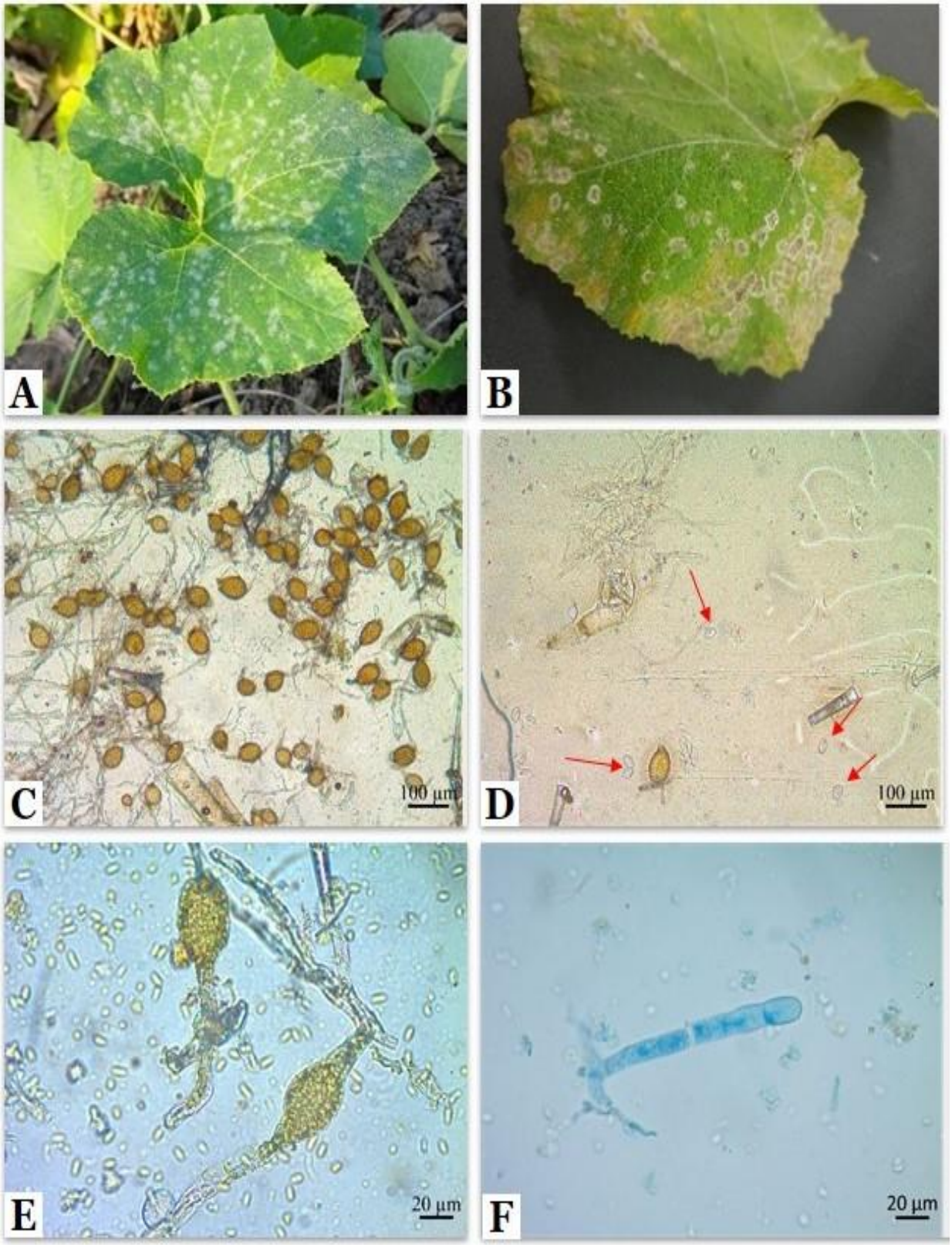

Figure 1. (A) Expression of powdery mildew symptom as a white powdery patch on the surface of *Cucurbita moschata* Duch. leaves (B) Intermixed infection of powdery mildew symptom in association with the mycoparasite, *Ampelomyces* sp. (C) Colony of *Ampelomyces* sp. pycnidia (D) An individual pycnidia along with powdery mildew fungal conidia (Arrow) around its vicinity. (E) Enlarged view, showing brownish pycnidia of *Ampelomyces* sp. (F) A hyphae of powdery mildew pathogen. Magnification=100x, Scale bar = 20 µm (C, D); 400x, 20 µm (E, F).

threats effecting wide range of economic crops (Petit et al., 2012), additionally, it also leads to imbalance and instability of the

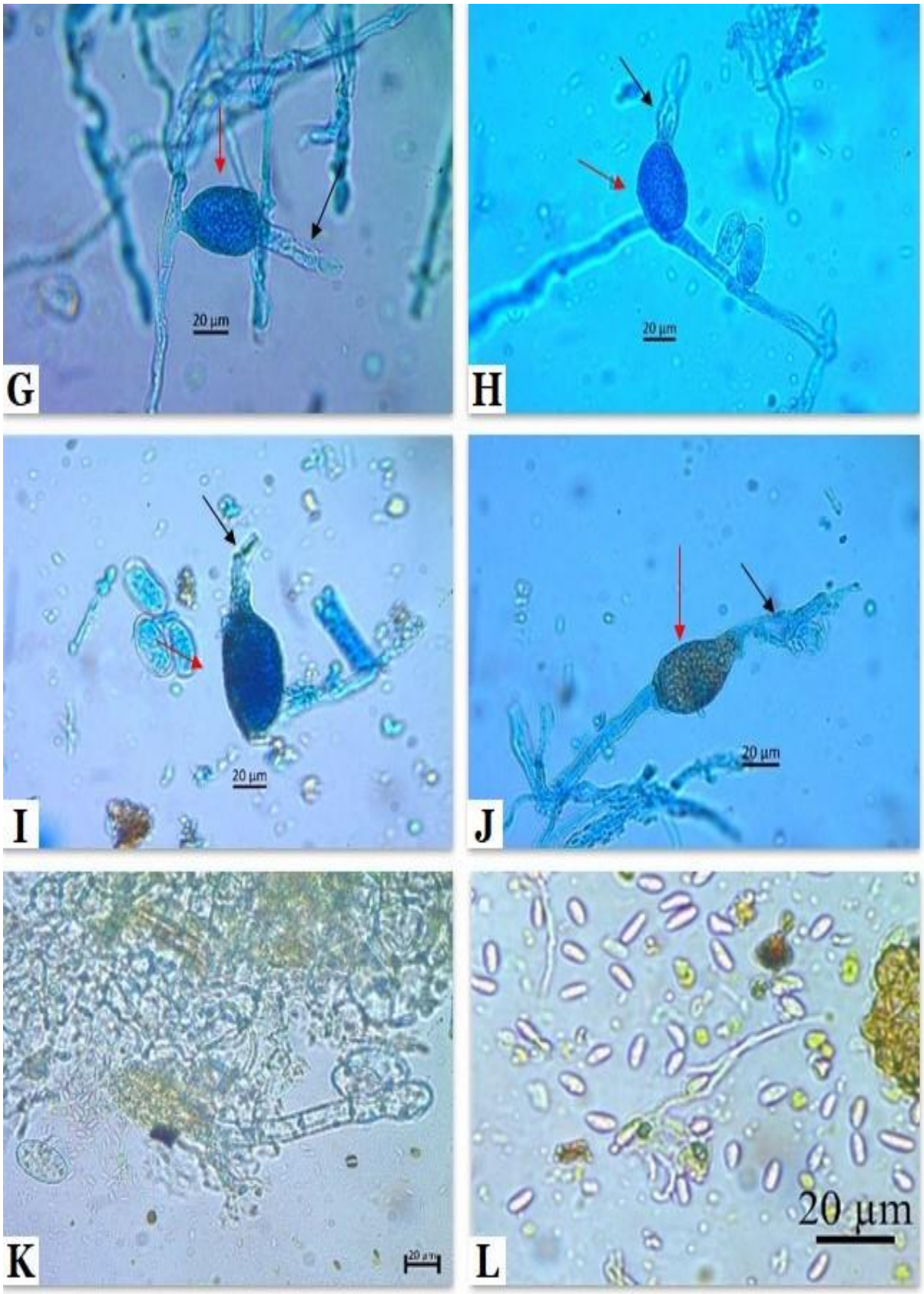

Figure 2. (G-J) Intracellular hyperparasitisation of pycnidia (Red arrow) of *Ampelomyces* sp. on the hypae (Black arrow) of powdery mildew fungal pathogen (K) Dehiscence of pycnidiophores of the mycoparasite on the conidiophore of the powdery mildew pathogen (L) Enlarged view of pycnidiophores of the mycoparasite. Magnification=400x, scale bar=20 µm (G-L)

biodiversity (Geiger et al., 2011). Treatments of powdery mildew using *Ampelomyces* have been reported to show considerable reduction of the powdery mildew disease, both in green house and natural field conditions. Therefore, in order to derive an eco-friendly substitute against chemical fungicides, especially to control the major destructive diseases in agriculture sector, certain beneficial microbes are being exploited in recent times, *Ampelomyces* is one such significant microbes adopted as a promising Biocontrol Agent against powdery mildew infection. Natural occurrence of *Ampelomyces* sp. is not only limited to pumpkin as documented in our present study, the scope of its occurrence and its application as a biocontrol agent transcends to wide range of other powdery mildew fungal pathogens which include several other host plants such as *P. xanthii* on zucchini (Carbo et al., 2021), *E. necator* on grapes (Falk et al., 1995) and *P. pannosa* on rose (Pasini et al., 1997), to mention a few. Besides its application as a biocontrol agent (BCA), an integrated approach to produce a combination of low dose chemical fungicides with compatible mycoparasite have also been obtained, which showed amplified rate of efficacy against the powdery mildew disease at much lower chemical concentration, a win-win scenario, where, both the environmental health is taken into consideration and the mitigation of the disease is also achieved at substantial scale (Sharma et al., 2009).

5. Conclusion

The present study confirmed the natural occurrence of mycoparasite, *Ampelomyces* sp. on fungal pathogen of powdery mildew of pumpkin (*Cucurbita moschata*. Duch), in Ziro, Lower Subansiri district of Arunachal Pradesh during winter season in temperature range of 17.20 to 20.85 °C. This report of occurrence of mycoparasite *Ampelomyces* sp. highlights the natural association of this fungus on fungal powdery mildew pathogen of pumpkin that paves a way for future researchers to initiate a holistic approach for its further isolation, screening, evaluation and exploration for application as a Biocontrol Agent (BCA) against powdery mildew disease along with several other fungal diseases in this region. Considering the time period of present survey, the presence of the mycoparasite, *Ampelomyces* sp., is certain to be encountered during the late harvest period (October-November) and the possibilities of its occurence during early cropping period (July-August) of pumpkin with temperature ranging between 20 to 25 °C in Ziro valley.

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Conflict of interest

Authors have no conflict of interests for publication of this research work.

Author's Contribution

Tenya Rina conceptualized, designed and supervised the experiments of this research work. Marbom Riba carried out the field survey, recorded the data and noted all the observations; and performed the microscopic examinations and prepared the manuscript.

References

Angeli D, Maurhofer M, Gessler C and Pertot I. 2012. Existence of different physiological forms within genetically diverse strains of *Ampelomyces quisqualis*. Phytoparasitica 40: 37-51.

Awad NGH. 2000. Reaction of some cucurbits against *Sphaerotheca fuliginea* in relation to their physiological and histopathological changes. Journal of Agriculture and Sciences 8: 829-851.

Carbó A, Teixidó N, Usall J, Solsona C and Torres R. 2021. Formulated *Ampelomyces quisqualis* CPA-9 applied on zucchini leaves: influence of abiotic factors and powdery mildew mycoparasitization. European Journal of Plant Pathology 161(1): 37-48.

Cesati VD. 1852. *Ampelomyces quisqualis* Ces. Botanische Zeitung 10: 301-302.

Chen L Honghai Z, Baodu L, Yingchen Z and Guozhong L. 2004. Biological characteristics of *Ampelomyces quisqualis* hyperparasite on tickseed powdery mildew.Journal of Yunnan Agricultural University 19(6): 648-652.

De Miccolis Angelini RM, Pollastro S and Faretra F. 2015. Genetics of fungicide resistance. In: H Ishii, D Hollomon (Eds.): *Fungicide Resistance in Plant Pathogens*. Springer, Tokyo. Pp 13-34. https://doi.org/10.1007/978-4-431-55642-8_2

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

Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB and Inchausti P. 2011. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland 11(2):97-105.

Hamzeh S, Naffaa W and Azmeh MF. 2022. Occurrence of the Hyperparasite *Ampelomyces quisqualis* on *Golovinomyces neosalviae* (Erysiphaceae), Causal Agent of Powdery Mildew on Common Sage (*Salvia officinalis*). Arab Journal of Plant Protection 40(2):158-163.

Hashioka Y and Nakai Y. 1980. Ultrastructure of pycnidial development and mycoparasitism of *Ampelomyces quisqualis* parasitic on Erysiphales. Trans Mycol Soc Jpn 21:329–338

Hewitt HG. 1998. *Fungicides in Crop Protection*. CAB International Publishing, Wallingford, UK.

Hussain A, Kausar T, Sehar S, Sarwar A, Ashraf AH, Jamil MA and Quddoos MY. 2022. Determination of total phenolics, flavonoids, carotenoids, β-carotene and DPPH free radical scavenging activity of biscuits developed with different replacement levels of pumpkin (*Cucurbita maxima*) peel, flesh and seeds powders. Turkish Journal of Agriculture-Food Science and Technology 10(8): 1506-1514.

Kamat MN and Patwardhan PG. 1966. Hyperparasite Ampelomyces and its role in the development of powdery mildews. The Proceedings of the Autumn School in Botany, Mahabaleshwar. Pp. 132-137.

Kanipriya R, Rajendran L, Raguchander T and Karthikeyan G. 2019. Characterization of Ampelomyces and its Potentiality as an Effective Biocontrol Agent against *Erysiphe cichoracearum* DC Causing Powdery Mildew disease in Bhendi (*Abelmoschus esculentus* (L.) Moench). Madras Agricultural Journal 106:267-278.

Kimura Y, Németh MZ, Numano K, Mitao A, Shirakawa T, Seress D, ... and Nonomura T. 2023. Hyperparasitic Fungi against Melon Powdery Mildew Pathogens: Quantitative Analysis of Conidia Released from Single Colonies of *Podosphaera xanthii* Parasitised by Ampelomyces. Agronomy 13(5): 1204.

Lee SY, Hong SK, Kim YK and Kim HG. 2007. Selection and identification of a hyperparasite, *Ampelomyces quisqualis* 94013 for biocontrol of cucumber powdery mildew. The Korean Journal of Mycology 35(2): 121-127.

Manjunatha L, Singh S, Ravikumara BM, Reddy GN, Senthilkumar M.2020. *Ampelomyces*. Beneficial Microbes in Agro-Ecology 44: 833-860.

Németh MZ, Mizuno Y, Kobayashi H, Seress D, Shishido N, Kimura Y and Nonomura T. 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. Publiv library of science one 16(5): 1-20.

Pasini C, D'Aquila F, Curir P and Gullino ML. 1997. Effectiveness of antifungal compounds against rose powdery mildew (*Sphaerotheca pannosa* var. rosae) in glasshouses. Crop Protection 16(3): 251-256.

Pérez-García A, Romero D, Fernández-Ortuño D, López-Ruiz F, De Vicente A, Torés JA. 2009. The powdery mildew fungus *Podosphaera fusca* (synonym *Podosphaera xanthii*), a constant threat to cucurbits. Mol. Plant Pathology 10: 153–160.

Petit AN, Fontaine F, Vatsa P, Clément C and Vaillant-Gaveau N. 2012. Fungicide impacts on photosynthesis in crop plants. Photosynthesis research 111: 315-326.

Sharma RR, Singh D and Singh R. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological control 50(3): 205-221.

Sharma SK. 2006. Integrated Management of Powdery Mildew of Apple, ICAR Adhoc Research Scheme, Final Report: 2003-2006.

Sletova ME. 2022. Species composition and identification of pathogens of real powdery mildew of pumpkin crops. Vegetable crops of Russia 4:91-97.

Sztejnberg A. 1979. Biological control of powdery mildews by *Ampelomyces quisqualis*. Phytopathology 69:1047.

Thite SV, Pise NM, Bagal SN and Chava CD. 2023. *Ampelomyces quisqualis* – a remarkable mycoparasite on Cucurbita maxima powdery from Satara (M. S) India. Plant Pathology and Quarantine 13(1): 71-74.

Yuan QL and Gao LQ.2006. Biological characteristics of *Ampelomyces quisqualis* parasitizing ladyslipper powdery mildew. Chinese Journal of Biological Control 22(3): 230-233.