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Morphological Characterization of Powdery Mildew of Pumpkin (*Cucurbita moschata* Duch.), in Two Agro-Climatic Regions of Arunachal Pradesh, India

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ABSTRACT

Pumpkin is an important vegetable crop grown in tropical and subtropical regions of the world. One of the most prevalent diseases common to pumpkin is powdery mildew, a widespread fungal disease leading to significant yield loss. To determine, the occurrence of powdery mildew disease and the morphology of the causal organism, a phytopathological survey was conducted in two agro-climatic regions namely: Ziro (1500 m) and Medo (250 m) in Arunachal Pradesh during October 2022. The mean temperature of Ziro and Medo ranged between 19-21℃ and 24-26℃ coupled with mean relative humidity ranging from 66-77 % and 90-96 % respectively. Occurrence of powdery mildew was encountered in open field conditions of both the sampling fields representing different agro-climatic zones. Microscopic study was performed to examine the anamorph and teleomorph of the causal organisms. Two types of conidial shapes were observed, three types of germ tubes were observed and the cleistothecia was observed at initial to mature stages. This morphological elucidation provided a convincible insight on presence of *Podosphaera xanthii* and *Erysiphe cichoracearum* as the causal organisms of powdery mildew of pumpkin in the natural field conditions of both the study sites ranging from subtropical to tropical Zones of Arunachal Pradesh, India.

Keywords: Powdery mildew, *Cucurbita moschata* Duch., Anamorphs, Cleistothecia, *Podosphaera xanthii*, *Erysiphe cichoracearum*

INTRODUCTION

Powdery mildew is a destructive fungal disease infecting wide range of vegetable crops, resulting in dire decline of crop production in India (Parameshwar and Kulkarni, 2018). It is prevalent in temperate and tropical to sub-trophical Zones of the world (Paul and Thakur, 2006; Mieslerova *et al.*, 2022). Hence, the present study was carried out in two agro-climatic Zones, ranging from tropical (Medo, 250 m) to subtropical Zone (Ziro, 1500 m), of Arunachal Pradesh, to determine the occurrence of powdery mildew of pumpkin (*Cucurbita moschata* Duch.).

Pumpkin is one of the important food crops grown in Arunachal Pradesh since time immemorial. One of the study sites, Ziro, is endowed with conducive climatic conditions suitable for agricultural farming. It is a renown farmland for production of organic vegetables including pumpkin, kiwi, apple, and several other agricultural products. Another region, Medo, is one of the leading commercial producers of pumpkin in the state. An Approximate record of 5000 tons of pumpkin harvest is exported annually from Medo to its neighboring regions (Arunachal Observer, 17th October, 2023).

Occurrence of powdery mildew was observed in natural field conditions of both the studysites. Powdery mildew corrupts the growth mechanism of the host, leaving it to produce low quality fruits, lessening up to 50% of the agricultural production (Reuveni and Reuveni, 1995). The two main causal organisms responsible for powdery mildew of pumpkin are *Podosphaera xanthii* and *Erysiphe cichoracearum*, the former being prevalent in tropical to subtropical climate and the later in temperate zones (Leao *et al.*, 2019), considering this aspect the present study also intends to analyze the differential population structure of causal organism from the respective study sites. The symptoms caused by both the reported pathogens are very similar (Block and Ritsma, 2005) but the species are distinguishable based on morphology of anamorph (Braun *et al.*, 2002) and the teleomorph characters (Sletova, 2022). This study presents the first report on study of powdery mildew of pumpkin in the state, since, no study has been reported till date.

Thus, the present study was conducted with an objective to determine: the occurrence of powdery mildew of pumpkin in the natural field conditions of two different agro-climatic regions, morphological characterization of the causal organisms and determination of differential population structures of the causal organisms in two agro-climatic regions ranging from tropical to subtropical Zones of Arunachal Pradesh, India

MATERIALS AND METHODS

A field survey was conducted in two agro-climatic regions of Arunachal Pradesh namely: Ziro (1500 m) located at 27.63ºN 93.83ºE, Lower Subansiri District and Medo (250 m) located at 28.11ºN 98.80ºE, Lohit District, during kharif season, October, 2022. To determine the occurrence of powdery mildew disease, three cultivation fields of pumpkin (*Cucurbita moschata* Duch.) from both Ziro and Medo were taken into consideration. Throughout the process of survey, primary meteorological data of the study sites were recorded: relative humidity was recorded using a hygrometer, temperature with digital thermometer and the geocoordinates and altitude with the help of Global Positioning System (GPS).

Sampling was implemented considering the severely infected pumpkin leaves, exhibiting about 70% of its surface area infected with powdery mildew symptoms. The collected samples consisted a total of 30 leaves, 5 from each location of both the study sites. It was transferred to a container in 4% formalin, moreover, for dry sampling, fresh leaves were collected in Zip-locker bag. Further, the Collected samples were duly labelled and brought to T.R. laboratory, Dept. of Botany, RGU, Doimukh for further analysis.

The morphology characterization was conducted using compound microscope, Carl Zeiss Axio Lab. A1 in the Central Instrumentation Facility (CIF), Department of Botany, Rajiv Gandhi University.

A small section of symptomatic leaf area from collected samples was scrapped into a clean slide using a needle, mounted with few drops of lactophenol and cotton blue to enhance the architecture of studied causal organism and further examined under microscope. A total of 120 conidia, 20 from each location of corresponding study sites, was studied to analyze the shape and size of the conidia of causal organism.

To determine the structure of germ tube, 10 infected leaves from both the study sites were cultured in 2% Agar-water media. It was incubated at 27±2℃ for 12 hours under fluorescent light in Laminar Air Flow Chamber. This procedure was performed by altering a slight modification to the method described by Aguiar *et al.*, (2012).

Further, for detection of fibrosin body, the conidia from symptomatic leafs were scraped into a clean slide and stained with 3% KOH (Kable and Bellantyne, 1963).

RESULTS

During the present survey, infection of powdery mildew was observed on aerial parts of pumpkin host (*Cucurbita moschata* Duch.) at natural field conditions of both the study sites located at different agro-climatic regions namely Ziro (1500 m), Lower Subansiri district and Medo (250 m), Lohit district of Arunachal Pradesh, India. Based on the primary data the mean temperature of Ziro and Medo ranged between 19-21℃ and 24-26℃ coupled with mean relative humidity ranging from 66-77 % and 90-96 % respectively.

SYMPTOMOLOGY

The symptom of powdery mildew of pumpkin (*Cucurbita moschata* Duch.) manifested as cool white granules, spreading extraneously all over the vines. These epiphyllous white granules characterize the ectoparasitic nature of the causal organisms. The infection was visible on the aerial parts of the vines, including the leaves, petioles and the stems, but less common in flowers and the fruit bodies. The degree of severity distinctly varied; the older leaves were more infected, suggesting they are more susceptible to the disease as compared to younger leaves. Initial infection appeared as tiny white spots sparingly scattered all over the surfaces, which latter coalesces and expands the proportion of disease severity. As the disease progresses, the color transitions from cool white to pale yellow, and eventually to dark gray at the threshold stage. With time, this superficial fungal mycelium grows and accumulates into thick layers over the surface of the interaction, and the powdery texture eventually turns into creamy base, apparently it interferes the physical contact between the stomata and the direct sunlight which is critical for the phenomenon of photosynthesis. The symptom was severe on leaf's surfaces than most other parts of the pumpkin plant. It was obvious on both sides of the leaves; however, much more severe on the adaxial surfaces and mild on abaxial surfaces of the leaves.

Morphological characterization

Perusal of microscopic observation demonstrated different morphological aspects that shows characteristic differences in shape and size of conidia, content of fibrosin bodies and structures of germ tube. Presence of chasmothecia was observed from initial to mature stages.

Conidia

A total of 120 conidia was microscopically evaluated from both the study sites, 20 from each location. The mycelium was hyaline; conidiophore, unbranched and septate; conidia, cylindrical and ovoid in shape, arranged in chain at the apical termini of upright conidiophore. All these studied morphologies were similar for both Ziro and Medo.

Seventy seven percent (77%) of the examined conidia from Ziro was ovoid in shape and the rest 23% was cylindrical in shape. Similarly, 63% was

ovoid in shape and 37% was cylindrical in case of Medo. The dimensional parameters drawn from the measurement of conidia, ranged between 26.81-35.95 µm in length and 15.33-26.99 µm in breadth for ovoid conidia and 27.27 -35.12 µm in length and 13.64 -16.28 µm in breadth for cylindrical conidia observed from Ziro. And for Medo, it ranged between 24.61-32.74 µm in length and 12.24-20.26 µm for ovoid conidia; 28.48-32.47 µm in length and 13.00-15.81 µm in breadth for the cylindrical conidia.

Germ tube

Evaluation of germ tube was carried out based on the total number of conidia germinated in PDA media under thetemperature (27±2 ℃). The rate of conidial germination of Medo was exceedingly higher than that of Ziro. Forty-six (46) germinated conidia were observed from Medo and only nine (9) from Ziro. Microscopic observation revealed its types of germ tube based on position (apical or lateral) and its morphology (simple or bifurcated). A bifurcated germ tube at lateral position, a simple germ tube at lateral position and at apical position were observed. Basically, the bifurcated germ tube was studied exclusively from ovoid conidia while simple germ tube was observed from both the ovoidal and cylindrical conidia. Also, the ovoid conidia had protrusion of germ tube originating at lateral position, while the protrusion of germ tube of cylindrical conidia originated from both apical and lateral position. Similar architecture of germ tubes was observed from both Ziro and Medo (**Table 3**).

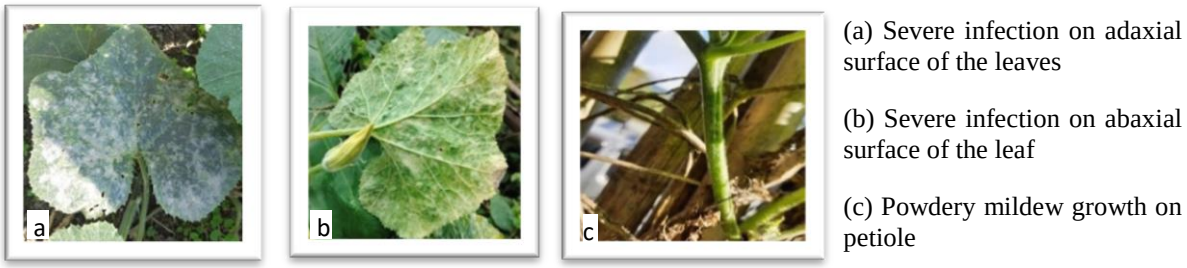
Fibrosin bodies

The conidia observed at 400x magnification in 3% potassium hydroxide solution under compound microscope inferred the presence of fibrosin bodies. As per the known sources of literature it is a conidial inclusion present in *Podosphaera-xanthii* but scarce in *Erysiphe cichoracearum*. The fibrosin bodies appeared as tiny crescent or rod-shaped elements immersed within the internal domain of the fungal conidia. It was detected in ovoid conidia and absent in cylindrical conidia examined from the powdery mildew samples of respective study sites.

Perfect Stage

Different developmental stages of cleistothecia were observed under the compound microscope, representing initial to mature stages. It was globose to sub-globose in shape, light to dark brown, covered with radiating myceloid appendages attached at its exterior surface. Among all the surveyed fields, presence of cleistothecia was observed in two fields of Ziro and in one field of Medo.

Two cleistothecia observed from one of the fields of Ziro (Old Ziro) was apparently in initial stage, measuring approximately 22.99 µm and 47.78 µm in diameters. Three matured cleistothecia were observed from another field of Ziro (Beere), measuring approximately 88.32 µm, 71.99 µm and 60.44 µm in diameter. One of the matured chasmothecium was in a ruptured state, releasing several ellipsoidal asci measuring about 21.11-30.11 x 15.51-23.29 µm. Further, two observations were made from Medo (Medo-6 kilo), measuring about 32.147 µm and 54.498 µm in diameter.



(a) Severe infection on adaxial surface of the leaves

(b) Severe infection on abaxial surface of the leaf

(c) Powdery mildew growth on petiole

Figure 1: Expression of cucurbit powdery mildew symptoms on pumpkin (*Cucubita moschata* Duch.) host plant in natural field conditions of two agro-climatic zones of Arunachal Pradesh, India

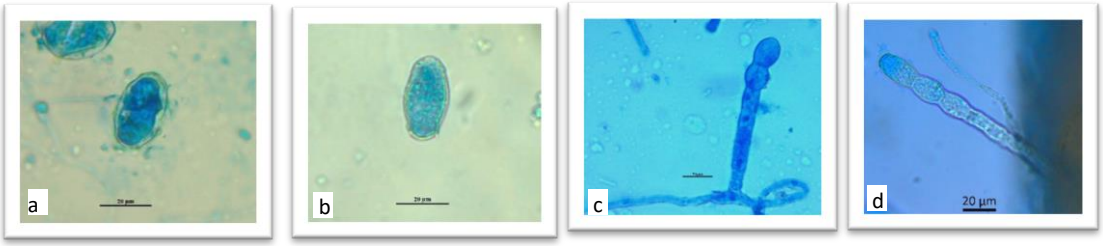


Figure 2: Morphology of conidia of powdery mildew causal organism on pumpkin plant plants (*Cucurbita moschata* Duch.). a, Ovoid conidium; b, Cylindrical conidium; c, Ovoid conidia on upright conidiophore; d, Chain of ovoid cylindrical conidia on upright conidiophore. Magnification = 40x; Scale bar=20 µm.

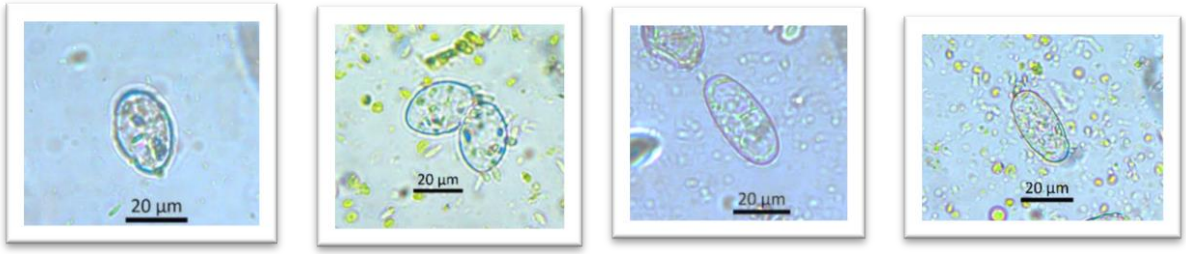


Figure 3: Observation of Fibrosin body in powdery mildew conidia. a and b, Ovoid conidia with rod shaped fibrosin bodies (Arrow); c and d, No residue of fibrosin bodies in cylindrical conidia.

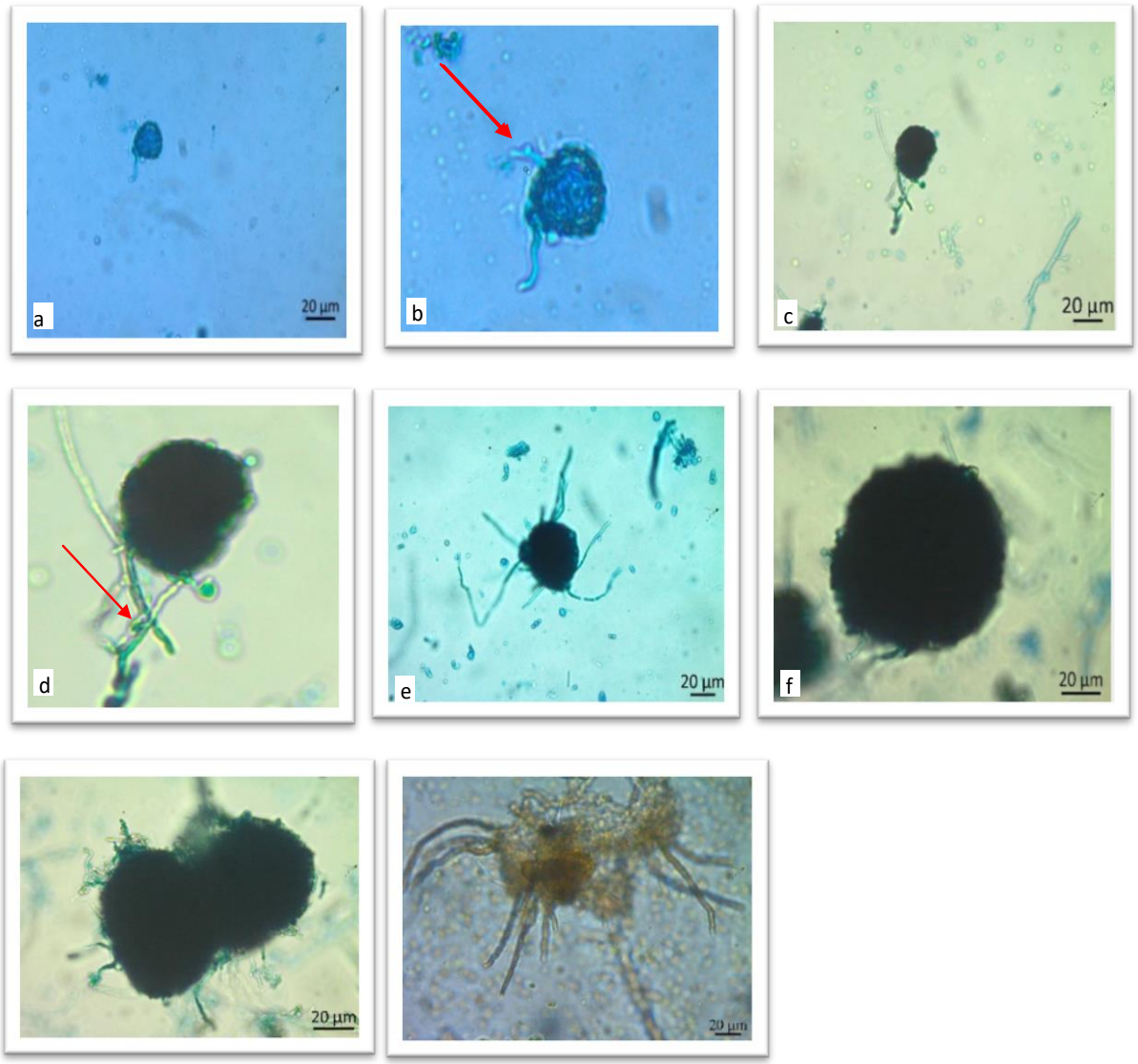


Figure 4: Morphological structure of perfect stage observed from powdery mildew samples collected from Ziro and Medo. a, Early stage cleistothecium of studied pathogen (~22.99 µm in diameter) observed under compound microscope at 40X (Scale bar = 20µm); b, Enlarged image of (a) showing closer view of dichotomously branched appendage at terminal end (arrow); c, Globose cleistothecia (~32.14 µm in diameter) at 40X (Scale Bar = 20 µm); d, Enlarged image of (c), showing closer view of dichotomously branched appendage (arrow); e, Early stage cleistothecium of studied pathogen (~47.782 µm in diameter) at 40X (Scale bar = 20µm); f, Matured cleistothecium (~88.323 µm) at 63x (Scale bar = 20 µm); g, Two globose cleistothecia (~71.99 µm and 60.440 µm in diameter) at 63X (Scale Bar = 20 µm); h, Ruptured chasmothecium with several visible asci (arrow), observed under compound microscope at 40X (Scale bar = 20 µm).

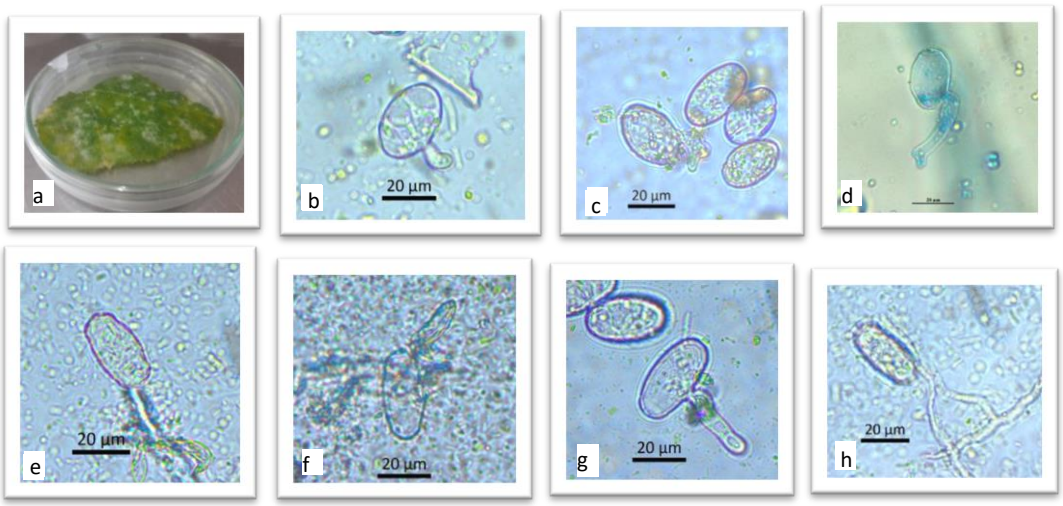


Figure 5: Leaf assay and microscopic observation of germ tube of powdery mildew conidia from collected samples of two agro-climatic zones of Arunachal Pradesh, India. a, Powdery mildew infected leaf cultured in 2 % Agar-water media; b, Ovoid conidia with simple lateral germ tube; c and d, Ovoid conidia with bifurcated lateral germ tube; e and f, Cylindrical conidia with simple apical germ tube; g and h, Cylindrical conidia with simple lateral germ tube.

Table 1: Conidial characterization of Powdery mildew samples collected from Ziro N=60 (20 from each experimental field)

Study site	Experimental Field	Ovoid Conidia		Cylindrical Conidial		No. of cylindrical conidia	No. of ovoid conidia
		Length (µm)	Width (µm)	Length (µm)	Width (µm)		
Ziro (1500 m) AMSL	Old Ziro	26.81-35.94	16.21-26.99	27.27-32.49	13.64-16.17	5	15
	Biiri	28.09-33.95	16.10-19.32	29.39-31.68	14.35-16.28	4	16
	Piyuli	27.16-32.87	15.33-18.61	27.55-35.12	13.82-15.55	5	15
Total percentage						23%	77%

N=60 (20 from each experimental field)

Table 2: Conidial characterization of Powdery mildew samples collected from Medo N=60 (20 from each experimental field)

Study site	Experimental field	Ovoid Conidia		Cylindrical Conidial		No. of cylindrical conidia	No. of ovoid conidia
		Length (µm)	Width (µm)	Length (µm)	Width (µm)		
Med0 (250 m) AMSL	Medo-6 kilo	27.88-32.74	15.80-20.26	28.48-31.29	13.00-15.21	11	09
	Medo-7 kilo	27.45-31.21	15.54-19.56	28.63-32.47	14.13-15.81	6	14
	Tissue	24.61-31.27	12.84-17.87	30.20-32.21	13.75-14.73	5	15
Total Percentage						37%	63%

N=60 (20 from each experimental field)

Table 3: Morphological study of germ tube observed from powdery, mildew samples collected from Ziro (n=9), Lower Subansiri District and Medo (n=46), Lohit District, Arunachal Pradesh, India

Structure of Germ tube	Ziro (1500 m)		Medo (250 m)	
	Ovoid	Cylindrical	Ovoid	Cylindrical
Simple Apical	-	22 %	-	13 %
Simple Lateral	67 %	-	76 %	7%
Bifurcated Lateral	11 %	-	4 %	-

DISCUSSION

This study confirms the occurrence of powdery mildew of pumpkin (*Cucurbita moschata* Duch.) in Ziro and Medo. The nature of superficial infection on entire aerial parts of the host, powdery layers, susceptibility of older leaves, necrosis, yellowing, collapsing of vines and higher frequency of incidence in shaded regions, all these patterns of infection conform with the symptom of powdery mildew disease as per the pre-existing literatures described by Guo *et al.* (2020); De Miccolis Angelini *et al.* (2019); Ali *et al.* (2014); Reuveni and Reuveni (1995). Detailed understanding of symptoms plays a vital role in the diagnosis of a disease. However, symptoms caused by causal organisms of powdery mildew disease appears very identical. Therefore, it entails detailed characterization of distinguishable morphological traits of causal organisms to ensure correct identification of the species.

Block and Ritesma (2005) reported that casual organism of powdery mildew cannot be distinguished based on mere symptoms and in absence of Chasmothecia. However, Garcia *et al.* (2010); Braun *et al.* (2009) stated that, it could be identified based on morphological differences in shape and size of conidia, structure of germ tube, the presence or absence of fibrosin bodies and the morphology of chasmothecia.

In the present study, the set of characters subjected to microscopic observation revealed different morphological aspects that show characteristic differences in shape and size of conidia, component of fibrosin bodies, structure of germ tube and the chasmothecia.

Two distinguishable shapes of conidia, one ovoidal and the other cylindrical both were hyaline, arranged in chains at upright conidiophore, and the size range (**Table 1**) signify the presence of *Podosphaera xanthii* and *Erysiphe cichoracearum* as the casual organism of powdery mildew of *Cucurbita moschata* Duchesne in both the study sites. Basically, the conidia of *P. xanthii* is spherical to ovoid in shape and the *E. cichoracearum* is cylindrical in shape (Boesewinkel, 1980; Wang *et al.*, 2013). These two pathogens were also emphasized by Landrón-García *et al.* (2023) as the prevalent causal organism of powdery mildew of *Cucurbita moschata* Duchesne, in purtorico. Similarly, McGrath and Thomas, (1996); Perez-García *et al.*, (2009) observed the presence *Podosphaera xanthii* and *Erysiphe cichoracearum* in susceptible pumpkins host affected by powdery mildew disease. It marks the presence of both the causal organisms and also the manifestation of dual infections of these causal organisms in both the respective study sites. Such mixed infection has also been reported from the Czech Republic, the Netherlands, Great Britain and Germany (Krístková *et al.*, 2009). However, either of the two species may occur as single causal organism infecting certain cucurbit fields, for they require different environment conditions for growth and development as reported by Lebeda *et al.* (2024). In Spain, *P. xanthii* was the only reported species which caused powdery mildew in cucurbits (Del Pino *et al.*, 2002).

Though, the observation of total percentage of ovoid conidia (77% in Ziro and 63% in Medo) exceeded the total percentage of cylindrical conidia (23% in Ziro and 37% in Medo), the correlation of mean temperature range, i.e. 24-26℃ in Medo has more percentage of cylindrical condia as compare to Ziro with mean temperature range of 19-21℃. Conversely, in Ziro, more percentage of ovoid conidia was observed as compared to Medo, it shows a sense of alignment with the report given by Nagy 1976, who reported that the optimal temperature for germination for *Erysiphe cichoracearum* is 25℃ and for *Podosphaera xanthii* it is 22 ℃.

Additionally, fibrosin body was exclusively detected from ovoid conidia and its was absent in cylindrical condia, it again highlights the presence of two distinct causual organisms. The conidia of *P xanthii* is ovoid in shape with conspicuous fibrosin bodies while *E. cichoracearum* are devoid of fibrosin bodies and are cylindrical in shape (Kiss, 2010; Landrón-García *et al.*, 2023), this similar observation was reported by Mukhtar *et al.*, 2012.

The ovoid conidia showed two distinct pattern of germ tubes, a simple and a forked germ tube, positioned laterally, which are in tandem with the findings of Muktar *et al.* (2012), defining the germ tube of P. *xanthii,* it is also backed by the statement of Hirta (1942, 1945) that germ tubes of this species are forked, simple and curved and the veracity of lateral position is also justified by Aguiar *et al.* (2012). Similarly, germ tubes observed from cylindrical conidia comprised of a simple germ tube but had its protrusion point at two different positions, either at apical or at lateral position, it aligns with the conclusion drawn by Landrón-García *et al.*, (2023) that the germ tube of *Erysiphe cichoracearum* are simple and not bifurcated; the apical position is validated by Aguiar *et al.* (2012).

Moreover, the frequency of simple lateral germ tube was common in ovoid conidia (67% in Ziro and 83% in Medo), and the bifurcated was rare (11% in Ziro and 4% in Medo). Similarly, the apical position of germ tube was common in cylindrical (22% in Ziro and 13% in Medo), and

the lateral position was rare and it was observed only from Medo (7%). Hence, of all the observed patterns of germ tube, simple lateral is common to *P. xanthii*, whereas, simple apical is common to *E. cichoracerum.* Along with morphology of conidia, the fibrosin contents, architecture of germ tubes and the presence of cleistothecia was also observed to add on the completeness of species identification.

The cleistothecia recorded from the sampling fields were globose to sub-globose in shape, dark brown with radiating appendages attached at its exterior surface, it shows morphological consistency with the different developmental stages of cleistothecia of Powdery mildew fungal pathogenas per the report, documented by David *et al.* (2010). The size of cleistothecia observed from Ziro ranged between 22.99 to 88.32 µm representing the occurrence of initial to mature stages, whereas only two cleistothecia were observed from Medo measuring about 32.147 µm and 54.498 µm in diameter. It is consistent with the existing literature (Braun and crook, 2012), where the authors described the marked differences in chasmothecia of both the causal organism depending on variation in size and number of asci and ascospores, for *Podosphaera xanthii* the diameter ranges between 65-98 µm comprising of one ascus with 8 hyaline ascospores, whereas for *Erysiphe cichoracearum* it ranges between 80-140 µm containing 10-15 asci with 2-3 ascospores. However, no ascus was observed from Medo but it was observed in one of the ruptured chasmothecium, releasing several ellipsoidal asci measuring about 21.11-30.11 x 15.51-23.29 µm, nearly exhibiting similar size range of ascospores of *E. cichoracearum* measuring about 18–30×11–20µm, reported by Braun, 1995.

Observation of perfect stage from both the sampling fields highlight the presence and production of cleistothecia during the autumn or end of the growing season (Braun, 1987), it is also in agreement with David *et al.*, (2010). It is also regarded as the primary inoculum responsible for initiation of powdery mildew disease (Nagy, 1976), so its presence indicates the certainty of reoccurrence of the disease in the next growing season.

To conclude, morphological elucidation of Laterally Bifurcated germ tube (Aguiar *et al.*, 2012; Hirta 1942, 1955), ovoid conidial structure (Lebeda, 1983), Presence of fibrosin bodies (Stadnik *et al.*, 2001; Vakalounakis *et al.*, 1994) and globose cleistothecia with dichotomously branched appendages (Paul and Thakur, 2006) extends the understanding of existence of *P. xantii* as of one of the causal organisms of powdery mildew of pumpkin in Ziro as well as in Medo.

Moreover, morphological traits which include simple apical germ tube (Aguiar *et al.*, 2012), cylindrical conidia, absence of fibrosin bodies (Homma, 1937; Hirta, 1942; Landrón-García *et al.*, 2023) and chasmothecium with multiple asci (Paul and Thakur, 2006; Gupta *et al.*, 2001; Tiwari *et al.*, 2018).) also indicates the occurrence of *Erysiphe cichoracearum as* causal organisms of powdery mildew of pumpkin in Ziro and Medo.

Considering the entire morphological facets, it is apparent that both *E. cichoracearum* and *P. xanthii* are the prevalent causal organisms of powdery mildew of pumpkin in the corresponding study sites. Moreover, the frequency of *P.xanthhii* was higher than *E. cichoracearum*, indicating it as the primary causal organisms of the respective study sites. Diagnosis of diseases and identification of causal organism is a preliminary step that give rise to scientifically sanctioned management strategies crucial for uplifting the economy of an agricultural arena.

CONCLUSION

This study substantiates the occurrence of powdery mildew disease of pumpkin in tropical to sub-tropical regions of Arunachal Pradesh, India.

It also, draws forth the distinct morphological traits of two causal organisms *P. xanthii* and *E. cichoracearum* of powdery mildew of pumpkin in both Ziro and Medo. The morphology of *P. xanthii* comprises of: ovoid conidia, hyaline, arranged in chain, with conspicuous fibrosin body; germ tubes are either simple lateral germ tube or bifurcated lateral. Another detected pathogen i.e. *Erysiphe cichoracearum* are cylindrical in shape and do not contain fibrosin bodies; germ tubes are either simple apical or simple lateral and; the cleistothecia comprises of several number of asci.

Since anamorph and teleomorph characters of causal organism of Powdery Mildew of both the study sites correspond well with the prior existing reports of the causal organisms *Podosphaera xanthii* and *Erysiphe cichoracearum*, both the organisms may be prevalent in such agroclimatic regions

Furthermore, observation of higher frequency of *Podosphaera xanthii* from the infected samples suggests it as the primary causal organism of powdery mildew of pumpkin in both Ziro and Medo.

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