PLANT SCIENCE TODAY
Vol 12(sp3): 01-14
https://doi.org/10.14719/pst.8959

eISSN 2348-1900

RESEARCH ARTICLE

Survey and morpho-molecular characterization of the bhendi powdery mildew incited by *Golovinomyces cichoracearum* (DC.) V.P. Heluta

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Received: 18 April 2025; Accepted: 02 July 2025; Available online: Version 1.0: 22 September 2025

Cite this article: Lokesh R, Sundaramoorthy S, Ajaydesouza VP, Mule HR, Sathiya AV. Survey and morpho-molecular characterization of the bhendi powdery mildew incited by *Golovinomyces cichoracearum* (DC.) V.P. Heluta. Plant Science Today. 2025;12(sp3):01-14. https:/doi.org/10.14719/pst.8959

Abstract

Abelmoschus esculentus L. (bhendi), commonly referred to as okra, is a significant vegetable crop extensively cultivated in India due to its high nutritional value and economic importance. It serves as an abundant source of vitamins, minerals and dietary fibre, establishing itself as a fundamental component in numerous Indian households. However, it is attacked by numerous biotic and abiotic factors, among which powdery mildew causes severe yield loss. Roving survey undertaken across key bhendi-growing regions of Tamil Nadu exposed that incidence of powdery mildew, expressed as Percent Disease Index (PDI), ranged from 22.37 % to 74.57 %. The top three villages with the PDI were Kannivadi in Dindigul district with 74.57 %, followed by Kollapatti in Dindigul district with 71.85 % and Neikarapatti in Dindigul district with 65.35 %. The village with the least disease incidence was Gengaveli in Attur, Salem district, with 22.37 %. Pathogenicity assays performed with fifteen *Golovinomyces* sp. isolates led to the manifestation of characteristic powdery mildew symptoms. Morphological studies of all fifteen isolates were performed using a stereo binocular microscope and a scanning electron microscope (SEM). The most virulent isolate, AUGC02, which exhibited the highest disease incidence, was molecularly characterized using ITS universal fungal primers and confirmed as *G. cichoracearum* (GenBank Accession: PP373832), providing valuable insights for disease management strategies.

Keywords: bhendi powdery mildew; disease incidence; *Golovinomyces cichoracearum*; molecular characterization

Introduction

A. esculentus (L.) Moench, commonly referred as bhendi or lady's finger, is a significant vegetable crop extensively cultivated throughout India. Valued for its immature green pods, the crop adapts well to tropical, subtropical and mild temperate climates. Belonging to the family Malvaceae, bhendi is widely used in culinary preparations, including both fresh cooking and canning. In addition to its culinary appeal, it holds notable nutritional, economic and medicinal importance. The pods are a good source of essential nutrients such as vitamin C, vitamin K1, folate and potassium and their high fiber content contributes to improved digestive function (1).

India leads in global bhendi production, accounting for about 60 % of the total, with Tamil Nadu playing a key role in cultivation (2, 3). However, the crop suffers significant yield losses due to powdery mildew caused by *G. cichoracearum*, which thrives under dry conditions and can lead to yield reductions ranging from 17 % to 86.6 % (4, 5). Over 9.8 million metric tonnes of bhendi were produced in 2021, with India leading the pack with roughly 60 % of the total (2). In India, bhendi is ranked fifth in terms of the total area used for vegetable growing (6). India produced 63.5 lakh metric tonnes of bhendi on 5.1 lakh hectares in 2021-2022, with an average productivity of 12.5 metric tonnes per hectare (3).

West Bengal, Bihar, Andhra Pradesh, Odisha, Gujarat, Tamil Nadu, Maharashtra, Karnataka and Uttar Pradesh are the states that produce the most bhendi (7). With over 18000 hectares under cultivation in 2021-2022, Tamil Nadu contributes significantly to India's bhendi production, yielding about 2.05 lakh metric tonnes. Major bhendi-producing districts in Tamil Nadu include Erode, Tirupur, Salem, Coimbatore, Dindigul, Dharmapuri, Krishnagiri and Cuddalore with Erode leading with 3000 hectares and a production of 36000 metric tons (8).

The decreased yield of bhendi is caused by a number of biotic and abiotic causes (9). One of the most damaging biotic factors in the world is powdery mildew, which is brought on by obligate biotrophic fungus *E. cichoracearum* DC., a member of the Erysiphaceae family. It affects about 10000 host plant species, including important horticultural crops and vegetables (9, 10). The causative organism of powdery mildew of bhendi, formerly identified as *E. cichoracearum* De Candolle, has been reclassified as *G. cichoracearum* (DC.) V.P. Heluta. The illness is common in Tamil Nadu's bhendi-growing districts and is strongly linked to yield

losses brought on by leaf defoliation. Studies show that yield loss from *G. cichoracearum* caused powdery mildew varies from 17.0 % to 86.6 % (5, 11).

This disease, which affects several taxa in the Asteraceae family, is common in tropical and subtropical areas. *Leveillula taurica, Podosphaera xanthii* and *G. cichoracearum* are the main pathogens to blame (12, 13). The hyphae of the fungus are septate, colourless, thin-walled and branching, displaying typical fungal shape. *G. cichoracearum* reproduces asexually by producing conidiophores, which are upright, hyaline structures that may or may not be branched. These conidiophores carry chains of oval-shaped, single-celled conidia, which are the main infectious propagules that travel in air currents. Chasmothecia are fruiting structures that contain ascospores and are another way that the fungus reproduces sexually. The fungus's ability to overwinter and survive depends on these ascospores, which develop under favourable circumstances (14). The present investigation aims to bridge the existing knowledge gap by undertaking a detailed molecular characterization of *G. cichoracearum* isolates collected from key bhendi-cultivating regions of Tamil Nadu. Through assessment of both morphological traits and molecular variability among the isolates, the study provides valuable insights into the pathogen's diversity and population structure-information that is essential for devising effective disease management strategies. Furthermore, pathogenicity assays were performed to determine the virulence of individual isolates, facilitating the identification of the most aggressive strain. The findings from this research are expected to support breeding programs focused on developing powdery mildew-resistant bhendi cultivars and to enhance integrated disease management approaches in bhendi cultivation.

Materials and Methods

Survey

To evaluate the prevalence of powdery mildew and collect diverse isolates of *G. cichoracearum* DC (V.P. Heluta), a comprehensive roving survey was undertaken from December 2022 to April 2023 across key bhendi-cultivating districts of Tamil Nadu including Dharmapuri, Dindigul, Salem, Krishnagiri and Cuddalore. Three villages were picked at random from each district and three farmers' fields were chosen from each village. Twenty plants were randomly chosen from each farmer's field at three different sites and PDI was computed (15) and the disease score chart (Fig. 1) provided (16). From these sites, infected samples were gathered and sent to the lab for further analysis. On the basis of anamorph and teleomorph traits, the causative pathogen was recognized (Table 1).

Collection of pathogen inoculum

Conidial suspensions of the pathogen *G. cichoracearum* were made from leaves infected with powdery mildew (17). In short, sterile distilled water was used to wash the affected leaves in order to collect the conidia. To ensure that there was a sufficient quantity of inoculum, this process was carried out three times. After passing through two layers of cheese cloth, the resultant prepared suspension underwent two rounds of centrifugation at 4000 rpm for 30 min each.

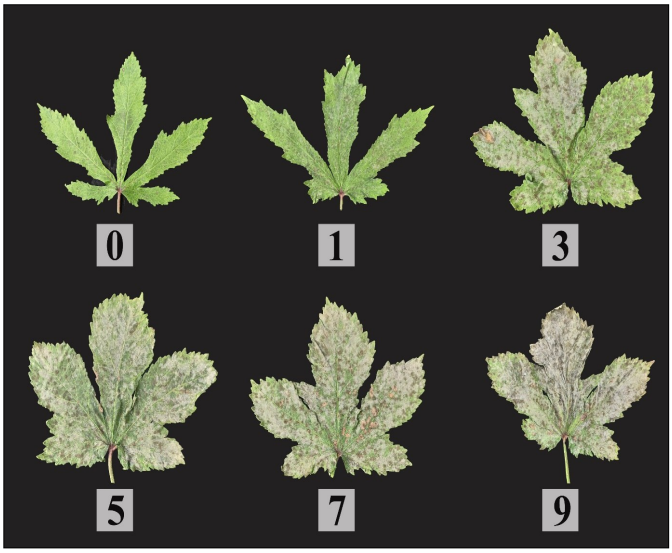


Fig. 1. Disease score chart for powdery mildew.

Table 1. Disease score chart for powdery mildew

Grade	Description	Affected leaf area
0	No observable symptoms	0
1	Minimal infection	0 - 10
3	Mild infection	11 - 15
5	Moderate infection	16 - 25
7	Severe infection	26 - 50
9	Highly severe infection	>50

Seed source

The seed variety 'Sakthi', procured from the Seed Sales Counter at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, was utilized in this research.

Pathogenicity of *G. cichoracearum*

The spore suspension was sprayed onto healthy 60-days-old bhendi plants of the Sakthi variety after making small pinpricks on the leaf surface. Prior to laboratory transfer for detailed identification and confirmation of the causal organism, the samples were incubated under conditions favourable for fungal colonization for a period of seven days. To promote optimal disease expression, the infected plant material was enclosed in polythene bags for 24 hr to keep high humidity, which facilitates proliferation of fungal pathogen (18). Sterile distilled water was used to adjust the conidial concentration of the pathogen to 5×10^6 mL^{-1} (19). The recovered isolates were designated as AUGC 01 through AUGC 15.

Morphological characterization of *G. cichoracearum*

Morphological features were analysed using a stereo binocular microscope (Euromex, Netherlands, IS 3153 PLFi/3) with 10× and 40× magnifications, housed in Department of Plant Pathology, Faculty of Agriculture, Annamalai University. For conidial morphological analysis, diseased samples were collected and hyaline fungal outgrowths were carefully scraped using a camel brush. Further investigations were conducted using the same microscope, equipped with a DC 20000i camera and differential interference contrast. Microscopic characteristics were assessed using Euromex Image-Focus-Alpha analysis software. Furthermore, in-depth morphological investigations were carried out at Annamalai University's Central Instrumentation Science Laboratory (CISL), Department of Physics, Faculty of

Science, utilising a SEM. Using a Polaron E-500 sputter coater, the conidia were adhered on aluminium stubs and carbon-sputter-coated. The samples were subsequently examined for pathogen morphological changes under a SEM set to 15 kV.

Molecular characterization of *G. cichoracearum*

DNA extraction

Conidial masses were delicately removed from the surface of infected leaves using a soft, moistened brush and subsequently macerated in a mortar and pestle to ensure uniform homogenization. Approximately 0.1g—0.2 g of the homogenized material were rapidly frozen using liquid nitrogen and ground thoroughly into a fine consistency. This powdered material was then transferred into sterile microcentrifuge vials containing 500 µL of pre-warmed CTAB extraction buffer (composed of 2 % CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl and 1 % PVP). The prepared tubes were maintained at 65 °C for 30 min with mild shaking at consistent intervals to promote efficient cellular lysis and DNA liberation. After incubation, chloroform:isoamyl alcohol (24:1) was added in equal proportion and the contents were gently mixed to form a stable emulsion. Phase separation was accomplished by centrifugation at 10000 rpm for 10 min and the upper aqueous phase was carefully transferred into new microcentrifuge tubes. Genomic DNA was isolated by adding an equal amount of ice-cold isopropanol and incubating the solution at -20 °C for 30 min. The precipitated DNA was recovered by centrifugation at 12000 rpm for 10 min, followed by a 70 % ethanol wash to eliminate residual contaminants. Once the pellet was completely dried, it was rehydrated in 50 - 100 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA) and stored at -20 °C for further molecular analysis. The quality and quantity of the extracted DNA were evaluated using spectrophotometric methods and agarose gel electrophoresis.

PCR amplification

Amplification of the Internal Transcribed Spacer (ITS) region of rDNA was executed using the universal primer set ITS-1 (5-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). These primers are intended to hybridize with conserved regions situated within the 5.8S ribosomal RNA gene and adjacent ITS1 and ITS2 sequences (20). The PCR reaction was conducted in a total volume of 25 µL, utilizing an Eppendorf Mastercycler Nexus Thermal Cycler (Germany), to maintain precise thermal cycling and accurate amplification of the desired DNA sequence. Each reaction mix comprised 2.5 µL of 10× Taq buffer, 0.5 µL of 25 mM $MgCl_2$, 2.0 µL of both forward and reverse primers (ITS-1 and ITS-4), 0.5 µL of 100 mM dNTPs, 0.125 µL of Taq polymerase, 14.375 µL of nuclease-free water and 3 µL of genomic DNA (~100 ng). To monitor for contamination, a negative control setup was included, where the DNA template was replaced with autoclaved distilled water. The PCR thermal profile began with an initial denaturation at 94 °C for 4 min, which allowed for strand separation. This was followed by 40 amplification cycles consisting of denaturation at 94 °C for 2 min, annealing at 52 °C for 2 min and extension at 72 °C for 2 min. The final step was a concluding extension at 72 °C for 7 min to ensure complete synthesis of the amplified products.

Gel electrophoresis

The amplified PCR fragments were separated using agarose gel electrophoresis (1.2 %) in 0.5 × TAE buffer, which contained 20 mM Tris-acetate (pH 8.0) and 0.5 mM EDTA to ensure stable pH and ionic strength. To visualize the nucleic acids, the gel was stained with ethidium bromide (10 mg/mL) and observed under UV light using a gel documentation system. The sizes of the DNA fragments were assessed by referencing a 1 kb molecular marker for comparison. The required ITS bands were meticulously cut and processed using a gel purification kit, with the DNA subsequently eluted in a low-salt buffer containing 5 mM Tris-Cl (pH 8.5) for further downstream analyses.

DNA sequencing analysis

Amplified product was sequenced by Medauxin (Bengaluru, India). Initial sequence analysis was performed using DNASTAR SeqMan Ultra (DNASTAR Inc., Madison, USA) to align the forward and reverse reads and generate a consensus sequence. Gene alignments were carried out using MEGA version 11 software. The ITS sequence obtained from the isolate was deposited to the NCBI GenBank repository, where it was given a specific accession number. To determine sequence similarity, the ITS rDNA sequence was aligned with nine reference sequences from NCBI database using BLASTn tool. For phylogenetic analysis, maximum likelihood (ML) approach was employed using MEGA version 11 software (21), with 1000 bootstrap replicates to ensure the reliability of the tree. *Rhizoctonia solani* served as the outgroup in the phylogenetic tree construction.

Satistical analysis

The collected experimental data were subjected to statistical analysis using the Web Agri Stat Package (WASP) version 2.0, developed by the ICAR-Central Coastal Agricultural Research Institute (CCARI), Goa. To evaluate the significance of treatment effects, an analysis of variance (ANOVA) was performed at a significance level of $P < 0.05$. Further, to identify statistically meaningful differences among treatment means, Duncan's Multiple Range Test (DMRT) was employed, enabling the classification of distinct treatment groups (22).

Results

Field survey on the incidence of powdery mildew in bhendi crops across Tamil Nadu

The roving survey conducted across key bhendi-cultivating regions of Tamil Nadu (Fig. 2, Fig. 3A - 3F) revealed considerable variation in the PDI of powdery mildew across different locations and crop growth stages (Table 2). The highest PDI was recorded in Kannivadi, Oddanchatram taluk (Dindigul district) with 74.57 % on the Sakthi variety, followed by Kollapatti, Vedasandur taluk (Dindigul district) with 71.85 % at the flowering stage of the same variety. Neikarapatti, Palani taluk (Dindigul district) also showed a significant incidence of 65.35 % during the flowering stage. In contrast, lower incidences were observed in Eriyur, Pennagaram taluk (Dharmapuri district) and Gengaveli, Attur taluk (Salem district), with PDIs of 31.88 % and 22.37 % respectively during the vegetative stage (Fig. 4).

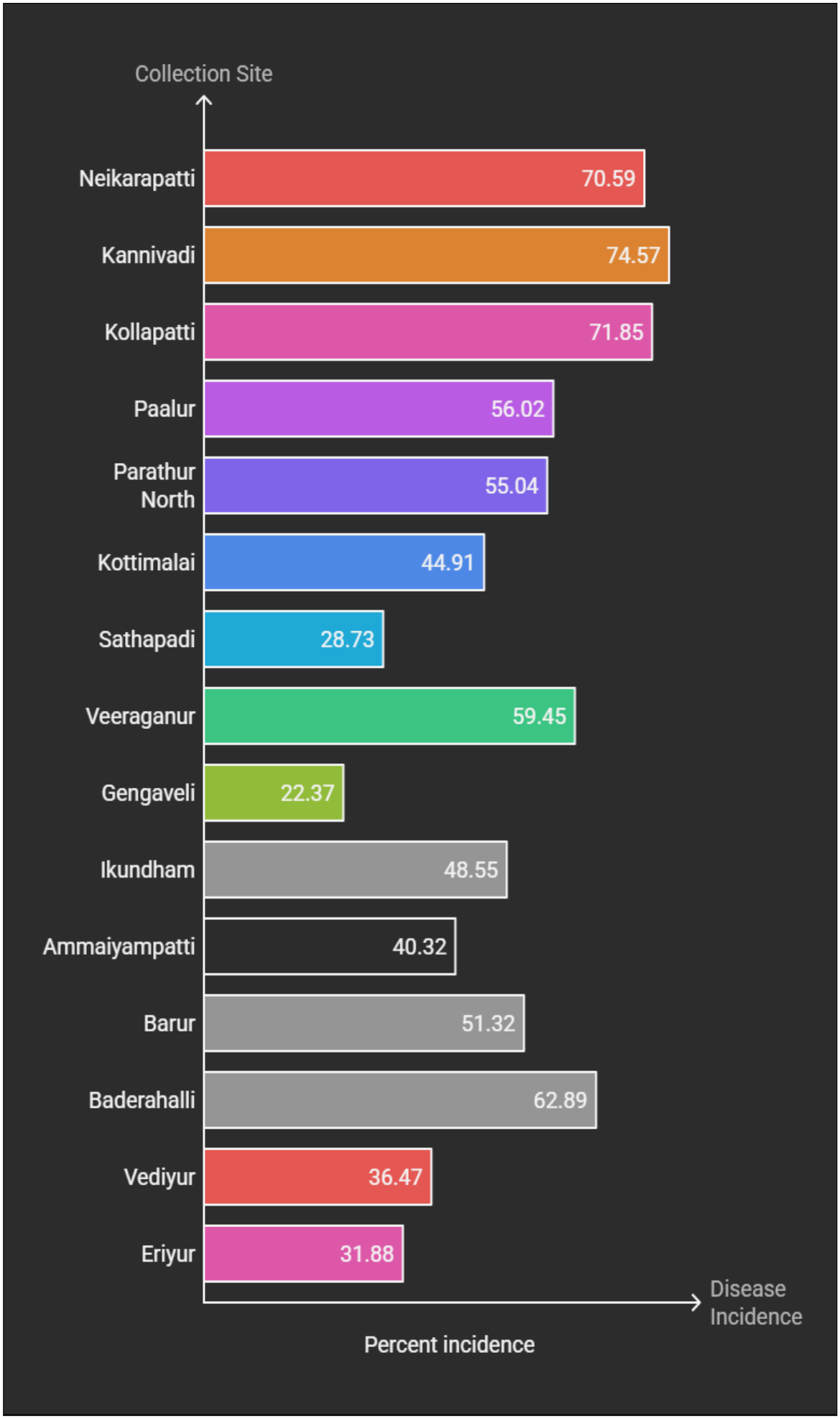


Fig. 2. Powdery mildew disease incidence across major bhendi growing districts in Tamil Nadu.

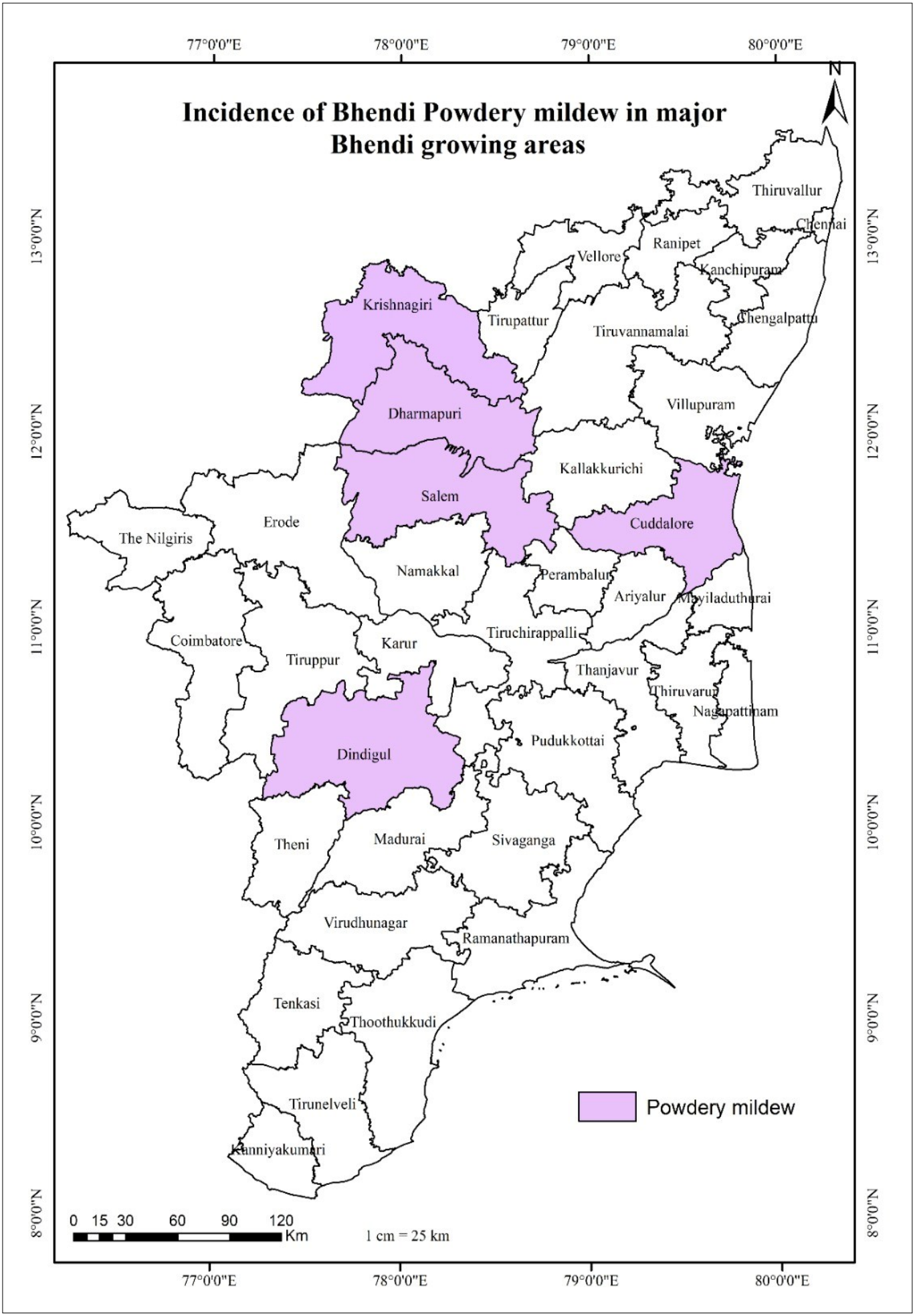


Fig. 3A. Survey on the distribution of powdery mildew in leading bhendi cultivation areas of Tamil Nadu.

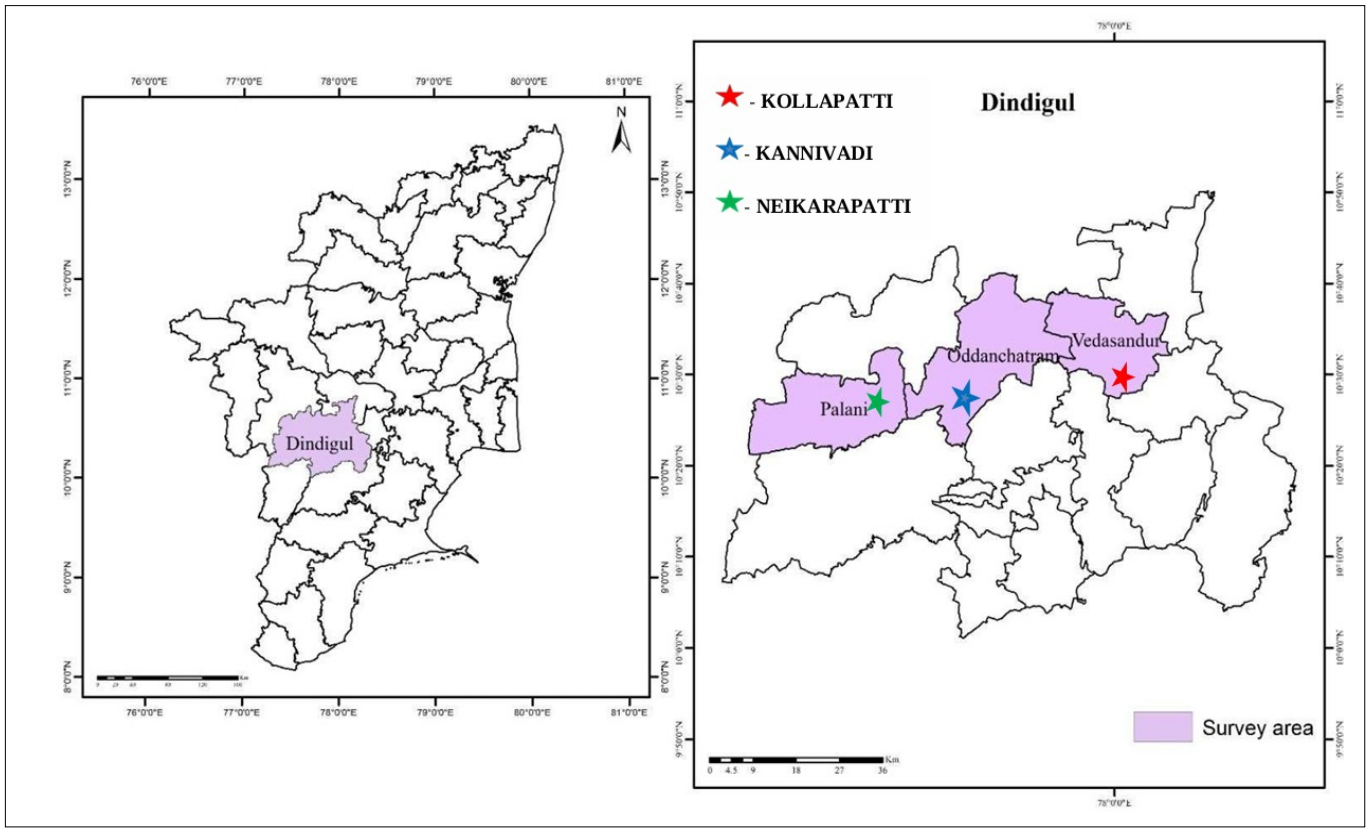


Fig. 3B. Assessment of powdery mildew incidence in bhendi— Dindugal district.

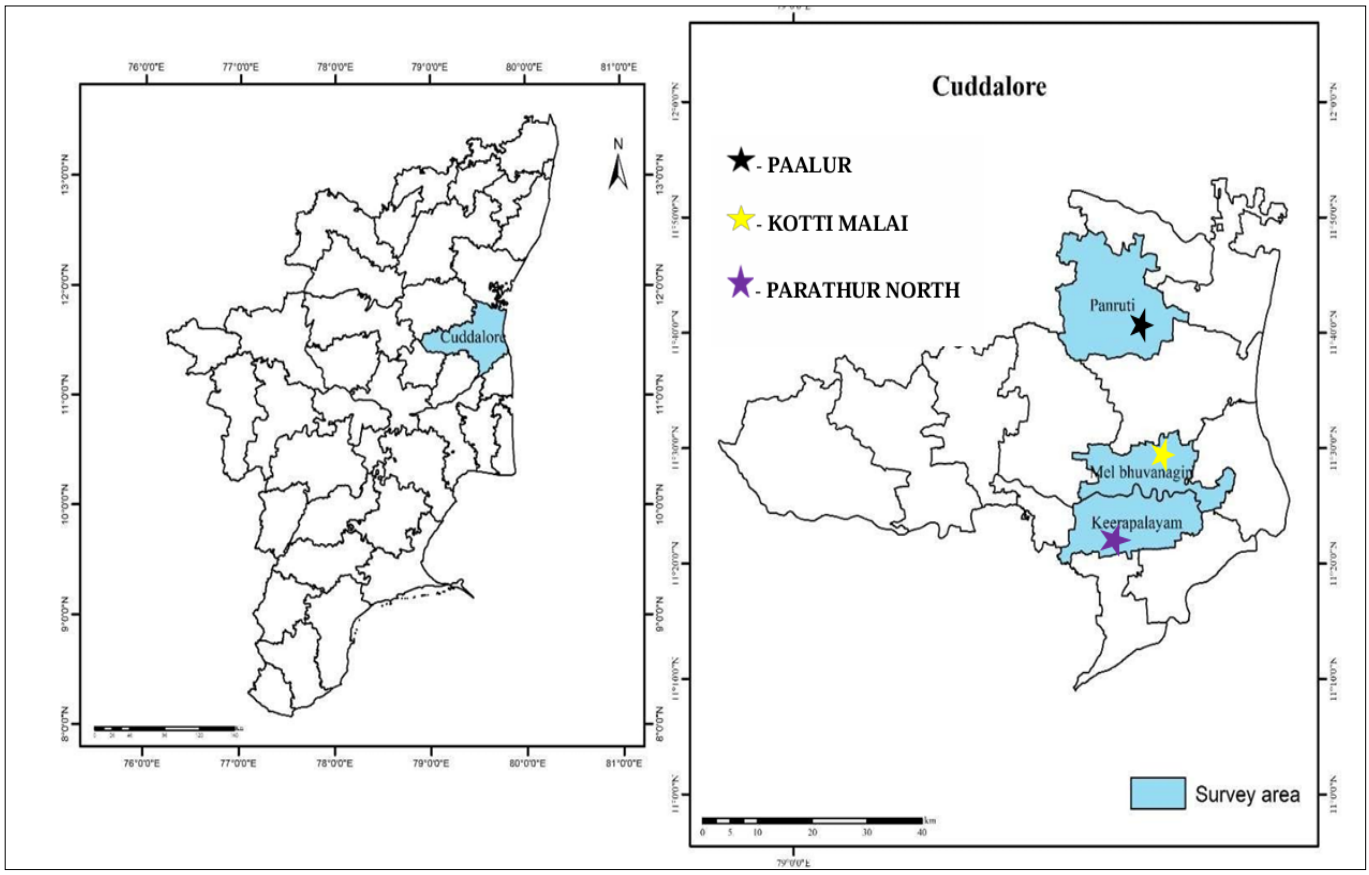


Fig. 3C. Assessment of powdery mildew incidence in bhendi— Cuddalore district.

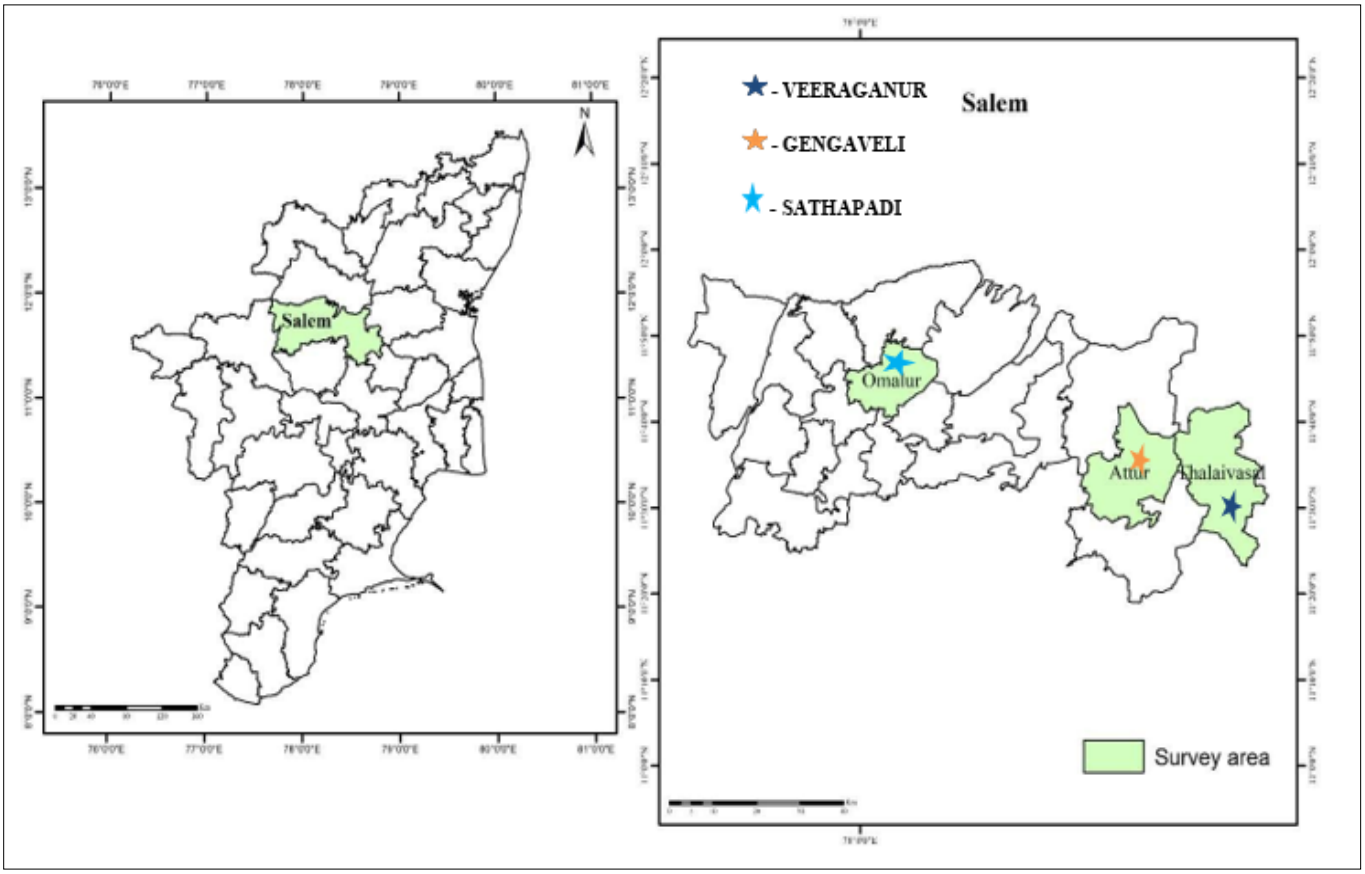


Fig. 3D. Assessment of powdery mildew incidence in bhendi— Salem district.

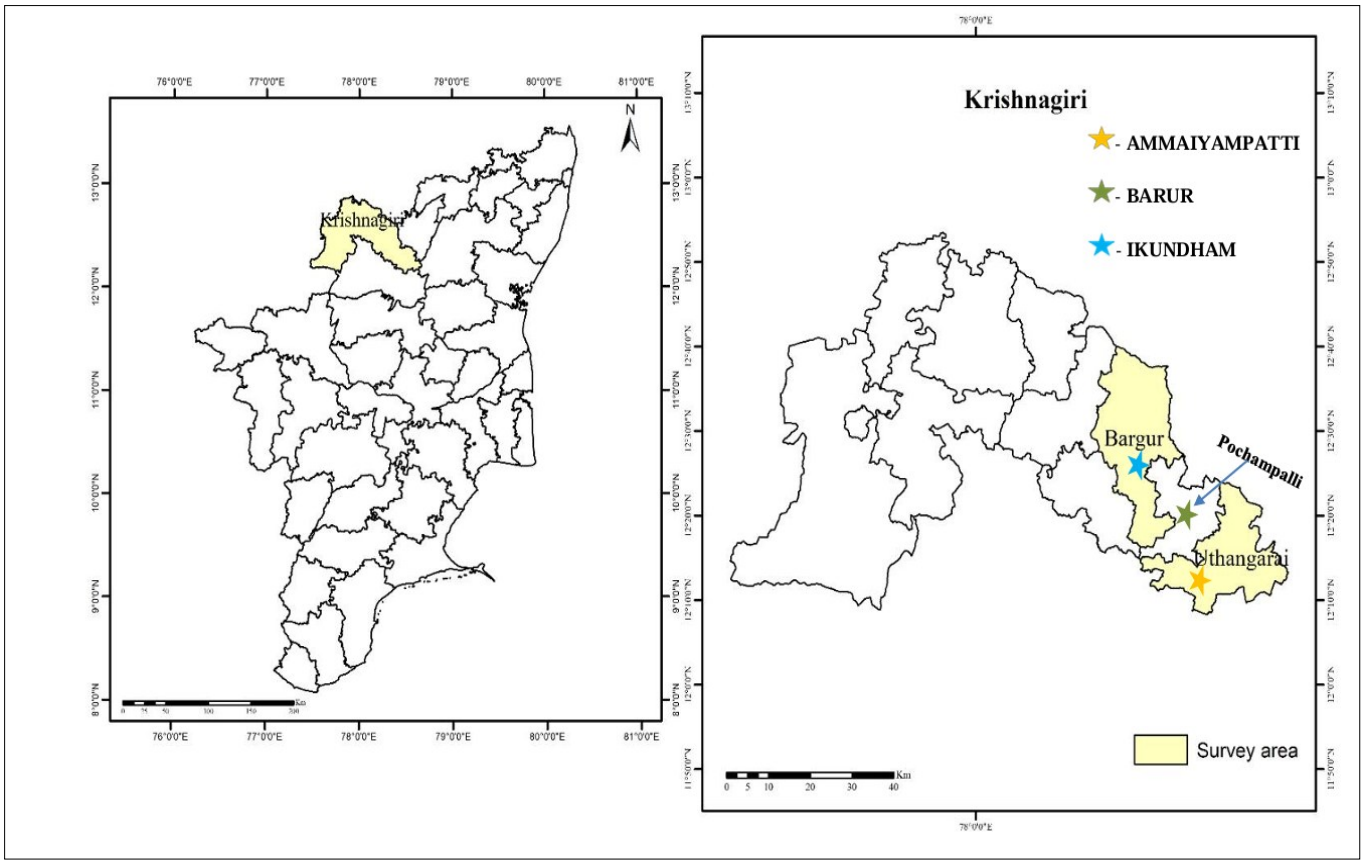


Fig. 3E. Assessment of powdery mildew incidence in bhendi— Krishnagiri district.

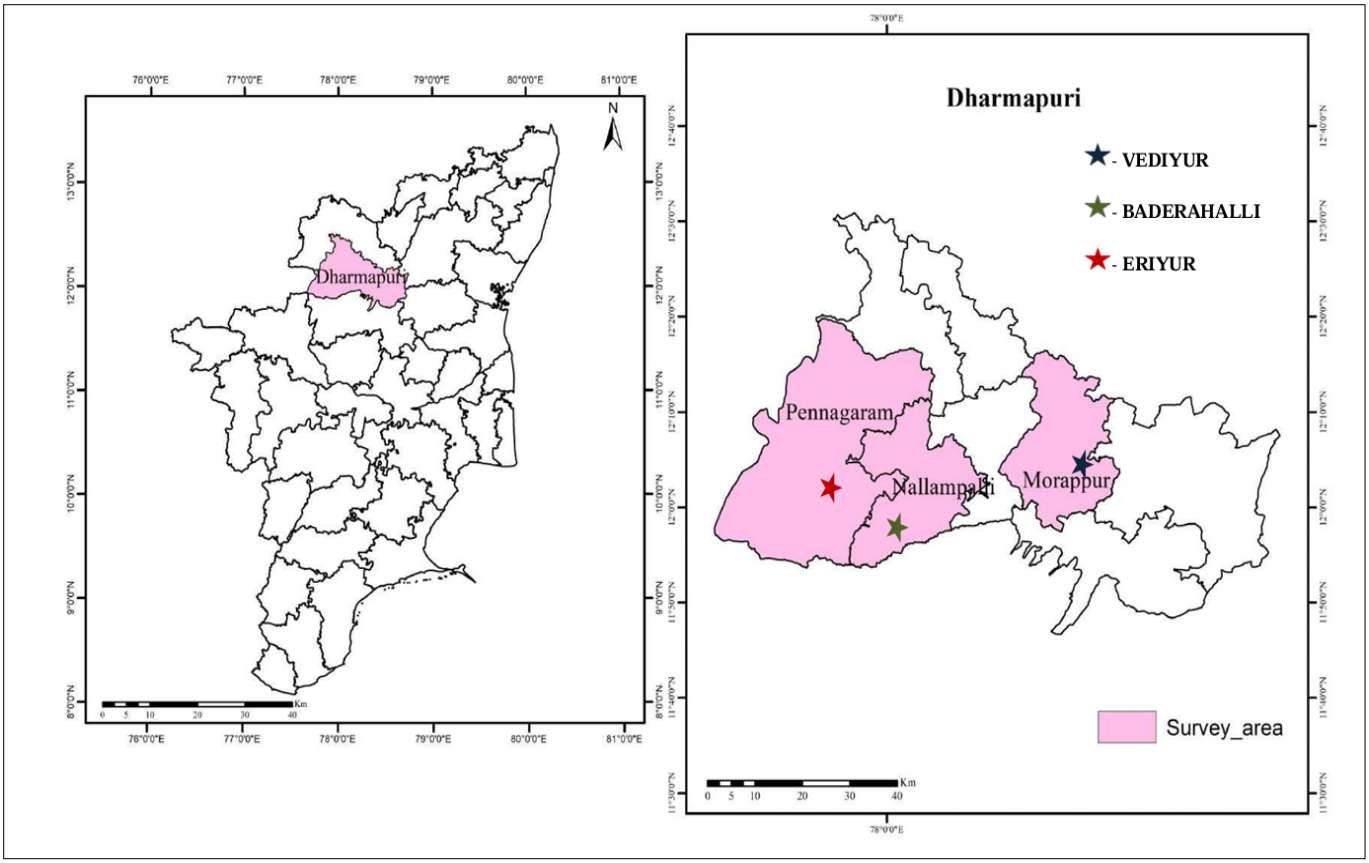


Fig. 3F. Assessment of powdery mildew incidence in bhendi- Dharmapuri district.

Table 2. Assessment of powdery mildew occurrence in bhendi across major growing districts in Tamil Nadu

Sl. No.	Place of collection	District	Taluk	Stage of the crop	Variety/ Hybrid cultivated	PDI	Mean disease incidence	Isolate code
1.	Neikarapatti	Dindugul	Palani	Flowering	Sakthi	65.35^{d} (53.95)	70.59	AUGC01
2.	Kannivadi		Oddanchatram	Maturity	Sakthi	74.57^{a} (60.05)		AUGC02
3	Kollapatti		Vedasandur	Flowering	Sakthi	71.85^{b} (57.96)		AUGC03
4.	Palur	Cuddalore	Panruti	Vegetative	COBH h 1	68.11^{c} (55.64)	56.02	AUGC04
5.	Parathur North		Chidambaram	Fruit formation	Ankur 41	55.04^{g} (47.89)		AUGC05
6.	Kottimalai		Bhuvanagiri	Fruiting	Sakthi	44.91^{j} (41.99)		AUGC06
7.	Sathapadi	Salem	Omalur	Fruit formation	Arka anamika	28.73^{n} (32.40)	36.85	AUGC07
8.	Veeraganur		Thalaivasal	Maturity	Ankur 41	59.45^{f} (50.44)		AUGC08
9.	Gengaveli		Attur	Maturity	Traditional variety	22.37^{o} (28.22)		AUGC09
10.	Ikundham	Krishnagiri	Bargur	Flowering	Sakthi	48.55^{i} (44.16)	46.73	AUGC010
11.	Ammaiyampatti		Uthangarai	Flowering	Farmers variety	40.32^{k} (39.57)		AUGC011
12.	Barur		Pochampalli	Vegetative	COBhH 1	51.32^{h} (45.75)		AUGC012
13.	Baderahalli	Dharmapuri	Nallampalli	Fruit formation	Sakata	62.89^{e} (52.47)	43.70	AUGC013
14.	Vediyur		Morappur	Fruiting	Arka anamika	36.47^{l} (37.14)		AUGC014
15	Eriyur		Pennagaram	Vegetative	Sakthi	31.88^{m} (31.88)		AUGC015

*Values represent the mean of three replications.

Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

a) Bird eye view of bhendi powdery mildew infected field.

b) Bhendi Powdery mildew infected individual diseased plant.

c) Powdery mildew infected on leaves and petiole.

d) Comparison of heathy and infected leaf of bhendi.

Fig. 4. Different symptoms of bhendi powdery mildew.

Collection details of *Golovinomyces* sp. isolates from different locations

The data on *Golovinomyces* sp. isolates demonstrated significant variation in conidial size, conidiophore length and foot cell length among different isolates (Table 3). Notably, isolates AUGC05, AUGC09 and AUGC12 exhibited the largest conidial sizes. AUGC05 had the largest conidia (29.5 µm), as well as the longest conidiophore (51.3 µm) and foot cell (8.9 µm). AUGC09 closely followed with a conidial size of 28.8 µm and a conidiophore length of 49.8 µm, while AUGC12 measured 29.1 µm in conidial size and 50.4 µm in conidiophore length. Conversely, isolates AUGC10 and AUGC15 had the smallest conidial sizes, measuring 21.9 µm and 23.2 µm respectively accompanied by shorter conidiophores and foot cells. These findings highlight substantial morphological variation among the isolates, particularly in conidial dimensions and structural traits.

Assessing the pathogenicity of *Golovinomyces* sp. isolates in greenhouse conditions

The pathogenicity assessment of 15 *Golovinomyces* sp. isolates (AUGC01 to AUGC015) under greenhouse conditions on the Sakthi variety of bhendi revealed significant variation in disease severity (Table 4). Isolate AUGC02 recorded the highest mean powdery mildew index (62.24), followed closely by AUGC07 (57.12) and AUGC08 (53.95). In contrast, AUGC09 exhibited the lowest mean powdery mildew index (13.85), followed by AUGC06 (17.89) and AUGC010 (23.40). For most isolates, disease severity progressively increased over time, with peak indices typically observed at 75 DAS (Days After Sowing). Due to its high virulence, AUGC02 was selected for further studies.

Table 3. Collection details of *Golovinomyces* sp. isolates from various locations

Sl. No	Location	Conidial size (µm)			Conidiophore (µm)	Foot cell Length (µm)	Isolate code
		Length	Breadth	L/B index	Length		
1.	Neikarapatti	24.8^{e}	11.2^{f}	2.21	42.5^{e}	7.2^{f}	AUGC01
2.	Kannivadi	28.8^{b}	13.5^{b}	2.10	48.7^{b}	8.5^{b}	AUGC02
3.	Kollapatti	22.6^{g}	10.8^{h}	2.09	39.2^{g}	6.8^{j}	AUGC03
4.	Palur	26.9^{c}	12.7^{d}	2.12	46.8^{c}	8.1^{c}	AUGC04
5.	Parathur North	29.5^{a}	13.9^{a}	2.12	51.3^{a}	8.9^{a}	AUGC05
6.	Kottimalai	23.7^{f}	11.6^{g}	2.04	41.6^{f}	7.1^{i}	AUGC06
7.	Sathapadi	27.1^{c}	12.9^{c}	2.10	47.9^{c}	8.2^{c}	AUGC07
8.	Veeraganur	25.3^{d}	11.8^{f}	2.14	44.1^{de}	7.6^{e}	AUGC08
9.	Gengaveli	28.8^{b}	13.7^{a}	2.09	49.8	8.6^{b}	AUGC09
10.	Ikundham	21.9^{h}	10.5^{h}	2.11	38.3^{h}	6.6^{k}	AUGC010
11.	Ammaiyampatti	26.2^{c}	12.4^{e}	2.13	45.6^{d}	7.9^{d}	AUGC011
12.	Barur	29.1^{a}	13.8^{a}	2.09	50.4^{a}	8.7^{a}	AUGC012
13.	Baderahalli	24.5^{e}	11.7^{f}	2.11	42.9^{e}	7.3^{f}	AUGC013
14.	Vediyur	27.6^{b}	13.1^{c}	2.11	48.2^{bc}	8.3^{c}	AUGC014
15.	Eriyur	23.2^{f}	11.1^{g}	2.09	40.7^{f}	7.0^{fi}	AUGC015

*Values represent the mean of three replications.

Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

Table 4. Assessing the pathogenicity of *G. cichoracearum* isolates in greenhouse conditions

Sl. No	Isolates	Powdery mildew PDI			Mean
		45 DAS	60 DAS	75 DAS	
1.	AUGC01	21.11	61.73	64.46	49.01^{de} (44.43)
2.	AUGC02	39.40	69.85	77.47	62.24^{a} (52.33)
3.	AUGC03	22.39	50.53	52.94	41.95^{f} (40.36)
4.	AUGC04	14.11	31.71	34.46	26.76^{j} (31.15)
5.	AUGC05	20.74	39.55	52.46	37.58^{gh} (37.80)
6.	AUGC06	66.30	13.70	33.69	17.89^{l} (25.02)
7.	AUGC07	33.33	65.68	72.35	57.12^{b} (49.09)
8.	AUGC08	37.83	57.04	66.98	53.95^{c} (47.26)
9.	AUGC09	11.63	13.56	16.36	13.85^{m} (21.84)
10.	AUGC010	17.41	20.37	32.44	23.40^{k} (28.92)
11.	AUGC011	18.96	38.78	40.11	32.61^{i} (34.82)
12.	AUGC012	15.33	34.81	54.95	35.03^{hi} (36.21)
13.	AUGC013	12.59	37.33	64.98	38.30^{g} (38.23)
14.	AUGC014	24.98	52.88	61.73	46.53^{e} (43.01)
15.	AUGC015	27.04	56.30	69.36	50.09^{d} (45.05)

*Values represent the mean of three replications.

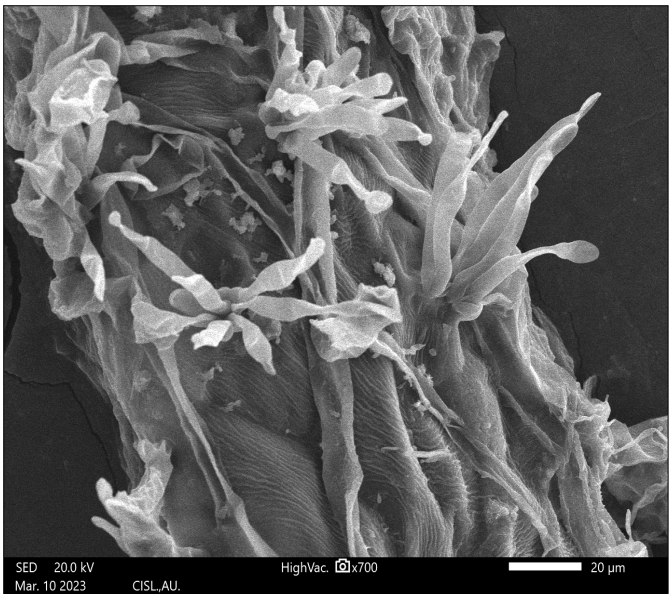
Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

Molecular characterization, confirmation and GenBank deposit (NCBI)

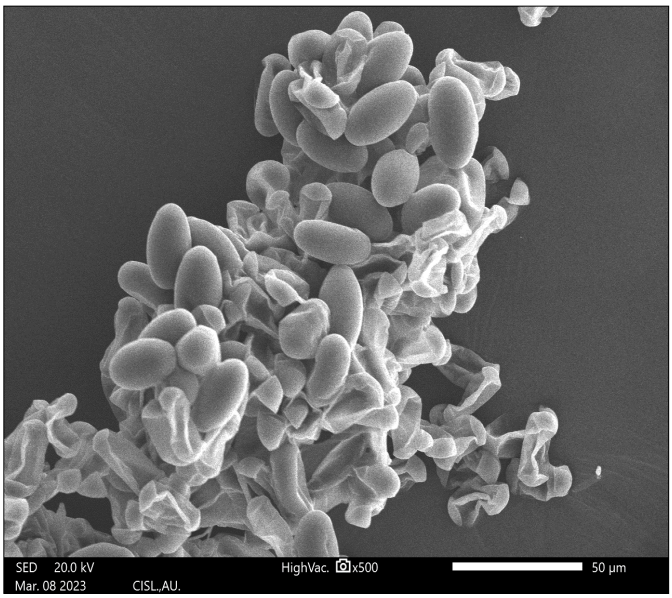
The isolate AUGC02 from Kannivadi was subjected to molecular characterisation following microscopic and SEM inspection (Fig. 5). It was determined to be *G. cichoracearum*, with GenBank accession number PP373832 (Fig. 6). The correctness of the pathogen diagnosis in this investigation is further confirmed by the molecular identification and matching GenBank deposit. In order to evaluate the genetic links between *Golovinomyces* isolates, a phylogenetic tree was also built using ITS 18S rDNA sequences (Fig. 7). Significant genetic homogeneity was found in the study, suggesting that the isolates were closely linked genetically. These findings validate the isolates' classification within the *Golovinomyces* genus by highlighting their striking similarity to other *Golovinomyces* species. This thorough molecular and phylogenetic investigation strengthens their taxonomic identification and improves our knowledge of their evolutionary history.

Discussion

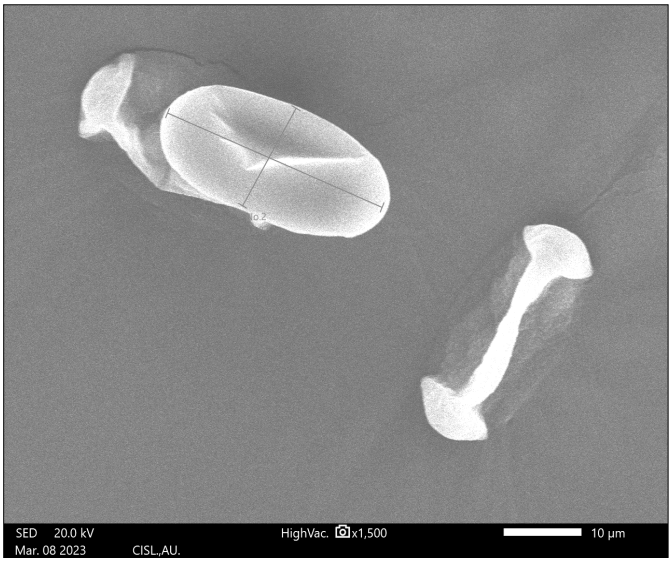
In the present study, the highest PDI was recorded in Dindigul district (Kannivadi at 74.57 and Kollapatti at 71.85), while the lowest PDI was observed in Gengaveli, Salem district (22.37). This variation in disease severity closely correlated with relative humidity (RH) levels. This study aligns with previous findings (23, 24) that *Sphaerotheca fuliginea* thrives well on high RH as moisture facilitates high germination and leads to increased disease severity. Similarly, RH levels of 70 % to 80 % are conducive to conidial germination and disease development in *Oidium tingitaninum* on citrus and *Leveillula taurica* on sweet peppers (25), which further consolidated our research. Higher PDI observed in Dindigul likely reflects the influence of low RH, while the lower PDI in Salem may be attributed to less favourable humidity conditions, highlighting the significant role of RH in disease incidence and progression. This study is unique since there were no survey reports on the prevalence of powdery mildew in Tamil Nadu's main bhendi-growing areas. However, for comparison study, pertinent records from various crops and other parts of India were employed.



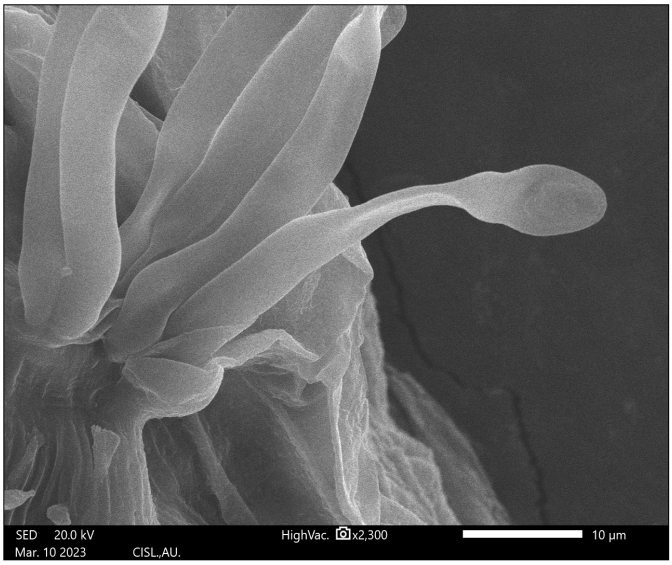
(a) (20µm)



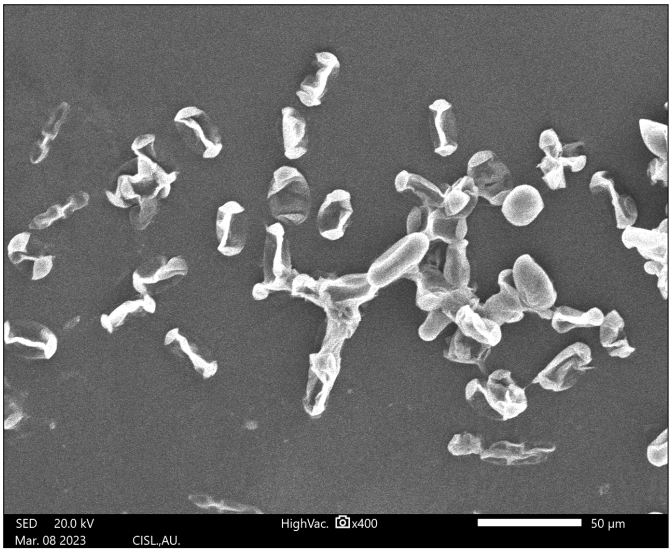
(b) (50µm)



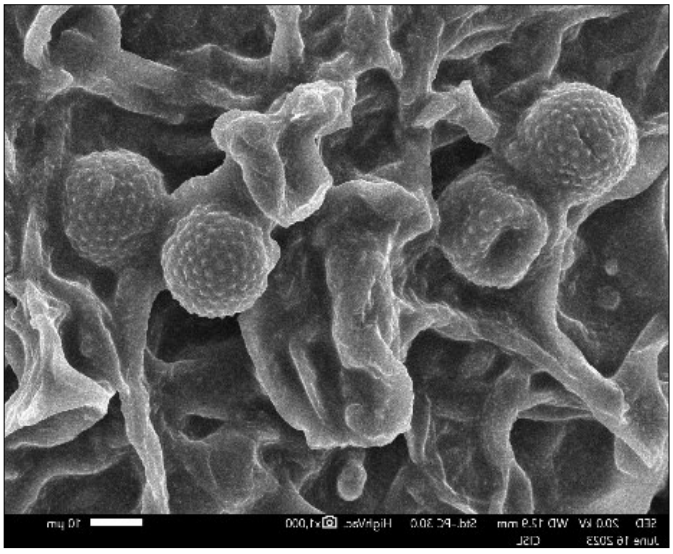
(c) (10µm)



(d) (10µm)



(e) (50µm)



(f) (10µm)

Fig. 5. SEM observation of conidial characters of *G. cichoracearum* (AUGC02).

(a) Conidiophore of *G. cichoracearum.*

(b) Cluster of conidia of *G. cichoracearum*.

(c) Conidial measurement of *G. cichoracearum*, (29.94 µm × 13.87 µm).

(d) Conidiophore bearing conidia of *G. cichoracearum*.

(e) Chains of conidia of *G. cichoracearum*.

(f) Group of chasmothecia of *G. cichoracearum*.

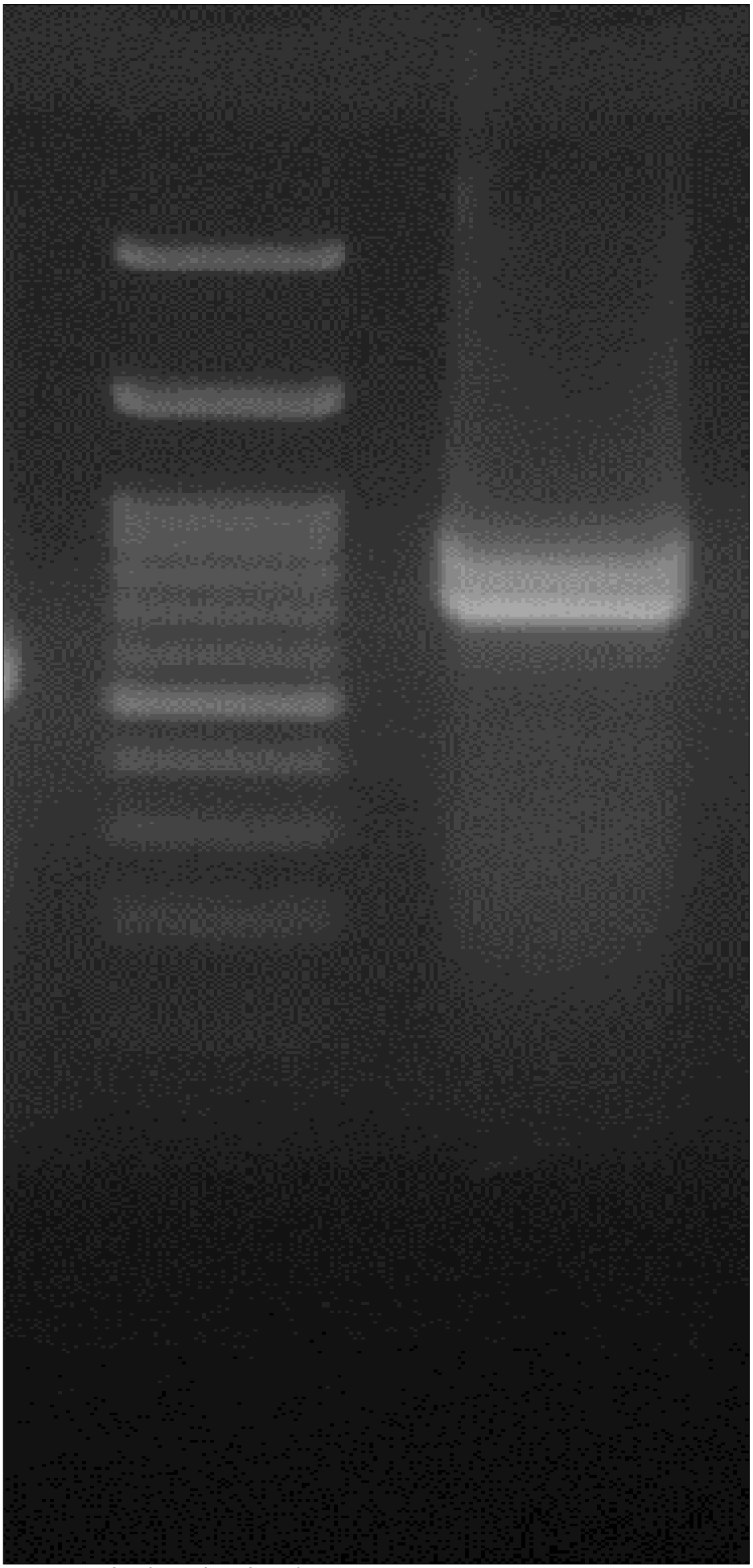


Fig. 6. Molecular characterization of virulent isolate of *G. cichoracearum.*

Agarose (1.5 %) gel electrophoresis of rDNA extracted from the *G. cichoracearum.*

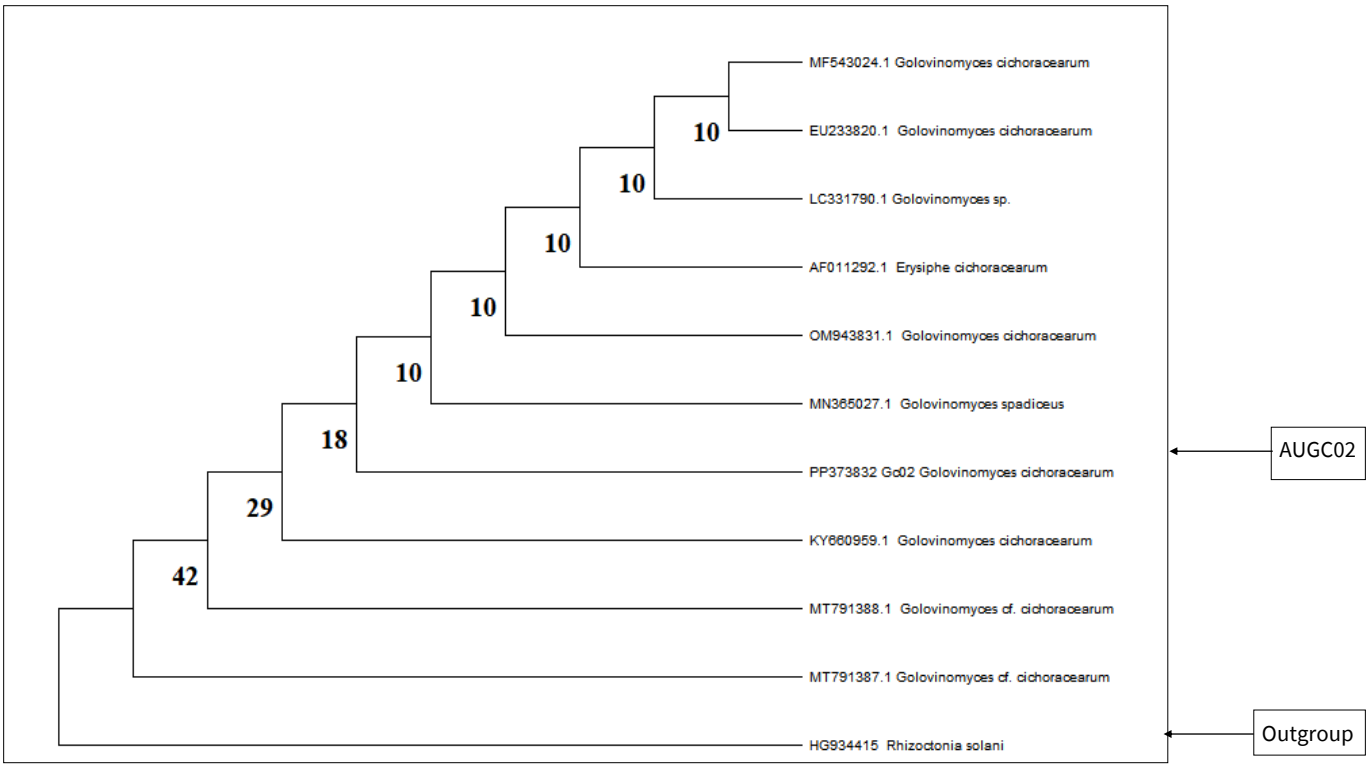


Fig. 7. Phylogenetic tree of *G. cichoracearum* species complex (AUGC02).

Disease incidence varied across locations, correlating with conidial size and conidiophore length. Kannivadi and Kollapatti in Dindigul had the highest PDI, with larger conidia and longer conidiophores. Gengaveli in Salem recorded the lowest PDI with the smallest conidia (21.9 µm) and shortest conidiophores (38.3 µm). Parathur North's AUGC05 isolate (29.5 µm × 13.9 µm, 51.3 µm) showed a moderate PDI. These findings align with other studies which found that increase in the RH have positive correlation with conidial metrics which supporting the link between pathogen morphology and disease severity (23, 26). The observed symptoms, including hyaline spots on the upper leaves, yellowing, drying and defoliation, were consistent with previous reports where they found hyaline to greyish superficial growth on leaf, stem, pods and this give rise to conidia and conidiophores (27, 28). Severe infections reduced yield proportionally. Pathogenicity assessments showed that isolates AUGC02 and AUGC07 exhibited the highest virulence (62.24 % and 57.12 %), while AUGC09 had the lowest (13.85 %). The higher virulence of specific isolates may be linked to increased conidial production and rapid colonization (29). Similar findings were reported, linking susceptibility with biochemical changes (30, 31). Isolate AUGC02 from Kannivadi was confirmed as *G. cichoracearum* (GenBank PP373832). Similarly, 100 % homology was reported for *P. xanthii* on watermelon (32) and *G. cichoracearum* on sunflower through a 418 bp amplicon (33).

Conclusion

The current investigation clearly demonstrates significant variation in the prevalence of powdery mildew on bhendi across the principal districts of Tamil Nadu, identifying *G. cichoracearum* as the principal pathogen through comprehensive morphological and molecular characterization. The pathogenicity assessments underscore isolate AUGC02's high virulence, which aligns with existing literature. This research not only reaffirms the significance of precise pathogen identification for disease management but also provides crucial insights into the pathogen's diversity and its potential impact on bhendi cultivation. The findings contribute valuable data for developing targeted control strategies and improving crop resilience against powdery mildew.

Authors' contributions

All authors read and approved the final manuscript.

Acknowledgement

The authors gratefully acknowledge the Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, for providing the platform to publish this article. They sincerely thank Mr. M. Keerthivasan, Agro Climate Research Centre, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, for his help in GIS mapping

Compliance with ethical standards

Conflict of interest: Authors declare that they have no financial or non-financial interests that could potentially be perceived as a conflict of interest related to the research.

Ethical issues: None

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