Crossref

IJBSM April 2026, 17(4): 01-12 Article AR6586

Research Article Stress Management

DOI: HTTPS://DOI.ORG/10.23910/1.2026.6586

Polyphasic Characterization of *Bipolaris maydis*: Insights into Pathogen Identity and Variability through Morpho-cultural and Molecular Traits

Monisha M.[1], Pankaja N. S.[1], Mummineni Sunitha[1], Mahadeva J.[2], Umashankar Kumar[3] and Supriya S.[1]

[1]Dept. of Plant Pathology, [2]Dept. of Forestry and Environmental Science, College of Agriculture, V.C. Farm, Mandya, University of Agricultural Sciences, Mandya, Karnataka (571 405), India

[3]Dept. of Plant Pathology, Organic Farming Research Station, Naganahalli, Mysuru, UAS, Mandya, Karnataka (560 065), India

Open Access

Corresponding nspanks@gmail.com

0000-0002-9056-0583

ABSTRACT

The experiment was conducted during the March–June, 2022 at College of Agriculture, V. C. Farm, Mandya, Karnataka, India to study Polyphasic characterisation of Maydis leaf blight (*Bipolaris maydis*) which accounts for 30–40% of yield loss. Isolates were collected in October 2021 during survey within four districts of Karnataka. Morphological studies revealed that mycelia and conidia of all the twenty isolates were brown in colour and septate. Conidial size among the isolates varied from 57.44–80.05×11.34–15.77 µm being either curved or fusiod. Variation in colony colour was observed *viz.*, grey to blackish grey. Two isolates BmCKD, BmMNG produced light grey, six isolates BmRDK, BmGLG, BmRCH, BmNYP, BmBDH, BmDHM produced grey, four isolates BmHSK, BmHNG, BmMDH, BmDHM produced dark grey and eight isolates BmZARS, BmMNY, BmHKP, BmADH, BmSVN, BmSNG, BmTNP, BmNVL produced greyish black color colony on PDA. Among nine different solid media used, maximum mycelial growth of 71.06 mm was observed in maize leaf decoction with PDA medium and minimum mycelial growth of 37.33 mm in Richard's Agar. Further, sporulation of conidia was earliest (48–84 hrs.) in maize leaf decoction with PDA and delayed (120–168 hrs.) in V8 Juice Agar media. Sequence analysis with ITS1 and ITS4 primers revealed that of all the isolates showed similarity with *B. maydis* and showed relatively low E-value of zero depicting the highest confidence level. Maximum of 100% similarity was observed in BmHKP, BmRDK, BmSNG, BmTNP, BmBDH and BmUPH isolates.

KEYWORDS: Isolates, colony growth, maize leaf decoction, ITS

***Citation* (VANCOUVER):** Monisha et al., Polyphasic Characterization of *Bipolaris maydis*: Insights into Pathogen Identity and Variability through Morpho-cultural and Molecular Traits. *International Journal of Bio-resource and Stress Management,* 2026; 17(4), 01-12. HTTPS://DOI.ORG/10.23910/1.2026.6589.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, author have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The author has declared that no conflict of interest exists.

RECEIVED on24th July 2025 RECEIVED in revised form on 18th March 2026 ACCEPTED in final form on 06th April 2026 PUBLISHED on 23rd April 2026

1. INTRODUCTION

Maize (*Zea mays* L.), originating from the highlands of Mexico and belonging to the family Poaceae, is the third most important cereal crop after rice and wheat. It is widely recognized as a global staple due to its nutritional value, adaptability, and diverse uses (Pavan and Shete, 2021). Maize serves as a major food source for humans and a high-quality feed and fodder for livestock (Haque et al., 2022). In India, it supports industries producing baby corn, sweet corn, and popcorn, highlighting its economic and agro-industrial importance (Layek et al., 2016). Globally, maize provides nearly 15% of protein and about 19% of calories derived from food crops (Kumari et al., 2018).

The United States is the largest maize producer (31.39% of global output), followed by China (23.28%) and Brazil (10.63%), while India ranks seventh with 3.07% (Anonymous, 2023). In India, maize is grown on 10.88 million hectares, yielding 38.08 million tonnes at 3.54 t ha^{-1}. Karnataka leads national production with 54.90 lakh tonnes, followed by Bihar (46.13 lakh tonnes) (Anonymous, 2023). The state's maize area is expanding due to favorable environments, high yield potential, and easy cultivation practices, covering 1.92 mha at 3.27 t ha^{-1} (Anonymous, 2023).

Despite its yield potential, maize faces major disease constraints, with nearly 65 reported in India. MLB, caused by *B. maydis* (Nisikado and Miyake; anamorph of *Cochliobolus heterostrophus*), is highly destructive (Pavan and Shete, 2021). The necrotrophic, polycyclic pathogen thrives in warm, humid Indian maize-growing regions (Soumya and Ramachandra, 2019). Field observations from Tamil Nadu and Karnataka show that higher rainfall and frequent rainy days significantly enhance disease intensity, while temperature and humidity influence sporulation and lesion development (Goudar and Harlapur, 2019; Raju et al., 2023).

Four physiological races-T, O, C, and S-are recognized. Race T caused the Southern corn leaf blight epidemic in the USA (1970s), while race O is prevalent in India. Race C affects cytoplasmic male sterile line 'C' in China, and race S remains poorly characterized (Singh et al., 2021). Race O symptoms start as small, diamond-shaped lesions that elongate into spindle-shaped necrotic spots (Singh and Srivastava, 2016; Li and Wilson, 2013; De Rossi et al., 2015). Lesion morphology varies with pathogen race and maize genotype, and severe infection reduces photosynthetically active leaf area, impairing growth and productivity (Sajeed and Chowdhury, 2014). Globally, biotic factors account for ~23% of annual maize yield losses, with foliar fungal diseases such as NLB, GLS, and SCLB contributing over 4% (Savary et al., 2019).

In India, MLB occurs in Punjab, Haryana, Delhi, Uttar Pradesh, Bihar, Madhya Pradesh, Gujarat, Jammu & Kashmir, Sikkim, Meghalaya, Rajasthan, Andhra Pradesh, and Maharashtra (Kumar et al., 2013). Yield losses may reach 60% under severe infection depending on varietal susceptibility (Kumar et al., 2011). Losses depend on crop stage: before silking, up to 40%; two to three weeks after pollination, ~50%; with reductions of 28–91% reported (Nwanosike et al., 2015). In Karnataka, MLB significantly affects hybrids and composites, causing similar losses (Harlapur et al., 2009).

Pathogen identification traditionally relied on culturing, re-inoculation, microscopy, and biochemical assays (Sharma and Sharma, 2016). Pal et al. (2015) reported differences in colony growth, conidial size, and sporulation. PCR and ITS sequencing (ITS1 and ITS4 primers) provide reliable molecular identification, enabling assessment of genetic. Neves et al. (2015) documented ITS-based genetic polymorphisms whereas Singh et al. (2021) observed physiological race diversity with differential virulence. Therefore, a comprehensive investigation of the cultural and morphological features of *B. maydis* isolates, coupled with ITS1 and ITS4 molecular characterization, was undertaken to confirm pathogen identity.

2. MATERIALS AND METHODS

The research was conducted during March, 2022 to June, 2022, Department of Plant Pathology, College of Agriculture, Mandya, Karnataka. In the Karnataka, Mandya accounts for major maize growing region, in order maintain productivity of crop disease and pest free conditions are necessary. Since Maydis leaf blight is one of the major diseases affecting the crop growth a detailed study of the causal organism is a necessity.

2.1. Sampling, isolation and identification of the pathogen

A total of 20 infected maize leaf samples which were obtained from four districts (Mandya, Hassan, Mysuru and Chamarajanagar) were used for isolation of the pathogen. The isolation of the pathogen was done by following a standard tissue isolation technique. The diseased leaves of about 0.5 cm were surface sterilized in 1% sodium hypochlorite solution for 60 seconds and washed with sterile water thrice to remove the traces of sodium hypochlorite. Later were transferred to the petri plates with Potato Dextrose Agar (PDA) medium and incubated at 27±1°C. Pure culture of the pathogen isolates was obtained by single hyphal tip isolation technique which on development was transferred to PDA slants and incubated at 27±1°C.

2.2. Pathogenicity test

To prove the Koch's postulates, the maize leaves were grown in pots under greenhouse conditions. Mycelial suspension

with 10^6cfu/ml was prepared by using 8 days old culture and sprayed on the plants (21 days old). Regular observations were made for the development of the symptoms (diamond shaped to rectangular lesions on leaves). Re-isolation was done from the infected leaf tissues and the isolate thus obtained was compared with the original culture for confirmation.

2.3. Morphological characterization

The morphological characters of the fungus were studied periodically by observing under microscope. The colour of the colony was determined with the help of Munsell's soil colour chart (Munsell Colour Company, *Inc.,* 1954). In addition, Time taken for mycelial initiation, centre of the colony (colour), Luster, texture of the colony was also determined. Conidial characteristics like length, width, length: width ratio, number of septa, type of hilum and conidial shape and colour of all the isolates of *B. maydis* was determined. Temporary slides from 14 days old culture were prepared in water mount. Data on length and width of conidia were recorded by ocular micrometer by using a precalibrated compound microscope.

2.4. Effect of media on pathogen colony growth

Colony growth of different isolates of *B. maydis* were studied on nine different media (Table 3, 4). These plates with PDA were inoculated with fungal discs (5mm) from 7 days old pure culture of *Bipolaris maydis* and incubated at 26±2°C. Four replications were maintained for each culture medium and the radial growth (mm) of the fungal mycelia was recorded diagonally 7 days after inoculation (Bhavani et al., 2016). In order to determine the disparity of the isolates, analysis of the experimental data was done by using completely randomised design (CRD) for the laboratory studies as suggested by Panse and Sukathme (1985).

2.5. Molecular characterization

The genomic DNA of twenty isolates *viz*., BmZARS, BmMNY, BmHKP, BmHSK, BmCKD, BmRDK, BmGLG, BmADH, BmSVN, BmHNG, BmRCH, BmSNG, BmNYP, BmMNG, BmTNP, BmBDH, BmNVL, BmMDH, BmUPH, BmDHM of *B. maydis* was extracted by following CTAB (Cetyl trimethyl ammonium bromide) extraction method (Dellaporta et al., 1983). Amplification of the internal transcribed spacer regions 1 and 4 (ITS 1 and 4) of the ribosomal DNA was done in a thermal cycle. A final volume of 50 µl reaction mixture was prepared with 5µl of template DNA, 2.5 µl of each primer, 25 µl Red Taq Master Mix and 15 µl of nuclease free water. Thermo cycling procedure includes initial denaturation (95°C) for 5min, followed by 35 different cycles with denaturation (95°C for 1min), annealing (53°C for 1min), extension (72°C 1min) and final extension (72°C 10 min). An aliquot (7 µl) of each amplification reaction was analysed on 3% w/v agarose gel cast and run in 1X TBE buffer (pH 8.3), gel was stained with ethidium bromide. 100 base pair markers were included on every gel. Later, the gel was visualized using gel documentation unit under UV light photographed using transmitted UV light. Result of band pattern was documented using Gel doc unit. The PCR of the samples were sequenced (by Eurofins Genomics India Pvt. Ltd., Bangalore, Karnataka) and FASTA format of both forward and reverse sequence data were cleaned for redundancy and error, edited and aligned using Bioedit software, and consensus sequences generated from each alignment was used for Basic Local Alignment Search Tool (BLAST) search. Later, the sequence similarity search was performed using web interphase of NCBI BLAST program.

3. RESULTS AND DISCUSSION

3.1. Morphological characterization

Mycelia and conidia of all the twenty isolates on PDA were brown in colour and septate. The conidia varied in size, ranging from 57.44–80.05×11.34–15.77 µm being brown with septation. The highest conidial length was noticed in BmMNY (80.05 µm) followed by BmNYP (76.75 µm) and BmMDH (57.44 µm) followed by BmUPH (58.26 µm) recorded lowest conidial length. The highest conidial width of 15.77 µm was recorded in BmADH followed by BmGLG (14.21 µm) whereas BmSNG recorded lowest conidial width of 11.34 µm followed by BmMDH (11.42 µm). Varied number of septation was observed in the conidia of the isolates ranging from 3 to 14. Maximum number of septation was observed in BmMNY (9–14) and Minimum number of septae was recorded in BmTNP (3–5) (Table 1) (Plate 2). Colony colour of all the isolates was initially whitish, after 7days of incubation colony colour varied from light grey to greyish black was noticed among the isolates. Two isolates BmCKD, BmMNG produced light grey, six isolates BmRDK, BmGLG, BmRCH, BmNYP, BmBDH, BmDHM produced grey, four isolates BmHSK, BmHNG, BmMDH, BmDHM produced dark grey and eight isolates BmZARS, BmMNY, BmHKP, BmADH, BmSVN, BmSNG, BmTNP, BmNVL produced greyish black colony. Two forms of colony were observed *i.e.,* smooth appressed or smooth raised with regular or irregular margin. The sporulation of isolates varied between 48 hrs to 84 hrs after incubation (Table 2).

Pal et al. (2015) reported similar results indicating that the isolates produced dark coloured, fusoid conidia, measuring 6.14-23.41 µm×2.15-5.70 µm were MDCh1 isolate was largest (19.6 µm×5.0 µm) and MUCh3 isolate produced smallest (9.7 µm×3.3 µm) conidia. The average number of septa varied from 2 to 8. The isolate, MLCh1, showed highest number of septa (5.0). Amorio and Cumagun

Table 1: Conidial characteristics of *Bipolaris maydis* isolates

Sl. No.	Isolates	Length (µm)	Width (µm)	Length: width ratio	Number of septa	Conidial shape	Hilum end	Conidial colour
1.	BmZARS	74.34	12.70	5.83	8-11	Curved	Slightly round	Brown
2.	BmMNY	80.05	13.51	5.93	9-14	Curved	Slightly round	Brown
3.	BmHKP	74.58	13.65	5.46	7-12	Slightly curved	Blunt round	Brown
4.	BmHSK	70.41	13.49	5.22	7-11	Curved	Blunt round	Brown
5.	BmCKD	70.10	12.53	5.59	7-10	Fusoid	Slightly round	Brown
6.	BmRDK	65.50	11.95	5.48	5-8	Slightly curved	Slightly round	Brown
7.	BmGLG	64.71	14.21	4.55	4-7	Slightly curved	Blunt round	Brown
8.	BmADH	75.84	15.77	4.81	8-13	Slightly curved	Blunt round	Brown
9.	BmSVN	65.51	11.72	5.59	5-8	Slightly curved	Blunt round	Brown
10.	BmHNG	71.39	12.93	5.52	7-11	Fusoid	Blunt round	Brown
11.	BmRCH	64.76	12.68	5.10	5-7	Spindle	Blunt round	Brown
12.	BmSNG	60.18	11.34	5.31	4-6	Slightly curved	Slightly round	Brown
13.	BmNYP	76.75	13.84	5.55	8-12	Curved	Slightly round	Brown
14.	BmMNG	63.47	13.89	4.57	5-7	Curved	Slightly round	Brown
15.	BmTNP	58.50	12.50	4.68	3-5	Slightly curved	Slightly round	Brown
16.	BmBDH	75.36	13.60	5.54	8-13	Curved	Slightly round	Brown
17.	BmNVL	61.28	12.86	4.77	5-6	Fusoid	Slightly round	Brown
18.	BmMDH	57.44	11.42	5.02	4-5	Curved	Slightly round	Brown
19.	BmUPH	58.263	11.81	4.93	4-5	Fusoid	Blunt round	Brown
20.	BmDHM	62.93	13.58	4.63	4-7	Spindle	Slightly round	Brown

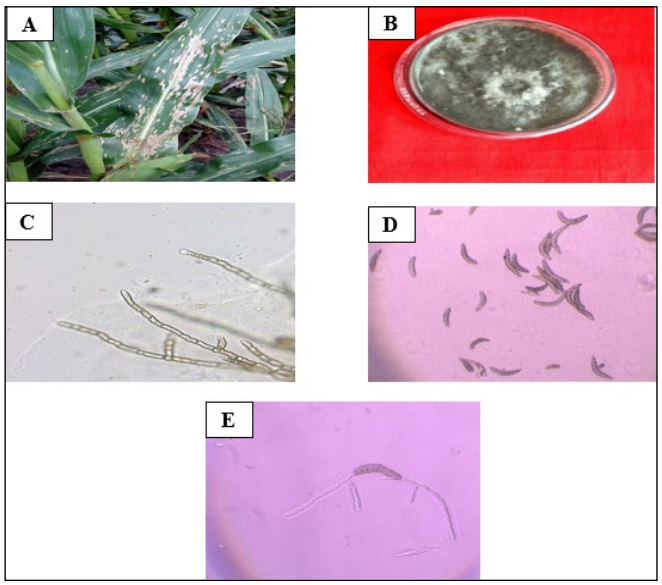

Plate 1: Symptomatology and Morphological characteristics of *B. maydis*; A: Rectangular lesions on infected leaf; B: Pure culture of *B. maydis*; C: Septate and brown mycelia under 40X; D: Conidia (septate) under 40X; E: Bipolar germination of conidia under 40X

(2017), reported that *Bipolaris maydis* produced slightly curved conidia with length of 70–160 µm and width of 15–20 µm bearing 7–11 distoseptate and rounded to pointed hilum. Sun et al. (2020) reported that conidia of isolates were curved or slightly straight with light brown to dark brown. The conidial septation ranged from 3–5 (Vignesh et al., 2023).

3.2. Effect of media on pathogen colony growth

In the nine different solid media tested, highest mean radial mycelial growth of 71.06 mm was recorded in maize leaf decoction with PDA followed by maize leaf decoction (69.40 mm), Oat Meal Agar (68.41 mm), potato dextrose agar (66.45 mm) and Czapek-Dox Agar (63.92 mm). The lowest mean radial mycelial growth of 37.33 mm was observed in Richard's Agar followed by Peptone Sucrose Agar (45.13 mm), V-8 juice agar (47.38 mm) and Malt Extract Agar (53.22 mm) (Table 3). Similarly, variation was observed in liquid media and the mean wet mycelial weight among the isolates varied from 1.03 g to 2.51 g. The highest mean wet mycelial weight of 2.51 g was recorded in leaf decoction with PDB followed by leaf decoction (2.50 g), Oat Meal broth (2.23 g), Potato Dextrose broth (2.06 g)

Table 2: Mycelial characteristics of *Bipolaris maydis* isolates

Sl. No.	Isolates	Mycelial initiation (hours)	Texture / edge	Zonations	Centre of the colony colour	Luster
1.	BmZARS	24	Irregular	No Zonation	Grey	Shiny
2.	BmMNY	24	Irregular	No Zonation	Grey	Shiny
3.	BmHKP	36	Irregular	No Zonation	Whitish grey	Shiny
4.	BmHSK	36	Irregular	No Zonation	Whitish grey	Shiny
5.	BmCKD	36	Regular	No Zonation	Whitish grey	Shiny
6.	BmRDK	24	Regular	No Zonation	White	Dull
7.	BmGLG	24	Irregular	No Zonation	White	Dull
8.	BmADH	36	Irregular	No Zonation	Light grey	Shiny
9.	BmSVN	48	Irregular	No Zonation	White	Shiny
10.	BmHNG	48	Irregular	No Zonation	White	Shiny
11.	BmRCH	24	Irregular	Zonation	Grey	Dull
12	BmSNG	24	Irregular	No Zonation	Blackish grey	Shiny
13.	BmNYP	48	Irregular	Zonation	Light grey	Dull
14.	BmMNG	36	Irregular	No Zonation	White	Dull
15.	BmTNP	36	Irregular	No Zonation	Grey	Shiny
16.	BmBDH	48	Irregular	Zonation	Grey	Shiny
17.	BmNVL	48	Irregular	No Zonation	Grey	Shiny
18.	BmMDH	36	Regular	No Zonation	Light grey	Dull
19.	BmUPH	24	Irregular	No Zonation	Grey	Shiny
20.	BmDHM	48	Irregular	No Zonation	Whitish grey	Shiny

Plate 2: Microscopic view of conidia of *B. maydis* isolates (40X)

and Malt Extract broth (1.14 g) out of nine different liquid media used and Czapek - Dox broth recorded lowest mean wet mycelial of 1.03 g followed by Richard's broth (1.04 g), V8 Juice broth (1.06 g), Peptone Sucrose broth (1.10 g) (Table 4) (Plate 3–11).

Under lab conditions it was observed that potato sucrose agar was suitable for the rapid growth of pathogen with a mean of 10.19 mm day^{-1}, followed by corn meal agar (8.56 mm day^{-1}) and potato dextrose agar (5.46 mm day^{-1}) (Kutawa et al., 2021). Harlapur (2017) stated that among the five solid media studied, pathogen produced maximum growth on Potato Dextrose Agar medium (79.95 mm) followed by Corn meal agar (77.02 mm). Bhavani et al. (2016) the colony diameter of 78.6 mm was observed on PDA whereas in Richard's Agar it was 66.0 mm.

3.3. Molecular characterization

The BLAST sequence similarity search with target nucleotide database showed that all the twenty isolates showed relatively low E-value of zero depicting the highest confidence level. In addition, they also showed highest sequence coverage and identity. The PCR produce was approximately 600 bp. The query coverage ranged from

Table 3: Variability of *B. maydis* isolates in different solid media pertaining to radial mycelial growth

Sl. No.	Isolates	PDA	Richard's agar	OMA	PSA	Czapek - Dox Agar	V8 Juice Agar	Malt extract agar	Leaf dec.	Leaf dec. +PDA
		Radial growth (mm)								
1.	BmZARS	80.67	54.00	75.67	53.67	83.00	61.67	39.30	72.00	80.67
2.	BmMNY	80.67	51.67	70.33	40.33	75.00	41.00	60.30	75.00	81.33
3.	BmHKP	78.00	54.00	73.00	32.00	82.00	52.33	56.00	62.30	75.00
4.	BmHSK	69.00	53.33	66.67	32.00	74.00	39.67	64.00	77.30	56.33
5.	BmCKD	53.00	31.00	64.00	43.33	55.00	25.00	36.70	78.70	73.00
6.	BmRDK	65.00	43.00	72.67	31.00	58.00	33.00	71.70	44.30	47.67
7.	BmGLG	44.00	33.00	63.00	29.00	73.00	50.00	48.30	54.30	43.00
8.	BmADH	69.00	20.67	71.00	53.00	49.00	50.67	49.70	76.30	68.33
9.	BmSVN	71.00	19.33	64.67	78.33	47.00	43.00	77.70	79.00	83.00
10.	BmHNG	77.33	49.00	65.00	35.00	75.00	52.00	44.30	81.00	73.00
11.	BmRCH	61.00	53.00	66.33	68.67	55.00	53.00	59.30	78.00	66.00
12.	BmSNG	61.33	36.33	65.00	54.00	73.00	51.67	37.70	39.67	75.00
13.	BmNYP	79.00	50.00	74.33	40.00	53.00	49.33	37.70	34.00	75.33
14.	BmMNG	62.00	23.00	55.00	48.67	43.00	62.00	50.00	82.00	83.00
15.	BmTNP	75.00	21.67	73.00	34.00	31.00	73.00	60.00	79.00	72.00
16.	BmBDH	60.00	18.67	53.67	31.67	42.67	21.33	34.00	70.00	72.33
17.	BmNVL	72.00	22.00	81.67	36.33	80.00	48.00	40.70	77.70	75.00
18.	BmMDH	33.00	40.00	66.33	68.33	65.00	50.00	75.00	76.00	61.00
19.	BmUPH	75.00	53.00	66.00	45.67	83.00	51.00	46.00	73.00	78.67
20.	BmDHM	63.00	50.00	81.00	47.67	81.67	40.00	76.00	78.30	81.67
Mean	66.15	37.33	68.41	45.13	63.92	47.38	53.22	69.40	71.06	
F						**				
SE m±		Media=0.02, Isolates=0.04, Media×Isolates=0.11								
CD *p*=0.01		Media=0.08, Isolates=0.13, Media×Isolates=0.39								
CV		2.66								

*OMA: Oat meal agar; PSA: Peptone sucrose agar; Leaf dec.: Leaf decoction

Table 4: Variability of *B. maydis* isolates in different liquid media pertaining to wet mycelial weight

Sl. No.	Isolates	PDA	Richard's broth	OMA	PSA	Czapek - Dox broth	V8 Juice broth	Malt extract broth	Leaf dec.	Leaf dec. +PDA
		Wet mycelial weight (gms)								
1.	BmZARS	1.92	1.17	2.28	1.52	2.00	1.66	1.82	2.58	3.42
2.	BmMNY	2.01	0.66	2.78	1.22	1.90	1.19	1.55	2.63	2.96
3.	BmHKP	2.18	0.86	2.07	0.77	0.84	1.04	1.00	2.82	2.33
4.	BmHSK	2.16	1.49	2.49	0.76	1.12	1.00	1.53	2.42	3.02
5.	BmCKD	1.95	1.27	2.03	1.18	1.26	0.86	1.31	2.23	3.22
6.	BmRDK	2.74	1.01	2.05	1.47	1.07	1.20	1.15	2.98	2.42
7.	BmGLG	2.26	1.04	2.03	0.84	1.33	1.66	0.55	2.76	2.24
8.	BmADH	2.04	0.76	1.98	0.56	0.99	0.49	0.95	2.44	2.14

Table 4: Continue...

Sl. No.	Isolates	PDA	Richard's broth	OMA	PSA	Czapek - Dox broth	V8 Juice broth	Malt extract broth	Leaf dec.	Leaf dec. + PDA
		Wet mycelial weight (gms)								
9.	BmSVN	1.98	0.94	2.36	0.36	1.03	0.50	0.55	2.40	2.12
10.	BmHNG	1.97	0.67	1.91	1.65	0.96	0.53	0.65	2.41	2.89
11.	BmRCH	1.95	0.67	2.28	1.37	1.17	1.22	1.43	2.10	2.03
12.	BmSNG	2.05	1.74	2.86	1.57	0.85	1.12	1.31	2.53	2.59
13.	BmNYP	2.25	0.55	2.03	0.78	0.79	0.92	0.43	2.93	2.97
14.	BmMNG	2.08	2.28	1.83	1.18	0.86	1.50	1.15	2.59	2.42
15.	BmTNP	2.43	1.07	2.54	1.07	0.77	0.38	1.49	2.48	2.12
16.	BmBDH	1.75	0.44	2.28	1.38	0.83	0.67	1.11	2.45	2.31
17.	BmNVL	1.96	2.18	2.44	1.40	0.86	1.24	0.97	2.62	1.88
18.	BmMDH	2.11	0.67	2.12	1.02	0.93	1.04	1.20	2.24	2.69
19.	BmUPH	1.55	0.67	1.94	0.95	0.35	1.15	0.60	2.07	2.13
20.	BmDHM	1.85	0.74	2.32	0.98	0.68	1.40	1.09	2.29	2.30
Mean	2.06	1.04	2.23	1.10	1.03	1.06	1.14	2.50	2.51	
F						**				
SEm±		Media = 0.006, Isolates = 0.01, Media × Isolates = 0.03								
CD @ *p*=0.01		Media = 0.02, Isolates = 0.03, Media × Isolates = 0.10								
CV		3.01								

*OMA: Oat meal agar; PSA: Peptone sucrose agar; Leaf dec.: Leaf decoction

Plate 3: Variability of mycelial growth of *B. maydis* isolates on Potato Dextrose Agar (PDA) at 8 days after incubation

Plate 4: Variability of mycelial growth of *B. maydis* isolates on Richard's Agar at 8 days after incubation

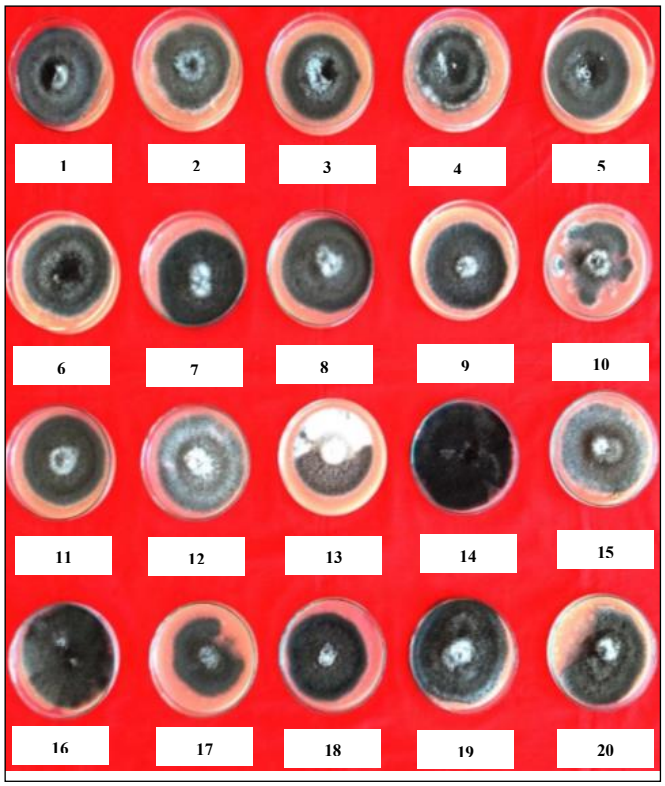

Plate 5: Variability of mycelial growth of *B. maydis* isolates on Oat meal Agar at 8 days after incubation

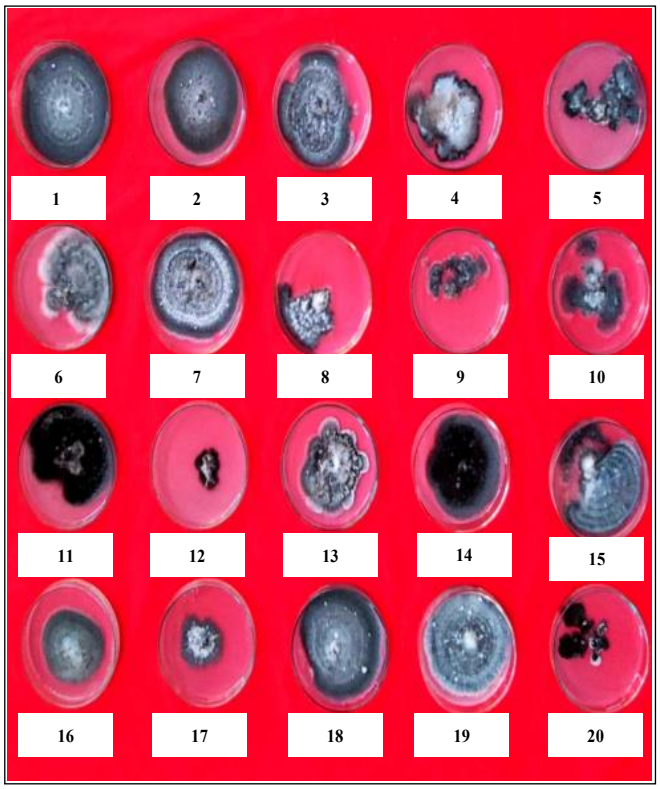

Plate 7: Variability of mycelial growth of *B. maydis* isolates on Czapek dox Agar at 8 days after incubation

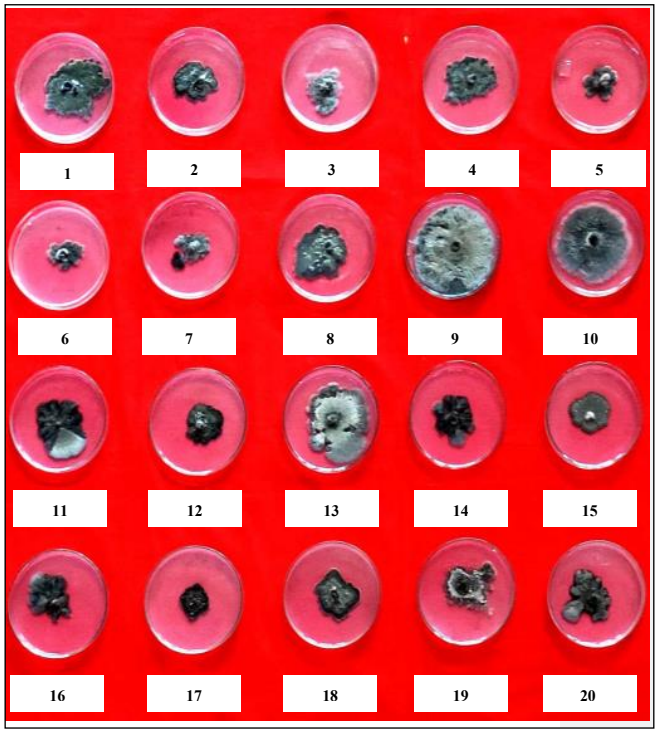

Plate 6: Variability of mycelial growth of *B. maydis* isolates on peptone sucrose Agar at 8 days after incubation

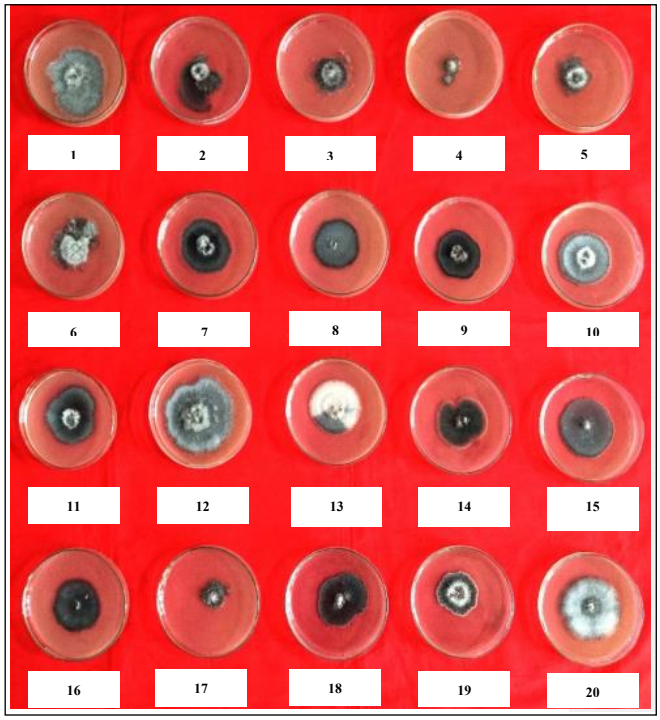

Plate 8: Variability of mycelial growth of *B. maydis* isolates on V8 Juice Agar at 8 days after incubation

Plate 9: Variability of mycelial growth of *B. maydis* isolates on Malt extract Agar at 8 days after incubation

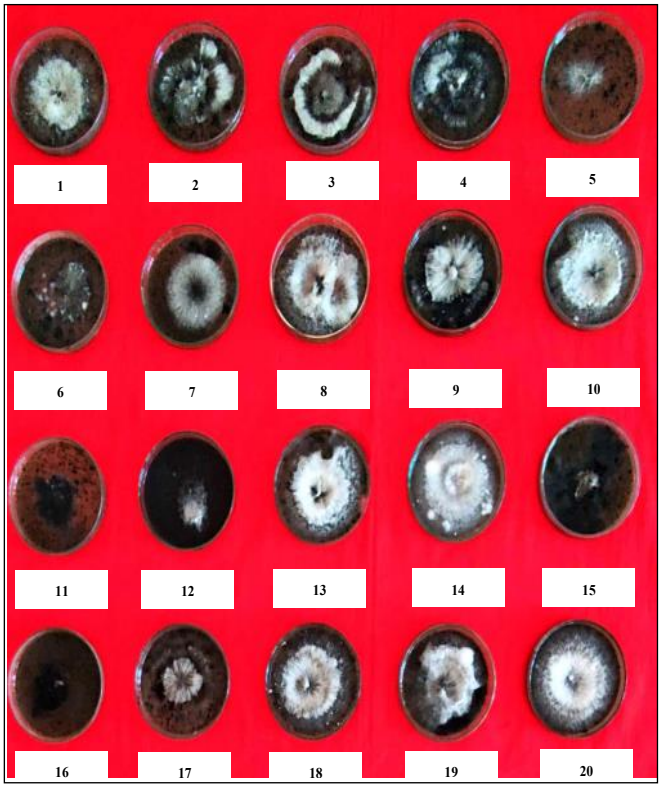

Plate 10: Variability of mycelial growth of *B. maydis* isolates on Leaf decoction at 8 days after incubation

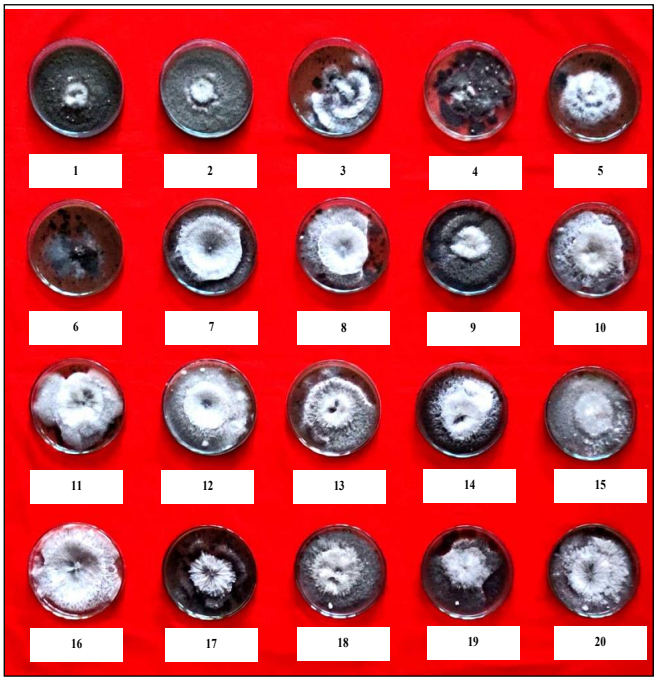

Plate 11: Variability of mycelial growth of *B. maydis* isolates on leaf decoction + PDA Agar at 8 days after incubation; 1. BmZARS; 2. BMMNY; 3. BmHSK; 4. BmCKD; 5. BmRDK; 6. BmGLG; 7. BmHKP; 8. BMADH; 9. BMSVN; 10. BMRCH; 11. BMSNG; 12. BMTNP; 13. BMMDH; 14. BMNVL; 15. BMHNG; 16. BMNYP; 17. BMBDH; 18. BMUPH; 19. BMDHM; 20. BMMNG

95% to 100% whereas, base pairs ranged from 559 bp to 1000 bp. The highest similarity of 100% with *B. maydis* was recorded in BmHKP, BmRDK, BmSNG, BmTNP, BmBDH, BmUPH isolates followed by BmMNY, BmHSK, BmCKD, BmHNG, BmRCH, BmNYP, BmMNG, BmNVL, BmMDH, BmDHM (99.82%), BmGLG, BmADH (99.81%), BmSVN (99.63%) and BmZARS (97.54%) (Table 5) (Plate 12).

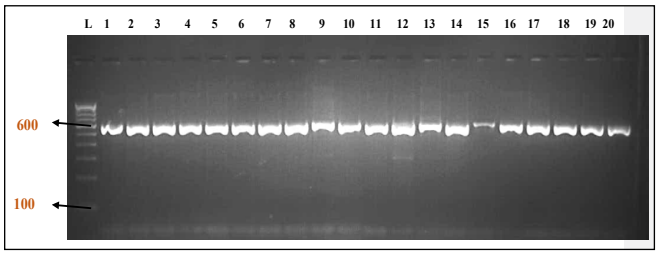

Plate 12. Gel electrophoretic profiles of ITS1 and ITS4 of amplicon from *B. maydis* isolates; Wells: L: 100bp ladder; 1. BmZARS; 2. BMMNY; 3. BmHSK; 4. BmCKD; 5. BmRDK; 6. BmGLG; 7. BmHKP; 8. BMADH; 9. BMSVN; 10. BMRCH; 11. BMSNG; 12. BMTNP; 13. BMMDH; 14. BMNVL; 15. BMHNG; 16. BMNYP; 17. BMBDH; 18. BMUPH; 19. BMDHM; 20. BMMNG

Table 5: BLAST analysis results of *B. maydis* isolates

Isolates	Isolates	Max score	Total score	Query coverage	E-value	Percent identity	Seq. length	Accession
1.	BmZARS	902	1770	95%	0.0	97.54%	710	OM281342.1
2.	BmMNY	1000	1817	100%	0.0	99.82%	570	KF922872.1
3.	BmHKP	1000	1771	98%	0.0	100.00%	561	KX834870.1
4.	BmHSK	1009	2007	99%	0.0	99.82%	563	MT130269.1
5.	BmCKD	992	1985	99%	0.0	99.82%	561	MT130270.1
6.	BmRDK	983	2156	97%	0.0	100.00%	595	MH864477.1
7.	BmGLG	959	1913	97%	0.0	99.81%	570	ON342865.1
8.	BmADH	977	1185	90%	0.0	99.81%	559	KU670348.1
9.	BmSVN	77	1799	99%	0.0	99.63%	563	KP211429.1
10.	BmHNG	992	1806	99%	0.0	99.82%	587	ON062997.1
11.	BmRCH	1000	1817	99%	0.0	99.82%	570	KF922872.1
12.	BmSNG	983	1863	97%	0.0	100.00%	595	MH864477.1
13.	BmNYP	1000	1823	99%	0.0	99.82%	562	KC315946.1
14.	BmMNG	1003	2000	99%	0.0	99.82%	587	ON062997.1
15.	BmTNP	994	1981	98%	0.0	100.00%	560	KP211430.1
16.	BmBDH	1000	2409	97%	0.0	100.00%	561	KX834869.1
17.	BmNVL	1002	1904	99%	0.0	99.82%	591	HM195266.1
18.	BmMDH	1002	1804	99%	0.0	99.82%	1000	ON062993.1
19.	BmUPH	983	1786	97%	0.0	100.00%	595	MH864477.1
20.	BmDHM	1007	1985	100%	0.0	99.82%	589	HM195267.1

*Seq.: Sequence

Similar to the present investigation Manzar (2022) mentioned that the ITS 1 and ITS 4 primers were used to detect the molecular characteristics of *Bipolaris maydis* isolates. According to Nadeem (2021), 15 isolates of was confirmed by sequencing the ITS region through BLASTn search was performed using nucleotide data base at NCBI and resulted that 98–100% identity of the isolates to other reported isolates of in the database. Sequence based species identification was in line with Dai *et al.* (2016) who conducted molecular identification studies on *Cochliobolus heterostrophus*.

4. CONCLUSION

All isolates exhibited consistent morphological traits such as brown, septate mycelia and conidia, with conidial size 57.44–80.05×11.34–15.77 µm and septation 3 to 14. Cultural variation was observed in colony color and morphology, with light grey to greyish black and two colony types: smooth appressed and raised. Among the medias tested, maize leaf decoction-based media supported the maximum mycelial growth and biomass. Molecular identification confirmed the identity of all isolates as *B. maydis.*

5. ACKNOWLEDGEMENT

The authors gratefully acknowledge the support of College of Agriculture, Mandya, Karnataka for providing laboratory facilities and technical assistance throughout the study.

6. REFERENCES

Amorio, D.J.H., Cumagun, C.J.R., 2017. Pathogenicity and cytological examination of adapted and non-adapted *Bipolaris* species on resistant and susceptible cultivars of rice and corn. Mycosphere 8(3), 377–381. DOI: 10.5943/mycosphere/8/3/3.

Anonymous, 2022–23. Area, production and productivity of maize in India. Available from: https://www.indiastat.com/data/agriculture/maize. Accessed date: 20/12/2024.

Anonymous, 2023. Faostat 2023, Statistical Database of the Food and Agriculture Organization of the United Nations. FAO. Available from: http://www.fao.org (2023). Agriculture production statistics, 2010-2023. Accessed date: 20/12/2024.

Bhavani, T.V., Gohil, V.P., Patel, J.K., 2016. Isolation, pathogenicity and culture media study of *Helminthosporium maydis* causing maydis leaf blight disease of maize. Advances in Life Sciences 5(1), 77–80. Available from: https://www.academia.edu/25444957/.

Dai, Y.L., Yang, X.J., Gan, L., Chen, F.R., Ruan, H.C., Du, Y.X., Shi, N.N., Gao, Z.M., 2016. First report of southern leaf blight caused by *Cochliobolus heterostrophus* on corn (*Zea mays*) in Fujian Province, China. Plant Disease 100(8), 1781. Available from: https://doi.org/10.1094/PDIS-12-15-1490-PDN.

Dellaporta, S.L., Wood, J., Hicks, J.B., 1983. A plant DNA minipreparation: version II. Plant Molecular Biology Reporter 1(4), 19–21. Available from: https://doi.org/10.1007/BF02712670.

De-rossi, R.L., Reis, E.M., Brustolin, R., 2015, Conidial morphology and pathogenicity of *Exserohilum turcicum* isolates of corn from Argentina and Brazil. Phytopathology, 41(1), 58–63. https://doi.org/10.1590/0100-5405/1948.

Goudar, S.V., Harlapur, S.I., 2019. Influence of weather parameters on development of maydis leaf blight of maize caused by *Bipolaris maydis* (Nisikado Shoemaker). International Journal of Current Microbiology and Applied Sciences 8(2), 2240–2245. https://doi.org/10.20546/ijcmas.2019.802.259

Haque, M., Marwaha, S., Deb, C.K., Nigam, S., Arora, A., Hooda, K.S., Soujanya, P.L., Aggarwal, S.K., Lall, B., Kumar, M., Islam, S., 2022. Deep learning-based approach for identification of diseases of maize crop. Scientific Reports 12(1), 1–14. http://dx.doi.org/10.1038/s41598-022-10140-z.

Harlapur, S.I., Kulkarni, M.S., Srikant Kulkarni, Patil, B.C., 2009, Assessment of crop loss due to turcicum leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs in maize. Indian Phytopathology 62(2), 144–154. Available from: https://epubs.icar.org.in/index.php/IPPJ/article/view/12539.

Harlapur, S.I., 2017. Morphological and cultural variability of *Drechslera maydis* (Nisikado) Subram and Jain causal agent of maydis leaf blight of maize. Environment and Ecology 35(1), 11–15. Available from: https://environmentandecology.com/wp-content/uploads/2025/02/MS3-1-4.pdf.

Kumar, P., Anshu, V., Kumar, S., 2011, Morpho-pathological and Molecular Characterization of *Bipolaris oryzae* in Rice (*Oryzae sativa*). Journal of Phytopathology, 159(1), 51–56. https://doi.org/10.1111/j.1439-0434.2010.01726.

Kumar, R., Hooda, K.S., Olakh, D.S., Kaur, H., Malik, V., Kumar, S., 2013. Reaction of QPM inbred lines against maydis leaf blight (MLB) and charcol rot. Electronic Journal of Plant Breeding 4(4), 1280–1283. Available from: https://doaj.org/article/4560433462484536a9a8701ffec8d83e.

Kumari, R., Singh, A.K., Sharma, V.K., 2018, Genetic divergence studies for morphoagronomical traits in maize (*Zea mays* L.) inbred lines. International Journal of Currrent Microbiology and Applied Sciences 7, 3589–3594. Avilable from: https://www.ijcmas.com/special/7/Ranju%20Kumari2,%20et%20al.pdf.

Kutawa, A.B., Ahmad, K., Bashir, A., Ali, A., Zobir, S.A.M., 2021. Identification and characterization of pathogen responsible for causing Southern Corn Leaf Blight (SCLB) disease in Malaysia. International Journal of Agriculture and Biology 26(5), 607–616. Available from: http://dx.doi.org/10.17957/IJAB/15.1873.

Layek, J., Ramkrushna, G.I., Suting, D., Ngangom, B., Krishnapp, A.R., de, U., Das, A., 2016. Evaluation of maize cultivars for their suitability under organic production system in the North eastern hill region of India. Journal of Hill Agriculture 29(2), 19–24. Available from: https://epubs.icar.org.in/index.php/IJHF/article/view/66678.

Li, B., Wilson, W.A., 2013. Composition and methods for enhancing resistance to northern leaf blight in maize. US Patent 20, 130, 061, 355. Available from: https://patents.google.com/patent/US20130061355A1/en.

Manzar, N., Avantika, A.S., Maurya, A., Rajawat, M.V.S., Sharma, P.K., Srivastava, A.K., Roy, M., Saxena, A.K., Singh, H.V., 2022. Multi-gene phylogenetic approach for identification and diversity analysis of *Bipolaris maydis* and *Curvularia lunata* isolates causing foliar blight of *Zea mays*. Journal of Fungi 8(8), 802. https://doi.org/10.3390/jof8080802.

Nadeem, A., Hussain, S., Fareed, A., Fahim, M., Iqbal, E., Ahmad, Z., Saeedullah, A., Karim, R., Akbar, A., 2021. Genetic variations among the isolates of *Bipolaris maydis* based on phenotypic and molecular markers. Brazilian Journal of Biology 84, 1–11. https://doi.org/10.1590/1519-6984.253147.

Neves, D.L., Silva, C.N., Pereira, C.B., Campos, H.D., Tessmann, D.J., 2015. *Cercospora zeina* is the main species causing gray leaf spot in southern and central Brazilian maize regions. Tropical Plant Pathology 40, 368–374. doi: 10.1007/s40858-015-0053-5.

Nwanosike, M.R.O., Mabagala, R.B., Kusolwa, P.M., 2015. Disease intensity and distribution of *Exserohilum turcicum* incitant of northern leaf blight of maize in Tanzania. International Journal of Pure and Applied Biosciences 3(5), 1–13. DOI: http://dx.doi.org/10.18782/2320-7051.2086.

Pal, I., Singh, V., Gogoi, R., Hooda, K.S., Bedi, N.,

2015. Characterization of *Bipolaris maydis* isolates of different maize cropping zones of India. Indian Phytopathology 68(1), 63–66. Available from: https://epubs.icar.org.in/index.php/IPPJ/article/view/46974.

Panse, V.G., Sukhatme, P.V., 1985. Statistical methods for agricultural workers. ICAR Publications, New Delhi, pp. 145–155. Available from: https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1814819.

Pavan, G.S., Shete, P., 2021. Symptomatology, etiology, epidemiology and management of southern corn leaf blight of maize (*Bipolaris maydis*) (Nisikado and Miyake) Shoemaker. The Pharma Innovation Journal 10(5), 840–844. Available from: https://www.academia.edu/75366890.

Raju, R., Veluchamy, S., Thambiyannan, S., Natesan, S. K., Vaithyanathan, S., Gandhi, K., 2023. Epidemiological studies on leaf blights of maize caused by *Exerohilum turcicum* (Pass.) leonard and suggs and *Bipolaris maydis* (Nisik. and Miyake). International Journal of Environmental Climate Change 13(11), 4472–4478. DOI: 10.9734/ijecc/2023/v13i113627.

Sajeed, A., Chowdhury, A.K., 2014. Histological studies on northern corn leaf blight disease of maize in hill agro-ecological zones of West Bengal. Journal of Mycology Research 113, 116. Available from: https://imskolkata.org/pdf/apr_14/20.%20sajeed%20ALI.pdf.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., Nelson, A. (2019). The global burden of pathogens and pests on major food crops. Nature Ecology and Evolution 3 (3), 430–439. DOI: 10.1038/s41559-018-0793-y.

Sharma, P., Sharma, S., 2016. Paradigm shift in plant disease diagnostics: a journey from conventional diagnostics to nano-diagnostics. Current Trends in Plant Disease Diagnostics and Management Practices Fungal Biology. Cham: Springer. DOI: 10.1007/978-3-319-27312-9_11.

Singh, R., Srivastava, R.P., 2016. Southern corn leaf blight-an important disease of maize: an extension fact sheet. Indian Research Journal of Extension Education 12(2), 324–327. Available from: https://api.seea.org.in/uploads/pdf/v12469.pdf.

Singh, S., Sharma, S., Ali, A., Kandan, A., Kumar, P., Akhtar, J., 2021. Morpho-molecular characterization of *Bipolaris* and *Exserohilum* spp. infecting various agricultural crops. International Journal of Agriculture and Applied Sciences 2(1), 110–117. Available from: http://dx.doi.org/10.52804/ijaas2021.2111.

Soumya, C., Ramachandr, Y.L., 2019. Assessment of pathogenicity in *Helminthosporium maydis* causing southern corn leaf blight disease in the region of Karnataka. Journal of Drug Delivery and Therapeutics 9, 146–154. Available from: https://jddtonline.info/index.php/jddt/article/view/2991.

Sun, X., Qi, X., Wang, W., Liu, X., Zaho, H., Wu, C., Chang, X., Zhang, M., Chen, H., Gong, G., 2020, Etiology and symptoms of maize leaf spot caused by *Bipolaris* spp. in Sichuan, China. Pathogens 9(3), 229. https://doi.org/10.3390/pathogens9030229.

Vignesh, K., Vengadeskumar, L., Sanjaygandhi, S., Sabesan, T., 2023. Morphological and molecular variation of *Bipolaris maydis* isolates causing maydis leaf blight in maize. Journal of Plant Disease Sciences 18(1), 5–10. http://dx.doi.org/10.48165/jpds.2023.1801.02.