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Prevalence of maydis leaf blight of maize in Tamil Nadu and assess the morphological character and virulence of *Bipolaris maydis* (NISIK.) shoemaker

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Abstract
Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adoptability under varied agro-climatic conditions. Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals. It is affected by several fungal, bacterial and viral diseases. Among these maydis leaf blight caused by the fungus *Bipolaris maydis*, is a disease that significantly affects maize productivity across the globe causes 40-45% yield loss. A survey was conducted to investigate the incidence and severity of maydis leaf blight incited by *B. maydis* in fifteen major maize growing areas of Tamil Nadu. The occurrence of wilt disease incidence ranged from 14 % to 45% was noticed. Plant showing typical symptoms were taken from 15 fields and identified based on symptom appearance as well as morphological characteristics. The result of the survey revealed that wide range of infection and severity of leaf blight were occurred in the major maize growing areas in Tamil Nadu. Isolation of the pathogen associated with maydis leaf blight was made from the diseased tissues in leaves of the plant on the Potato dextrose agar (PDA) medium. Bm_7 recorded the maximum blight incidence followed by Bm_8 and the minimum blight incidence was recorded in Bm_2. The pathogenicity of the fungal pathogen was also proved after artificial inoculation of the maize crop.

Keywords: Maize, survey, maydis leaf blight, *Bipolaris maydis*

1. Introduction
Maize (*Zea mays* L.) is known as queen of cereals belonging to the family, Gramineae. It is the most versatile crop, adapted to different agro-ecological and climatic conditions. It contributes a lot to nutritional food security of the society and is an important cereal crops of the world, grown in more than 166 countries across the globe, including tropical, subtropical and temperate regions, from sea level to 3000 m above sea level. Use of maize as feed, food, fodder and specialty corn as pop-corn, sweet corn, baby corns makes it one of the main crops par excellence for industrial use adapted to different agro-ecological and climatic conditions (Vardhan *et al*. 2020) [28].

Maize is one of the largest consumable cereals in the world. It is cultivated on nearly 197 m ha production of 1148 m tonnes and productivity of 582.8 kg/ha all over the world having wider diversity of soil, climate, biodiversity and management practices, contributing 37 per cent in the global grain production (FAO STAT 2020-21). India produced 30 million tonnes in an area of 9.9 million hectares in 2020-21 (agricoop.nic.in). In the country, more than three-fourths of the maize is grown in Andhra Pradesh, Karnataka, Tamil Nadu, Madhya Pradesh, Maharashtra, Rajasthan, Bihar, Uttar Pradesh, Telangana and Gujarat. The predominant maize growing states that contributes more than 80% of the total maize production are Andhra Pradesh (20.9%), Karnataka (16.5%), Maharastra (9.1%) and Tamil Nadu (7.5%). In Tamil Nadu, area, production & productivity of Maize were 3.44 L ha, 17.81 L. MT and 5176 Kgs/ha at 2020 – 21 (www. tn.gov.in).

The worldwide yield losses due to diseases in maize crop have been estimated at 4 to 14% of annual global harvest (Oerke 2006). Maize crops are susceptible to various diseases caused by different agents such as Bacteria, Viruses, Nematode, Fungi and Abiotic factors (Sahu *et al*. 2013) [23]. Among the fungal diseases, Maydis leaf blight also named as southern corn leaf blight caused by *B. maydis* (*Helminthosporium maydis*) is a serious fungal disease of maize throughout the world where warm and humid conditions exists during crop growing season.

Southern corn leaf blight occurs worldwide and important in regions of warm damp climate of 20-30^{0}C temperature (Agrios, 2005) [2].

Almost 70% yield loss is worldwide recorded due to SCLB reported by Ali *et al*. (2011) [6]. Prasoon *et al*. (2020) [21] mentioned that the Maydis leaf blight (MLB) caused by *Helminthosporium maydis* an important foliar disease in almost all the maize growing regions in India which leads to potential losses even up to 60% under severe disease conditions. However, various workers have reported yield loss up to 41 per cent (Sharma and Rai, 2000) [24] and 40 to 70% (Hussain *et al*. 2016) [13] depending on the variety, severity and stage of infection.

In this present study to conduct the survey on the occurrence of maydis leaf blight incidence in various maize growing areas in Tamil Nadu and collect the diseased leaves for further studies. Similar the present study, Akshaya (2018) [4] reported that the survey of maydis leaf blight incidence ranging from 5.61 – 26.41% in various maize growing areas in Tamil Nadu. Hulagappa *et al*. (2013) [12] reported that the survey for severity of maydis leaf blight of maize in northern Karnataka at the range of 33.88 – 56.26%. Yadav *et al*. (2013) [29] reported the occurrence of MLB in six districts with incidences ranging from 2.5 - 4.5 disease rating scale with more severe in Udaipur, Durgapur and Banswara districts in southern Rajasthan.

2.1 Materials and Methods

2.1.1 Survey to assess the incidence of maydis leaf blight (MLB) on maize

An intensive roving survey was conducted to find out the occurrence of disease incidence and severity of MLB in Maize growing areas of Tamil Nadu. In each districts three major maize growing villages were selected. In each village, five fields were selected randomly when the crop was in flowering to grain filling stage. Such fields were assessed for MLB severity by recording the disease on 0 - 9 disease rating scale. Further PDI was calculated by using the following formula proposed by Wheeler, 1969.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{Total number of leaves observed}} \text{ X } \frac{100}{\text{Maximum disease grade}}$$

Disease Scale

Grade	Scale
0	No visible symptom
1	Very slight to slight infection, one or two to few scattered lesions on lower leaves
3	Light infection, moderate number of lesions on lower leaves only
5	Moderate infection, abundant lesions on lower leaves, few on middle leaves
7	Heavy infections abundant lesions on lower and middle leaves, extending to upper leaves
9	Very heavy infection, lesions abundant on almost all leaves; plants prematurely dry or killed by the disease

2.1.2 Isolation and identification of pathogen

The fungus was isolated using the below-mentioned conventional tissue isolation procedure. The necrotic leaf fragments and some healthy pieces were surface sterilised in 1% sodium hypochlorite solutions for 30 seconds, and then the sodium hypochlorite residues were thoroughly removed by washing three times in distilled water. The leaf fragments were then aseptically added to the PDA-containing petriplate and cultured for 7 days at room temperature (28±2 °C), with occasional checks for fungus growth on the medium. By looking at the conidia and conidiophore under a powerful microscope, the pathogen was confirmed. Observed septa's length, breadth, and number, which were then supported by conventional literature (Alexopoulos *et al*. 1996) [5].

2.1.3 Morphological characters of *Bipolaris maydis* isolates

Using a sterile cork borer, a nine mm culture disc from a pathogen PDA culture that had been in existence for 10 days was inserted in the middle of sterilised Petri dishes holding 15 ml of PDA under aseptic conditions. The mycelial development and physical characteristics of the isolates were noticed seven days after incubation at room temperature (28±2 °C). Colony colour, sporulation, conidial population, and number of septa were observed, counted, and recorded as morphological features (CMI 1980) [8].

2.1.5 Pathogenicity Test

The susceptible cultivar of maize NK6240 was grown from surface-sterilized seeds in cement pots with soil that had undergone autoclave sterilization. A single plant was kept in each pot. The plant was inoculated with B. maydis isolates at the 4-6 leaf stage using the conventional sorghum grain inoculation method (Shekhar and Kumar 2012) [25]. Using a 0–5 scale suggested by Payak and Sharma, the incidence of MLB illness was noted 21 days after immunization (1985). Re-isolating the virus from the artificially infected plants allowed researchers to compare it to the initial isolate kept in the lab.

2.1.6 Scanning Electron Microscopy

A fungal culture that was actively developing was fixed overnight at 28°C in a phosphate buffer solution that contained 4% glutaraldehyde. The following day, ethanol was used to dehydrate the sample for 15 minutes after the fungal mat had been rinsed three times with phosphate buffer. After being dried with CO_2 for 5 minutes, the samples that had been fixed and dehydrated were then fixed on aluminium stubs, sputtered coated with carbon polaron E-500, and immediately viewed under a scanning electron microscope at 15 KV.

2.1.7 Statistical Analysis

Statistical evaluation was done using analysis of variance (ANOVA) followed by Duncan' s Multiple Range Test (DMRT). The statistical significance was expressed at $p < 0.05$.For analyzing the result of the medicinal studies, the results obtained are expressed as means ± S. D of six rates in each group.

3.1 Result and Discussion

An extensive survey conducted in major maize growing areas of Tamil Nadu in different locations during the year 2021 revealed, the endemic nature of the disease with maydis leaf blight incidence ranging from 14 to 45% (Table 1 & Fig. 1). The survey revealed that, the incidence and severity of the disease varied from locality to locality. Among the different locations of Tamil Nadu surveyed for maydis leaf blight incidence, Kalangani in Namakkal district registered the maximum incidence of the disease (44.88%) followed by Pavithiram with (42.65%) and the minimum maydis leaf blight incidence of (14.46%) was recorded at Nellikuppam in Cuddalore district. The variation in the extent of the disease incidence might be due to the prevalence of the isolates of the pathogen differing in their virulence (Fig. 2). Also the

susceptibility of the variety NK6240 and their extent of cultivation and favourable conditions could be the reason for the higher incidence.

Zishan Gul *et al*. (2021) [30] conducted a survey of maydis leaf blight incidence in Abottabad district and the maximum per cent incidence was recorded in Sultanpur and Takia Sheikan (93.33%), followed by Jangra (86.67%), however location Diwal showed least incidence (46.67%) of *Bipolaris maydis*. Similarly Akonda *et al*. (2015) [3] revealed that the higher southern corn leaf blight incidence was observed in Mymensingh (30.47%) followed by Rangpur (21.53%) in Bangladesh. Similar such endemic nature of MLB disease of maize in Karnataka was reported by Sunaina Bisht (2015) [27] with incidences ranging 27.88-56.26% in major maize growing regions of north Karnataka.

B. maydis was isolated from the diseased samples of maize plants in fresh PDA plates. The results pertaining to the morphological characters of *B. maydis* has exhibited by different isolates are mentioned in table 2.

The morphological characteristics of isolates of *B. maydis* were examined, including colony character, colony colour, colony diameter, sporulation, conidial population, and number of septa. Information on the isolates with the Bm_1 to Bm_{15} designations. The morphological characteristics of conidial population, number of septa, sporulation, and solely the colour of the isolates, which ranged from greyish to dark black to olivaceous green in colour, varied among the *B. maydis* isolates. The mean colony diameter was ranging from 65.24 to 90.00 mm. The culture colour varied from grey, light grey, dark grey, grey to black, olivaceous green to grey colour. The conidial population ranged from 0.5 to 2.7 /ml ($\times 10^6$) and septation ranged from 3-5 were observed under microscopic field (Fig. 3 & Fig. 4).

Bipolaris maydis produced light grey to black colour colony on PDA (Zishan Gul *et al*. 2021) [30]. Agag *et al*. (2021) [1] mentioned that the colony colour in various isolates of *B. maydis* were white grayish to dark olive in colour. Likewise, El-Bakery (2010) found three different colors but white, dirty white and grayish white. Nadeema *et al*. (2021) [17] reported that *B. maydis* mycelium was grayish white in colour initially but turning grayish black at maturity, extensive conidiophores arising singly or in small groups, straight or flexuous, septate, simple or branched and brown in colour. Conidia were light to dark brown, short or long, straight or slightly curved, septate and smooth. Likewise, the highest conidial concentration was recorded for isolate Bm11 (110.33 x 103ml-1). Kutawa *et al*. (2021a) [14] resulted that *B. maydis* conidial shape were elongated and it length and width ranges from 42-133 µm and 6-21 µm respectively.

The data depicted in table 3 revealed that varied levels of pathogenicity with difference in isolates. Among the fifteen isolates of *B. maydis* collected from different maize growing areas of Tamil Nadu, the isolate (Bm_7) collected from Kalangani was found to be more virulent and recorded the maximum incidence of 65.23 per cent followed by Bm_8 (63.65%) collected from Pavithiram. The isolate Bm_2 collected from Nellikuppam was the least virulent which recorded the minimum (25.18%) maydis leaf blight disease incidence (Fig. 5).

Ferreira *et al*. (2021) [11] mentioned that all lines showed lesions in different parts of the inoculated leaves, showing high pathogenicity of the isolate used in the experiment, which can be confirmed with the high disease incidence symptoms of plants and the different levels of average severity. Likewise Kutawa *et al*. (2021b) [15] resulted that the symptoms progressed to form single, fusiform, elongated, elliptical and long lesions or blighted zones. Amorio *et al*. (2017) mentioned that susceptible corn cultivar inoculated with *B. maydis* were observed to produce the highest mean lesion density, lesion size and percentage disease severity among all treatments.

Table 1: Survey on the incidence of maydis leaf blight of maize incited by *Bipolaris maydis* in major maize growing areas in Tamil Nadu

Sl. No.	Isolate Name	Location	District	Soil type	Variety	Disease Incidence (%)
1.	Bm_1	Sivapuri	Cuddalore	Clay loam	NK 6240	29.45 (32.86) ^{hi}
2.	Bm_2	Nellikuppam		Sandy Loam	MRM405	14.46 (22.35) ^{n}
3.	Bm_3	Vadalur		Sandy loam	Local	33.39 (35.29) ^{fg}
4.	Bm_4	Attur	Salem	Clay	900 M gold	40.21 (39.35) ^{bc}
5.	Bm_5	Vaazhappadi		Sandy loam	NK 6240	17.17 (24.47) ^{m}
6.	Bm_6	Thalaivasal		Clay loam	900 M	25.96 (30.63) ^{jk}
7.	Bm_7	Kalangani	Namakkal	Clay	MRM405	44.88 (42.06) ^{a}
8.	Bm_8	Pavithiram		Red soil	NK 6240	42.65 (40.77) ^{ab}
9.	Bm_9	Veppadai		Clay loam	Local	35.67 (36.67) ^{ef}
10.	Bm_{10}	Alangudi		Clay loam	NK 6240	31.89 (34.38) ^{gh}
11.	Bm_{11}	Thirumayam	Pudukottai	Sandy Loam	MRM405	38.58 (38.39) ^{cd}
12.	Bm_{12}	Illupur		Sandy loam	Local	20.87 (27.18) ^{l}
13.	Bm_{13}	Anaiyur		Clay	900 M gold	37.74 (37.90) ^{de}
14.	Bm_{14}	Vandiyur	Madurai	Sandy loam	MRM405	23.73 (29.15) ^{k}
15.	Bm_{15}	Melamadai		Clay loam	NK 6240	27.15 (31.40) ^{ij}

Table 2: Isolation and cultural characteristics of various isolates of *Bipolaris maydis* (Bm) from major maize growing areas of Tamil Nadu

Sl. No.	Isolate Name	Cultural characteristics	Mycelial growth (mm)	Conidial population/ml (×106)	No. of septa
1.	Bm_1	Mycelium with elevated, regular margins that range from grey to dark black growth	76.29 ^{hi}	1.5	4-5
2.	Bm_2	Poor growth regular margin in olivaceous green	65.24 ^{n}	0.5	2-4
3.	Bm_3	Good expansion normal development Mycelium, dark grey in hue	80.02 ^{fg}	1.8	3-5
4.	Bm_4	Moderate, consistent growth Later, white cottony growth turns greyish in colour	87.25 ^{bc}	2.4	4-5
5.	Bm_5	With a regular edge, the colour is olivaceous green to grey. silky-smooth growth	68.19 ^{m}	0.7	3-5
6.	Bm_6	Poor growth regular margin in olivaceous green	73.11 ^{jk}	1.1	3-5

7.	Bm_7	Mycelium with elevated, regular margins that range from grey to dark black growth	90.00 [a]	2.7	7-8
8.	Bm_8	Irregular growth of woolly hue dark grey to black	89.45 [ab]	2.5	4-6
9.	Bm_9	Good expansion normal development Mycelium, dark grey in hue	81.78 [ef]	2.0	2-4
10.	Bm_{10}	With a regular edge, the colour is olivaceous green to grey. silky-smooth growth	78.89 [gh]	1.6	2-4
11.	Bm_{11}	Excellent expansion flat, dark grey to black, round edge	85.78 [cd]	2.3	3-5
12.	Bm_{12}	Moderate, consistent growth Later, white cottony growth turns greyish in colour	70.68 [l]	0.8	4-5
13.	Bm_{13}	With a regular edge, the colour is olivaceous green to grey. silky-smooth growth	83.47 [de]	2.1	3-5
14.	Bm_{14}	Irregular growth of woolly hue dark grey to black	72.35 [k]	1.0	2-4
15.	Bm_{15}	Mycelium with elevated, regular margins that range from grey to dark black growth	74.26 [ij]	1.3	3-5

Table 3: Effect of *Bipolaris maydis* on the incidence of Maydis leaf blight in maize (Pot Culture)

Sl. No.	Isolate Name	% disease incidence
1.	Bm_1	43.62 (41.33) [hi]
2.	Bm_2	25.18 (30.11) [n]
3.	Bm_3	47.12 (43.34) [fg]
4.	Bm_4	60.27 (50.93) [bc]
5.	Bm_5	29.23 (32.72) [m]
6.	Bm_6	38.58 (38.39) [jk]
7.	Bm_7	65.23 (53.86) [a]
8.	Bm_8	63.65 (52.86) [ab]
9.	Bm_9	52.07 (46.19) [ef]
10.	Bm_{10}	45.29 (42.29) [gh]
11.	Bm_{11}	57.11 (49.08) [cd]
12.	Bm_{12}	31.72 (34.27) [l]
13.	Bm_{13}	55.43 (48.11) [de]
14.	Bm_{14}	34.78 (36.13) [k]
15.	Bm_{15}	42.04 (40.41) [ij]

Fig 1: Survey on the incidence of Maydis leaf blight in various Maize growing Areas

Fig 3: Axenic culture of *Bipolaris maydis* (Bm_7)

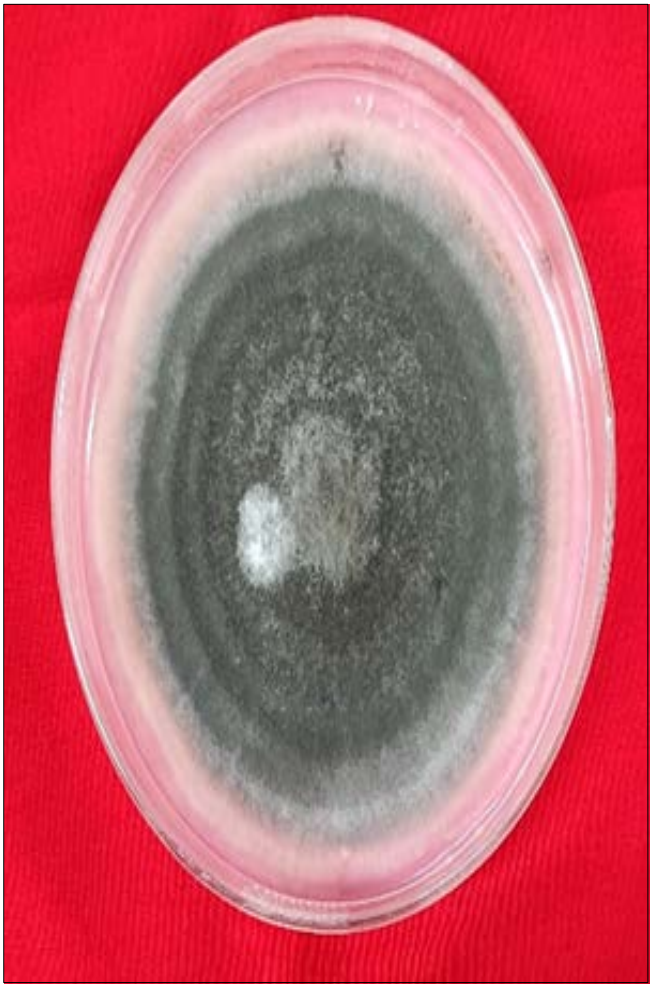

Fig 3: Axenic culture of *Bipolaris maydis* (Bm_7)

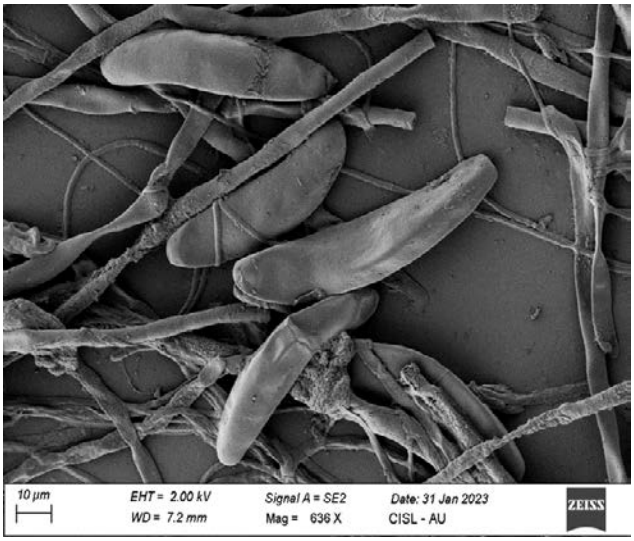

Fig 4: Scanning Electron Microscopy observation of *Bipolaris maydis*

Fig 5: Inoculation of *Bipolaris maydis* in healthy Maize plant (Pathogenicity Test)

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