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Study on the response of different maize cultivars to various inoculum levels of *Bipolaris maydis* (Y. Nisik & C. Miyake) shoemaker under field conditions

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Abstract
In the present study, the response of three maize varieties viz. Iqbal, Azam, and Jalal to four inoculum levels of *Bipolaris maydis* (i.e $1.5x10^4$, $3x10^4$, $4.5x10^4$ and $6x10^4$ conidia/ml) and one control was evaluated in a field trial carried out at New Developmental Farm of the University of Agriculture, Peshawar during summer 2013. The experiment was laid out in a split plot design with varieties in the main plot and inoculum levels in the sub-plots. The three maize varieties and different inoculum levels of B. maydis differed significantly ($P \leq 0.05$) from one another in all parameters. Similarly, interaction between the maize varieties and pathogen inoculum levels was also significant. Variety Jalal was found to be the most resistant and gave disease severity of 2.00 on a 0-5 scale. This variety registered the maximum plant height (155.07cm), fresh ear weight (1838.8 g), number of grain per ear (381.20), cob length (17.82cm), number of grain rows per cob (12.53), two hundred grains weight (45.78) and grain yield (2506.4 g). The highest inoculum level of B. maydisi.e, 6x104 conidia/ml caused maximum disease severity (3.33) and minimum plant height (145.14 cm), fresh ear weight (1678.8 g), number of grains/ear (335.67), cob length (13.38 cm), number of grain rows/cob (8.88), 200 grains weight (40.08 g) and grain yield/sub-plots (2322.0 g). Similarly, inoculation of variety Iqbal with the highest inoculum level of 6 x 104 conidia/ml resulted in the most severe disease (4.00) and the lowest plant height (141.10 cm), fresh ear weight (1644.0 g), number of grains/ear (329.00). Cob length (10.53 cm), number of grain rows/cob (7.33), 200 grains weight (38.30 g) and grain yield (2259 g).

Keywords: Maize, *Bipolaris maydis*, Cultivars, Pathogens, Inoculum levels

1. Introduction
Maize (*Zea mays* L.) is an important cereal crop of the world. It is mostly grown in rainfed and well irrigated areas. Maize gives good result in deep well aerated, warm, loam soil full of organic matter and with high nitrogen, phosphorus and potassium content. Optimum crop performance can be achieved from the areas where there is moderately high summer temperature with warm nights and adequate rainfall that is evenly distributed during the growing season. Maize is extensively grown in the world with an annual worldwide production of 822 and 817 million tons in 2008 and 2009, respectively [8].
It has been estimated that more than half of the increased demand in the world food in term of cereals as a whole will be produced from maize farmers and consumers [22]. It is predicted that by 2050, 9 billion people in the world will require almost 70% more food than today's population in which a large proportion of increased demand will come from developing countries [8]. Total production of maize has been affected by many factors, such as balance use of inputs, cultivation of hybrids, weather conditions and the use of adopted objective techniques of yield estimation. Despite of good achievements in the field of maize breeding, yield in the developing countries is very low as compared to developed countries.
In Pakistan, after wheat and rice maize occupies third position, 98% of which is grown in Punjab and Khyber Pakhtunkhwa. Pakistan is also a major maize producing country, contributing an area of 974200 ha with a total annual production of (3707000 tones) while Khyber Pakhtunkhwa contributes an area of 422900 ha with a total production of (740500 tones) [1]. Maize grain has high nutritional value as it having 72% starch, 10% protein, 4.8% oil, 8.5% fiber, 3% sugar and 1% ash [6].

Although maize gives high yield potential but there are some major limiting factors which makes it sensitive to several diseases. The most important diseases causing severe losses in maize yield are Southern leaf blight (SLB), northern leaf blight (NLB), gray leaf spots (GLS) and different types of rust. As many as 65 pathogens infect maize crop [17]. SCLB or Maydis leaf blight (MLB) is one of the most important disease of maize and constitutes a major threat to maize production worldwide. Southern corn leaf blight (SCLB) or maydis leaf blight (MLB) caused by *B. maydis* (Drechslera maydis; teleomorph: Cochliobolus heterostrophus) occurs widely on maize [4]. *B. maydis* is the sac fungi and is a member of ascomycetes, produces a toxin that attacks the mitochondria and destroys the ability of plants to capture energy from metabolism. Three races of B. maydis have been described [5][21]. Race 'O' is most common in those areas where SCLB occurs more. This infects the leaves only, forms small (0.6 x 2.5 cm), tan and parallel-side lesions with buff or brown borders. While on the other hand race 'T' caused SCLB epidemic in 1970 in North America. It is specifically virulent on Taxas male sterile cytoplasm (cmsT) maize because it produces a polypeptide toxin (T toxin) to which cmsT maize is sensitive [5]. It attacks all above ground parts of maize plant and produce spindle shaped lesions surrounded by green or chlorotic halos [2][16]. Race C of B. maydis has been reported from China, specifically virulent on C male sterile cytoplasm (CMSC) maize, but is not known to occur elsewhere [10, 21].

In maize growing regions of Khyber Pakhtunkhwa, maize leaf blight is more prevalent and share 20% or more yield losses to the crop in Pakistan [11]. Other scientists reported 28.3-29.5, 30.5-32.5, and 9.2-11.6% reduction in grain yield, number of kernels, ear and 200 kernel weights, respectively [18]. In addition to host susceptibility, pathogen virulence and favorable environmental conditions; inoculum load may play an important role in determining the level of disease severity and reduction in yield parameters. Therefore, this study was undertaken with the objectives to study the response of different maize cultivars to various inoculum loads of *B. maydis* under field conditions and to work out a standard inoculum level for screening maize germplasm against maydis leaf blight under field conditions.

2. Materials and Methods

This study was carried out at New Developmental Farm of The University of Agriculture, Peshawar during summer 2013.

Experimental design

Three maize cultivars i.e Azam, Jalal and Iqbal and five inoculum levels of *Bipolaris maydis* were tested in a split-plot design in RCBD as shown in the layout. Varieties were allotted to the main plots and inoculum levels to the sub-plots. Each sub-plot consisted of 6 rows 3m long and 75cm apart. Each treatment was replicated three times.

Isolation of pathogen

Inoculum of *B. maydis* was prepared by isolation of the pathogen from previous year infected leaves. The infected leaves were cut out into small pieces (about $1cm^2$) and surface sterilized with 0.1% $HgCl_2$ solution for 15-30 seconds. The pieces were then rinsed three times in sterile distilled water, blotted dry with sterile tissue paper and plated aseptically on fresh PDA medium (4-5 pieces/ petri dish). The petri dishes were sealed with parafilm and incubated at $25C^0$ for culture development.

Inoculum preparation and method of inoculation

The isolated pathogen was sub-cultured on PDA medium to obtain enough inoculum. Conidia from the fully grown culture were washed with sterile water and collected in a beaker. The different inoculum levels were determined with the help of a hemocytometer by adding more sterile water for diluting the inoculum suspension. A few drops of Tween-20 were added to the inoculum suspension before inoculating the maize crop.

The central two rows of each sub-plot were inoculated with the respective inoculum level of *B. maydis* at 12-16 leaf stage using a hand sprayer. Control sub-plots were sprayed with sterile water only. The un-inoculated two rows on both sides of the inoculated rows served as buffer rows to prevent the spread of the disease between treatments. Inoculation was done in the late afternoon to prevent the inoculum from desiccation in the warm weather.

Data collection and statistical analysis

Data were recorded as follows:

Disease severity

Data on disease severity were recorded on a 0-5 scale suggested by Sharma (1983) [19]. According to this scale 0 = no disease, 1 = one or two to few scattered lesions on lower leaves only, 2 = Moderate number of lesions on lower leaves only, 3 = Abundant number of lesions on lower leaves few on middle leaves, 4 = Lesions abundant on lower leaves and middle leaves, extending to upper leaves, 5 = Lesions abundant on almost all the leaves, plants prematurely dried or killed by the disease.

Plant height (cm)

Data on plant height were recorded with the help of inch tape. Eight plants from two central rows were randomly selected for this purpose and then average plant height was calculated.

Cob length (cm)

Cob length was determined by using inch tape. Eight cobs were randomly selected from central two rows for measuring their cob length and the average cob length was then calculated.

Kernel Rows per ear

Rows per ear were counted by selecting eight plants randomly from two central rows and then average number of rows per ear was counted.

Grain yield (kg/ha)

Data on grain yield per sub-plot were recorded by harvesting the central two rows and their weight was taken by digital balance. The weights of plastic bags were tarred off before the weighing.

Number of grains per ear

Number of grains per ear was counted by selecting eight ears randomly from two central rows and then their average was counted.

Two hundred grains weight (g)

Two hundred grains were taken randomly from each sub plot and were weighed with the help of a digital balance. The average two hundred grains weight was then calculated for each sub plot.

Fresh ear weight (g)

Fresh ear weight was taken just after the harvesting of crop. Eight ears were taken randomly from each plot and then were weighed with the help of a digital balance. The average fresh ear weight was then calculated.

Data analysis

Data were subjected to analysis of variance technique with inoculum levels and varieties as independent variables and different parameters as dependent variables using Statistix 8.1 statistical package. Means showing significant variations were separated by Least Significant Difference test [20].

3. Results

Disease severity

The different maize cultivars and inoculum levels of *Bipolaris maydis* exhibited significant (P = 0.0005) differences among themselves (Table 1). Cultivar Iqbal registered the highest disease severity (2.86) while Jalal showed the lowest disease severity (2.00).

Inoculum level of $6x10^4$ conidia/ml resulted in the highest disease severity (3.33) while the lowest disease severity (1.00) was recorded in the control sub plots where the maize plants were sprayed with sterile water only. A significant reduction in disease severity was noticed with the reduction of inoculum level of *B. maydis*. The interaction between maize cultivars and inoculum levels of *B. maydis* was also significant (P = 0.0000). The highest disease severity (4.00) was recorded in cultivars Iqbal inoculated with $6x10^4$ conidia/ml while the lowest disease severity (1.00) was observed in all the three cultivars where no inoculum was used.

Plant height

The different maize cultivars and inoculum levels of *B. maydis* exhibit significant (P = 0.001) differences among themselves (Table 2). Cultivar Iqbal gave the lowest plant height (148.76cm) while the highest plant height (155.07cm) was obtained from Jalal. The lowest plant height (145.14cm) was observed when an inoculum level of $6x10^4$ conidia/ml was applied while the control sub-plots registered the highest plant height where sterile water was applied. A significant reduction in plant height was noticed with increasing inoculum levels. Similarly, the interaction between maize cultivars and inoculum levels was also significant (P = 0.0000). The lowest plant height (141.10cm) was obtained from the cultivar Iqbal inoculated at $6x10^4$ conidia/ml while the cultivar Jalal gave the highest plant height (160.33cm) where no inoculum was applied.

Table 1: Effect of different inoculum levels of *Bipolaris maydis* on the maydis leaf blight disease severity of different maize cultivars**.**

Inoculum levels	Varieties			Mean
	Iqbal	Azam	Jalal	
Control (sterile water)	1.00 e	1.00 e	1.00 e	1.00 e
$1.5x10^4$	3.00 bc	2.00 d	2.00 d	2.33 d
$3x10^4$	3.00 bc	2.66 c	2.00 d	2.55 c
$4.5x10^4$	3.33 b	3.00 bc	2.00 d	2.77 b
$6x10^4$	4.00 a	3.00 bc	3.00 bc	3.33 a
Mean	2.86 a	2.33 b	2.00 c	

LSD value for varieties 0.1851
LSD value for inoculums level 0.2051
LSD value for interaction (varieties × inoculum levels) 0.3553

Table 2: Effect of different inoculum levels of *Bipolaris maydis* on plant height (cm) of different maize cultivars.

Inoculum levels	Varieties			Mean
	Iqbal	Azam	Jalal	
Control (sterile water)	153.67 de	156.00 bc	160.33 a	156.67 a
$1.5x10^4$	153.17 ef	154.00 de	156.67 b	154.61 b
$3x10^4$	149.03 h	152.07 fg	154.83 cd	151.98 c
$4.5x10^4$	146.83 i	148.57 h	152.67 ef	149.36 d
$6x10^4$	141.10 k	143.50 j	150.83 g	145.14 e
Mean	148.76 c	150.83 b	155.07 a	

LSD value for varieties 0.8851
LSD value for inoculums levels 0.6599
LSD value for interaction (varieties × inoculum levels) 1.3386

Fresh ear weight (g)

The different maize cultivars and inoculums levels of *B. maydis* exhibited significant (P = 0.0000) differences among themselves (Table 3). The lowest fresh ear weight (1757.1g) was recorded on cultivar Iqbal while Jalal gave the highest fresh ear weight (1838.8g).

Inoculum level of $6x10^4$ conidia/ml significantly reduced the fresh ear weight (1678.4g) whereas the highest fresh ear weight (1954.1g) was noticed in the sub-plots where only sterile water was applied. A significant reduction in fresh ear weight was noticed with the increasing of inoculum levels of *B. maydis.*

The interaction between maize cultivars and inoculum levels of *B. maydis* was also significant (P = 0.0000). The highest fresh ear weight (2004.0g) was recorded on cultivar Jalal where no inoculum was applied and the lowest fresh ear weight (1644.0g) was observed on cultivar Iqbal when an inoculum load of $6x10^4$ was applied.

Table 3: Effect of different inoculum levels of *Bipolaris maydis* on fresh ear weight (g) of different maize cultivars.

Inoculum levels	Varieties			Mean
	Iqbal	Azam	Jalal	
Control (sterile water)	1913.0 c	1945.3 b	2004.0 a	1954.1 a
$1.5x10^4$	1787.7 h	1814.0 f	1868.7 d	1823.4 b
$3x10^4$	1748.7 i	1792.7 g	1827.3 e	1789.6 c
$4.5x10^4$	1692.3 l	1734.0 j	1784.0 h	1737.1 d
$6x10^4$	1644.0 n	1682.3 m	1709.0 k	1678.4 e
Mean	1757.1 c	1793.7 b	1838.8 a	

LSD value for varieties 2.6887
LSD value for inoculums levels 1.8274
LSD value for interaction (varieties × inoculum levels) 3.8634

Cob length (cm)

The different maize cultivars and inoculums levels of *B. maydis* exhibited significant (P = 0.0000) differences among themselves (Table 4). Minimum cob length (12.61cm) was recorded in cultivar Iqbal while the maximum cob length (17.82cm) was recorded in variety Jalal.

Different inoculum levels showed significant differences among themselves. Maximum cob length (16.97cm) was obtained from those sub-plots where only sterile water was applied. The inoculum level of $6x10^4$ conidia/ml caused the lowest cob length (13.38cm).

The interaction between maize cultivars and inoculum levels of *B. maydis* was also significant (P = 0.0000). The lowest cob length (10.53cm) was noticed on cultivar Iqbal where an inoculum load of $6x10^4$ conidia/ml was applied, while maximum cob length (19.33cm) was observed in cultivar Jalal where no inoculum was applied.

Table 4: Effect of different inoculum levels of *Bipolaris maydis* cob length (cm) of different maize cultivars.

Inoculum levels	Varieties			Mean
	Iqbal	Azam	Jalal	
Control (sterile water)	15.26 e	16.33 d	19.33 a	16.97 a
$1.5x10^4$	13.46 g	15.43 e	18.53 b	15.81 b
$3x10^4$	12.33 h	14.30 f	17.50 c	14.71 c
$4.5x10^4$	11.46 i	13.46 g	17.26 c	14.06 d
$6x10^4$	10.53 j	13.16 g	16.46 d	13.38 e
Mean	12.61 c	14.54 b	17.82 a	

LSD value for varieties 0.0513
LSD value for inoculums levels 0.2188
LSD value for interaction (varieties × inoculum levels) 0.3425

Yield/plot (Kg/ha)

Significant differences were observed among maize cultivars and inoculum levels of *B. maydis* (Table 5). Cultivar Jalal gave maximum yield (5569.8kg/ha) while minimum yield was obtained from cultivar Iqbal (5301.3kg/ha).

Inoculum levels affected the yield/sub-plot and significant differences were observed among the different inoculum levels. Increasing inoculum levels significantly reduced the yield/sub-plot. An inoculum level of $6x10^4$ conidia/ml resulted in minimum yield (5160.0kg/ha) while maximum yield (5654.1kg/ha) was obtained where no inoculum was applied.

The interaction between maize cultivars and inoculum levels was found significant. Lowest grain yield (5020.0kg/ha) was recorded on cultivar Iqbal with the application of $6x10^4$ conidia/ml while the highest (5822.2kg/ha) was recorded in cultivar Jalal where no inoculum was applied.

Table 5: Effect of different inoculum levels of *Bipolaris maydis* on yield (kg/ha) of different maize cultivars.

Inoculum levels	Varieties			Mean
	Iqbal	Azam	Jalal	
Control(sterile water)	5222.2 e	5617.8 c	5822.2 a	5654.1 a
$1.5x10^4$	5473.3 g	5580.0 d	5680.0 b	5577.8 b
$3x10^4$	5348.0 h	5486.7 f	5577.8 d	5471.1 c
$4.5x10^4$	5142.2 k	5348.9 h	5491.1 f	5327.4 d
$6x10^4$	5020.0 l	5182.2 j	5277.8 i	5160.0 e
Mean	5301.3 c	5443.1 b	5569.8 a	

LSD value for varieties 4.3911
LSD value for inoculums levels 5.6178
LSD value for interaction (varieties × inoculum levels) 9.7303

4. Discussion

This research was carried out to evaluate the response of three maize cultivars against different inoculum levels of *B. maydis* and to work out a standard inoculum level for breeding program under field condition. Three locally available varieties (Jalal, Azam and Iqbal) and four inoculum levels ($1.5x10^4$, $3x10^4$, $4.5x10^4$ and $6x10^4$conidia/ml) were used.

Among the three varieties Jalal was found to be more resistant against maydis leaf blight while Iqbal was found to be the least resistant. Similarly, an increase in disease severity was recorded with the increasing inoculum level. Variety Azam showed a moderate resistance against applied inoculum levels. Variety Jalal may have some advanced defensive mechanism against maydis leaf blight as compared to Azam and Iqbal.

This Study showed that the highest inoculum level ($6x10^4$ conidia/ml) caused the maximum disease severity showing a positive correlation between the two factors. Several researchers have reported a close association between inoculum levels and disease severity. Baijnath (2012) [3]. found that with highest inoculum concentration, Black scurf of potatoes was most severe on tubers. Significant differences in disease severity and between inoculum levels were observed. Similarly, Raziq and Ahmad (1992) [18], working with *Maydis leaf blight*, reported that the highest inoculum level ($4x10^4$ conidia/ml) caused the maximum disease severity suggesting a positive correlation between the two factors. Probably high inoculum densities depressed the defensive mechanism of the host easily and resulted in intensive disease severity. This may also be due to the decrease in photosynthetic area of leaves, reduced efficiency of carbon fixation and decrease in dry weights resulted in alteration of growth pattern and decline in photosynthetic activity of infected plants [15]. Higher inoculum density resulted in highest disease severity, which may be due to the fact that higher inoculum always enhanced the chances of disease infection. Similar results have been reported by several workers [7]. Muthukumar and Venkatesh (2013) [14] found that amongst the tested levels, 5% inoculum load of *Sclerotium. rolfsii* caused maximum incidence of collar rot, which was followed by inoculum load of 4%. Similarly, an increase in the incidence (upto 4%) of Sclerotium wilt of potato was recorded with higher concentration of inoculum level. The results were similar to the findings of Mallesh *et al* (2006) [13]. While working with root rot in ground nut. Direct relationship between the inoculum density and disease incidence was noticed in Groundnut caused by *Fusarium solani*. Different inoculum levels were found to be significant. The root rot incidence increased with increasing inoculum levels. Disease incidence of 100 per cent was recorded at six per cent inoculum level. However, this was only one-third when the inoculum load was applied at the rate of two percent.

A negative effect between inoculum levels and different plant parameters was found. All tested inoculum levels significantly affected the agronomic features such as yield, fresh ear weight and plant height etc. However, a gradual intensity in the disease establishment was found when an incremental increase in the inoculum load occurred. Various amount of yield/plot was recorded with the application of different inoculum levels ranging from $1.5x10^4$ to $6x10^4$conidia/ml. The minimum yield was obtained from the plots treated with highest inoculum load. Conversely, maximum yield was recorded in the control plots where no inoculum of *B. maydis* was applied. Similarly, plant height was also affected by the increasing inoculum levels. All tested varieties were reached to their maximum height when received the minimum inoculum load. Maximum plant height was recorded at $1.5x10^4$conidia/ml, while minimum height was recorded when inoculum levels increased to $6x10^4$ conidia/ml. Similar results were observed for other parameters (Cob length, fresh ear weight, number of Cobs/plant, number of grains/cob and 200 grains weight).

These results corroborated well with the findings of Fouzia and Shahzad (2005) [9]. Who reported a negative correlation between the inoculum level of *S. rolfsii* in soil and plant growth parameters. They observed a gradual decrease in plant length, weight and shoot weight in both the host plants with increase in population of *S. rolfsii* in soil. Khalequzzaman (2003) [12]. Reported similar results where soybean plants were inoculated with different inoculum levels of *S. rolfsii* and *Meloidogyne javanica*. They found a gradual reduction in plant growth, nodulation and yield per plant with a gradual increase in inoculum levels. Disease incidence and severity

may also be deeply correlated with fluctuation in the environmental conditions. Optimum temperature, higher moisture content, leaf wetness duration and presence of pre inoculum load in the fields are the main sources of disease establishment.

5. Conclusion and Recommendations

Disease severity increased with increasing inoculum levels. Highest disease severity was recorded at the inoculum load of $6x10^4$. Variety Jalal was more resistant followed by Azam and Iqbal. Variety Jalal gave good yield under disease stress conditions. In order to get maximum yield, disease resistant varieties like Jalal should be used by the farmers. Further research is necessary to evaluate as many as possible cultivars against Maydis leaf blight. For effective evaluation of resistance of maize varieties, the inoculum levels of $6x10^4$ conidia/ml should be used.

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