COMPARATIVE EFFECTIVENESS OF FUNGICIDES ON *HELMINTHOSPORIUM MAYDIS* IN THE MANAGEMENT OF SOUTHERN MAIZE LEAF BLIGHT

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Abstract:

Leaf blight, a significant threat to maize crops caused by the pathogen *Helminthosporium maydis*, leads to substantial agricultural losses nationwide. In an effort to mitigate this impact, a study was undertaken to evaluate the effectiveness of various fungicides in combating this affliction. Utilizing the susceptible variety Malaka-16 as a baseline for disease proliferation during the 2022-23 season. Propiconazole was found highly effective with inhibition of mycelial growth of H. maydis. The investigation revealed that Propiconazole, marketed as Tilt, was the most effective treatment, curtailing disease progression by 79.20%. This was closely followed by Difenoconazole, known commercially as Score, which achieved a 70.40% efficacy rate. Conversely, the fungicides Thiophanate Methyl (Topsin-M) and Copper Oxychloride (Blitox-50) were the least effective, with control rates of 48.8% and 44.70% over the baseline, respectively.

Keywords: Fungicide, Propiconazole, Maize, Tebuconazole, Maize, Blitox-50, Southern leaf blight

Introduction:

Maize ranks as the world's third most important cereal crop, following wheat and rice. Believed to have originated in Central America, maize spread to as far north as Canada and as far south as Argentina. This versatile crop is cultivated in approximately 166 countries, thriving in a variety of agro-climatic zones. Often referred to as the "queen of cereals," maize boasts a high yield potential. It serves as a vital source of nutrients for both humans and animals and is a key raw material in industrial manufacturing. Additionally, maize is utilized as a biofuel. The grain is rich in essential nutrients, including nicotinic acid, riboflavin, vitamin E, and vitamin A.

Research by Saritha *et al*., 2020 highlighted its use in producing syrup, starch, linoleic acid, and oils. Rehman *et al*., 2020 noted that during the 2022-23 season, maize was planted over 1.6 million hectares, yielding 9.5 million tons of produce. According to the Food and Agriculture Organization of the United Nations (FAO), the world's total maize production has been on a steady rise over the years. In 2022, the global maize production reached a staggering 1,163 million metric tons, marking a significant increase from the 318 million metric tons recorded in 1973. This growth reflects an average annual rate of 3.04% over the past decades. The United States has consistently been the top producer of maize, contributing a substantial portion of the world's supply. In the 2023/2024 period, it is expected that the U.S. will produce approximately 389.7 million metric tons of maize. Following closely are countries like China, Brazil, Argentina, and Ukraine, which together with the U.S., account for nearly 75% of the global maize production. Year-wise data over the last five years reveal a pattern of incremental growth in maize production. The FAO's statistical data indicates a 4.1% increase in maize production from 2020 to 2021, driven by favorable conditions and advancements in agricultural practices. This uptrend is a part of the broader agricultural expansion needed to meet the demands of a growing global population while striving for sustainable practices as outlined in the UN's 2030 Agenda. The distribution of maize production across continents is also noteworthy. The Americas lead with a significant margin, followed by Asia, Europe, and Africa. This distribution is influenced by a variety of factors, including climate, soil fertility, technological advancement, and economic policies. The versatility of maize, both as a food source and a raw material for various industries, underscores its importance and the need for continued investment in its production. Efforts to increase maize yield per hectare, improve resistance to pests and diseases, and adapt to climate change are ongoing. These efforts are crucial for ensuring food security and economic stability in many regions. The data and trends observed in maize production not only inform agricultural policies but also guide research and development in the field, aiming for a balance between productivity and sustainability. Maize is susceptible to a range of diseases caused by fungi, bacteria, and viruses, with fungal infections being the most significant. These include brown spot, blended leaf, sheath blight, stalk rots, smuts, and charcoal rots. Maydis leaf blight, in particular, is a severe disease impacting all growth stages of the maize plant, as reported by Atif et al. in 2019. *Helminthosporium maydis*, the pathogen responsible for Southern leaf blight in maize, poses a significant threat to maize production, leading to considerable yield losses globally. This fungal disease, characterized by tan to brown lesions on leaves, thrives in warm, humid conditions, which are prevalent in many maize-growing regions. The impact on yield can be severe, with losses reported up to 70% under favorable conditions for the pathogen. In Pakistan, crop losses can escalate to 60% under extreme conditions. The disease initially manifests as lesions on the leaves, bordered by a brownish color. Microscopic examination reveals the spore of *Helminthosporium maydis*. Several races of the pathogen exist, but races O, T, and C are the most noteworthy, with race T responsible for approximately 99% of infections. Race O is commonly seed-borne. H. maydis predominantly affects regions with warm and humid climates. The race T of *H. maydis* disease causes outbreak that spread rapidly in growing areas of maize in USA. Race T caused an epidemic in maize in 1970 in USA. However, the most important race of the USA is O. The incidence of Southern Leaf Blight of was first time reported by Drechsler *et al* (1925) from United States. In India this disease was reported by Kapoor. This disease is most critical if infection occurs prior to silky stage weather condition are favorable for the development during the reproductive growth stages. Management strategies include the use of resistant maize varieties, crop rotation, and fungicide applications. Research has shown that seed treatment and foliar application of fungicides can effectively reduce disease severity and increase yield. Moreover, understanding the genetic resistance against H. maydis is crucial, as it can lead to the development of maize varieties with durable resistance, minimizing reliance on chemical controls. Quantitative trait loci (QTL) mapping has been instrumental in identifying genetic markers associated with resistance, which are valuable for breeding programs. The integration of these approaches resistant varieties, cultural practices, and chemical management forms a comprehensive strategy to mitigate the effects of H. maydis on maize yield. Keeping in view the above facts, field trial was conducted in the research area of Plant Pathology Research Institute, Faisalabad during 2022-23.The main objective of the study was to sort out the effective fungicide against the southern leaf blight of maize.

Material and Methods:

During the Kharif Season of 2022-23, maize plants exhibiting symptoms of southern leaf blight were harvested and stored in paper bags. These specimens were collected from a nursery within the research domain of PPRI, Faisalabad, which had shown signs of the affliction. In order to cleanse the samples of any adhering soil, they were thoroughly rinsed with water.

Subsequently, the samples were dissected into diminutive fragments measuring 2-3 mm in size. These fragments were then submerged in a 0.1% mercuric chloride solution for a duration of 30 seconds, followed by another rinse in water to eliminate any residual chemical. The prepared samples were then methodically placed onto potato dextrose agar within Petri dishes, utilizing sterilized needles for the transfer. Incubation at a consistent temperature of 25°C resulted in the emergence of fungal growth six days post-inoculation. The pathogen was purified using the Hyphal Tip Technique, ensuring a single strain of the fungus was obtained. Upon the manifestation of the fungal colony, its identification was conducted using the taxonomic key developed by Koneman & Roberts in 1985. The study on southern leaf blight involved cultivating the pathogen in a potato Dextrose Agar medium. This culture was then incubated at a consistent temperature of 25 ± 2°C across twenty petri dishes, each heavily inoculated with H. maydis. To create a suspension, the culture was pulverized with sterile water for a duration of twenty seconds, yielding approximately 4-5 liters. This suspension was then evenly dispersed over the experimental plants using a compressed air apparatus. The trial aimed to assess the effectiveness of various fungicides, with evaluations commencing at the onset of disease symptoms. Spanning two consecutive years, 2022 and 2023, the research was conducted at the PPRI's Faisalabad facility, employing a Randomized Block Design (RBCD) with triplet replications. The maize plants were spaced 20 cm apart. Among the treatments, four different fungicides were tested alongside a control, which was the susceptible maize variety 'Malaka 2016'. The experimental plots were structured in three rows, each extending 3 meters. Except for the control, all plots received fungicide applications. The control plot remained untreated to serve as a benchmark. Inoculation against the blight was carried out on maize plants aged 35 days, followed by a subsequent fungicide application fifteen days later.

Table 1: The fungicides used in the experiment was given below:

Sr. No.	Common Name	Trade Name	Active Ingredient	Formula
1.	Score	Difenoconazole	25%	EC
2.	Tilt	Propiconazole	25%	WP
3.	Nativo	Tebuconazole	25%	EC
4.	Topsin-M	Thiophanate Methyl	70%	WP
5.	Copper Oxychloride	Blitox- 50	50%	WP

For the first appearance of disease the percentage disease incidence was recorded by using the formula of Wheeler, 1969 and severity of disease was noted by using the scale given below (T-2).

$$\% \text{ incidence of disease} = \frac{\text{Number of affected/diseased plants} \times 100}{\text{Total Number of plants}}$$

Table 2: The severity of disease was recorded by using the scale 1-9 (Wheeler, 1969)

Rating scale	Degree of infection (Per cent DLA*)	PDI**	Disease reaction
1.0	Nil to very slight infection (≤ 10%).	≤11.11	Resistant (R) (Score: ≤3.0) (DLA: ≤ 30%)PDI: ≤33.33)
2.0	Slight infection, a few lesions scattered on two lower leaves (10.1-20%).	22.22	
3.0	Light infection, moderate number of lesions scattered on four lower leaves (20.1-30%).	33.33	
4.0	Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%).	44.44	Moderately resistant (MR) (Score: 3.1- 5.0) (DLA: ≤ 30.1-50%) PDI: 33.34 -55.55)
5.0	Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%).	55.55	
6.0	Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and a few lesions on two leaves above the cob (50.1-60%).	66.66	Moderately susceptible (MS) (Score: 5.1- 7.0) (DLA: ≤ 50.1-70%) PDI: 55.56 -77.77)
7.0	Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%).	77.77	
8.0	Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaves (70.1-80%).	88.88	Susceptible (S)(Score: > 7.0) (DLA :> 70%) PDI: >77.77)
9.0	Very heavy infection, lesions abundant scattered on almost all the leaves, plants prematurely dried and killed (>80%).	99.99	

* DLA- Diseased leaf area; **Per cent disease index (PDI)

Results and Discussion

Helminthosporium maydis, the pathogen responsible for Maydis leaf blight, poses a significant threat to maize crops, potentially leading to substantial yield losses. This fungal disease, also known as Southern corn leaf blight, primarily affects the foliage of the maize plant, manifesting as tan to brown lesions on the leaves which can coalesce, causing extensive damage to the photosynthetic area of the plant. The severity of the infection can lead to a reduction in the plant's ability to produce food through photosynthesis, ultimately resulting in a decrease in kernel development and overall crop yield. The disease outbreak is influenced by environmental conditions, particularly high humidity and warm temperatures, which favor the pathogen's proliferation. Recent studies have explored the efficacy of biocontrol agents, plant extracts, and elicitors in managing the disease, with some showing promising results in reducing disease severity and improving crop yield. Management of Maydis leaf blight has traditionally involved the use of fungicides. The damage caused by *maydis* leaf Blight of maize can be saved by the used of foliar fungicides and resistant varieties. Sanjeev Kumar *et al*; (2009) reported that at 250, 500 and 1000 ppm, different fungicides like Tilt (Propiconazole), Copper Oxychloride (Blitox-50), Mancozeb (Dithane M-45), Thiophanate Methyl (Roko), Carbendazim (Bavistin) and Carbendazim + Mancozeb (Companion) inhibited the growth of *Helminthosporium maydis* in vitro conditions by food poisoned technique. Harlapur *et al*; (2007) reported that Mancozeb 0.25 % followed by Carboxin powder 0.1% gave the maximum mean percent inhibition of mycelia growth of *E.Turcicum*. Waghe *et al* ;(2015) revealed that seed treatments with fungicides like SAAF + two sprays of Mancozeb at 30 and 45 DAS recorded highest disease over control in the field conditions.

Different fungicides at recommended doses were sprayed against *maydis* leaf blight and it was observed that foliar spray of fungicides were found better to control *maydis* leaf blight. The observations showed that all the fungicides significantly reduced the disease over check. In the table 3, it was concluded that Tilt 25% EC was found to be most efficient in controlling the *H.maydis* by 79.20% followed by Score 25% EC which control the disease by 70.40%. The fungicide which showed poor performance were Topsin-M 70% WP & Copper Oxychloride 50% WP which control the disease by 48.8 % & 44.79% respectively. These results resembled with Sanjeev *et al* (2009) results who concluded that Tilt 25%EC was recorded best to control *H. maydis*. Similarly another scientist Bhavani *et al* (2016) also revealed that Tilt 25% EC was highly competent to control the *H.maydis* followed by Chlorothalonil. Nine (9) different fungicides were tested by Jha *et al* (2004) against southern leaf blight of maize and it was found that Tilt 25% EC (0.1%) was useful in controlling the disease. Five (5) fungicides like Tilt, Bavistin, Blitox-50, Kavach and Dithane M-45 were tested by Bharti *et al* (2020) against southern leaf blight of maize and concluded that these fungicides control the disease more efficiently. Naz *et al* (2013) tested twelve (12) fungicides against southern leaf blight of maize at National Agricultural Research Center (NARC) Islamabad. They reported that Ridomil Gold & Mancozeb was found best inhibiting the colony growth of the fungus. Kumar *et al* (2019) tested that two sprays of Tilt (Propiconazole) 25% EC at the rate 0.1% and two sprays of Mancozeb (Dithane M45) at the rate 0.2% were found most effective in reducing the percent disease index of southern leaf blight by (18.51% & 29.62% respectively). Two sprays of Hexaconazole 5% EC at the rate of 0.1% and seed treatment with SAFF (Mancozeb 63% + Carbendazim 12%) at the rate 3.00 gm/kg seed was found least effective in reducing the percent disease index (66.66% and 74.06% respectively) in comparison to control. Similarly Javed *et al* (2023) tested four fungicides against southern leaf blight of maize and it was concluded that Propiconazole (Tilt 25 % WP) was found to be the more efficient to control the disease by 80.0% .The second was Dithane M-45 (Mancozeb 80%WP) which control by 70.83% and third was Kavach (Chlorothalonil 75%WP) control the disease by 59%. The least one was Blitox-50 % WP(Copper Oxychloride 50) control the disease by 46.00% over check. Propiconazole is a systemic triazole fungicide known for its protective and curative action. Chemically, it is classified as a demethylation inhibitor (DMI), which interferes with the biosynthesis of ergosterol, an essential component of fungal cell membranes. Its molecular formula is $C_{15}H_{17}Cl_2N_3O_2$, indicating it contains carbon, hydrogen, chlorine, nitrogen, and oxygen atoms. The mode of action of propiconazole involves inhibiting the enzyme 14α-demethylase, which hinders the conversion of lanosterol to ergosterol, leading to the disruption of cell membrane formation and function in fungi. This disruption halts the growth and proliferation of the fungal pathogen, thereby protecting the plant from disease. Propiconazole is effective against a broad spectrum of fungi, including those that cause leaf spots, rusts, and powdery mildew in a variety of crops. It is absorbed by the plant and translocated acropetally, which means it moves upwards from the point of application, providing protection to new growth. This fungicide is often used in agriculture for its broad-spectrum activity and its ability to combat fungal pathogens that have developed resistance to other fungicides. Its use is critical in integrated pest management programs, where it contributes to the overall health and yield of crops. For optimal effectiveness, propiconazole is applied before the onset of disease or at the first sign of infection, as it cannot reverse damage already caused by fungal infections. It is also important to note that the use of propiconazole should be carefully managed to prevent the development of resistance in fungal populations. This involves rotating it with fungicides that have different modes of action, adhering to recommended application rates and timings, and integrating non-chemical disease control measures. The strategic use of propiconazole, in conjunction with other management practices, helps maintain its efficacy and ensures sustainable crop protection. The second most effective fungicide was difenoconazole. Difenoconazole is a systemic fungicide belonging to the triazole chemical group, known for its broad-spectrum activity against a variety of pathogens. Its chemical composition includes two chiral carbons, resulting in a pair of cis-trans diastereoisomers, which are not stereo-selectively distinguished by current analytical methods. The fungicide operates by inhibiting the demethylation process during ergosterol synthesis in fungal cell membranes, a critical component for their growth and function. This mode of action, classified as sterol demethylation inhibition (DMI), effectively halts the development of fungi by interfering with the biosynthesis of sterols, thus protecting plants from a wide range of diseases caused by Ascomycetes, Basidiomycetes, and Deuteromycetes. Difenoconazole is applied through foliar spray or seed treatment and is valued for its efficacy in controlling foliar, seed, and soil-borne diseases across various crops. Analytical methods for detecting difenoconazole residues in plant materials involve refluxing with methanol-ammonia and high-speed homogenisation with an acetone/water mixture, followed by solid-phase extraction and detection using LC-MS/MS or HPLC-MS/MS, with a limit of quantification (LOQ) set at 0.01 mg/kg.

Table 3: Effectiveness fungicides in control of Disease

Sr. No.	Fungicides Name	R1	R2	R3	Mean	% Disease decrease over control
		Mean PDI	(%)Disease reduction over control	Mean grain yield (q/ha.)	% Yield Increa seover control	
1	T1 Tilt (Propiconazole)	14	21	17	17.33	79.20 a
2	T2 Score (Difenoconazole)	22	23	29	24.66	70.40 b
3	T3 Nativo (Tebuconazole)	35	30	34	33	60.39 c
4	T4 Topsin-M (Thiophanate Methyl)	40	46	42	42.66	48.8 d
5	T5 Copper Oxychloride (Blitox- 50)	50	47	41	46	44.79 e
6	T6 (Control)	80	90	80	83.33	-

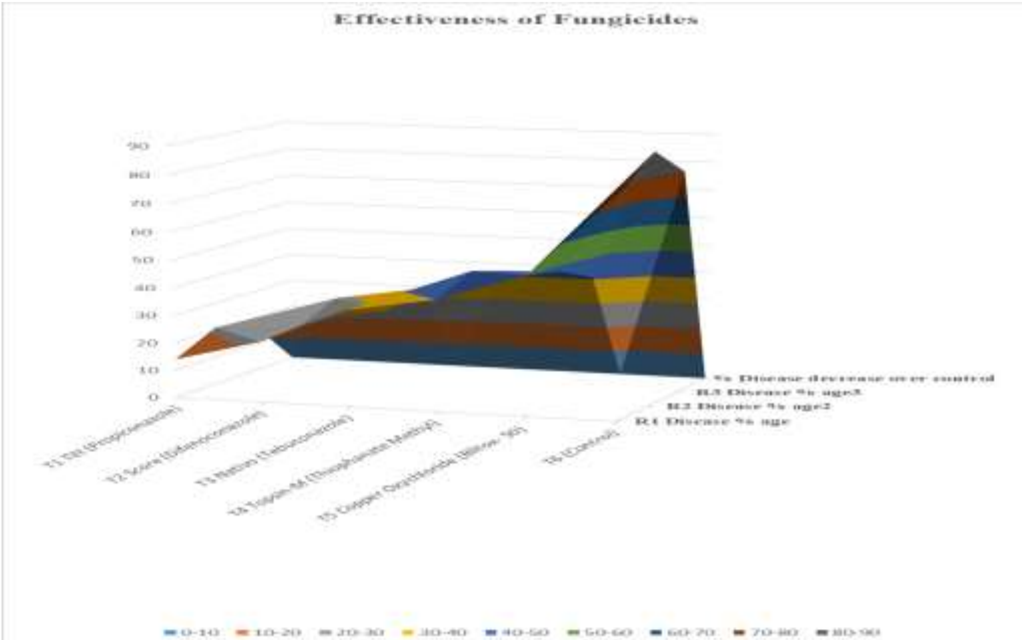
Table 4: Inhibitory Effect of Different Fungicides On Growth Of *H. Maydis* (At 9 Days)

Fungicides	50 ppm	100 ppm	150 ppm	200 ppm	250 ppm
Propiconazole	93.89 (75.78)	95.77 (78.23)	100 (90.00)	100 (90.00)	100 (90.00)
Difenconazole	67.30 (55.09)	71.07 (57.44)	74.63 (59.73)	78.63 (62.44)	79.96 (63.38)
Tebuconazole	60.47 (51.03)	67.47 (55.21)	73.18 (58.79)	74.33 (59.54)	76.41 (60.92)
Copper Oxychloride	57.41 (49.23)	60.07 (50.79)	64.47 (53.39)	72 (58.03)	72.96 (58.65)
Control	0	0	0	0	0
CD at 5%	0.993		0.702		2.221
SE(m)±	0.353		0.250		0.790
CV%	1.923				

Table 5: Effect of fungicides on the Per cent disease index of *Maydis* leaf blight disease, grain yield of maize

	Treatment details	Mean PDI	Disease uction over trol	Mean grain yield (q/ha.)	Yield Increase over control	ICBR
T1	Propiconazole 25 EC @ 0.1%	18.51 (25.38)	78.07 (62.18)	35.15	44.513 (41.77)	22.60
T2	Difenconazole 25 EC @ 0.1%	55.55 (48.18)	34.58 (35.88)	32.90	34.977 (36.23)	-7.59
T3	Tebuconazole 25 EC @ 0.1%	59.25 (50.33)	30.16 (33.11)	30.85	26.407 (30.87)	1.04
T4	Topsin-M (Thiophanate Methyl) 50% WP@ 0.1%	33.33 (35.17)	61.07 (51.41)	31.15	27.847 (31.78)	14.51
T5	Blitox- 50 Copper Oxychloride 50% WP @ 0.3%	48.14 (43.91)	43.23 (41.04)	31.50	29.320 (32.64)	5.32
T6	Control	85.17 (67.45)	0.00 (0.00)	24.50	0.000	-
	SE(m)±	3.359	3.897	1.305	2.252	
	CD at 5%	10.056	11.669	3.909	6.744	
	CV (%)	11.301	17.115	7.413	15.248	

Figure 1: Disease Control



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