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Exploring the Modern Fungicides for the *In vitro* Management of Pokkah Boeng Disease of Maize Caused by *Fusarium verticillioides* (SACC.) Nirenberg

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pokkah boeng disease caused by *Fusarium verticillioides* is an emerging fungal disease affecting maize recently leading to substantial yield losses. The increasing resistance of pathogens to older fungicides, along with environmental concerns, underscores the need for new management strategies that incorporates next-generation fungicides. In response to this challenge, a comprehensive and systematic survey was conducted in 2022-23 across the Dharwad district of Karnataka. The survey documented disease symptoms such as chlorosis at the base of leaves, malformation and twisting of the foliage. The pathogen was isolated and confirmed as *F. verticillioides* through molecular diagnosis. In the present study, the pathogen was tested for *in vitro* efficacy of modern fungicides at three different concentrations *viz*., 0.1, 0.15 and 0.2 per cent using poisoned food technique. *In vitro* testing of new generation fungicides revealed that, Tebuconazole 25.9%EC and combinations fungicides *viz*., Carbendazim 12%+Mancozeb 63%WP and Trifloxystrobin 25% + Tebuconazole 50%WG resulted in 100 per cent inhibition of the mycelial growth of the pathogen. Hexaconazole 5%SC, Azoxystrobin 23%SC, Trifloxystrobin 25%SC, Tricyclazole 18%+Mancozeb 62% WP and Azoxystrobin 18.2%+Difenoconazole 11.4%SC inhibited mycelial growth by 99.15, 85.49, 85.57 93.08 and 86.87 per cent, respectively. The mycelial growth in fungicide-treated media appeared either fluffy or cottony. Among the fungicides tested, Carbendazim 12% + Mancozeb 63% WP, Trifloxystrobin 25% + Tebuconazole 50% WG, and Tebuconazole 25.9%EC demonstrated 100 per cent efficacy in inhibiting pathogen mycelial growth, even at a low concentration of 0.1%. These results suggest a promising direction for disease management, while the other fungicides tested were found to be less effective.

Keywords: Pokkah boeng; maize; fusarium verticillioides; fungicides; management.

1. INTRODUCTION

"Maize (*Zea mays* L.) is one of the world's most prominent cereal crops, originating primarily from Central America and Mexico. Belonging to the Poaceae family, maize is a highly versatile crop, known for its adaptability to diverse agro-ecological and climatic conditions. Globally, maize is often referred to as the "Queen of Cereals" because it can be grown throughout the year due to its photo-thermo insensitive character and has the highest genetic yield potential among all cereals" [1]. "It is highly valued for its multifarious use as food, feed, fodder and raw material for large number of industrial products. Maize with its wide adaptability, it can be grown with elevation ranging from sea level to up to 3000 m above mean sea level. Maize serves as a staple food in many regions across the globe and ranks as the third most important crop following rice and wheat for India" [2]. Since 2005, India ranks 4th in terms of area with 9.89 million ha land under maize. However, India remained among the top 10 producers of maize in the World since 1961 and presently ranks 6th with annual output of 31.65 million MT. The productivity of maize in India is approximately 3.19 t/ha, which is just over half of the global average yield of 5.6 t/ha (https://iimr.icar.gov.in/?page_id=51). "Various diseases, including downy mildews, leaf blights, stalk rots, ear rots and rusts are notable which have an impact on crop productivity" [3]. "Pokkah boeng is one of the emerging diseases caused by *Fusarium* spp. complex that causes significant economic losses. It was first reported in Andra Pradesh (India) as *Fusarium luffae"* [4]. "Later, in Karnataka it was reported as *Fusarium verticillioides"* by Harlapur et al. [5]. "Current management strategy of Pokkah boeng disease includes spraying of Carbendazim 50% WP (1ml/l), Mancozeb 75%WP (2g/l) and biocontrol practices of applying *Trichoderma harzianum etc*., according to the recent reports made for the management of sugarcane Pokkah boeng disease, in which the complete management is not achieved" [6]. Considering the limitations in the practical usage of new generation fungicides *i.e.,* Triazoles, Strobilurins and combination fungicides in the management of such pathogens. So, there is an urgent need for developing a new management strategy with new generation fungicides. Accordingly, to the above facts and research gaps, the present study was conducted with the main objective to identify the effective fungicide for the management of this emerging disease, Pokkah boeng.

2. MATERIALS AND METHODS

Isolation of the pathogen, purification and maintenance of culture: The infected samples collected from different maize growing areas were isolated using the standard procedure for tissue isolation. The isolated cultures were purified by single spore technique [7]. The purified cultures were maintained on Potato Dextrose Agar (PDA) slants. The pathogenicity was proved by artificial inoculation of *Fusarium verticillioides* onto the healthy plants at 20 days after sowing.

2.1 Screening of Fungicides

The *Fusarium verticillioides* was tested with different commercially available new generation fungicides *i.e.* two triazoles (Tebuconazole and Hexaconazole), two strobilurin (Trifloxystrobin and Azoxystrobin) and three combination fungicides (Carbendazim+Mancozeb, Trifloxystrobin+Tebuconazole, Tricyclozole+ Mancozeb and Azoxystrobin+Difenoconazole) by using poisoned food technique (Nene and Thapliyal 1993) at three different concentrations *viz*., 0.1, 0.15 and 0.2 per cent (Table 1).

Fusarium verticillioides was allowed to grow in the sterile petri dishes containing PDA medium for seven days. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted media to obtain the required concentrations. About 20 ml of poisoned medium was poured in each sterilized Petri plates. Suitable check was maintained without addition of fungicides. “The amended molten medium was poured into the sterile Petri dishes and allowed to solidify. This method was repeated for the different concentrations of each treatment. After the solidification of the medium, mycelial discs of 5 mm diameter were cut by using the sterile cork-borer from the seven-day old culture plate. The mycelial discs were taken by flame sterilized inoculation needle and placed on the centre of the solidified PDA medium amended with the fungicide. Three replications for each concentration of different fungicides were maintained. A control plate inoculated with the pathogen alone and without the fungicide was maintained as control. The Petri dishes were then wrapped and incubated at the room temperature of 28±1°C. The radial growth of the pathogen was recorded when the pathogen in the control plate was fully grown and the per cent mycelial inhibition by the fungicide was calculated” [8]. Per cent inhibition of the pathogen by the fungicide over the control was calculated by the formula Vincent, [9] and the data on the per cent mycelial inhibition of the pathogen was also converted into angular values and analysed statistically using OPSTAT. The mean values were evaluated using Duncan's multiple range test Duncan, [10] for interpretation of the results.

Per cent inhibition = C-T/C ×100

Where C= Radial growth of the pathogen in control plate in cm

T= Radial growth of the pathogen in the treatment plate

3. RESULTS AND DISCUSSION

The *In vitro* screening aimed out to find out the most effective fungicide against *Fusarium verticillioides* using poisoned food technique. The experiment tested various fungicides *viz*., Tebuconazole 25.9 % EC, Hexaconazole 5 % SC, Azoxystrobin 23 % SC, Trifloxystrobin 25 % SC, Tricyclazole 18 % + Mancozeb 62 % WP, Trifloxystrobin 25 % + Tebuconazole 50 % WG, Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC and Carbendazim 12% WP + Mancozeb 63% WP. These fungicides were evaluated at three different concentrations of 0.1, 0.15 and 0.2 per cent. The results of the *in vitro* screening revealed that, triazole fungicide Tebuconazole 25.9 % EC as well as the combination fungicides, Carbendazim 12% + Mancozeb 63% WP and Trifloxystrobin 25% + Tebuconazole 50% WP were found to be significantly effective with cent per cent mycelial inhibition of the pathogen at all the tested concentration. Hexaconazole 5 % SC was the next effective fungicide (99.15%) followed by Tricyclazole 18 % + Mancozeb 62 % WP which achieved inhibition of 93.08 per cent and both of them showed 100 per cent inhibition at 0.2% (higher) concentration. (Table 2 and Fig. 1 and Plate 1). On the other hand, Azoxystrobin 23% SC was the least effective fungicide with an inhibition of 85.49 per cent. The table indicated that the effectiveness of these fungicides increased with higher concentrations. Among them, Trifloxystrobin 25% SC exhibited the greatest variability in inhibition rates across the different concentrations. This finding aligns with the study by Golakiya et al. [11], which reported that Tebuconazole 25.9% EC exhibited

maximum inhibition (89.19%) of *Fusarium oxysporum* f. sp. *ciceri* at all tested concentrations compared to other fungicides. Similarly, Gadhave et al. [12] tested the efficacy of various fungicides *in vitro* against Fusarium wilt of tomato and found that Carbendazim 12% + Mancozeb 63% WP was the most effective among the combination fungicides, achieving 100 per cent inhibition. Other studies, such as those by Song et al. [13] and Sahoo et al. [14], also confirmed the efficacy of Carbendazim 12% + Mancozeb 63% WP in inhibiting Fusarium wilt of tomato. Additionally, Sahool et al. (2023) demonstrated that Tebuconazole 50% + Trifloxystrobin 25% WG (90.57 %) was also effective in controlling Fusarium wilt, further supporting the findings of this study.

Table 1. Different fungicides used in the invitro efficacy

Sl. No.	Fungicide	
	Chemical name	**Trade Name**
1.	Azoxystrobin 23 % SC	Amistar
2.	Tebuconazole 25.9 % EC	Folicure
3.	Trifloxystrobin 25 % SC	Flint
4.	Hexaconazole 5 % SC	Contaf
5.	Carbendazim 12 % + Mancozeb 63 % WP	Saaf
6.	Tricyclazole 18 % + Mancozeb 62 % WP	Merger
7.	Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC	Amistar Top
8.	Trifloxystrobin 25 % + Tebuconazole 50 % WG	Nativo

Table 2. *In vitro* evaluation of fungicides against *Fusarium verticillioides*

Sl. No.	Fungicide	Mycelial inhibition over control (%)			
		Concentration (%)			Mean
		0.1	0.15	0.2	
1	Azoxystrobin 23% SC	82.64 ^{d} (65.37) *	86.18 ^{de} (68.17)	87.65 ^{c} (69.42)	85.49 ^{d} (67.61)
2	Hexaconazole 5% SC	98.65 ^{b} (83.32)	98.82 ^{b} (83.78)	100.00 ^{a} (90.00)	99.15 ^{b} (84.70)
3	Trifloxystrobin 25% SC	76.63 ^{e} (61.09)	82.42 ^{e} (65.21)	97.66 ^{b} (81.21)	85.57 ^{d} (67.67)
4	Tebuconazole 25.9% EC	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)
5	Carbendazim 12%+ Mancozeb 63% WP	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)
6	Tricyclazole18%+Mancozeb 62% WP	87.20 ^{c} (69.04)	92.03 ^{cd} (73.60)	100.00 ^{a} (90.00)	93.08 ^{c} (74.75)
7	Azoxystrobin 18.2% +Difenoconazole 11.4% SC	85.75 ^{cd} (67.82)	87.09 ^{de} (68.95)	87.76 ^{c} (69.52)	86.87 ^{d} (68.76)
8	Trifloxystrobin 25 % + Tebuconazole 50 % WG	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)	100.00 ^{a} (90.00)
	Mean	89.56 (71.15)	93.88 (75.68)	89.97 (71.54)	-
			S. Em (±)		**C.D at 1 %**
	Fungicides (F)		0.08		0.24
	Concentration (C)		0.05		0.15
	F×C		0.14		0.41

**Values in the parentheses are arc sine transformed*

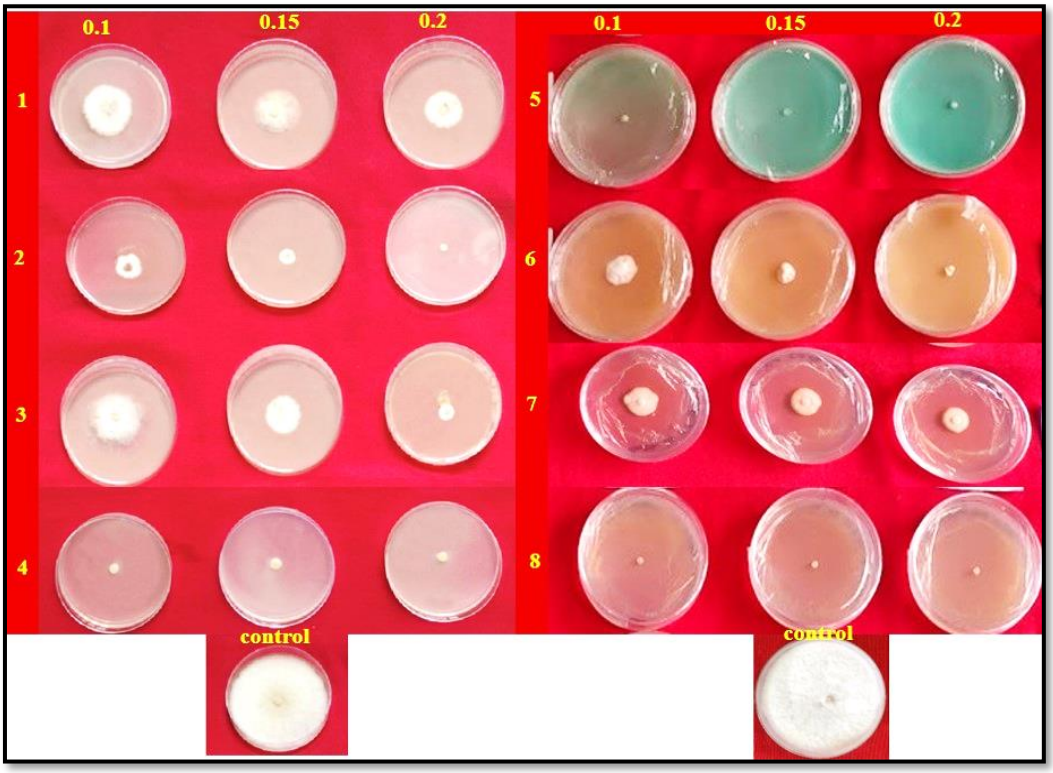

Plate 1. *In vitro* evaluation of fungicides against *Fusarium verticillioides*

1. *Azoxystrobin 23 % SC*	2. *Hexaconazole 5 % SC*
3. *Trifloxystrobin 25 % SC*	4. *Tebuconazole 25.9 % EC*
5. *Carbendazim 12 % + Mancozeb 63 % WP*	6. *Tricyclazole 18 % + Mancozeb 62 % WP*
7. *Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC*	8. *Trifloxystrobin 25 % + Tebuconazole 50 % WG*

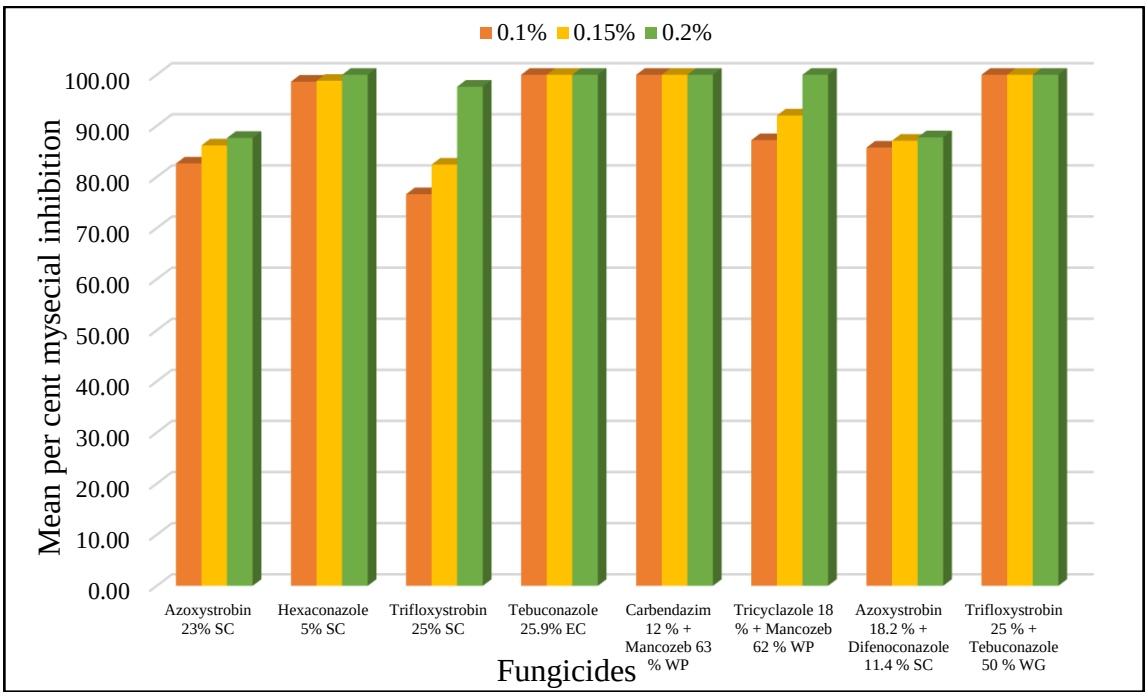

Fig. 1. *In vitro* evaluation of fungicides against *Fusarium verticillioides*

The effectiveness of Tebuconazole and other triazole fungicides observed in this study might be due to their inhibition of ergosterol biosynthesis, a critical component of fungal cell membranes necessary for growth [15]. However, the use of systemic fungicides like these poses a risk of resistance development, as they typically target one or two specific functions within the fungus, making them vulnerable to mutations or selection of resistant individuals. Combination fungicides, which include both systemic and non-systemic components, offer a more robust strategy for long-term management of fungal pathogens [16]. While systemic fungicides disrupt key processes like the electron transport chain and cell membrane integrity, non-systemic protectant fungicides impact multiple aspects of fungal physiology, making it more difficult for the pathogen to develop resistance. This dual action enhances immediate efficacy and helps prevent or delay resistance, making combination fungicides a more sustainable option for disease management.

4. CONCLUSION

In the present study, among the eight new-generation fungicides tested, Tebuconazole 25.9%EC was found to be the most effective systemic fungicide. Among the combination products, Carbendazim 12%WP+Mancozeb 63%WP and Trifloxystrobin 25%+Tebuconazole 50%WP were also highly effective in inhibiting the pathogen, even at lower concentrations. Though, Tebuconazole demonstrated strong efficacy, its use as a standalone treatment poses a risk due to its specific mode of action, which targets particular biochemical pathways within the fungus. This specificity makes the fungicide vulnerable to resistance development, as a single mutation or the selection of resistant individuals within the fungal population could significantly reduce its effectiveness or render it obsolete. In contrast, the use of combination fungicides offers better management of the pathogen. The dual action provided by combination products not only enhances the immediate efficacy of the treatment but also helps in preventing or delaying the development of fungicide resistance within the pathogen population. This approach ensures a more sustainable and effective strategy for disease control.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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