RESEARCH ARTICLE

Screening of systemic fungicides against *Fusarium verticilloide* initiating stalk rot of maize

Kashf Wajid[1], Gull-e-Laala[1*], Nayla Haneef[1], Abd-ur-Rehman Khalid[1], Gulshan Irshad[2], Shawal Shakeel[1] Nigarish Saghir[1]

Wajid K, Laala Ge, Haneefet N, et al. Screening of systemic fungicides against *Fusarium verticilloide* initiating stalk rot of maize. AGBIR. 2024;40(3):1160-1163.

Maize is an important cereal crop with huge global attraction and significant role as human food and animal feed. Various phytopathogenic fungal rots infect quality and quantity of maize before and after harvesting. The present study was designed intensively to combat the major objective of screening systemic fungicides against *Fusarium verticilloide* initiating stalk rot of maize. Various concentrations (20 ppm, 40 ppm) of synthetic fungicides *viz*., dithiocarbamate, ebuconazole 50%+trifloxystrobin 25% and prochloraz were evaluated *in vitro* against *F. verticilloide*. It was revealed that tebuconazole 50%+trifloxystrobin 25% at 60 ppm showed the strongest anti-mycotic potential 83.6% followed by prochloraz showing 75.19% mycelial inhibition of fungal rot, whereas dithiocarbamate was least effective at 7th day post inoculation respectively. Summarization of our results signified that trifloxistrobin 25%+tebuconazole 50% precisely controlled the maximum radial growth of *F. verticilloide*. It is therefore suggested that timely use of these fungicides may help in enhancing overall maize yield with good economic returns.

Keywords: *Fusarium verticilloide*; Dithiocarbamate; Tebuconazole; Trifloxystrobin; Prochloraz

INTRODUCTION

Maize (*Zea mays* L.) from family Gramineae, is a globally important cereal crop that ranks third after rice and wheat according to area and production. Maize was first domesticated for about 9,000 years ago in southern Mexico/Meso America. The global maize area (for dry grain) accounts 197 M ha, including substantive areas in Sub-Saharan Africa (SSA), Asia and Latin America. The global maize area is primarily located in the America and Asia, with over a third each, followed by Africa with a fifth and Europe with a tenth. Maize is also called queen of cereals due to high yield population among all cereals [1].

In Pakistan, the average productivity of maize is 2,850 kg/ha, which is the highest among all cereals grown in the country. The bulk (99%) of total production comes from two major provinces, NWFP accounting for 52% of the total area and Punjab contributing 48%. It is pertinent to mention that maize is also an important cereal crop of Azad state of Jammu Kashmir Pakistan with about 0.122 million ha cultivated in autumn. It is an established and important human food crop in a number of countries, especially in SSA, Latin America, and a few countries in Asia, where maize consumed as human food contributes over 20% of food calories. The share of maize in grain production is 1.8 million tons per annum [2]. US are the largest producer of maize crop, and it dominates world maize trade.

Major fungal diseases affecting quality and quantity of maize crop include *Fusarium* root, root rot, stalk rot, seedling blight, head blight, charcoal rot, etc., *Fusarium* is one of the major devastating groups of plant pathogenic fungi affecting a huge diversity of crops in all climatic zones across the globe. Various *Fusarium* species *viz*., *F. verticillioides*, *F. graminiacum*, *F. sporotrichioides*, *F. sambucinum*, etc., infect maize grains after harvesting in storage and during transportation. Maize is particularly susceptible to *F. verticillioides* infection due to the large amounts of fumonisins produced, whereas *F. verticilliodes* is a toxigenic fungus with ability to survive under harsh weather and high temperature.

Among which major rots observed and reported are *F. proliferatum*, *F. graminearum* and *F. anthophilium*. *Fusarium* spp. produces gibberella ear rot, kernal rot, seedling blight, seedling rot, wilt and smut respectively and are considered the largest group of seed born fungal rots [3]. *Fusarium* species invade 50% of maize grain before harvest and it produces mycotoxins. Fungi are the second most cause of deterioration and loss of maize. *Fusarium* species produces mycotoxins such as monilliformin, fumonisin and fusaric acid. A high incidence of stalk rot has been reported in several maize fields all over the world. Maize stalk rot is associated with *Fusarium temperatum*, *Fusarium subglutinas* and *Fusarium verticilloide*. With the introduction of high yielding hybrids both indigenous, exotic and use of fertilizers, there is a phenomenal increase in the area and production, but at the same time, it is prone to several foliar and stalks rot diseases. *Fusarium* rot can infect any part of maize straight starting from beginning to end of growing season. Stalk rot is the major and severe fungal rot on maize causing reduced growth, rotted leaf sheaths, internal stalk tissue and brown streaks in lower internodes. It causes pink to salmon discoloration of the internal stalk pith tissues. *Fusarium* stalk rot occurs in warm and dry region and produces black perithecia (sexual fruiting bodies) in infected maize stalk. The infection results in premature death of maize plant as pathogen interferes with the translocation of water and nutrients.

This study was aimed to determine the most prevailing pathogen of stalk rot of maize crop field located at national agriculture research center Islamabad and to screen the efficacy of various fungicides against Stalk rot of maize [4]. Different fungicides were evaluated *in vitro* against stalk rot disease where the most effective synthetic fungicide was further suggested for future use in enhancing overall maize yield production with good economic returns.

MATERIALS AND METHODS

The research was conducted at national agriculture research centre Islamabad, Pakistan. Maize stalks with clearly visible rot symptoms were brought to the fungal plant pathology laboratory in polythene bags from maize fields and were air dried for 24 h. Later on, stalks were pierced into 5 mm pieces and were surface sterilized with 0.5% sodium hypochlorite solution for 3 minutes and were washed with 70% ethanol for 2 minutes followed by three washings with sterile distilled water [5]. The pieces were placed on potato dextrose agar separately and were incubated at 25°C for 72 hours. Morpho-molecular studies were conducted under 40x Nikon microscope.

[1]*Department of Plant Pathology, University of The Poonch, Rawalakot, Pakistan*

[2]*Department of Plant Pathology, PMAS Agriculture University, Rawalpindi, Pakistan*

Correspondence: *Gull-e-Laala, Department of Plant Pathology, University of The Poonch, Rawalakot, Pakistan; E-mail: gulelalah.khan@yahoo.com*

Received: *09-May-2023, Manuscript No. AGBIR-23-98243;* ***Editor assigned:*** *11-May-2023, PreQC No. AGBIR-23-98243 (PQ);* ***Reviewed:*** *24-May-2023, QC No. AGBIR-23-98243;* ***Revised:*** *10-July-2023, Manuscript No. AGBIR-23-98243 (R);* ***Published:*** *17-May-2024, DOI: 10.35248/0970-1907.24.40.1160-1163*

Pathogenicity assay was conducted by harvesting conidia of *F. verticilloide* by scraping the surface of 3-day-old culture plate flooded with Sterile Distilled Water (SDW) using the bottom of a 1.5 ml micro centrifuge tube. Mycelial fragments were removed by filtration through two layers of Miracloth. The conidia were pelleted by centrifugation, washed and diluted to approximately 4 × 10^6 conidia/ml in distilled water. A total of 20 seeds of maize were planted in pots containing sterilized soil and placed in a greenhouse. A conidial suspension (1 ml/ was injected into stalks of the 3-week-old maize plant using toothpick method. Isolated fungal rot was later preserved in 15% glycerol solution and stored at -20°C [6].

In-vitro evaluation of fungicides against *Fusarium verticilloide*

To screen the inhibition percentage of *Fusarium verticilloide* inoculum poisoned food technique was followed by using different fungicides along with various concentrations. PDA was prepared and autoclaved at 121°C for 20 min. Different concentrations of dithiocarbamate (20 ppm, 40 ppm) tebuconazole 50%+trifloxystrobin 25% (20 ppm, 40 ppm) and prochloraz (20 ppm, 40 ppm) were added to the PDA media made in a volume of 100 ml. PDA was poured into 5 petri plates (90 mm) of each concentration and were solidified [7]. A small disc of 7 days old culture of *Fusarium verticilloide* was placed perpendicularly at the center of each petri dish and was incubated at 25°C for 7 days. Readings were taken after 5 and 7 odd day interval. Percentage Inhibition of radial growth of *Fusarium verticilloide* was calculated according to formula [8].

I (%) = (C-T/C) × 100

RESULTS AND DISCUSSION

Morphological features of isolated fungi were observed with yellowish-white colonies on PDA and the average size of micro conidia ranged from 4.0-33.0 µm × 2.4-3.3 µm. The shape varied from oval-club with flattened base having no septations. The micro-conidia were abundant in aerial mycelia, formed long chains with false head attached at monophialides branches [9]. The average size of macro conidia ranged from 37.0-55.0 µm × 4.0-4.2 µm with curved-tapered shape showing a pointed notched or foot shape showing 3-5 septations. The fungus produces a copious amount of single-celled micro-conidia and plentiful amount of septate macro-conidia [10]. In another study of microscopic features of *Fusarium* chains of micro-conidia were present whereas chlamydospores were totally absent.

Colonies are white to pale salmon colored, with low and often ropy mycelium and a powdery texture due to production of micro conidia (Figure 1) [11].

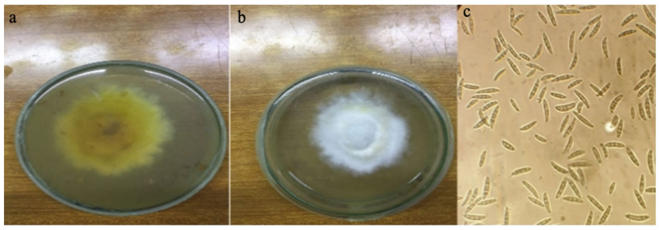

Figure 1: Pure culture of *Fusarium verticilloide* A) Lower surface of culture on petri-dish, B) Upper surface of culture on petri-dish (C) Spores observed under 40X Nikon Microscope Magnification.

In vitro evaluation of fungicides against *Fusarium verticilloide*

Results of *in vitro* evaluation of systemic fungicides revealed that all the tested three systemic fungicides exhibited significant effect on radial mycelial growth of *Fusarium verticilloide* where the radial growth was decreased with increase in concentrations of test fungicides [12]. Similarly, in another study reported by Cotton and Munkvold in 1998, *Fusarium subglutinans*, *Fusarium temperatum* and *Fusarium graminearum* were reported as major pathogens causing stalk rot on maize in various countries.

In the present study stalk rot of maize was observed associated with *Fusarium verticilloide*. *Fusarium stalk* rot occurs in warm and dry region and produces black perithecia (sexual fruiting bodies) in infected maize stalk [13]. The infection results in premature death of maize plant as pathogen interferes with the translocation of water and nutrients. To investigate the effects of different fungicides on *F. verticilloide* three selective systemic fungicides were applied [14].

In another similar study, significant efforts in the development and use of fungicides against radial growth inhibition of *Fusarium verticillioides* and to maximize yield in cereals several reports focus on evaluation of synthetic fungicides (tolylfluanid m, pencycuron, captan, thiram, thiabendazole, iprodione, carboxin, thiram) where it was observed that thiabendazole was the most effective in *F. verticillioides* control [15].

In present study three fungicides (dithiocarbamate, tebuconazole 50% + trifloxystrobin 25% and prochloraz) were tested against *Fusarium verticilloide* under *in vitro* conditions through poison food technique (Table 1) [16]. The data regarding the percent growth inhibition by these fungicides proved that tebuconazole 50%+trifloxystrobin 25% is the most toxic fungicide against *F. verticilloide* which inhibit 69.1% in C1 followed by C2 72.7% at 5^{th} DPI, whereas C1 and C2 inhibited mycelial growth of phytopathogenic fungi on 7^{th} DPI 75.11% and 83.6% respectively (Figures 2-4).

TABLE 1

Measurement of mycelial inhibition of *F. verticilloide* after 5^{th} and 7^{th} dpi (days post inoculation)

S. no.	Fungicides	5^{th} DPI (%)		7^{th} DPI (%)	
		Conc. 1	Conc. 2	Conc. 1	Conc. 2
1	Dithiocarbamate	67.21	69.11	69.43	71.2
2	Tebuconazole 50%+ Trifloxystrobin 25%	69.1	72.7	75.11	83.6
3	Prochloraz	69	69.47	70.1	75.19
4	Control	0	0	0	0

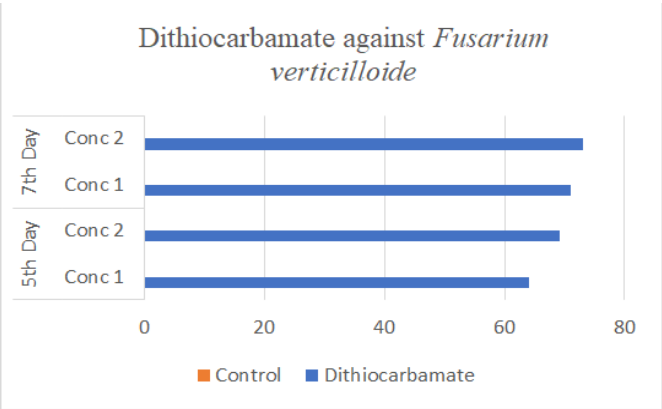

Figure 2: Mycelial inhibition of *F. verticilloide* by C1 after 5th day, C2 after 5th day, C1 after 7th day, C2 after 7th day. Control* no visible mycelial inhibition recorded.

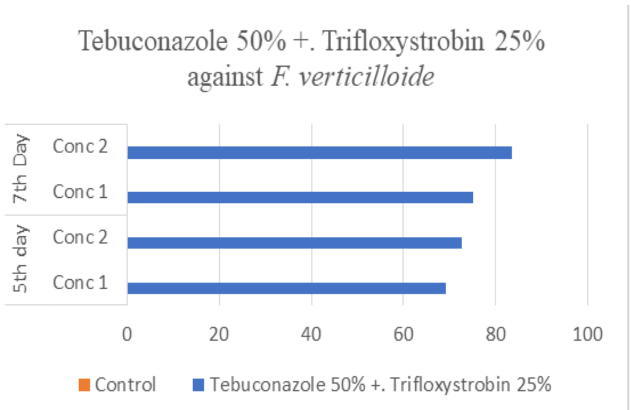

Figure 3: Mycelial inhibition of *F. verticilloide* by C1 after 5th day, C2 after 5th day, C1 after 7th day, C2 after 7th day. Control* no visible mycelial inhibition recorded.

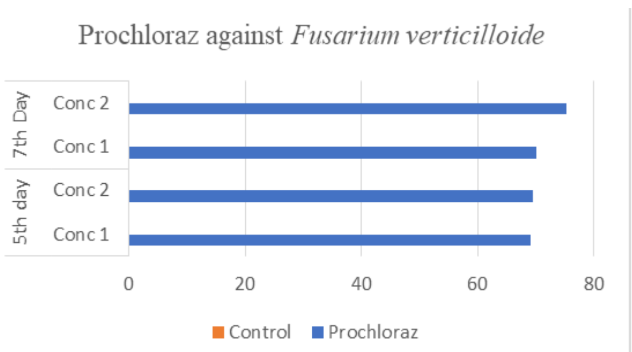

Figure 4: Mycelial inhibition of *F. verticilloide* by C1 after 5th day, C2 after 5th day, C1 after 7th day, C2 after 7th day. Control* no visible mycelial inhibition recorded.

Maize crop contributes toward economy and food security around the globe [17]. Maize fungal diseases preferably caused by *Fusarium* spp., are not specifically immense to the plant but to the entire biotic community also. In various studies many fungicides were tested against stalk rot of maize by other researchers and found many of them were extremely effective [18]. Fungicides have played a major role in the management of devastating crop diseases and thereby realizing avoidable yield loss. It is known fact that their application is unavoidable in control of more dreaded plant pathogens against which host resistance is not easily available or is unstable, especially against the polycyclic pathogens [19]. Further, most of the crops cannot remain disease free during the cropping season and hence greatly dependent on the use of fungicides. At present more than 200 chemicals of diverse classes are in use in the world as fungicides [20]. Hence, it is summarized by various experiments that novel chemical formulations could be an important component of integrated disease management against globally threatening maize crop fungal rots.

CONCLUSION

Knowledge of the sensitivity of different fungal species to active substances is extremely important during planning of protection systems and to choose the most effective fungicides. Accurate diagnosis of the etiology of crop diseases, as well as the correct identification of *Fusarium* pathogen and determination of its area, are necessary to predict the effect of used fungicides. In present study *in vitro* efficacy of three systemic fungicides against *Fusarium verticillioides* revealed that mean radial mycelial growth of the test pathogen was strongly inhibited by application of applied systemic fungicides. Summarization of our results signified that trifloxistrobin 25% +tebuconazole 50% precisely controlled the maximum radial growth of *F. verticilloide*. It is therefore suggested that timely use of these fungicides may help in enhancing overall maize yield with good economic returns.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

REFERENCES

1. Abate T, Shiferaw B, Menkir A, et al. Factors that transformed maize productivity in Ethiopia. Food Secur. 2015;5(7):965-981.
2. Afolabi CG, Ojiambo PS, Ekpo EJ, et al. Novel Sources of Resistance to Fusarium Stalk Rot of Maize in Tropical Africa. Plant Dis. 2008;92(5):772-780.
3. Ahmad N, Sinha DK, Singh KM, Mishra RR. Growth performance and resource use efficiency of maize in Bihar: An economic perspectives. 2017;1-12.
4. Ajayi AM. Seed dressing with aqueous *Allium sativum* L. extracts enhanced the tolerance of maize plant to stalk and ear rot disease caused by *Fusarium verticillioides* (Sacc.) Nirenberg. J Agric Crops. 2020;5(2):160-173.
5. Bacon CW, Yates IE, Hinton DM, et al. Biological control of *Fusarium moniliforme* in maize. Environ Health Perspect. 2001;109(Suppl 2): 325-332.
6. Cotten TK, Munkvold GP. Survival of *Fusarium moniliforme, F. proliferatum*, and *F. subglutinans in Maize Stalk Residue.* Phytopathology. 1998;88(6):550-555.
7. Czembor E, Stepien L, Waskiewicz A. Effect of Environmental Factors on *Fusarium* Species and Associated Mycotoxins in Maize Grain in Poland PloS One. 2015;10(7):e0133644.
8. Geiser DM, Aoki T, Bacon CW, et al. One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. Phytopathology. 2013;103(5):400-408.
9. Hawksworth DL, Sutton BC, Ainsworth GC. Ainsworth and Bisby's dictionary of the fungi, 7th ed. Commonwealth Mycology Institute, Kew, Surrey, England; 1983.
10. Kasetsart J. *Fusarium* mycotoxin contention. Isogenic traditional maize and genetically modified maize grown in France and Spain. 2018;50(4):728-731.
11. Hussain S, Ali F, Muhammad F, et al. Impact of organic and inorganic fertilizers on maize crop an experimental study. Biosci Res. 2022;19(3):1390-1395.

12. Kenganal M, Patil M, Nimbaragi Y. Management od stalk rot caused by *Fusarium moniliforme* 9Sheldon). Int J Curr Microbiol Appl Sci; 2017;6(7).
13. Kumar A. Maize Production Systems for Improving Resource-Use efficiency and Livelihood Security. Vol; 123. New delhi-110012: DMR. 2013.
14. Karlsson I, Persson P, Friberg H. Fusarium Head Blight From a Microbiome Perspective. Front Microbiol. 2021;12:628373.
15. Munkvold GP, Proctor RH, Moretti A. Mycotoxin Production in Fusarium According to Contemporary Species Concepts. Ann Rev Phytopathol. 2021;59:373-402.
16. Musmade SA, Musmade NA, Ranjale SR. Studies on stalk rot of maize caused by *Fusarium moniliforme* Sheldon. Int J Plant Prot. 2013;6(2): 390-393.
17. Nelson PE, Toussoun TA, Marasas WF. *Fusarium* species: An illustrated manual for identification. 2013.
18. Nuss ET, Tanumihardjo SA. Maize: A Paramount Staple Crop in the Context of Global Nutrition. Compr Rev Food Sci Food Saf. 2010;9(4):417-436.
19. Payak MM, Sharma RC. An Inventory and Bibliography of Maize Diseases in India. Indian Agricultural Research Institute, New Delhi, 1980; pp. 44.
20. Ranum P, Pena-Rosas JP, Garcia-Casal MN. Global maize production, utilization, and consumption. Ann N Y Acad Sci. 2014;1312:105-112.