Emerging Research Nexus eISSN 3078-5278

Research Article

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Bio-Fungicides Efficacy Evaluation against *Colletotrichum Falcatum* Causing Red Rot of Sugarcane

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Accepted: 13 January 2025
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DOI: https://doi.org/10.70788/ern.2.1.2025.17

Abstract: Sugarcane is an economically important crop commercially cultivated worldwide owing to huge nutritional and medicinal purposes. Annual sugarcane yield in Pakistan is rapidly minimizing due to attack of various fungal rots. Red rot of sugarcane is an important constraint to sugarcane production worldwide. To combat these threatening losses, present study was designed intensively to screen the substantial efficacy of selected systemic fungicides against red rot of sugarcane. It is pertinent to mention that at planting, seed treatment application of fungicides along with pesticides, reduce crop losses by minimizing fungal attack. Our findings revealed *Colletotrichum falcatum*, upon morphological studies of isolated fungal rot from sugarcane crop. Two selective fungicides viz Mancozeb and Nativo along with three different concentrations viz; 60, 80 and 100 ppm were evaluated *invitro* against *C. falcatum* of sugarcane. Study findings revealed that among selected fungicides, Nativo was most effective (92.31%) in inhibiting mycelial growth of *C. falcatum* following poisoned food technique. The results showed that both applied fungicides significantly affect against red rot of sugarcane, whereas no mycelial growth inhibition was recorded in control treatment. It is therefore, recommended that timely application of systemic fungicides may enhance crop yield by minimizing effect of phytopathogenic fungal rots.

Keywords: Nativo, Mancozeb, Red Rot of Sugarcane

Introduction

Sugarcane (Saccharum officinarum L.) from family Poaceae is an important crop with global annual production recorded 531.3 million metric tons, which is almost four times higher (Shahbandeh, 2023). Grown in tropical and subtropical regions, sugarcane is the world's largest crop by in terms of production, accounting about2.1 billion tones in 2021, with Brazil accounting for about 40% of the total production.

Published Online: 15 January 2025
How to cite this paper: Saghir, N., Hafeez, I., Rasheed, M. N., Jabeen, Z., and Shakeel, S. (2025). Bio-Fungicides Efficacy Evaluation against *Colletotrichum Falcatum* Causing Red Rot of Sugarcane. *Emerging Research Nexus*. *2*(1). 79-84. https://doi.org/10.70788/ern.2.1.2025.17

Pakistan contributes 0.9% sugarcane production share in Gross Domestic Product (GDP) (GOP, 2022). Sugarcane is one of the most significant cash crops of Pakistan contributing enormous role in the economy of Pakistan. It is grown in the tropical and subtropical regions of world. Due to its wide range of adaptability, it supplies more than 60% of world sugar and basic raw material for Pakistan sugar industry, which is second to textile industry in the country. Molasses is used for the synthesis of alcohol, bio-compost etc., while bagasse is used as fodder for farm animals during the winter season, which helps alleviating the shortage of green fodder. Cane crushed material are used as fuel in Pakistan and its juice is very popular among people of Pakistan during the hot summer season. Besides producing a vast agro-industrial base, sugarcane is an important contributor sharing (5.2%) to Gross National Product (Government of Pakistan, 2022).

Among various factors responsible for low yield, diseases are the major cause. About more than100 fungal, 20 bacterial, 12 viruses and 55different nematode species are observed pests of sugarcane around the globe (Singh and Waraitch, 1981). Red rot of sugarcane causes severe losses in sucrose yield in many cane growing areas of the world (Singh & Singh, 1989). In Pakistan *C. Falcatum* caused serious losses in past and achieved the status of the most threatening and destructive fungal rot of sugarcane (Chaudhry et al., 1999; Subhani et al., 2008; Bharti et al., 2012). *Colletotrichum falcatum* can infect all parts of the plant (Viswanathan 2021) and may be ubiquitous on plants in a field.

The endless struggle between varieties and the complexity of disease have led to the development of correspondingly a variety of approaches for control. Fungicides are often a vital part of disease management as they control many diseases satisfactorily (McGrath, 2004). Red rot is a major problem for sugarcane production and is responsible for the eradication of numerous best varieties from the cultivation due to the constant evolution of the newer species (Malathi et al., 2010). Red rot pathogen hydrolyzed the stored sucrose by producing the enzyme invertage which breaks the sucrose molecule into its components namely glucose and fructose. As a result, the quantity of molasses increases and it is aptly called the cancer of sugarcane (Khan et al., 2011). As this has become major constraint in the profitable cultivation of sugarcane (Mohan and Sangeeta, 2009).

The role of fungicides in modernizing and changing the condition of agriculture is quite significant. Keeping in view the seriousness of disease, present investigations were conducted to study the efficacy of native and mancozeb on red rot of sugarcane.

Material & Methodology

The study was designed with the aim to identify the threatening phytopathogenic rot of sugarcane crop in fields of National Agriculture research Centre Islamabad (NARC). A survey was conducted, and diseased specimens (sugarcane leaves and stalks) were brought to Fungal Plant Pathology Lab NARC. Diseased specimens were air dried for 24 hours and were pierced into 5mm pieces. After drying diseased specimens were surface sterilized with 70% ethanol and 0.5% Sodium hypochlorite solution for 10 minutes. Sterilized distilled water was applied for washing the specimen and were again surface sterilized with chlorax. Small 2mm portion of diseased specimen was isolated on potato dextrose agar and petri plates were incubated at 27° C for one week in incubator. After week of incubation growing edges of fungal hyphae developing from

tissues were transferred to PDA. Pure cultures were preserved in 15% glycerol solution and were stored at -20° C. Morphological and microscopic studies were conducted to exactly identify the pathogen. Moreover, Pathogenicity assay was conducted to confirm Koch's postulates. Isolated fungi were inoculated into 6-7weeks old sugarcane plant grown in greenhouse. Fungal rot was isolated on PDA petri dishes for 7 days 27°C in incubator and was further re-inoculated firstly, by conidial suspension sprayed on the insertion of leaves and secondly by inserting two mycelial plugs of 5mm diameter taken from the fungal colonies into the sugarcane stalk. The inoculated stalks were placed in the trays and kept in laboratory at room temperature. Diseased symptoms were observed daily after post inoculation and were compared to fungal taxonomic keys for exact disease identification. After 9 days stalks were cut down longitudinally to observe internal symptoms.

In vitro Management of *Colletotrichum falcatum*

Poisoned food technique assay was conducted to screen efficacy of applied fungicides against isolated fungal pathogen. Percentage Mycelial Inhibition was recorded after all odd DPI (Days Post Inoculation) for one week. Fungicides (Nativo and Mancozeb) were applied at three concentrations (60, 80 and 100 ppm) whereas, sterile distilled water was applied as control treatment. All the three concentrations of selected fungicides were individually added into PDA and media was poured into 5 Petri plates of 90mm diameter. Once media gets solidified into petri plates, a small disc of 7 days old pure culture of isolated fungi was and placed in centre of at equilibrium into each poisoned media plates. Petri dishes were incubated for a week in incubator at 25 C. Readings were noted after all odd DPI's. Mycelial Percentage inhibition of fungus was calculated by following formula.

$$I\ (\%) = (C\text{-}T/C) \times 100.$$

Data was subjected to analysis of variance and separation of means among the treatments were determined using LSD test at 5% level of significance using Statistical Packages for Social Science (SPSS 16.0 version) and MS Excel (2010).

Results & Discussion

Morphological and microscopic studies identified the fungal pathogen as *Colletotrichum falcatum*. Among a total of 10 isolates, 4 were observed 89white, gray-colored colonies with a dark and gray conidial mass in the center. The results revealed that the isolates of *Colletotrichum falcatum* produced hyaline cylindrical conidia. 3 isolates were recorded having creamish-white colonies with a salmon-gray colored conidial mass exactly at the center and fusiform tapered to a point in both ends. Remaining 3 isolates were observed showing pure creamish to light orange mycelium with reverse side of plate showing blackish to light, gray-colored conidial mass at the center with cylindrical conidia having obtuse to slightly rounded ends. The length of conidia ranged from 10.7-15.5 µm (Table 1).

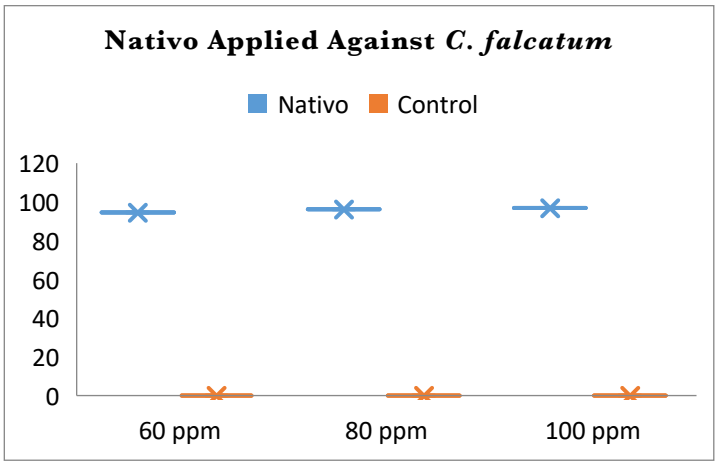

Figure 1: Nativo applied at three concentrations (60 ppm, 80 ppm, 100 ppm) Control* No mycelial inhibition observed

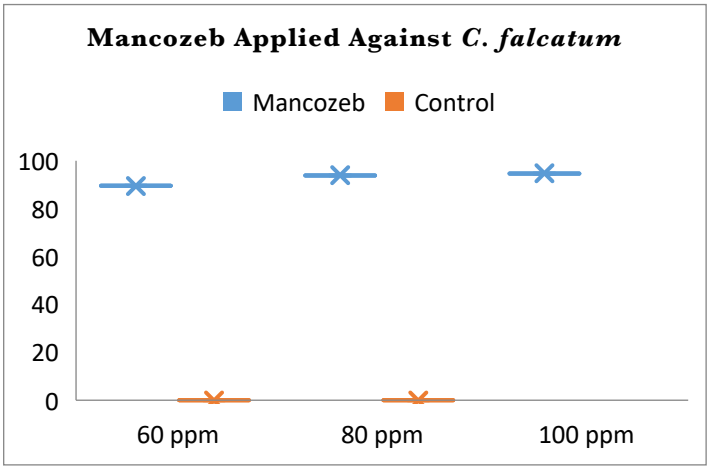

Figure 2: Mancozeb applied at three concentrations (60 ppm, 80 ppm, 100 ppm) Control* No mycelial inhibition observed.

Table 1. Morphological Characteristics of *Colletotrichum falcatum*

S. No	Isolates	Conidia Shape	Appressoria Shape	Size	Color	Pathogenic
1	CFM1	Falcate	Ovoid-irregular	10.1-11.1 µm	Whitish to Gray color	Moderately Pathogenic
2	CFM2	Sickle Shaped	Ovoid-irregular	10.5-13.4 µm	Whitish to Gray color	Severely Pathogenic
3	CFM3	Sickle Shaped	Ovoid-irregular	10.1-11.4 µm	Whitish to Gray color	Highly pathogenic
4	CFM4	Sickle Shaped	Ovoid-irregular	10.6-15.4 µm	Gray-colored	Highly pathogenic
5	CFM5	Sickle Shaped	Ovoid-irregular	8.4-13.5 µm	Creamish-light orange	Severely Pathogenic

6	CFM6	Falcate	Ovoid-irregular	9.4-14.1 µm	Creamish-white	Severely Pathogenic
7	CFM7	Falcate	Ovoid-irregular	10.2-11.4 µm	Creamish-white	Highly pathogenic
8	CFM8	Falcate	Ovoid-irregular	10.2-12.4 µm	Creamish-light orange	Severely Pathogenic
9	CFM9	Falcate	Ovoid-irregular	10.5-11.6 µm	Creamish-white	Moderately pathogenic
10	CFM10	Falcate	Ovoid-irregular	10.5-15.4 µm	Creamish-light orange	Highly pathogenic

Efficacy of Fungicides against *Colletotrichum falcatum*

Fungicides are specific in their action and specificity for various genus and species. Analysis of variance showed significant difference in the effectiveness of both tested fungicides, their doses and the interaction between doses and fungicides for the suppression of radial growth of *C. falcatum.* Results of poisoned food technique showed that maximum inhibition of *C. falcatum* was observed by Nativo viz; 94.51%, 96.22% and 96.91% at all applied concentrations (60, 80 and 100 ppm) after one week of incubation. Whereas application of Mancozeb at all applied concentrations also significantly inhibited fungal mycelium viz; 89.45&, 93.91% and 94.72%, respectively (Figure 1 & 2).

Conclusion

Present research summarizes that Nativo is the best effective systemic fungicide against red rot of sugarcane initiated by *C. falcatum*. All the three applied concentrations of native and mancozeb significantly suppresses fungal mycelium at all odd DPI (Days Post Inoculation). The growth and proliferation of red rot pathogen is affected by various environmental and nutritional conditions also, therefore timely applications of particular dose of systemic fungicides may play an excellent inhibitory role against fungus and promote enhanced yield.

Conflict of Interest: No potential conflict of interest is declared by any author.

Author (s) Contribution: All the authors contributed equally to the manuscript.

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