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SURVEY AND MOLECULAR DETECTION OF POKKAH BOENG (*FUSARIUM MONILIFORME* SHELD.) OF SUGARCANE OF MEERUT AND ITS *IN-VITRO* MANAGEMENT

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ABSTRACT

Pokkah Boeng, a significant disease of sugarcane caused by *Fusarium moniliforme* Sheld., poses a substantial threat to cane yield and quality in several sugarcane-growing regions, including Meerut, Uttar Pradesh. Field surveys were conducted during the cropping season across different blocks of Meerut district to assess disease incidence and symptomatology. Infected samples were collected and the pathogen was isolated and purified. Morphological and cultural characteristics confirmed the pathogen as *Fusarium moniliforme*, which was further validated through molecular characterization using ITS-rDNA sequencing. *In-vitro* evaluation of fungicides and bio-agents was carried out using the poisoned food technique and dual culture method, respectively. Among the tested treatments, Carbendazim 50% WP showed maximum inhibition (100%) of mycelial growth amongst all tested fungicide at each concentration whereas, in case of bioagents *Trichoderma viride* shown the maximum inhibition (70.74%) indicating strong antifungal activity against *F. moniliforme*. This study highlights the prevalence of Pokkah Boeng in Meerut, confirms the pathogenic identity using molecular tools (PCR amplification & DNA sequencing) and suggests effective *in-vitro* management options. The findings provide a foundation for developing integrated disease management strategies to mitigate the impact of Pokkah Boeng in sugarcane.

Key words : Pokkah Boeng, *Fusarium moniliforme*, *Trichoderma viride*, Carbendazim.

Introduction

Sugarcane (*Saccharum officinarum*) is the most significant sugar-producing crop globally, accounting for over 70% of the total sugar output (Gawade *et al.*, 2012). Propagated vegetatively, it is cultivated in more than 80 countries and contributes approximately 11.8 million tonnes of sugar annually (Ali and Sonar, 1984). India ranks as the second-largest sugarcane producer after Brazil and holds the top position as the world's largest sugar consumer. Within India, sugarcane is a major crop, representing about 7.5% of the total value of the country's agricultural production (Yonzone and Devi, 2018). According to the ICAR-Sugarcane Breeding Institute (2023-2024), the crop is grown on 56.48 lakh hectares, yielding 466.43 million tonnes with an average productivity of 79.03 tonnes per hectare.

Pokkah boeng disease in sugarcane is caused by the fungal pathogen *Fusarium moniliforme*, whose sexual stage is known as *Gibberella fujikuroi* (Sowada). Although initially considered a minor disease, it has the potential to significantly impact high sugar-yielding varieties, reducing sugar production by approximately 40.8% to 64.5%, depending on the cultivar (Duttamajumder, 2004). The disease not only leads to substantial losses in cane weight but also adversely affects the quality of the cane (Ali and Sonar, 1984). Pokkah boeng has emerged as a serious threat across India, particularly in both southern and northern sugarcane-growing regions, with subtropical areas being especially vulnerable (Karuppaiyan *et al*, 2015). Pokkah boeng

disease manifests in four distinct phases: chlorotic phases I and II, top rot (acute) phase, and the knife-cut phase (Vishwakarma *et al*., 2013). Early symptoms include yellowish patches at the base of young leaves. As the disease progresses, affected leaves may exhibit various deformities such as twisting, wrinkling, and shortening. The top rot phase is particularly severe, marked by the death of the growing point, resulting in the death of the plant's top and distortion of stalks with characteristic ladder-like lesions both internally and externally. The knife-cut phase is considered the most destructive stage, often accompanying the top rot phase, and is identified by one or more sharp, horizontal cuts in the stalk, resembling incisions made with a knife (Zhang and Jeyakumar, 2018). Additional symptoms include reddish streaks and necrotic lesions on the leaves. Sugarcane plants aged three to seven months are especially vulnerable to the disease, which tends to become more pronounced when a hot and dry period is followed by a wet season (Lin *et al*., 2014).

The present investigation was undertaken with an aim to isolate the pathogen associated with Pokkah boeng disease of sugarcane for molecular identification and testing the efficacy of fungicide and biocontrol agents which will help in the management of the disease.

Materials and Methods

Cropping Survey of sugarcane

The survey of sugarcane field of six blocks of Meerut District was conducted during July, 2024 to September, 2024. Five villages were randomly selected from sugarcane cultivated area in selected blocks from five farmer's field was selected from each village and leaf & cane samples were collected from farmer's fields during survey and approximately 20% sample was collected from 5% area. The presence of the disease was initially confirmed based on symptomatology.

Molecular identification of pathogen

Fungal isolates were obtained through isolation procedures and maintained as pure cultures. Genomic DNA from *Fusarium moniliforme* isolates was isolated at Centre of Excellence for Sanitary and Phytosanitary, Department of Plant Pathology, SVPUA&T, Meerut, using CTAB (Cetyletrimethylammonium Bromide) method (Doyle and Dolye, 1987). Mycelial mats were collected from 5-7 days old culture used as the source material. The DNA pellet was washed with 70% ethanol, air-dried at room temperature and dissolved in 50 µL of nuclease free water. DNA quantity and purity were evaluated through agarose gel electrophoresis and spectrophotometric analysis using Nano-spectrophotometer.

Further confirmation of the isolates was done by cultural characterization and molecular identification using universal and specific primers;

Universal Primers: ITS1 Forward 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 Reverse 5'-TCCTCCGCTTATTGATATGC-3' (White *et al*., 1990).

Specific Primers: EF1 Forward5'-ATGGGTAAGGA(A/G) GACAAGAC-3' and EF2 Reverse 5'-GGA(G/A) GTACCAGT (G/C) ATCATGTT-3' (Fourie *et al*., 2009).

PCR amplification was conducted in a total volume of 40 µL using genomic DNA as the template. The reaction mixture included 2 µL of DNA, 20 µL of PCR master mix containing Taq polymerase, dNTPs, $MgCl_4$ and buffer components, 1 µL each of forward and reverse primers and 16 µL of nuclease free water. Thermal cycling conditions were; an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension step at 72°C for 10 minutes (Sidique, 2007).

In-vitro efficacy of biocontrol agents and fungicide

Assessment of *in-vitro* antagonistic efficacy of bio control agents against *Fusarium moniliforme* was done using dual culture method in which two bacterial (*Pseudomonas fluorescence*, *Bacillus subtilis*) and two fungal (*Trichoderma harzianum* and *Trichoderma viride*) biocontrol agents were used observation was recorded for seven days at every 24 hours. *In-vitro* efficacy of fungicides (Propiconazole, Hexaconazole, Azoxystrobin and carbendazim) at different concentrations (100, 200 & 300 ppm) was checked against *Fusarium moniliforme*using poison food technique and the observation were recorded for seven days at every 24 hours.

The mycelial growth and its inhibition percent were calculated by the following formula.

$$I = \frac{C - T}{C} \times 100$$

I = Per cent Growth inhibition

C= Radial growth of pathogen in control plate (mm)

T = Radial growth of pathogen in treated plate (mm)

Results

In this study, we conducted a cropping survey of disease incidence of pokkah boeng disease sugarcane

Table 1 : Disease incidence percentage of six blocks of Meerut District.

S. no.	Block	% Disease Incidence (2024-25)
1.	Daurala	8.88
2.	Meerut Sadar	10.21
3.	Sardhana	9.00
4.	Rohta	8.60
5.	Mawana	9.23
6.	Parikshitgarh	9.40

Table 2 : Efficacy of biocontrol agents against *Fusarium moniliforme* under *in-vitro* condition.

Tr. no.	Treatment name	Mycelial growth	% Inhibition
T_1	*Trichoderma harzianum* Isolate-TS004	34.66	61.48
T_2	*Trichoderma viride* Isolate-TS006	26.33	70.74
T_3	*Pseudomonas fluorescens* Isolate-PF008	44.66	50.37
T_4	*Bacillus subtilis* Isolate-B005	55.66	39.26
T_5	Control	90	0

cultivars of 6 blocks of Meerut District (Table 1). During survey the maximum incidence of 10.21% was observed in Meerut block followed by Parikshitgarh (9.40%), Mawana (9.30%), Sardhana (9.00%) and Daurala (8.88%) whereas, the least incidence was observed in Rohtablock (8.60%) (Table 1).

Morphological characterization of *Fusarium moniliforme*

The morphological characteristics of *Fusarium moniliforme* were examined on Potato Dextrose Agar (PDA). The colonies displayed moderate to rapid growth, reaching 85-90 mm in diameter within 7 days of incubation at 26 ± 2°C. Initially, colonies appeared white, gradually developing a pale pink to violet as they aged. The aerial mycelium was cottony and fluffy in texture, with the reverse side of the culture plates showing pigmentation ranging from light yellow to violet.

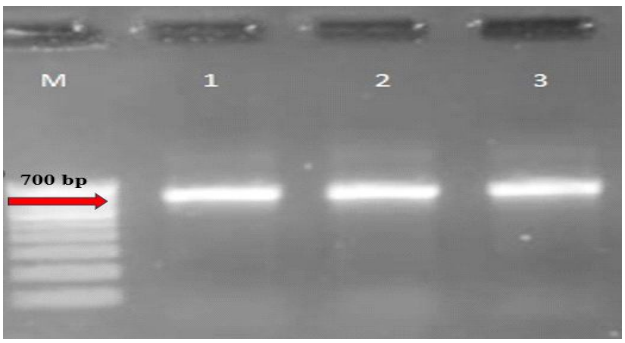

Fig. 1 : Agarose gel electrophoresis of PCR product amplification using EF1 and EF2 primers; L- 100 bp DNA ladder.

Under microscopic observation, conidiophores were found to be branched and produced both microconidia and macroconidia. Microconidia were numerous, single-celled, oval to ellipsoidal in shape whereas, macroconidia were less frequent, slightly curved or sickle-shaped, generally comprising 3 to 5 septa. Chlamydospores were either absent or rarely observed.

These morphological traits matched the diagnostic features of *F. moniliforme* as described by Omar (2006), thereby confirming the identity of the isolates.

Molecular characterization using PCR Analysis and Sequencing of Target genes

The genomic DNA was isolated from the pure culture of the collected diseased sample. DNA of fungal isolates from each block were run under PCR and gel electrophoresis. Later on, PCR products were sent for the sequencing report (Biokart India Pvt. Ltd., Bangalore), there was no variability found among the samples from different blocks. The sequence was then submitted to NCBI for BLAST. NCBI BLAST comparison results for sequences confirmed their identity as *Fusarium moniliforme,*10 similar sequences were selected for multiple sequence alignment and Phylogenetic tree was constructed using MEGAX (Fig. 1).

Efficacy of biocontrol agents and fungicide against *Fusarium moniliforme*

An *in-vitro* study was conducted to assess the antagonistic effectiveness of four biocontrol agents, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma harzianum* and *Trichoderma viride* against *Fusarium moniliforme* using the dual culture technique. All biocontrol agents demonstrated significant suppression of the pathogen's radial growth when compared to the untreated control.

Among the tested agents, *Trichoderma viride* showed the highest antagonistic activity, inhibiting fungal growth by 70.74%, and displaying strong microparasitic interactions. *Trichoderma harzianum* followed closely, restricting growth by around 61.48% followed by *Bacillus subtilis* (50.37%). *Pseudomonas fluorescens*was the least effective, showing (39.26%) suppression. Both bacterial agents produced observable inhibition zones, suggesting the release of antifungal compounds. Overall, *Trichoderma* species, especially *T. viride*, emerged as most effective antagonists of *F. moniliforme*, indicating their potential for inclusion in sustainable sugarcane disease management programs.

Table 3 : Efficacy of fungicides against *Fusarium moniliforme* under *in-vitro* condition.

Tr. no.	Treatment Details	Concentration (ppm)	Mycelial growth	% Inhibition
T_1	Carbendazim 50%WP	100	0.00	100.00
T_2	Carbendazim 50%WP	200	0.00	100.00
T_3	Carbendazim 50%WP	300	0.00	100.00
T_4	Propiconazole 25%EC	100	23.00	74.44
T_5	Propiconazole 25%EC	200	8.00	91.11
T_6	Propiconazole 25%EC	300	0.00	100.00
T_7	Azoxystrobin 25% WS	100	19.33	78.52
T_8	Azoxystrobin 25% WS	200	0.00	100.00
T_9	Azoxystrobin 25% WS	300	0.00	100.00
T_{10}	Hexaconazole 5%SC	100	29.01	67.77
T_{11}	Hexaconazole 5%SC	200	24.00	73.33
T_{12}	Hexaconazole 5%SC	300	19.50	78.33
T_{13}	Control	-	90.00	0.00

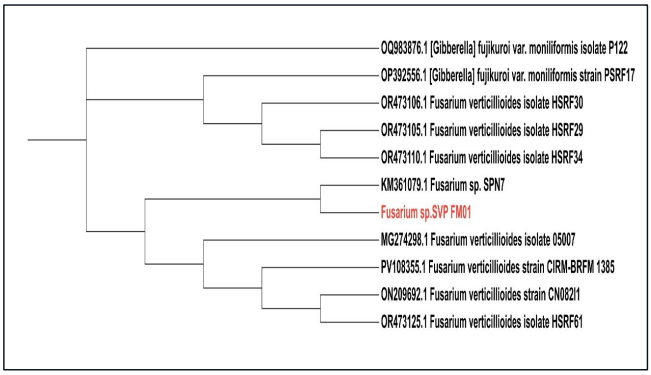

Fig. 2 : The phylogenetic tree of *Fusarium moniliforme* constructed based on its nucleotide sequence reported in different places. Red one represents the collected sample from survey.

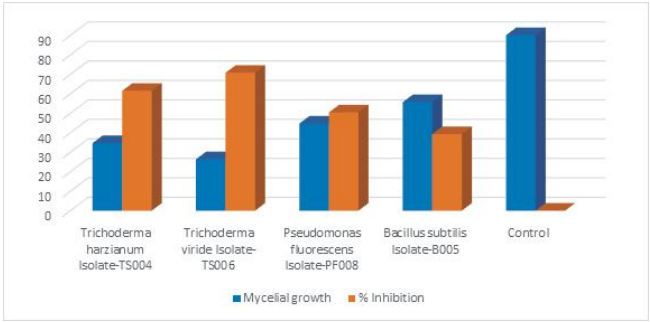

Fig. 3 : Graphical representation of *in-vitro* efficacy of biocontrol agents.

The *in-vitro* efficacy of four systemic fungicides Propiconazole, Hexaconazole, Azoxystrobin, and Carbendazim were evaluated against *Fusarium moniliforme* at three different concentrations: 100 ppm, 200 ppm and 300 ppm, using the poisoned food technique. Observations were recorded after 7 days of incubation at 26 ± 2°C, focusing on radial mycelial growth inhibition.

Among the tested fungicides, Carbendazim was most effective among the four, with inhibition percentages of 100% at 300 ppm, 200 ppm and 100 ppm followed by Azoxystrobin displayed the efficacy, with growth inhibition rates of 100% at 300 ppm, 100% at 200 ppm and 78.52% at 100 ppm. Propiconazole showed efficacy of 100 % inhibition at 300 ppm, while at 200 ppm and 100 ppm, it recorded inhibition percentages of approximately 91.11% and 74.44%, respectively. Hexaconazole also showed the least antifungal activity, achieving 78.33% inhibition at 300 ppm, 73.33% at 200 ppm and 67.77% at 100 ppm.

Overall, Carbendazim and Azoxystrobin proved to be the most potent fungicides against *F. moniliforme* at all

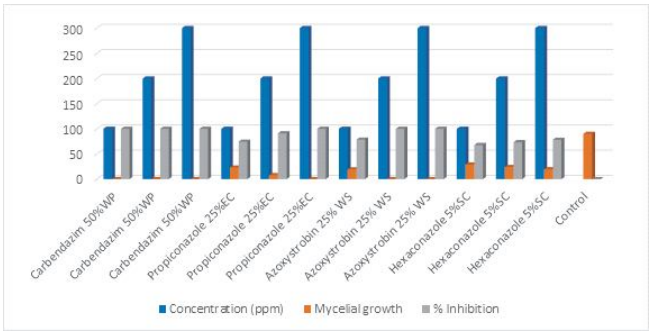

Fig. 4 : Graphical representation of *in-vitro* efficacy of fungicides.

tested concentrations, highlighting their potential role in integrated disease management strategies for controlling Pokkah boeng disease in sugarcane.

Discussion

Various *Fusarium* species have been associated with causing Pokkah Boeng disease across the globe (White *et al.*, 1990). The pathogen responsible for PB was initially isolated in Java by Bolle in 1927 and was identified as *Fusarium moniliforme* (Vigmassa *et al.*, 2021). Since then, the disease has been reported in multiple countries, with different studies attributing its cause to several *Fusarium* species, either individually or in combination.

The field survey carried out across six blocks of Meerut district highlighted differing levels of Pokkah Boeng (PB) disease occurrence in sugarcane varieties. Among the surveyed areas, the Meerut block exhibited the highest infection rate at 10.21%, followed by Parikshitgarh (9.40%) and Mawana (9.30%), while the Rohta block showed the lowest incidence at 8.60%.

These differences in disease prevalence may be influenced by several factors, including local environmental conditions, the susceptibility of specific cultivars, farming practices, and the level of pathogen presence, as previously reported by (White *et al*., 1990). The consistent detection of PB in all surveyed regions indicates its wide distribution in this key sugarcane-producing zone, underscoring the need for continuous surveillance and targeted disease management approaches.

Morphological analysis of the pathogen isolated from infected samples grown on Potato Dextrose Agar (PDA) revealed characteristics typical of *Fusarium moniliforme*, now classified within the *Fusarium fujikuroi* species complex. The fungal colonies showed moderate to fast growth, along with a pinkish hue and cottony mycelial texture. Microscopic observations confirmed branched conidiophores, numerous ovals to ellipsoidal microconidia, and a smaller number of sickle-shaped macroconidia with 3 to 5 septa. Chlamydospores were rarely seen or absent, which aligns with the morphological descriptions provided by Nelson *et al*. (1983) and Lin *et al*. (2014).

Further validation was achieved through molecular analysis. Genomic DNA extracted from isolates obtained from each block underwent PCR amplification, sequencing, and BLAST comparison. The results revealed no significant genetic variation among the isolates, suggesting a uniform or clonally distributed population of *F. moniliforme* in the study area. Similar genetic consistency in PB-causing *Fusarium* species under specific environmental conditions has also been reported by Dewing *et al*. (2022). The phylogenetic analysis, performed using MEGAX, showed a close genetic relationship between the local isolates and previously identified *F. moniliforme* sequences in the NCBI database, corroborating the morphological findings (Vignassa *et al*., 2021).

The *in vitro* study demonstrated that *Trichoderma viride* was the most effective biocontrol agent against *Fusarium moniliforme*, followed by *T. harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* (Ayele *et al*., 2021), given the similar report demonstrating that *T. viride* inhibits the growth of *F. oxysporumf*. sp. *lycopersici* with 76.94% growth inhibition.

Their effectiveness is linked to mechanisms like mycoparasitism and antifungal metabolite production. Among fungicides, Carbendazim showed complete inhibition at all concentrations, with Azoxystrobin also highly effective. However, concerns over resistance development from repeated Carbendazim use highlight the need for integrated management, tested fungicides, azoxystrobin and tebuconazole showed similar effects against *Fusarium* spp. (Omar *et al*., 2006) reported that carbendazim significantly reduced disease symptoms by 84% compared with inoculated controls and by 77% compared with carbendazim treatment alone.

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Conflict of interest

Authors declare no competing interests.

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