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RESEARCH PAPER

Assessing the effectiveness of commercially available botanicals and bioagents against *Fusarium verticillioides* causing Pokkah boeng disease in maize

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Abstract: Maize (*Zea mays* L.) is one of the important cereal crops in the world which stands next to wheat and rice. It is the source of food security and economic development in India. However, maize is prone to several diseases. Climate change has led to the emergence of a new disease Pokkah boeng caused by *Fusarium* spp. in India. In this study, the causal agent of Pokkah boeng disease in maize, was isolated and cultured on potato dextrose agar (PDA) medium. The pathogen exhibited white mycelial growth that developed into a pinkish-white colony, with characteristic microconidia and macroconidia. The ITS region of r DNA was sequenced, revealing 100% homology with *F. verticillioides* MK264336. The effectiveness of twelve botanicals and six bioagents against *F. verticillioides* was assessed using poison food and dual culture techniques. Among the botanicals, Elixir and Crop Verdict achieved complete inhibition (100%) of the pathogen, significantly outperforming others. Among bioagents, *Trichoderma harzianum* (IOF strain) exhibited the highest inhibition (86.27%), followed by the Raichur strain of *T. harzianum* (83.95%) and *T. viride* (81.40%). The study under scores the potential of using botanicals and bioagents as sustainable alternatives to chemical pesticides in managing *F. verticillioides*, with *Trichoderma* spp. showing strong antagonistic activity through mechanisms such as mycoparasitism, antibiosis and competition.

Key words: Botanicals, Maize, *Fusarium verticillioides,* Pokkah boeng, *Trichoderma*

Introduction

Maize (*Zea mays* L.) belongs to *Poaceae* family is highly adaptable and versatile crop that thrives in diverse agro-ecological and climatic conditions. Globally recognized as"Queen of Cereals," maize boasts remarkable genetic yield potential and photo-thermo-insensitivity compared to other cereals allowing for year-round cultivation (Troyer, 2006). Maize suffers from multitude of challenges attributed to fungi, bacteria and nematodes (Payak and Sharma, 1985). A new and an emerging disease Pokkah boeng affecting maize has been reported in India (Harlapur *et al*., 2023) affecting the yield and quality of the crop. The management of this disease through conventional technology such as growing resistant varieties, fungicide treatment alone cannot provide a remedy for disease control (Gade *et al*.,2007) as the non -judicious use of synthetic fungicides over the years led to several problems related to human and animal health besides environmental issues (Arya and Sharma, 2017).Therefore, there is growing need for an alternative approach that are economically feasible and eco-friendly to manage the disease. Hence, the study was undertaken for an eco-friendly management using bioagent and commercially available botanicals to control the pathogen. The present study was under taken to identify effective bioagent and plant extracts for the eco-friendly and economical management with increase in agricultural production and sustaining food and nutritional security without any hazardous effect on human and environment health.

Material and methods

Pathogen

The symptomatic maize leaves were collected from the infected fields in Belagavi district of Karnataka resembling the Pokkah boeng disease of sugarcane (Vishwa karma *et al*., 2013). The pathogen was isolated from the specimen using standard tissue isolation procedure on potato dextroseagar (PDA) medium. The morphological and sporulation patterns were studied. The DNA from pure culture isolate was extracted using the CTAB protocol (Doyle and Doyle, 1987). The ITS region of r-DNA was amplified with ITS1/ITS4 primers and sequenced. BLAST analyses of sequences of pathogen at NCBI database revealed 100% homology with *Fusarium verticillioides* MK264336 (Lin *et al*., 2016). PCR amplification with *Fusarium verticillioides* specific primers VER1/VER2 (Mule *et al*., 2004) confirmed the organism.

Collection of plant extracts

The commercially available plant-based products (botanicals) were collected from the market (Table 1) and bioagents were collected from Institute of Organic Farming (IOF), University of Agricultural Sciences, Dharwad (Table2).

In-vitro evaluation of botanicals

The antifungal activity of twelve plant-based products were assayed in the laboratory for their efficacy against the *Fusarium verticillioides* using poisoned food technique (Nene and

Table1. List of commercially available botanicals with their contents

Product name	Contents/ingredients
Jojoba oil	Jojoba Oil (raw)
Pongamia oil (Karanjin)	Pongamia oil (raw)
Shefaneem	*Azadirachtin* 0.15%
Kaneem	*Azadirachtin* 0.03%
Neemba	*Azadirachtin* 1%
Multineem	*Azadirachtin* 0.03%
Vijayneem	*Azadirachtin* 0.03%
Cropverdict	Cinnamomum zylenicum oil 10% + herbal extract 23%
Margoneem	*Azadirachtin* 0.15%
Elixer	Turmeric extract 25% + Neem extract 50%
Dischek	*Ficusbengalensis* 0.001%, *Ficusreligiosa* 0.001%, *Ficusretusa* 0.001% and *Aqua solvent* 99.97%
Nimbicidine	*Azadirachtin* 0.03%

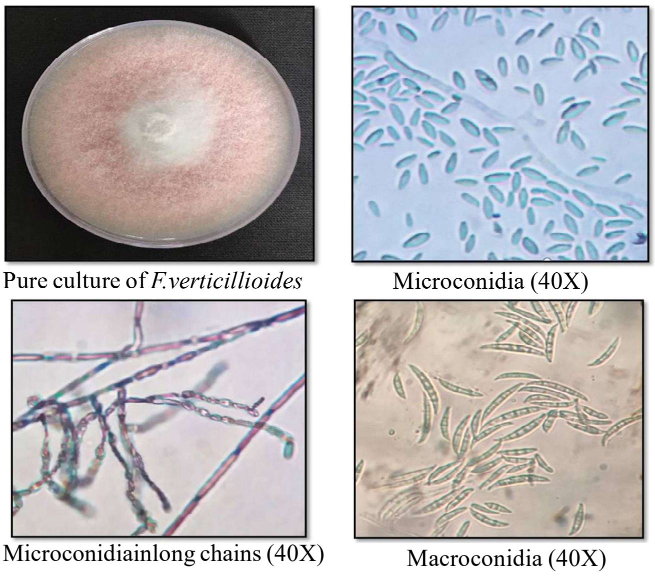
Thapliyal, 1982). All the botanicals were tested at concentrations of 0.25, 0.50 and 1.0 per cent using PDA as the basal media. The bioagents were tested using dual culture technique (Dennis and Webster,1971). The experiment was planned with completely randomized design (CRD) and all the treatments replicated thrice. The observations on colony diameter of the fungus were recorded when untreated control Petri plate was fully covered with mycelial growth and the per cent mycelial inhibition of the test fungus was calculated using the formula (Vincent, 1947; Cramer, 2000)

$$I= \frac{(C-T)}{100C} x$$

Where, I=Per cent inhibition of mycelia growth.

C=Growth of mycelium in control.

T=Growth of mycelium in treatment.



Pure culture of *F.verticillioides* Microconidia (40X)

Microconidiainlong chains (40X) Macroconidia (40X)

Plate 1. Pureculture, micro and macroscopic images of *Fusarium* verticillioides

Table 2. Details for bioagents used for evaluation

Bioagent	Source
Trichoderma harzianum	Institute of Organic Farming UAS, Dharwad
Pseudomonas fluorescens	
Bacillus subtilis	
Actinobacteria (AUDT502)	MGL UAS, Dharwad
Trichoder maviride	Kerala Agricultural University (KAU)
Trichoderma harzianum	UAS, Raichur

Statistical analysis

The experimental data was subjected to statistical analysis as per the procedures given by (Gomez and Gomez, 1984). One way ANOVA by SPSS version 23, Factorial CRD using OPSTAT were used. The mean values were evaluated using Duncan's multiple range test (Duncan,1955) for interpretation of the results.

Results and discussion

The pathogen was isolated from diseased maize samples and cultured on potato dextrose agar (PDA) medium for further analysis. Initially, the growth of white mycelia was observed, which later developed into a pinkish-white colony. Microconidia were formed in long chains and clusters with oval to club-shaped without septa, monophialide borne microconidia, Macroconidia were slightly curved with 3-4 septation (Plate 1). The ITS region of r-DNA was amplified with ITS1/ITS4 primers at 500 bp and sequenced (Plate 2). BLAST analyses of sequences of pathogen at NCBI database revealed 100 per cent homology with *Fusarium verticillioides* MK264336 (Lin *et al*., 2016). Similar spore observation was made by Kaur *et al*. (2020). In nature, there are several plants and microorganisms which are known to exhibit anti-fungal properties against various fungi. Present experiment aimed to assess the effectiveness of various commercially available botanicals (plant extracts) and effective bioagents against Pokkah boeng pathogen *Fusarium verticillioides.* Twelve botanicals and six bioagents were subjected to testing using

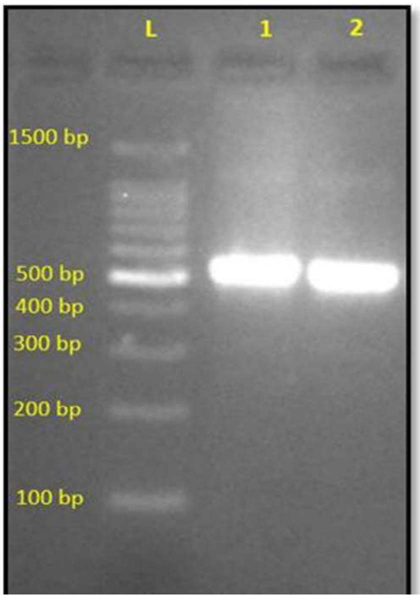


Plate 2. *Fusarium verticillioides* amplification using TS1/ITS4

Table 3 . Effect botanical on mycelia growth of *Fusarium verticillioides*

Treatment detail	Radial mycelial growth (mm)			Mean mycelial growth (mm)
	Conc.(0.25%)	Conc.(0.5%)	Conc.(1.0%)	
Jojoba oil	80.27	80.13	80.07	80.16 [b]
Pongamia oil	62.04	60.13	58.01	50.06[d]
Sheefa neem	49.02	40.33	28.05	39.13 [ef]
Kaneem	42.00	37.00	36.96	38.65[f]
Neemba	47.01	38.06	33.99	39.69[ef]
Multineem	57.00	50.02	37.08	48.03[d]
Vijayneem	62.31	57.03	46.01	55.12[c]
Cropverdict	0.00	0.00	0.00	0.00[g]
Margoneem	62.34	57.03	55.03	58.13[c]
Elixer	0.00	0.00	0.00	0.00[g]
Dischek	50.42	45.04	30.02	41.83[e]
Nimbicidine	60.07	53.02	53.02	55.37[c]
Control	90.00	90.00	90.00	90.00[a]

Poison food and dual culture technique, respectively and the results were analysed. Analysis of variance revealed significant impact of plant extracts, their concentration and interaction between them ($p < 0.01$). Results of botanicals revealed the complete inhibition (100%) of pathogen in Elixir and Crop verdict which proved significantly superior over other treatments as these products contain the polyphenols exhibiting antifungal and antibacterial effects. Followed by Kaneem (57.05%), which was on par with Sheefa neem (56.52%) and Neemba (55.90%). Conversely, Jojoba oil exhibited the least inhibition (10.94%) of the test fungus over control. This trend indicated a consistent reduction in mycelial growth of *Fusarium verticillioides* with increased absorption of plant extract (Table 3-4, Plate 4 Fig. 1). Previous studies demonstrated neem's effectiveness in inhibiting the growth of *Fusarium oxysporum* f.sp. *gladioli* (Kadam *et al.*, 2014) while another study reported maximum mycelial inhibition of *Fusarium oxysporum* f.sp. *dianthi* using neem extract (Sunderrao *et al.*,2017). Additionally, crude extracts of neem (*Azadirachta indica*) leaf showcased promising results

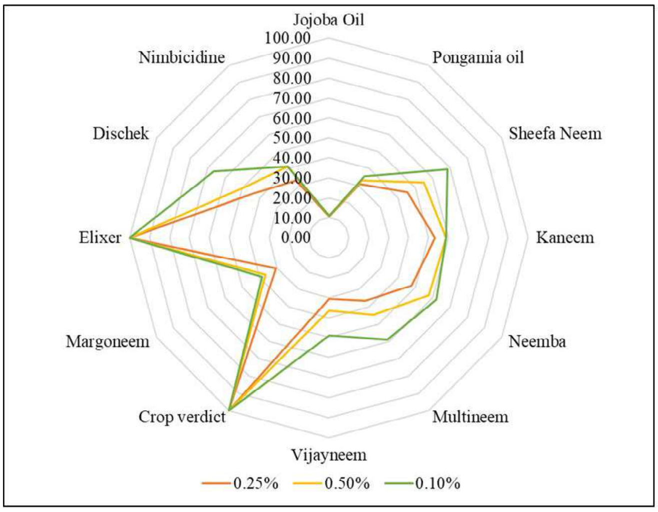


Fig 1. *In vitro* efficacy of commercially available botanicals against *Fusarium verticillioides*

Plate 3. *Invitro* efficacy of bioagents against *Fusarium verticillioides*

Table 4. Per cent age inhibition of mycelia growth by botanicals against *Fusariumverticillioides*

Treatment details	Per cent mycelia inhibition (%)			Mean mycelial inhibition (%)
	Conc.(0.25%)	Conc.(0.5%)	Conc.(1.0%)	
Jojoba Oil	10.81*(19.20)**	10.96 (19.34)	11.04 (19.40)	10.94 [f](19.31)
Pongamia oil	31.07(33.88)	33.19 (35.17)	35.55 (36.60)	33.27[e](35.22)
Sheefa Neem	45.54 (42.44)	55.19 (47.98)	68.84 (56.07)	56.52[b] (48.75)
Kaneem	53.33(46.91)	58.89 (50.12)	58.93(50.14)	57.05[b](49.05)
Neemba	47.76 (43.72)	57.71 (49.43)	62.23(52.08)	55.90[b](48.39)
Multineem	36.67 (37.27)	44.43 (41.80)	58.81(50.07)	46.64[c](43.07)
Vijayneem	30.76 (33.69)	36.64 (37.25)	48.88 (44.36)	38.76[d](38.50)
Cropverdict	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00[a](90.00)
Margo neem	30.74 (33.67)	36.64 (37.25)	38.86 (38.56)	35.41[de](36.52)
Elixer	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00[a](90.00)
Dischek	43.98 (41.54)	49.95 (44.97)	66.65 (54.72)	53.53[b](47.02)
Nimbicidine	33.26 (35.22)	41.09 (39.87)	41.09 (39.87)	38.48[d](38.34)
Mean	46.99 (43.28)	52.06 (46.18)	57.57 (49.36)	-
S.Em.(±)	C.D.@1%			
Botanical(B)	0.361	0.95		
Concentration(C)	0.180	0.48		
Botanical (B) ×Concentration(C)	0.625	1.65		

*Mean of three replications

**The values in the parent heses are arcsine transformed values

Table 5. *In vitro* efficacy of bioagents against *Fusarium verticillioides*

Bioagents	Per cent mycelia inhibition over control
Trichoderma harzianum (IOF Strain, Dharwad)	86.27 *(68.25)[a]**
Trichoderma harzianum (Raichur)	83.95 (66.38)[b]
Trichoderma viride (KAU)	81.40 (64.45)[c]
Pseudomonas fluorescence (IOFStrain,Dharwad)	76.42 (60.95)[d]
Bacillus subtilis (IOFStrain,Dharwad)	71.88 (57.98)[e]
Actinobacteria (AUDT502)	65.02 (53.74)[f]
S.E m(±)	0.14
C.D.at 1%	0.42

*Mean of four replications
**The values in the parentheses are arcs in evalues

against *Fusarium oxysporum* f. sp. *ciceris* (Abdulle *et al*., 2022). Biological control using antagonistic microorganisms offers a potential non-chemical method for combating plant diseases by reducing the pathogen inoculum level. This approach helps prevent pollution and health hazards. Microbial biocontrol agents are eco-friendly and provide a long-lasting effect on plants compared to chemical pesticides in disease management. Among the bioagents tested *Trichoderma harzianum* (IOF, strain) was most effective achieving 86.27 per cent inhibition. The next best was *T harzianum* (Raichur, strain) with an inhibition of 83.95 per cent followed by *T. viride* (KAU) with 81.40 per cent mycelial inhibition of the pathogen. However, the least inhibition was noticed in actinobacteria (AUDT502) with mycelia inhibition of about 65.02 per cent (Table 5, Plate 3 and Fig. 2). The strains of *Trichoderma* spp. are opportunistic invaders known for their rapid growth, spore production and antibiotic activity (*e.g*.,viridin). Their primary mechanisms of

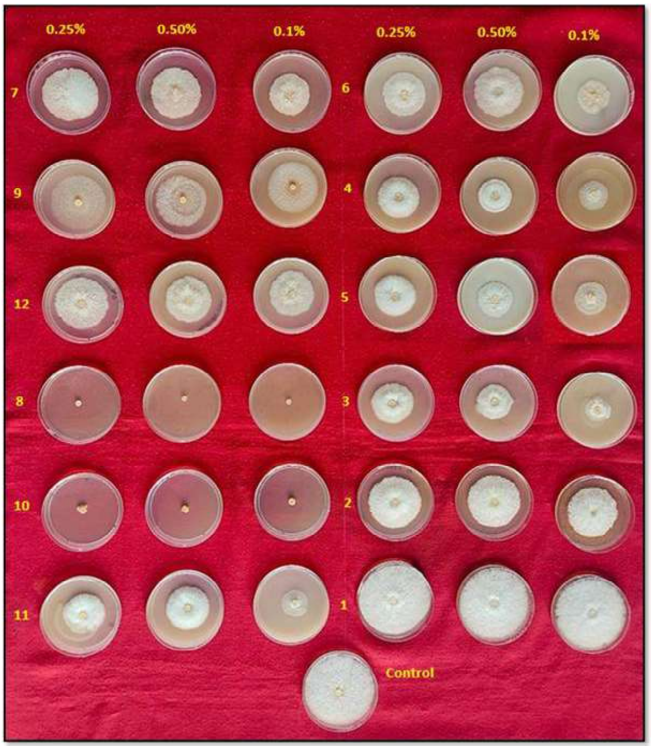


Plate 4. *In vitro* efficacy of commercially available botanicals against *Fusarium verticillioides*

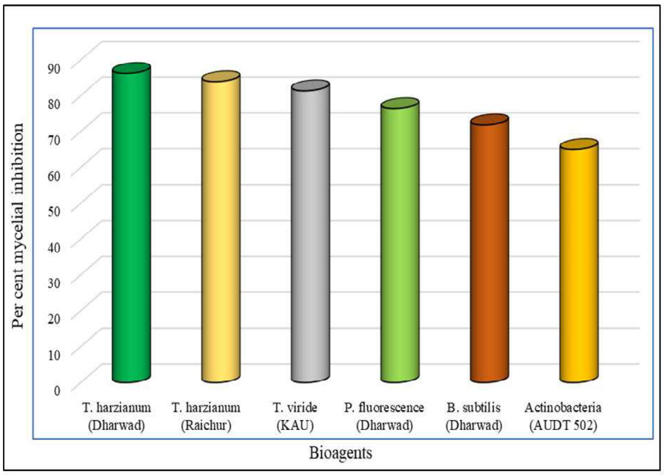


Fig.2. *Invitro* efficacy of bioagents against *Fusarium verticillioides*

action include mycoparasitism (involving antibiosis and cell wall-degrading enzymes like chitinases and glucanases), antibiosis, competition, induced resistance and inactivation of host enzymes. Studies suggest that signal transduction pathways, including G proteins, cAMP and MAP kinase, regulate extra cellular enzyme production and hosthy phaecoiling in *Trichoderma* spp. Evidence shows that G-á proteins play a role in coiling around host hyphae, as observed by increased coiling after G-protein activators were introduced. Inhibitory effects on *Fusarium moniliforme* were reported by Srivastava *et al*. (2019), who found that *T. viride* (81.5% inhibition) and *T.harzianum* (70.76%) were the most effective bioagents, while *T.hamatum*, *A.niger* and *A.flavus* were less effective. Sobowale *et al*. (2009) evaluated three *T.harzianum* strains against *F.verticillioides*, finding that strain IMI380934 was most effective, primarily through nutrient competition and mycoparasitism.

Conclusions

Fusarium verticillioides, the causal agent of Pokkah boeng disease, produces chains of microconidia and sickle-shaped macroconidia. Among the botanicals tested, Elixer and Crop Verdict demonstrated complete inhibition (100%) of *F. verticillioides*, even at low concentrations (0.25%), making them the most effective. Kaneem, Sheefa Neem and Neemba also exhibited strong antifungal activity. Among bioagents, *Trichoderma* spp. emerged as the most effective antagonist against the pathogen. Validating the efficacy of top-performing botanicals through field studies is essential for their successful integration into Integrated Disease Management (IDM) strategies. Before advancing to field trials, further research is needed to identify the active compounds and elucidate their mechanisms of action. The discovery of novel natural compounds from medicinal plants offers a promising alternative to synthetic chemicals, fostering sustainable disease management. Recent advances in controlling *Fusariums* pp.through botanical products and antagonistic microorganisms underscore the potential of these natural solutions. Plant extracts, particularly those rich in polyphenols, have exhibited potent antifungal, antibacterial, biodegradable, and selectively toxic properties. However, the excessive use of synthetic chemicals has resulted in environmental pollution and the emergence of more virulent pathogen strains. Thus, botanicals and bioagents represent a sustainable and effective alternative for disease management.

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