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Biopriming of maize seeds with bio-inoculants reveals the suppression of post flower stalk rot incited by *Macrophomina phaseolina*

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Abstract
Maize is being an important cereal crop next to rice and wheat, its cultivation is hampered due to the infection of fungal and bacterial pathogens. Among the fungal pathogens, post flowering stalk rot caused by *Macrophomina phaseolina* remains as a challenging issue in maize cultivation. Hence, to assess the efficacy of bio inoculants on the management of *Macrophomina phaseolina*, maize seeds COHM(8) were bioprimed with antagonists *Trichoderma asperellum, Trichoderma koningiopsis*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, and *Brachybacterium paraconglomeratum* during flowering stage pathogen was artificially inoculated in maize plants. Biopriming of maize seeds indicated that, priming with *T. asperellum*, *T. koningiopsis* at 1%, *B. amyloliquefaciens* at 5% significantly reduced the incidence on post flowering stalk rot of maize but there is no significant difference in growth parameters.

Keywords: Seed, biopriming, bio inoculants, post flowering stalk rot-*Macrophomina phaseolina*

Introduction
Maize is popularly known as Queen of cereals. Maize is not only used as food material but also used as an important raw material in poultry and animal feed manufacturing industries. Owing, to the significance of maize, globally it is cultivated over an area of 193.7 million ha with an average productivity of 5.75 t/ha (FAOSTAT, 2020). In India, it is cultivated over an area of 80.38 lakh hectares. Area under maize cultivation during 2019-2020, in Tamil Nadu was around 3.905 lakh ha, with a productivity of 7,257kg/ha (Department of agriculture, Government of Tamil Nadu, 2020).

However maize cultivation is being limited due to the outbreak of Anthracnose stalk rot (*Colletotrichum graminicola*), Post flowering stalk rot/Charcoal rot of maize (*Macrophomina phaseolina*), Corn grey leaf spot disease (*Cercospora zeae-maydis*), Aspergillus ear and kernel rot (*Aspergillus flavus*), Corn smut (*Ustilago maydis*), Southern corn leaf blight disease (*Bipolaris maydis*) etc. [1]. Intensive cultivation of maize, predisposed it to the attack of *M. phaseolina* in Tamil Nadu. The disease caused by *M. phaseolina* is also referred as charcoal rot/ dry root rot/ post flowering stalk rot. The pathogen attack at leaf and stem portion which leads to 20-30% of yield loss [2].

M. phaseolina is both soil and seed borne. Pathogen has the capability to survive even for a period of 12 months in maize seeds, and spread the disease rapidly if the contaminated seeds are used [3]. Abiotic stress including moisture stress and increased soil temperature ranging from 36 ºC to 38 ºC predisposes the disease outbreak [4, 1].

Maize crop infected by post flowering stalk rot at physiological maturity results in prematured death, produce light weight ears having poorly filled kernals and also leads to lodging of infected plants thus renders harvesting as a difficult process [5]. Seed treatment with carbendazim aided in the management of post flowering stalk rot of maize. But, the Pesticide bill act passed during 2021 has recommended to ban carbendazim. In this condition, it is highly imperative to innovate a attractive alternative for the management of post flowering stalk rot of maize.

Seed priming is an emerging technique of seed treatment that involves application of beneficial microorganism followed by seed hydration, which enhance germination, provide protection before seedling emergence and protect from pathogen attack. Thus, in recent times, bio-priming with microbial inoculants gains a prime position in the management of plant pathogens as alternate option to chemical fungicides. Hence, attempts were made to address the issue through bio-priming with bio-inoculants to suppress *M. phaseolina*.

Materials and Methods

Seed material

Maize COHM (8) hybrid seeds with 8% seed moisture content and 92% germination was obtained from Department of Millets, Tamil Nadu Agricultural University, Coimbatore, for the present investigation.

Pathogen and Bioinoculant culture

Five bioinoculants *viz*., *B. amyloliquefaciens* VB7 MG241252, *B. licheniformis* COEH6 MG241257, *B. paraconglomeratum* YEBPT2 MK263736, *T. asperellum* TRI 15 KX533985, *T. koningiopsis* TRI 41 MF423101 were used to induce *inplanta* resistance against *M. phaseolina*. Pure mother culture of bioinoculants and *M. phaseolina* were collected from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. Fungal and bacterial cultures were sub cultured and maintained on potato dextrose agar (PDA) and Luria-Bertani medium respectively.

Preparation of bioinoculants

Sterilzed molten PDA was dispensed into sterile Petriplates @ 15 ml/plate and incubated to solidify. After solidification, a 5mm disc of *T. asperellum* and *T.* koningiopsis was placed at the centre of the plate and incubated at room temperature 28±2°C for five days till complete sporulation. For bio-priming of seeds, liquid suspension of fungal Conidia of *T. asperellum, T.koningiopsis* were prepared from five days old cultures maintained in PDA. Spores were re-suspended in 100 ml potato dextrose broth, spore concentration maintained to 10^6 Cfu/ml. Liquid suspension of bacterial bioinoculants were prepared in Luria- Bertani broth for 48 hours and the concentration adjusted to give 10^9cfu/ml [6]. Seeds of maize hybrid COHM (8) were bioprimed with *T. asperellum*, *T. koningiopsis* at 1%, *B. amyloliquefaciens* and *B. licheniformis* at 5%, *Brachybacterium paraconglomeratum* at 10% by 12 hr soaking in an ratio of 1:2 between seed and water. After 12 hours, seeds were dried back to original seed moisture content [7].

Tooth pick inoculation

Tooth pick were boiled in water for one hour and loosely packed in glass jars containing sterile potato dextrose broth. Autoclaved and cooled sterile glass jars with tooth picks were inoculated with 5 days old *M. phaseolina* culture. After 10 days of inoculation, tooth picks were examined for the colonization of fungal mycelium. The fungal mycelium of *M. phaseolina* colonized the tooth picks and the colonized tooth picks were inserted into maize plants at first internode on 50 and 60 days after sowing. In field trail, 40 plants per replication pertaining to different treatments were artificially inoculated with fungal pathogen

Field experiment

To assess the efficacy of antagonist againt *M. phaseolina* field trial was conducted with bioprimed seeds in Department of Seed Science and Technology, Tamilnadu Agricultural University, Coimbatore during March to June, 2020. The treatments were comprised with four replications and each replication pathogen inoculation was comprising of 10 plants per replication. Similarly, uninoculated control was also maintained and irrigated regularly. The plots are artificially inoculated at flowering stage with *M. phaseolina* through tooth pick method. Growth parameters and disease score observation were recorded.

Growth parameters

Germination

Germination was recorded by counting the number of hills germinated in each plot at after seven days of sowing.

Plant height

The plant height was measured randomly in selected 10 plants from each replication, from the base to the tip of the leaf and the mean values were expressed in cm. Plant height was observed on 15, 30, 45 and 60 days after sowing.

Leaf Area

The length and breadth of the 3rd leaf from the peak of the leaf were measured at 45 DAS. Leaf area was calculated by using the following formula.

Leaf Area = L xW x K x Number of leaves per plant

Where,
L-Maximum length of the 3rd leaf (cm)
W-Maximum width of the 3rd leaf (cm)
K-Constant factor (0.747)

Leaf Area Index (LAI)

The length and breadth of the 3rd leaf from the peak of the leaf were measured at 45 DAS. The leaf area index was calculated as per the procedure by using the following formula.

$$LAI = \frac{L\ xW\ x\ K\ x\ Number\ of\ leaves\ per\ plant}{Spacing\ (cm^2)}$$

Where

L-Maximum length of the 3rd leaf (cm)
W-Maximum width of the 3rd leaf (cm)
K-Constant factor (0.747)

Chlorophyll content

Chlorophyll content was estimated by DMSO method as prescribed by Hiscox & Israelstam. The absorbance was read in a Spectrophotometer at 645 and 663 nm against DMSO blank total chlorophyll was calculated.

$$\text{Total chlorophyll} = (8.02 \times OD@663) + (20.02 \times OD@645) \times \frac{V}{1000 \times W}$$

Disease score

The disease reaction was recorded individually at the time of maturity, by split open the lower internodes longitudinally to see the extent of pith damage in the form of shredding and pinkish discoloration on a 1-9 rating scale [5]

Statistical analysis

Data from field experiments were analysed using analysis of variance (ANOVA), for randomized block design using IBM SPSS statistics 26 software. Each treatment were replicated thrice. Treatment means were compared with least significant difference (LSD).

Results and Discussion

In the present study, on *inplanta* resisance of bioinoculant against post flowering stalk rot pathogen fungal bioinoculants are effective compared to bacterial bioinoculants. Minimum

amount of disease score was observed in plots treated with *Trichoderma asperellum, Trichoderma koningiopsis* at 1% and *Bacillus amyloliquefaciens* at 5% which significantly reduce the disease severity by 95% over the inoculated control, followed by *Bacillus licheniformis* at 5%.

Among the growth parameters, germination, plant height, chlorophyll content, leaf area, and leaf area index, chlorophyll content shows a significant differences. In chlorophyll content there was a significant differs at ($F_{6,18}$ =73.56, P< 0.05), in which *Trichoderma koningiopsis* at one% shows higher chlorophyll content compared to other treatment followed by *Brachybacterium paraconglomeratum* at 10% (Fig.1). Other growth parameters *viz.*, germination percentage, leaf area, leaf area index and plant height were not showed any significant differences among the treatments (Table 1).

Disease score observation shows a significant difference at ($F_{6,18}$ =55.15, P< 0.05), in which two fungal bioinoculants *Trichoderma asperellum, Trichoderma koningiopsis* at one %, one bacterial inoculants *Bacillus amyloliquefaciens* at 5%, shows a lower score of disease incidence. Three treatments means are on par (fig. 2,3).

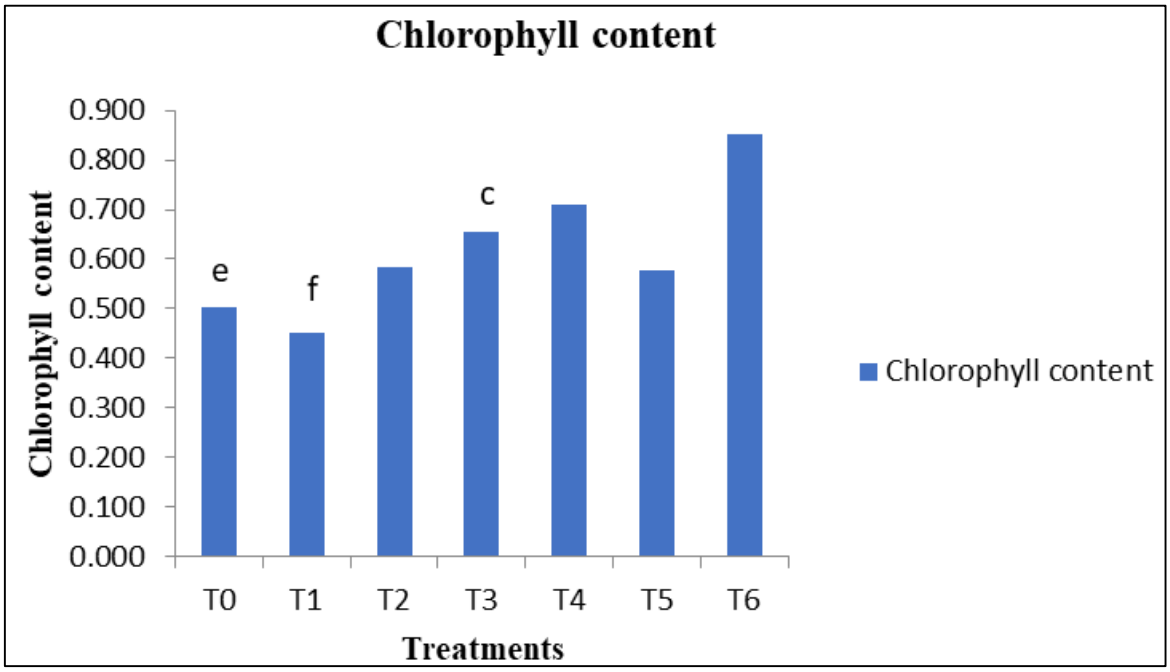


Fig 1: Effect of bioinoculants on chlorophyll content

T_0 – Uninoculated control; T_1 – Inoculated control; T_2 – 5% Bacillus amyloliquefaciens + Fungal inoculation; T_3 – 5% Bacillus licheniformis + Fungal inoculation; T_4 – 10% Brachybacterium paraconglomeratum + Fungal inoculation; T_5 – 1% Trichoderma asperellum + Fungal inoculation; T_6 – 1% Trichoderma koningiopsis + Fungal inoculation. Bar indicated a means of chlorophyll content of different treatments. Same letters are on par not differ significantly at 0.01 level

Table 1: Effect of bioinoculants in growth characters

Treat-ments	Field Emerg-ence (%)	Plant height @ 60 DAS (cm)	Leaf area (cm^2)	Leaf Area Index
T0	79	164.1	518.37	4.65
T1	85	144.2	470.17	4.69
T2	88	167.5	550.84	5.87
T3	79	181.2	503.56	5.03
T4	81	153.1	555.29	4.99
T5	84	154.7	505.05	5.38
T6	91	157.9	468.19	4.92
SEd	4.74	14.27	60.92	0.60
CD(0.05)	9.97	29.98	128.00	1.26

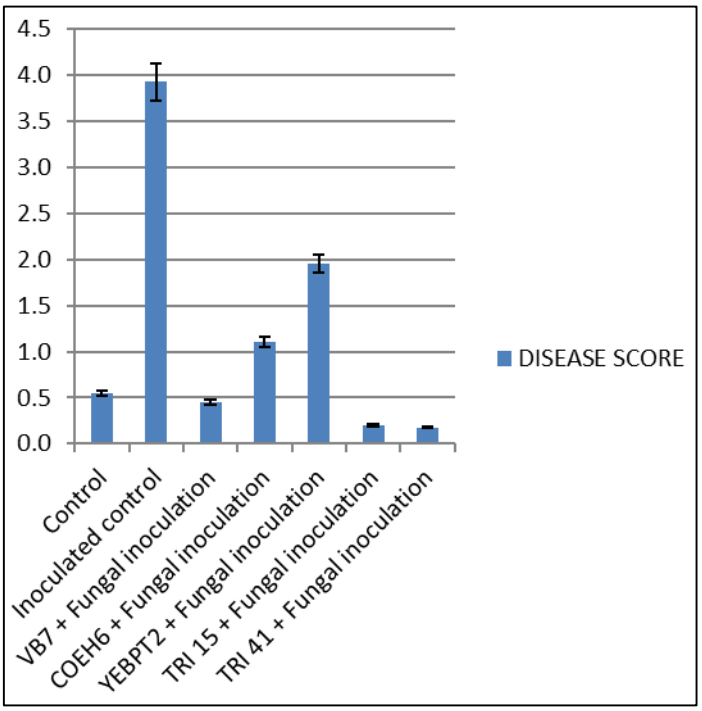


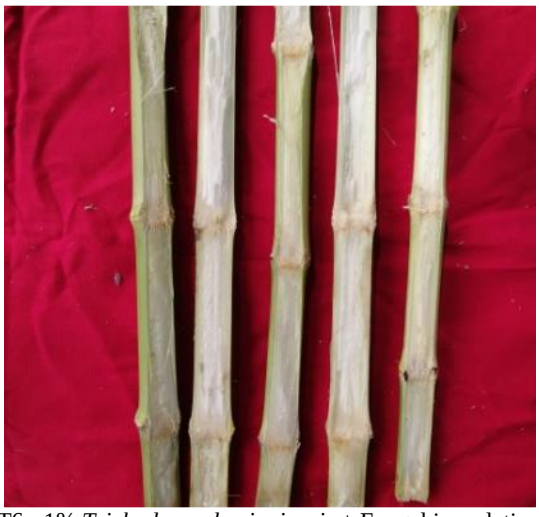
Fig 2: Effect of bioinoculants on disease incidence

T0 – Control

T1 – Inoculated Control

T2 - 5% *Bacillus amyloliquefaciens* + Fungal inoculation
T3 - 5% *Bacillus licheniformis* + Fungal inoculation

T4 - 10% *Brachybacterium paraconglomeratum* + Fungal inoculation
T5 - 1% *Trichoderma asperellum* + Fungal inoculation



T6 - 1% *Trichoderma koningiopsis* + Fungal inoculation

Fig 3: Disease Score observation

Similar findings were reported that *Trichoderma* may be used as a potential biocontrol agent for reducing post flowering stalk rot incidence in maize [8]. *Trichoderma* found to be effective potential biocontrol agent was due to the fact that growing condition of pathogen *Macrophomina phaseolina* and antagonist were the same, hence there is more competition leading to production of growth regulation factors [9]. Bacterial bioinoculants of the genus *Bacillus* were recorded to be effective antagonists of the phytopathogenic fungus M. phaseolina in *invitro* study [10]

Mechanism of bioinoculants to induce a resistance against pathogen are antibiosis, lysis and siderophore. Antibiotics are considered to be organic compounds of low molecular weight produced by microbes, which play an active role in the bio-control of plant diseases and often acts in concert with competition and parasitism [11]. *Trichoderma* spp. are rich and important sources of secondary metabolites (SMs) used for biological control of plant diseases [12]. It was stated that antibiosis occurs during the interactions between a host plant, pathogens, and *Trichoderma* spp. that resulted in the production of antibiotics by *Trichoderma* to inhibit the growth of phytopathogenic fungi. Like fungi most important mechanism of antagonist bacteria was expressed by antibiosis, production of antibiotic compounds and inhibition of other microbes [13]. In *Bacillus* spp. reported that the production of antibiotics by the *Bacillus* spp. and their uses in the biological control of plant pathogens [14]. *Tricoderma* spp. induced systemic or localized resistance in plants may be the reason for reducing the colonization of *M. phaseolina* [15]. *Bacillus* spp. inoculation with *M. phaseolina*, bursting of hyphae tips of pathogen, digestion of sclerotia, hypertrophy of germ tube cells and lysis of hyphae. *Bacillus* in association with seed and soil microbes produce IAA, which promote plant growth, by controlling the disease causing microorganism [16]. The production of considerable quantity of HCN in bioinoculated seeds make a plant to develop with genetic *inplanta* resistance [17]. Hydrogen cyanide (HCN) plays a role in blocking the cytochrome oxidase pathway, which is highly toxic to all aerobic microorganisms and also suppress the rot pathogens at pico molar concentrations [18]. *Trichoderma* and *bacillus* induced the systemic resistance in plants.

Conclusion

In the view of above data, seed priming with *Trichoderma asperellum, Trichoderma koningiopsis* at 1% which induces the systemic resistance or *inplanta* resistance against post flowering stalk rot pathogen.

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Reference

1. Rehman F, Adnan M, Kalsoom M, Naz N, Husnain MG, Ilahi H *et al.* Seed-borne fungal diseases of Maize (Zea mays L.): A review. Agrinula: Jurnal Agroteknologi dan Perkebunan 2021;4(1):43-60.
2. Mueller DS, Wise KA, Sisson AJ, Allen TW, Bergstrom GC, Bosley DB *et al.*, Corn yield loss estimates due to diseases in the United States and Ontario, Canada from 2012 to 2015. Plant health progress 2021;17(3):211-222.
3. Savita P, Jain BL, Pareek S. Survival of *Macrophomina phaseolina* on seed and diseased stem pieces of maize. J. Phytol. Res 1996;9:75-76.
4. Kaiser SAKM, Das SN. Physical factors that influence the growth and spread of charcoal rot pathogen (Macrophomina phaseolina) infecting maize. Journal of Phytopathology 1988;123(1):47-51.
5. Hooda KS, Khokhar MK, Sekhar JC, Bagaria PK, Sharma SS, Harleen K *et al.*, Identification of resistance sources against post flowering stalk rot in maize. Indian Phytopathology 2017;70(4):432-439.
6. El-Mougy NS, Abdel-Kader MM. Long-term activity of bio-priming seed treatment for biological control of faba bean root rot pathogens. Australasian plant pathology 2008;37(5):464-471.
7. Anbalagan A, Lakshmi S, Nakkeeran S, Renganayaki PR. Biopriming with bacterial inoculants on seed quality parameters of maize hybrid COH (M) 6 seeds. Journal of Pharmacognosy and Phytochemistry 2020;9(4):3340-3343.
8. Shekhar M, Kumar S, Sharma RC, Singh R. Sources of resistance against post-flowering stalk rots of maize. Archives of Phytopathology and Plant Protection 2010;43(3):259-263.
9. Windham MT, Elad Y, Baker R. A mechanism for increased plant growth induced by Trichoderma spp. Phytopathology 1986;76(5):518-521.
10. Marroni IV. Screening of bacteria of the genus Bacillus

for the control of the plant-pathogenic fungus Macrophomina phaseolina. Biocontrol Science and Technology 2015;25:302-315.
11. Rajendran, Raguchander. Detection of antibiotic compounds from liquid formulation of Pseudomonas fluorescens (Pf1) by TLC and HPLC analysis. Journal of Pure and Applied Microbiology 2014;8(5):4025-4030.
12. Mukharjee B, Khatua DC, Sen C. Potential antagonists of Macrophomina phaseolina and bio-control of seedling blight of Jute. Abstract of papers presented in the workshop on Biological Control of Plant Diseases,10-12 March, TNAU, Coimbatore, 1987, 21.
13. Intana W, Yenjit P, Suwanno T, Sattasakulchai S, Suwanno M, Chamswarng C. Efficacy of antifungal metabolites of Bacillus spp. for controlling tomato damping-off caused by Pythium aphanidermatum. Walailak Journal of Science and Technology (WJST), 2008;5(1):29-38.
14. Abaidoo RC, Killani AS, Akintokun AK, Abiala MA. Antagonistic effect of indigenous Bacillus subtilis on root-/soil-borne fungal pathogens of cowpea 2011.
15. Harman GE. Overview of mechanisms and uses of Trichoderma spp. Phytopathology 2006;96(2):190-194.
16. Jharia HK, Khare MN. Biological control of Rhizactonia bataticola causing disease in soybean. Indian phytopathology 2006;39:148.
17. Wahyudi AT, Astuti RI, Giyanto. Screening of Pseudomonas sp. isolated from rhizosphere of soybean plant as plant growth promoter and biocontrol agent. American Journal of Agriculture and Biological Science 2011;6:134-141.
18. Jan Mohd Junaid, Nisar Ahmad Dar, Tariq Ahmad Bhat, Arif Hussain Bhat, Mudasir Ahmad Bhat. Review commercial bio-control agents and their mechanism of action in the management of Plant Pathogens. Internatioanl Journal of Modern Plant and Animal Sciences 2013;1(2):39-57.