



# Eco-Friendly Biocontrol through Seed Biopriming and Induced Systemic Resistance against Maydis Leaf Blight of Maize Caused by *Bipolaris maydis*

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## Authors' contributions

This work was carried out in collaboration among all authors. Author SLP designed the study, drafted the manuscript, performed the statistical analysis. Author PJ designed, guided the experiment, proof reading of the manuscript. Author AMP overlooked the draft, proof reading of the draft. Authors RV and SSB have checked the manuscript and helped to manage the literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

Though chemical seed treatment is usual and affluent, using the prevailing beneficial microorganisms in nature eases the detrimental effect of chemicals on nature and also human beings in many possible ways. This study highlights the biocontrol potential of seed biopriming as an eco-friendly alternative to chemical seed treatments for managing Maydis leaf blight caused by *Bipolaris maydis*. So, seed biopriming is preferable which is a biological seed treatment and involves seed hydration followed by inoculation with useful microorganisms which adds improvement to seeds in terms of viability, vigor indices and germination. Induced systemic resistance (ISR) is a resistance mechanism in plants that is activated by infection with biological agents. In the present study two fungal and two bacterial biocontrol agents used individually and also in combination along with pathogen and control and maximum seed vigour index was found in *P. fluorescens* + *B. subtilis* (3644.04). Host defense responses were activated through induced systemic resistance and observed that antioxidant enzymes were higher in *P. fluorescens* + *B. subtilis* and *T. harzianum* colonized seedlings. Total phenols and polyphenol oxidases were higher after 3<sup>rd</sup> day of pathogen inoculation whereas, peroxidase and phenylalanine ammonia lyase were higher after 5<sup>th</sup> day of pathogen inoculation and superoxide dismutase was activated in transient manner. This clearly depicts that bio-priming enhances the seed vigour, antioxidant enzymes production and accumulation which helps in overcoming the biotic stress tolerance by reducing the infection process of the pathogen during the plant growth through inducing systemic resistance. Induced systemic resistance (ISR) mediated by biocontrol agents was due to the upregulation of defense related enzymes and by the accumulation of phenolic compounds.

**Keywords:** Biopriming; seed vigour index; host defense; induced systemic resistance.

## 1. INTRODUCTION

“Maize or Corn (*Zea mays* L.) is one of the most important cereal crops in the world. Globally, it is also known as ‘Miracle crop’ or ‘Queen of the Cereals’ because of its highest genetic yield potentiality among the cereals. It is a dual-purpose crop which is mainly utilized for human consumption as well as livestock/poultry feed. Its demand has steadily increased over the past few years for value-added products such as glucose, sorbitol, dextrose, starch-based products, and oil, as well as for use as a basic raw material as an ingredient in thousands of industrial products, including those in the food sweeteners, alcoholic beverages, pharmaceutical, cosmetic, film, textile, gum, packaging, and paper industries” (Rao et al., 2014). “An estimated 201.98 million hectares of maize are grown worldwide, with an average yield of 5.75 tons per hectare and a production of 1162.35 million tons” (FAO, 2021). India is fourth in terms of area and seventh in terms of production among the nations that grow maize; it accounts for around 4% of the global maize area and 2% of the overall production.

“Of the 115 maize diseases known to exist worldwide, 35 have been documented in India. These include Downy mildew, Charcoal rot,

Fusarium stalk rot, Turcicum leaf blight, Curvularia leaf spot, Maydis leaf blight, and Banded leaf and sheath blight. Since airborne fungus likely cause the most losses, foliar infections are one of the biotic variables that economically hinder the production of tropical maize. Many leaf blights are caused by various pathogens such as *Bipolaris/Helminthosporium*, *Excerohilum*, *Curvularia* etc. Among this, maydis leaf blight (MLB) is one of the biotic stresses caused by *Bipolaris maydis* (Syn. *Helminthosporium maydis* (Nisikado and Miyake) Shoemaker), (Telomorph: *Cochliobolus heterostrophus*) is a serious fungal disease of maize throughout the world where maize is grown under warm and humid conditions” (White, 1999). MLB is currently one of the most common and serious illnesses in China, Vietnam, Indonesia, Pakistan, India, Nepal, Kampuchea, and the Philippines (White, 1999). MLB can cause the yield loss of upto 40% or more depends upon the environmental conditions (Aggarwal et al., 2024).

With growing awareness of the detrimental effects of chemical inputs, the adoption of sustainable management strategies, such as biological control, has become increasingly important. Biological control not only enhances

plant growth parameters and strengthens defense mechanisms against pathogens but also plays a pivotal role in improving soil health and fostering microbial diversity. By promoting a balanced and resilient agroecosystem, these approaches contribute to sustainable crop production, environmental conservation, and long-term agricultural productivity.

“Seed Biopriming is one of the newest method of seed treatment which integrates biological aspects of disease management where seed is inoculated with beneficial/biological organism which eventually defends the seed from various diseases. Biopriming utilizes a variety of beneficial microorganisms, such as bacteria and fungi, including species of *Trichoderma*, *Pseudomonas*, and *Bacillus* spp. which increases plant resistance to biotic and abiotic stresses while improving crop production and productivity with improved seed quality. Beneficial microorganisms used in biopriming can increase the percentage of seed germination, improve the vigor of seedlings and manages soil and seedborne pathogens” (Kumar et al., 2019).

“Seed biopriming with *Pseudomonas fluorescens* @  $1 \times 10^7$  cfu/seed provide better protection against damping off disease which is caused by fungal pathogen *Pythium ultimum* in maize” (Callan et al., 1990). “Biopriming with different isolates of *Pseudomonas fluorescens* showed higher germination, seedling vigour and elicited resistance against downy mildew disease in pearl millet” (Raj et al., 2004). “Plant growth promoting rhizobacteria (PGPR) form biofilm on roots of plant and also compete with other soil microorganism in rhizosphere for nutrition and colonization to protect from harmful pathogens” (Rudrappa et al., 2008; Walker et al. 2003). “They also limit plant pathogens growth through secretion of siderophores and lytic enzymes” (Compant et al. 2010). “In maize, biopriming with *Pseudomonas aeruginosa* strain MF-30 enhanced shoot and root biomass, plant growth, and antioxidant content. It also reduced the severity of disease against banded leaf and sheath blight, suggesting the potential for an environmentally friendly and cost-effective method of producing healthier, antioxidant-rich maize plants” (Singh et al. 2020). Hence an effort was made to study the seedling vigour index and accumulation of various defence related antioxidant enzymes in the plants with various biocontrol agents and its significance in disease management.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material, Microorganism and Culture Conditions

Seeds of the maize (cv. CM 202), susceptible to *B. maydis* infection (Southern corn leaf blight), were procured from Main Maize Research Station, Godhra, Gujarat. Seeds were surface sterilized, using 1.0% sodium hypochlorite for 60 -90 seconds. *Bipolaris maydis* (Accession No. ON329785) was maintained on Potato Dextrose Agar (PDA) slants and used for further studies. The spores of *B. maydis* from 10 days-old-culture was suspended in a sterile distilled water at a concentration of  $4 \times 10^4$  conidia/ml, using a hemocytometer and used for greenhouse experiments.

Biocontrol agents viz., *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were isolated and maintained in Department of plant pathology, Navsari Agricultural University, Navsari, Gujarat and found suppressing various plant pathogens as well as *B. maydis* in *in vitro* (Prasanna and John, 2022). The fungi and bacteria were routinely subcultured and grown on potato dextrose agar and nutrient agar, respectively throughout the experimental period.

### 2.2 Seed Biopriming

Fungus cultures grown on Potato Dextrose Broth (PDB) for 10 days and after incubation conidia were dispersed in the solution and adjusted to a concentration of ( $1 \times 10^8$  cfu/ml) whereas bacterial cultures, grown on nutrient broth (NB) for 36 h at room temperature on a rotary shaker at 150 rpm, was subjected to centrifugation at 8000 rpm for 10 min. Obtained pellet was washed by distilled water (twice) and adjusted to a concentration of ( $1 \times 10^8$  cfu/ml). Washed and air-dried seeds of maize were soaked in culture suspensions of biocontrol agents. Carboxymethyl cellulose (CMC) used at 0.4% concentration aided in the adherence of the biocontrol agent to seeds. Incubation was carried out in a rotary shaker at 150 rpm for 6 h at  $28 \pm 2$  °C along with control seeds which soaked in sterile distilled water and amended with CMC. Further, the seeds were aseptically air-dried and used for further analyses.

### 2.3 Effects of Seed Bio-Priming on Seed Germination and Seedling Vigour

The seeds of susceptible variety CM-202 were treated (seed priming) with four different

biocontrol agents with eight treatments in three replications in Completely Randomized Design (CRD).

Under laboratory circumstances, the germination and vigor of seeds were monitored using the paper towel method (ISTA 2005). The maize cultivar CM-202 seeds were incubated for 14 days on a presoaked paper towel with three replicates of 50 seeds each. Following the incubation period, the proportion of seeds that germinated was determined, and the rate of germination was represented in percentage. Seedling vigor (Abdul Baki and Anderson, 1973) as a measure of mean root length and shoot length was calculated, using the following formula.

$$\text{Vigour index} = (\text{Mean root length} + \text{Mean shoot length}) \times \% \text{ germination}$$

## 2.4 Effects of Seed Bio-Priming on the Accumulation of Defense-Related Enzymes

The potting mixture (soil: sand: farmyard manure in the ratio, 2:1:1 w/w/w), which was autoclaved repeatedly for 2 days was filled in plastic pots (25 cm diameter). The pots were arranged randomly in a greenhouse. Bioprimered maize seeds and control seeds were sown equidistantly with eight seeds per pot.

Experiment was laid in Completely Randomized Design (CRD) with three replications. Inoculation of *B. maydis* was done by spraying conidial suspension ( $4 \times 10^4$  spores/ml) on maize variety CM-202 at twenty five day old plant. After the challenge inoculation with pathogen, the pots were kept at the proper humidity level with the help of polythene cover and moistened cotton for two days in order to allow for conidial germination, penetration, and symptom development. Control plants were treated with water only.

## 2.5 Induction of Defense Mechanism

Seeds were bioprimered and grown and challenge inoculated as explained earlier. To study the ISR, eight types of treatments were maintained viz., T<sub>1</sub>- seeds bioprimered with *T. asperellum* alone, T<sub>2</sub>- seed bio-primed with *T. harzianum* alone, T<sub>3</sub>- seed bio-primed with *P. fluorescens*, T<sub>4</sub>- seed bio-primed with *B. subtilis*, T<sub>5</sub>- seed bio-primed with *T. asperellum* + *T. harzianum*, T<sub>6</sub> - seed bio-primed with *P. fluorescens* + *B. subtilis*, T<sub>7</sub> - seed

bio-primed with *B. maydis* pathogen, T<sub>8</sub>-Control (untreated). Seedlings (1 g) were carefully uprooted without causing any damage to root and leaf tissues at different day intervals (first, third, fifth, seventh and ninth day) and washed under running tap water, blot-dried, and used for the extraction of the enzyme.

## 2.6 Estimation of Peroxidase (POX) Activity

One gram of seedlings was ground into a powder, mixed with five milliliters of phosphate buffer (0.1 M, pH 7.0), and centrifuged for ten minutes at 10,000 rpm at 4°C. Supernatant/enzyme extract (0.1 ml) was added to 2.9 ml of substrate buffer containing [125 µl guaiacol (0.05 M) and 153 µl 30% H<sub>2</sub>O<sub>2</sub> in 50 ml phosphate buffer (0.1 M, pH 7.0)] in order to estimate peroxidase activity (POX). The change in optical density at 470 nm/min/mg protein was used to express the specific activity of POX (Hammerschmidt et al. 1982).

## 2.7 Estimation of Polyphenol Oxidase (PPO) Activity

A cold potassium phosphate buffer (5 ml, 0.1 M, pH 6.5) was used to homogenize one gram of seedlings, which were then centrifuged for 10 minutes at 10,000 rpm at 4 °C. The enzyme extract (100 µl) was successively mixed with 1.5 ml of sodium phosphate buffer (0.1 M, pH 6.5), and the reaction was initiated by adding 200 µl of catechol (0.01 M). The specific activity of polyphenol oxidase activity (PPO) was measured as the change in OD at 420 nm/min/mg protein (Mayer et al. 1965).

## 2.8 Estimation of Phenylalanine Ammonia Lyase (PAL) Activity

One gram of seedlings was ground into powder and homogenized in five ml of cold Tris buffer (100 mM, pH 8.8) containing β-mercaptoethanol (1.2 mM) and centrifuged for 10 min at 10,000 rpm. The enzyme activity was measured by incubating enzyme extract (0.3 ml) with 1.2 ml of Tris buffer (25 mM, pH 8.8) and 1.5 ml of L-phenylalanine (12 mM). The conversion rate of L-phenylalanine to t-cinnamic acid was measured at 290 nm, and the enzyme activity was expressed as nmol t-cinnamic acid/min/mg protein (Dickerson et al. 1984).

## 2.9 Estimation of Super Oxide Dismutase (SOD) Activity

In accordance with Beauchamp and Fridovich's (1971) description, the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm was measured to determine the SOD activity. The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM L-methionine, 75 µMNBT, 10 µMEDTA, 2 µM riboflavin, and 0.3 ml of enzyme extract. The test tubes containing the reaction mixture were maintained at 35 oC for 20 minutes under 4000 lux. One unit SOD activity was defined as the quantity of enzyme needed to produce 50% inhibition of the rate of NBT reduction measured at 560 nm.

## 2.10 Estimation of Total Phenols

The Bray and Thorpe (1954) method was used to estimate the total phenols in the challenge-inoculated leaves. Ten milliliters of 80% ethanol were mixed with one gram of fresh leaf material, which was then heated for thirty minutes at fifty degrees Celsius. After passing through cheesecloth and Whatman No. 41 filter paper, the extracts were centrifuged for 10 minutes at 8000 g. Ethanol was added to get the volume up to 10 ml. An aliquot of one ml was taken in a series of boiling tubes and made up to 3 ml with distilled water. To this, one ml of Folin-ciocalteu reagent and two ml of 20% sodium carbonate were added. The tubes were heated for one min in a boiling water bath and cooled in running water. The solution was diluted to 10 ml with distilled water and the intensity of the blue colour was measured at 660 nm in a spectrophotometer against a blank (a blank was maintained with three ml of distilled water instead of the extract and the colour was developed as described above) for which three replications were

maintained. Catechol was used for preparing the standard graph from which the amount of phenol in the given sample was calculated. All the enzyme activities and the content of total phenols were expressed as Katal/mg of total proteins.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Biopriming with Biocontrol Agents on Seed Vigour Index of Maize cv. CM-202

Seed vigor is another important quality parameter which determines the crop productivity. Seed vigor index was determined by considering germination percentage and seedling length. The result revealed that germination percentage was found sound in all treatments as compared to the pathogen primed seed and maximum germination percentage was seen in *T. harzianum* (88%) followed by *P. fluorescens*+ *B. subtilis* and *P. fluorescens* with 86.67 percentage. Shoot and root length was also measured and maximum was observed in *P. fluorescens*+ *B. subtilis* with 19.35 cm at par with *B. subtilis* (18.64 cm) and minimum was found in *B. maydis* treated seeds with 14.79 cm. Maximum root length was observed in *P. fluorescens*+ *B. subtilis* with 22.69 cm at par with *T. asperellum* (22.20 cm) and minimum was found in *T. asperellum*+ *T. harzianum* with 18.09 cm followed by *B. maydis* (18.69 cm) bio primed seeds.

Vigour index was recorded and presented in the Fig. 1 and maximum vigour index was found in *P. fluorescens*+ *B. subtilis* (3644.04) followed by *P. fluorescens* (3401.96) and the minimum vigour index was observed in *B. maydis* (2768.23) bio primed seeds followed by *T. asperellum*+ *T. harzianum* (2993.49).

Table 1. Effect of biopriming with biocontrol agents on vigour index of maize cv. CM-202

Sr. No.	Biocontrol agents	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1.	<i>T. asperellum</i>	86.00 <sup>b</sup>	17.07	22.20	3376.93
2.	<i>T. harzianum</i>	88.00 <sup>a</sup>	17.88	19.65	3302.93
3.	<i>P. fluorescens</i>	86.67 <sup>b</sup>	17.42	21.83	3401.96
4.	<i>B. subtilis</i>	84.00 <sup>d</sup>	18.64	20.28	3269.28
5.	<i>T. asperellum</i> + <i>T. harzianum</i>	85.33 <sup>c</sup>	16.99	18.09	2993.49
6.	<i>P. fluorescens</i> + <i>B. subtilis</i>	86.67 <sup>b</sup>	19.35 <sup>a</sup>	22.69 <sup>a</sup>	3644.04
7.	<i>B. maydis</i>	82.67 <sup>e</sup>	14.79	18.69	2768.23
8.	Control	86.00 <sup>b</sup>	17.29	18.77	3101.73
	SEm ±	0.33	0.28	0.27	
	CD at 5%	1.00	0.84	0.80	
	CV%	1.33	2.78	2.24	



**Fig. 1. Effect of seed biopriming on maize cv. 202 with various biocontrol agents**  
 A. *T. asperellum*, B. *T. harzianum*, C. *P. fluorescens*, D. *B. subtilis*, E. *T. asperellum*+ *T. harzianum*, F. *P. fluorescens* + *B. subtilis*, G. *Bipolaris maydis*, H. Control

### 3.2 Effects of Seed Bio-Priming on the Accumulation of Defense-Related Enzymes

#### 3.2.1 Peroxidase (POX) activity

The maximum POX activity (1.235  $\Delta A/mg/min$ ) was measured in *T. harzianum* treated plants at fifth day after pathogen inoculation which was at par with *P. fluorescens* + *B. subtilis* (1.215  $\Delta A/mg/min$ ) treated plants followed by *B. subtilis* (1.138  $\Delta A/mg/min$ ) treated plants. In *B. maydis* pathogen treated plants and control showed minimal activity (0.515  $\Delta A/mg/min$ ) and (0.583  $\Delta A/mg/min$ ), respectively and activity of peroxidase was always lower than biocontrol agent treated plants. POX activity was reduced gradually after fifth day which was observed even at seventh and ninth day after challenge inoculation with pathogen in all the treatments.

#### 3.2.2 Polyphenol oxidase (PPO) activity

The maximum PPO activity was found in *P. fluorescens*+ *B. subtilis* (0.121  $\Delta A/mg/min$ ) followed by *T. harzianum* (0.107  $\Delta A/mg/min$ ) and *T. asperellum* + *T. harzianum* (0.101  $\Delta A/mg/min$ ) after third day of inoculation. Minimum PPO activity was observed in *B. maydis* treated and control with (0.039  $\Delta A/mg/min$ ) and (0.058  $\Delta A/mg/min$ ) respectively after third day of pathogen inoculation. After this PPO activity was reduced gradually at fifth, seventh and ninth day after challenge inoculation in all the treatments.

#### 3.2.3 Phenylalanine ammonia lyase (PAL) activity

The maximum PAL activity was found in *T. harzianum* (0.332  $\mu mol$  cinnamic acid/ $mg/min$ ) which was at par with *P. fluorescens*+ *B. subtilis* (0.325  $\mu mol$  cinnamic acid/ $mg/min$ ) and *T. asperellum* + *T. harzianum* (0.321  $\mu mol$  cinnamic acid/ $mg/min$ ) after fifth day of inoculation with pathogen. Minimal activity of PAL was found in *B. maydis* (0.127  $\mu mol$  cinnamic acid/ $mg/min$ ) and control (0.129  $\mu mol$  cinnamic acid/ $mg/min$ ) treated plants which were statistically at par with each other. After the raise in PAL activity at fifth day it was reduced gradually in seventh and ninth day after challenge inoculation with pathogen in all the treatments which was similar to peroxidase activity.

#### 3.2.4 Superoxide Dismutase (SOD) activity

The results revealed that plants treated with biocontrol agents showed SOD activity in a transient manner. Among the biocontrol agents, the maximum SOD activity (0.158 Unit Activity) was observed in *P. fluorescens*+ *B. subtilis* treated plants after third day of inoculation. Later, at seventh day after inoculation maximum activity of SOD was observed in *P. fluorescens* + *B. subtilis* (0.185 Unit Activity) followed by *T. asperellum* + *T. harzianum* (0.165 Unit Activity).

#### 3.2.5 Total phenolics

Plants treated/primed with biocontrol agents, the level of phenolics contents began to increase

after pathogen inoculation and became maximum on third day after inoculation and then starts decreasing. At third day after inoculation the maximum accumulation of phenolics content (366.0  $\mu\text{g/gFW}$ ) was observed in *P.*

*fluorescens*+ *B. subtilis* followed by *P. fluorescens* (354.3  $\mu\text{g/gFW}$ ) and minimum was observed in *B. maydis* treated plants (182  $\mu\text{g/gFW}$ ) followed by control (253  $\mu\text{g/gFW}$ ).

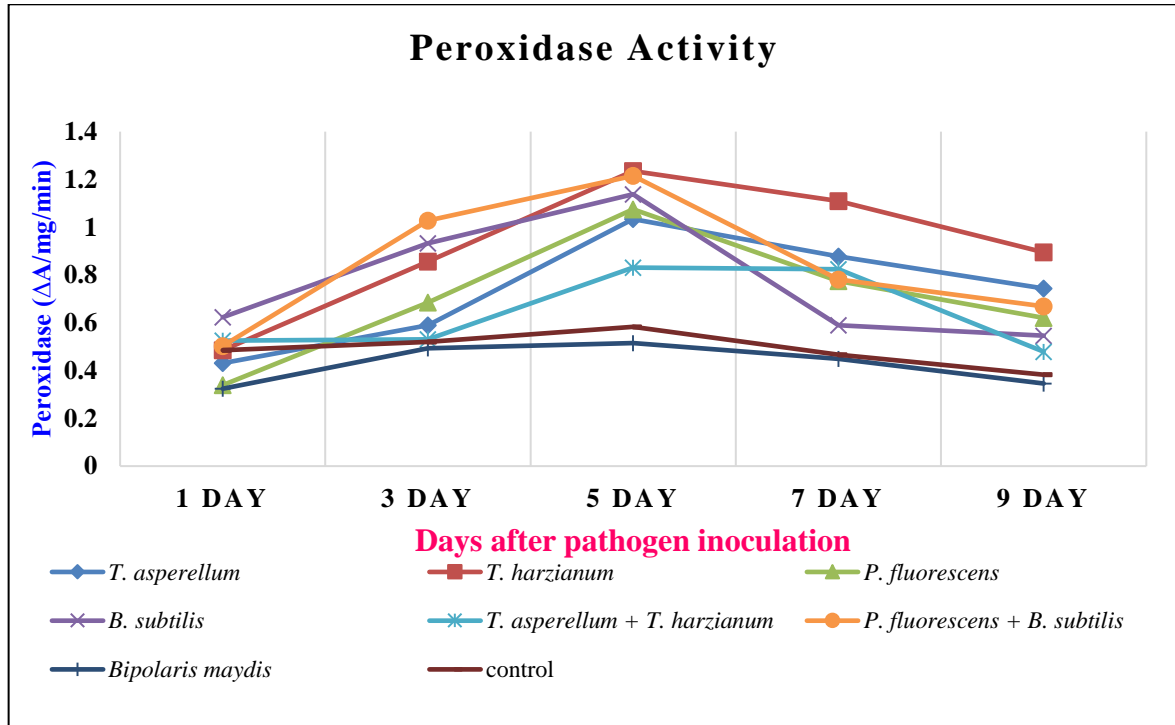


Fig. 2. Peroxidase activity ( $\Delta\text{A/mg/min}$ ) in maize leaves after inoculation of *B. maydis* at various intervals

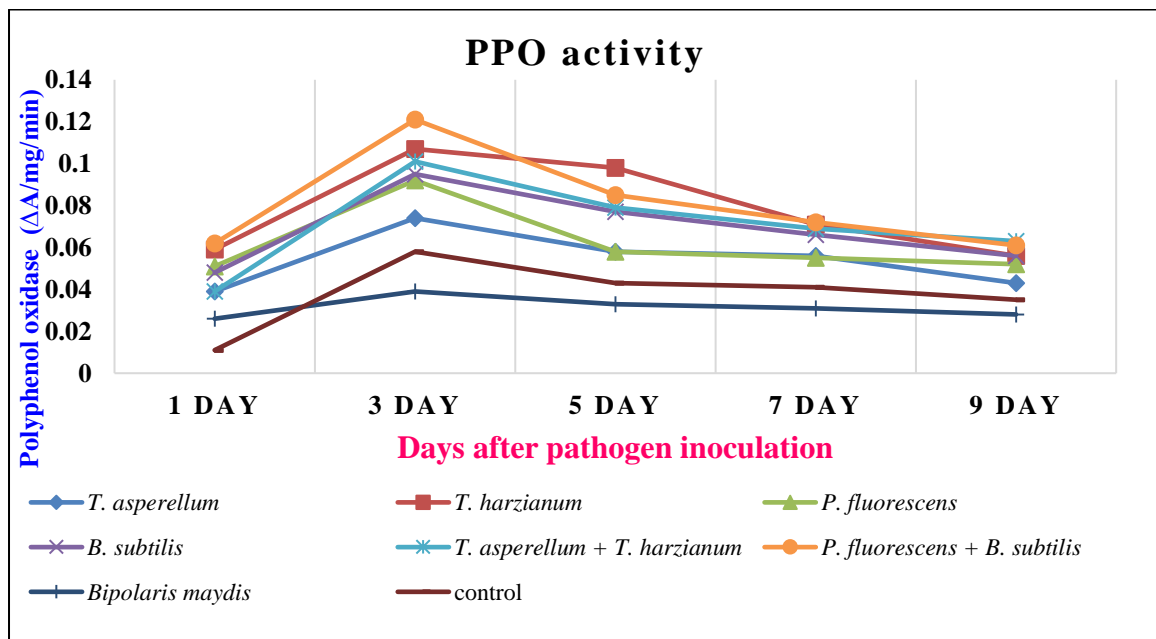


Fig. 3. Polyphenol Oxidase activity ( $\Delta\text{A/mg/min}$ ) in maize leaves after inoculation of *B. maydis* at various intervals

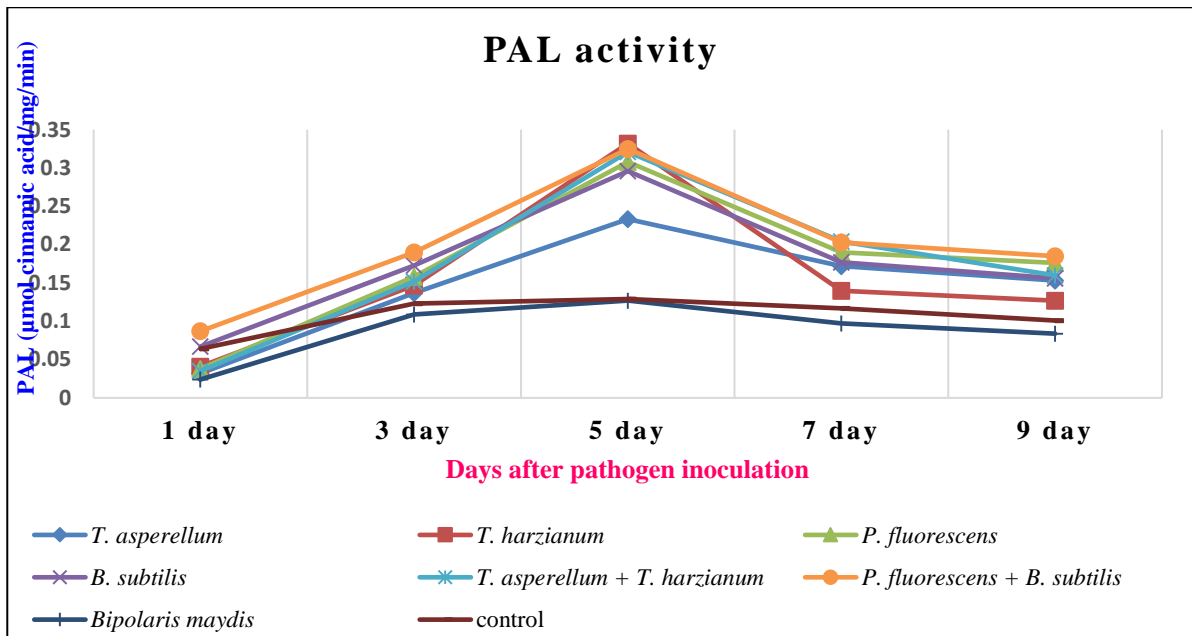


Fig. 4. Phenylalanine Ammonia Lyase ( $\mu\text{mol}$  cinnamic acid/mg/min) in maize leaves after inoculation of *B. maydis* at 2 days interval

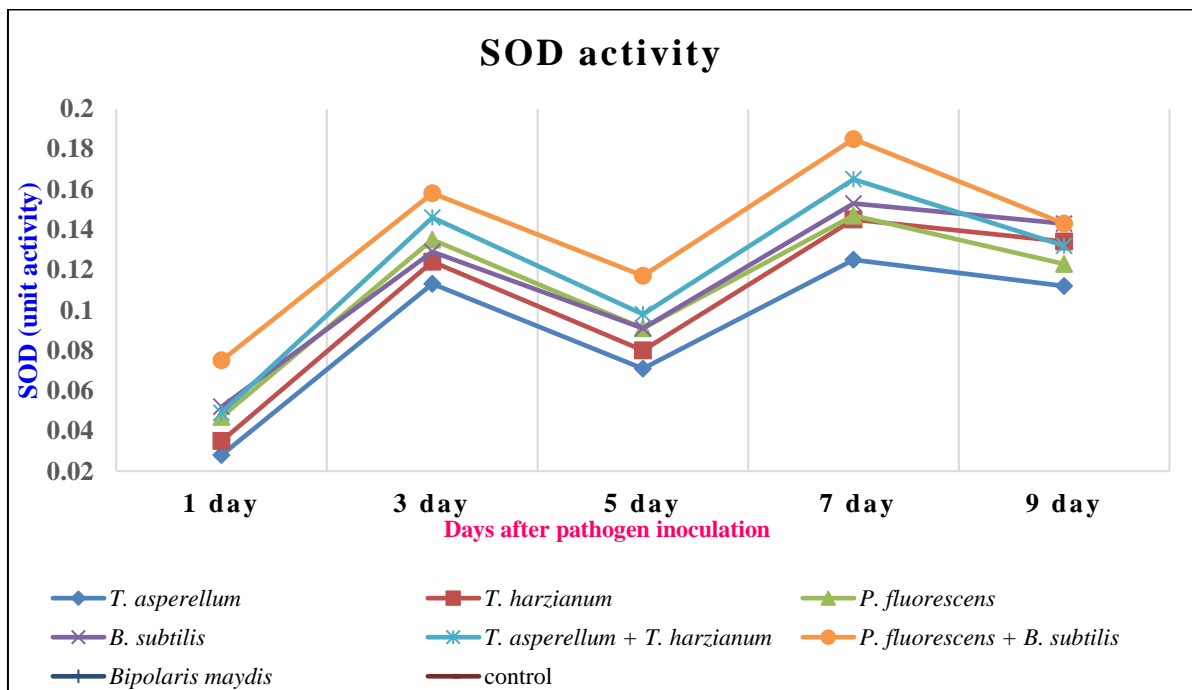


Fig. 5. Superoxide dismutase (Unit Activity) in maize leaves after inoculation of *B. maydis* at 2 days interval

### 3.3 Discussion

The present study demonstrates the impact of biopriming on seed vigour index and accumulation of defence related enzymes/antioxidants which provide resistance

against various phytopathogens especially *Bipolaris maydis* leaf blight in maize susceptible cultivar CM-202. The biocontrol agents which were used in the study were exhibited antagonistic activity against *Bipolaris maydis* in *in vitro* (Prasanna and John, 2022). So, the study was carried out to

know the effect on seed vigour index and induced systemic resistance and observed the higher vigour index in biocontrol agent primed seeds showed enhanced seed germination concerning mean shoot length (MSL) and mean root length (MRL) and vigor index (VI) when compared to control as shown in table and it is in harmony with Ananthi et al. (2014), who studied the seed vigour index in chilli through bio priming with the bio-control agents *T. asperellum* and *P. fluorescens*. Similar results were also found by Iswariya et al. (2019) who analyzed the enhancement of seedling vigour through bio priming in barnyard millet var. MDU 1 where the seeds bio primed with 20 per cent Azophos + *P. fluorescens* for eight hours have recorded 100per cent germination, high root length (15.2 cm), shoot length (8.8 cm) and vigour index (2400). “The protection by biocontrol agents such as *Trichoderma* spp. and bacteria like *Pseudomonas fluorescens* and *Bacillus subtilis* against foliar pathogens was by induced systemic resistance (ISR) and also by the production of antimicrobial compound such as chitinases, plant growth hormones, siderophores etc., Through a variety of mechanisms, including defense enzymes, beneficial microorganisms like plant growth-promoting fungi (PGPF) are known

to induce systemic resistance against a wide range of plant diseases” (Jogaiah et al. 2018). Similarly, in the present study, protection of maize plants against *B. maydis* infection was by the ISR as the PGPR and pathogen are spatially separated.

“There are similarities between pathogen-driven systemic acquired resistance (SAR) and induced systemic resistance (ISR) in plants. ISR, which covers a wide spectrum of pathogens and provides resistance in multiple plant species, causes resistance in uninfected plants. The application of PGPR is therefore a more effective biocontrol technique for disease management and cropping system enhancement. Elevated chitinase, PAL, POX, PPO, LOX, and total phenol content activities have been considered key elements in the systemic and local resistance” (Radjacommare et al. 2004 and Anupama et al. 2015). “Plants produce secondary metabolites called antioxidant enzymes. Under various stress situations, higher levels of antioxidant chemicals were seen; phenolics serve as signaling and defense molecules, shielding the plant from oxidizing agents and ultraviolet rays” (Winkel-Shirley, 2002; Murthy et al., 2014).

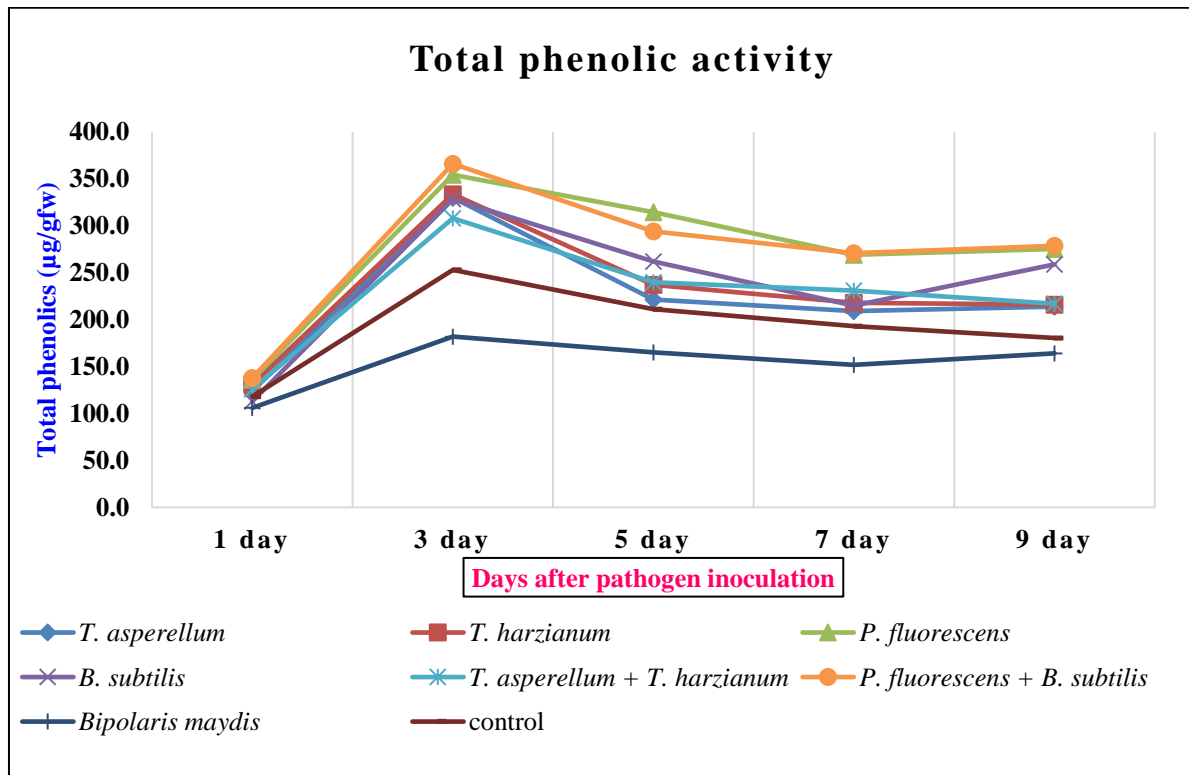


Fig. 6. Level of total phenolics (µg/gFW) in maize leaves after inoculation of *B. maydis* at 2 days interval

“An increase in phenolics correlates to the increase in activity of enzymes involved in metabolism of phenolic compounds. Increased biosynthesis of phenolic compounds like tannic, gallic, caffeic, chlorogenic and cinnamic acid was associated with induction of PAL” (Rahman *et al.*, 2012; Christopoulos and Tsantili, 2015). Nassimi and Taheri, 2017 also found the activation and accumulation of plant defence responses such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and antioxidants such as superoxide dismutase (SOD) and guaiacol peroxidase (GPX) in plants inoculated with endophytic fungus *Piriformospora indica*, *Rhizoctonia solani* and *P. indica-R. solani* and the results revealed that *P. indica* not only increased the plant biomass, but also delayed the infection process of *R. solani* and decreased sheath blight severity. Reduced levels of H<sub>2</sub>O<sub>2</sub> and increased SOD activity was associated with decreased severity of the disease

“PGPR-induced systemic resistance was studied in chilli seedlings which are raised from bioprimes seeds that were challenge inoculated. The activities of PAL, POX, PPO, LOX, phenolics, and chitinase activity were found to be higher in seedlings raised from bioprimes seeds, followed by challenge inoculation. Furthermore, even at the end of the assessment period, the bioprimes + challenge infected seedlings had the highest concentration of phenolic chemicals. When compared to other treatments, phenolics accumulation was shown to be lower and consistent throughout the study period in control and pathogen-inoculated seedlings. Therefore, seed bioprimes can successfully be employed against chili anthracnose. Increased levels of defense-related enzymes and phenolics in bioprimes chilli seedling under greenhouse conditions were well correlated with the decreased incidence of chili anthracnose disease under laboratory conditions” (Jayapala *et al.*, 2019; Yadav *et al.*, 2021).

#### 4. CONCLUSION

The present study on vigour index and biochemical changes through defence related antioxidant enzymes suggest that all the treatments were effective in inducing systemic resistance and biochemical defense in maize plants against maydis leaf blight. Biochemical defense in plants is multi-level process which involves multitude of enzymes and metabolites. From the present study it was concluded that vigour index and induced systemic resistance activity was higher in *P. fluorescens* + *B. subtilis*

bioprimes seeds. Biochemical studies exposed that peroxidase and phenylalanine ammonia lyase increased after pathogen inoculation and reached highest level on fifth day in most of the treatments whereas total phenols and polyphenol oxidase reached highest level on third day and superoxide dismutase was recorded in transient manner. Among the biocontrol agents, *P. fluorescens* + *B. subtilis* showed maximum production of various antioxidant enzymes followed by *T. harzianum* and minimum activity was found in *B. maydis* treated seeds and control. Bioprimes also provides protection against wide range of soil borne and seed borne pathogens of a particular crop up to some days after germination in reducing disease infection and severity and induces antioxidant enzymes whenever pathogen attacks during the crop growth stage. Hence, usage of PGPR is more efficient as biocontrol method to manage disease and to improve cropping systems together with improving the soil health and soil fertility. So, this is useful practice for crop protection and also can be easily adopted in integrated disease management practice to increase the yield and crop productivity.

#### DISCLAIMER

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

- Abdul-Baki, A. A. and Anderson, J. D. (1973). Vigor Determination in Soybean Seed by Multiple Criteria. *Crop Sci.*, 13: 630-633.
- Aggarwal, S. K., Hooda, K. S., Kaur, H., Gogoi, R., Chauhan, P., Bagaria, P. K. and Lal, M. K. (2024). Comparative evaluation of management modules against Maydis leaf

- blight disease in maize (*Zea mays*). *Europ. J. Plant Pathol.*, 168(3): 485-495.
- Ananthi, M., Selvaraju, P. and Sundaralingam, K. (2014). Effect of bio-priming using bio-control agents on seed germination and seedling vigour in chilli (*Capsicum annuum* L.) 'PKM 1'. *J. Hortic. Sci. Biotechnol.*, 89 (5): 564-568.
- Anupama, N. B., Jogaiah, S., Ito, S., Nagraj, K. A. and Tran, L. S. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Sci.*, 231:62–73
- Beauchamp, C. and Fridovich, I. (1971). Superoxide dismutase: improved assays and applicable to acrylamide gels. *Anal. Biochem.*, 44: 276-287
- Bray, H. G. and Thorpe, M. V. (1954). Analysis of phenolic compounds of interest in metabolism. D. Glick (Ed.), *Methods of Biochemical Analysis*, Interscience Publications, New York. 1: 17-52
- Callan, N. W., Mathre, D. and Miller, J. B. (1990). Bio-priming seed treatment for biological control of *Pythium ultimum* pre-emergence damping-off in sh-2 sweet corn. *Plant Dis.*, 74: 368-372.
- Christopoulos, M. V. and Tsantili, E. (2015). Participation of phenylalanine ammonialyase (PAL) in increased phenolic compounds in fresh cold stressed walnut (*Juglans regia* L.) kernels. *Postharvest Biol. Technol.*, 104: 17-25.
- Compant, S., Clement, C. and Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.*, 42(5): 669-678.
- Dickerson, D. P., Pascholati, S. F., Hagerman, A. E., Butler, L. G. and Nicholson, R. L. (1984). Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiol. Plant Pathol.*, 25(2): 111-123.
- Hammerschmidt, R., Nuckles, E. M. and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.*, 20(1): 73-82.
- International Seed Testing Association (ISTA). (2005). International rules for seed testing. *Rules 1999* (No. Suppl).
- Iswariya, S., Sujatha, K. and Subhashini, R. (2019). Enhancement of seedling vigour through bio-priming for Barnyard millet var. MDU 1. *Int. J. Cur. Microbiol. Appl. Sci.*, 8(4): 2254.
- Jayapala, N., Mallikarjunaiah, N. H., Puttaswamy, H., Gavirangappa, H. and Ramachandrappa, N. S. (2019). Rhizobacteria *Bacillus* spp. induce resistance against anthracnose disease in chili (*Capsicum annuum* L.) through activating host defense response. *Egy. J. Biol. Pest con.*, 29(1): 1-9.
- Jogaiah, S., Abdelrahman, M., Tran, L. S. P. and Ito, S. I. (2018). Different mechanisms of *Trichoderma virens*-mediated resistance in tomato against Fusarium wilt involve the jasmonic and salicylic acid pathways. *Mol. plant pathol.*, 19(4): 870-882.
- Kumar, G., Kumar, A. and Kumar, V. (2019). Seed bio-priming: Step toward disease management. *Think India Journal.*, 22(34): 699-704.
- Mayer, A. M. (2006). Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry*, 67: 2318-2331.
- Murthy, H. N.; Lee, E. J. and Paek, K. Y. (2014). Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell, Tissue and Organ Cult.*, 118(1): 1-16.
- Nassimi, Z., and Taheri, P. (2017). Endophytic fungus *Piriformospora indica* induced systemic resistance against rice sheath blight via affecting hydrogen peroxide and antioxidants. *Biocont. Sci. Technol.*, 27(2): 252-267.
- Prasanna, S. L., and John, P. (2022). Exploring the proficiency of botanicals, bioagents and plant activators against maydis leaf blight of maize caused by *Bipolaris maydis*. *J. Pharma Innov.*, 11(7): 31-36
- Radjacommar, R., Kandan, A., Nandakumar, R. and Samiyappan, R. (2004). Association of the hydrolytic enzyme chitinase against *Rhizoctonia solani* in rhizobacteria-treated rice plants. *J. Phytopathol.*, 152(6): 365-370.
- Rahman, M. H.; Begum, L. A.; Alam, K. B. and Khan, A. L. (1993). Efficacy of fungicides to control turcicum leaf blight of maize. *Bangladesh J. Plant Pathol.*, 9(1-2): 35-36.

- Rao, P. V., Subbaiah, G. and Veeraraghavaiah., R. (2014). Agronomic responses of maize to plant population and nitrogen availability-a review. *Int. J. Plant, Animal Environ. Sci.*, 4 (1): 107-116.
- Rudrappa, T., Czymbek, K. J., Pare, P. W. and Bais, H. P. (2008). Root-secreted malic acid recruits beneficial soil bacteria. *Plant physiol.*, 148(3): 1547-1556.
- Singh, S., Singh, U. B., Malviya, D., Paul, S., Sahu, P. K., Trivedi, M. and Saxena, A. K. (2020). Seed biopriming with microbial inoculant triggers local and systemic defense responses against *Rhizoctonia solani* causing banded leaf and sheath blight in maize (*Zea mays* L.). *Int. J. Environ. Res. Public Health*, 17(4): 1396.
- Walker, T. S., Bais, H. P., Grotewold, E. and Vivanco, J. M. (2003). Root exudation and rhizosphere biology. *Plant Physiol.*, 132(1): 44-51.
- Whetten, R.W. and Sederoff, R. R. (1992). Phenylalanine ammonia lyase from loblolly pine: purification and isolation of complementary. *Plant Physiol.*, 98: 380-386.
- White, D. G. (1999). Compendium of corn diseases, Ed.
- Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Cur. Opin. Plant Biol.*, 5: 218-22
- Yadav, M., Dubey, M. K. and Upadhyay, R. S. (2021). Systemic resistance in chilli pepper against anthracnose (caused by *Colletotrichum truncatum*) induced by *Trichoderma harzianum*, *Trichoderma asperellum* and *Paenibacillus dendritiformis*. *J. Fungi*, 7(4): 307.

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