**Biocontrol potential through seed biopriming and activated host defence response against maydis leaf blight caused by *Bipolaris maydis***

**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. 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 3rd day of pathogen inoculation whereas, peroxidase and phenylalanine ammonia lyase were higher after 5th 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

**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, mainly utilized for human consumption and livestock/poultry feed. During the last few years, there has been a progressive escalation in its demand for the value-added products, like glucose, sorbitol, dextrose, starch-based products and oil. It also serves as a basic raw material as an ingredient to thousands of industrial products that include starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package, paper industries *etc*. (Rao *et al.*, 2014). Global maize area of about 201.98 million hectares stands with the production of 1162.35 million tons and average productivity is about 5.75 tons/ha (FAO, 2021). Among the maize growing countries, India ranks 4th in area and 7th in production, representing around 4% of the world maize area and 2% of total production.

On global basis, out of 115 diseases recorded on maize, 35 have been reported in India such as Maydis leaf blight, Turcicum leaf blight, Curvularia leaf spot, Banded leaf and sheath blight, Charcoal rot, Fusarium stalk rot, Bacterial stalk rot, Downy mildew *etc*. Among the biotic factors, foliar diseases are economically important constraints in tropical maize production since, the airborne fungi probably account for the greatest losses. 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 has now become one of the most prevalent and severe diseases in Pakistan, India, Nepal, Kampuchea, Philippines, Indonesia, Vietnam and China (White, 1999).

Seed Biopriming is a new age technique of seed treatment which integrates biological aspects of disease management where seed is inoculated with beneficial organism which ultimately protects the seed from various diseases. Biopriming provide resistance to plant against biotic and abiotic stresses while improving the production and productivity of crop with seed quality improvement. It uses different beneficial microorganisms to increase plant growth promoting activity and managing diseases. Microorganisms used for biopriming includes beneficial fungi and bacteria involve different species of *Trichoderma*, *Pseudomonas*, *Bacillus* spsand others can improve seed germination percentage, enhance seedling vigour and manage soil and seed borne pathogens (Kumar *et al*., 2019).

Seed biopriming with *Pseudomonas flourescens* @ 1 × 107 cfu/seed provide better protection against damping off disease in maize plant which is caused by fungal pathogen *Pythium ultimum* (Callan *et al*., 1990). Biopriming with different isolates of *Pseudomonas fluorescens* showed enhanced germination, seedling vigour and elicited resistance against downy mildew disease in pearl millet (Raj *et al*., 2004). 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 and Sessitsch 2010). Biopriming with *Pseudomonas aeruginosa* strain MF-30 in maize increased the shoot and root biomass and also plant growth and antioxidant content, as well as decreased disease severity against banded leaf and sheath blight suggested the possibility of an eco-friendly and economical means of achieving antioxidants-rich, healthier maize plants (Singh *et al*. 2020). Hence an attempt 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.

**Material and Methods:**

**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 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 × 104 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, 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.

### **Seed biopriming**

Fungus cultures grown on Potato dextrose broth for 10 days and after incubation conidia were dispersed in the solution and adjusted to a concentration of (1 × 108 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 × 108 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 ± 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.

**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).

Paper towel method (ISTA [2005](https://link.springer.com/article/10.1186/s41938-019-0148-2#ref-CR17)) was employed to monitor the germination and vigor of seeds under laboratory conditions. Seeds of maize cultivar CM-202 were allowed to germinate on a presoaked paper towel at incubation conditions for 14 days. The experiment was carried out with 3 replicates of 50 seeds each. The number of germinated seeds after the incubation period was calculated and the rate of germination was represented in percentage. Seedling vigor (Abdul Baki and Anderson, [1973](https://link.springer.com/article/10.1186/s41938-019-0148-2#ref-CR1)) as a measure of mean root length and shoot length was calculated, using the following formula.

#### 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. Bioprimed maize seeds and control seeds were sown equidistantly (8 seeds/pot).

Experiment was laid in Completely Randomized Design (CRD) with three replications. Inoculation of *B. maydis* was done by spraying conidial suspension (4×104 spores/ml) on maize variety CM-202 at twenty five day old plant. Proper humidity was maintained in the pots with the help of polythene cover and moistened cotton after inoculation of the pathogen and continuously upto 2 days for conidial germination, penetration and development of symptoms. Control plants were treated with water only.

### **Induction of defense mechanism**

Seeds were bioprimed and grown and challenge inoculated as explained earlier. To study the ISR, eight types of treatments were maintained viz*.*, T1- seeds bioprimed with *T. asperellum* alone, T2- seed bio-primed with *T. harzianum* alone, T3- seed bio-primed with *P. fluorescens*, T4- seed bio-primed with *B. subtilis*, T5- seed bio-primed with *T. asperellum* + *T. harzianum,* T6 - seed bio-primed with *P*. *fluorescens + B. subtilis*, T7 - seed bio-primed with *B. maydis* pathogen, T8-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.

**Estimation of Peroxidase (PO) activity**

Seedlings (1 g) were grinded into powder and homogenized with 5 ml of phosphate buffer (0.1 M, pH 7.0) and centrifuged at 10,000 rpm for 10 min at 4 °C. Peroxidase activity (POX) was estimated by adding supernatant/enzyme extract (0.1 ml) to 2.9 ml of substrate buffer containing [125 μl guaiacol (0.05 M) and 153 μl 30% H2O2 in 50 ml phosphate buffer (0.1 M, pH 7.0)]. The specific activity of POX was expressed as change in optical density at 470 nm/min/mg protein (Hammerschmidt *et al*. [1982](https://link.springer.com/article/10.1186/s41938-019-0148-2#ref-CR14)).

**Estimation of Polyphenol Oxidase (PPO) activity**

Homogenization of ground seedlings (1 g) was done using a cold potassium phosphate buffer (5 ml, 0.1 M, pH 6.5) and centrifuged at 10,000 rpm at 4 °C for 10 min. Successively, 1.5 ml sodium phosphate buffer (0.1 M, pH 6.5) was added to the enzyme extract (100 μl), and the reaction was started by adding 200 μl catechol (0.01 M) and the specific activity of polyphenol oxidase activity (PPO) was expressed as change in OD at 420 nm/min/mg protein (Mayer *et al*. [1965](https://link.springer.com/article/10.1186/s41938-019-0148-2#ref-CR22)).

**Estimation of Phenylalanine Ammonia Lyase (PAL) activity**

Seedlings (1 g) were ground into powder and homogenized in 5 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. Supernatant served as an enzyme source and the activity was determined 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 recorded at 290 nm and the enzyme activity was expressed as nmol *t*-cinnamic acid/min/mg protein (Dickerson et al. [1984](https://link.springer.com/article/10.1186/s41938-019-0148-2#ref-CR8)).

**Estimation of Super oxide Dismutase (SOD)activity**

Super oxide Dismutase (SOD) activity was assayed by measuring the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm as described by Beauchamp and Fridovich (1971). The reaction mixture (3 ml) consisted of 50 mM sodium phosphate buffer (pH 7.8), 13 mM L-methionine, 75 µMNBT, 10 µMEDTA, 2 µM riboflavin and 0.3 ml enzyme extract. The test tubes containing reaction mixture were kept under 4000 lux at 35 ºC for 20 min. One unit SOD activity was defined as the amount of enzyme required to cause 50 per cent inhibition of the rate of NBT reduction measured at 560 nm.

**Estimation of Total phenols**

The total phenols present in the challenge inoculated leaves was estimated following the procedure of Bray and Thorpe (1954). Fresh leaf samples of 1.0 g weight were blended with 10 ml of 80% ethanol and boiled at 50°C for 30 min. The extracts were filtered through cheese cloth and then with Whatman No. 41 filter paper and centrifuged at 8000 g for 10 min. The volume was made up to 10 ml with ethanol. 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.

**Results:**

**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).

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**Fig 1: Effect of seed biopriming on maize cv. 202 with various biocontrol agents.**

1. *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

**Table I: 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.** | ***T. asperellum*** | 86.00b | 17.07 | 22.20 | 3376.93 |
| **2.** | ***T. harzianum*** | 88.00a | 17.88 | 19.65 | 3302.93 |
| **3.** | ***P. fluorescens*** | 86.67b | 17.42 | 21.83 | 3401.96 |
| **4.** | ***B. subtilis*** | 84.00d | 18.64 | 20.28 | 3269.28 |
| **5.** | ***T. asperellum*+**  ***T. harzianum*** | 85.33c | 16.99 | 18.09 | 2993.49 |
| **6.** | ***P. fluorescens* + *B. subtilis*** | 86.67b | 19.35a | 22.69a | 3644.04 |
| **7.** | ***B. maydis*** | 82.67e | 14.79 | 18.69 | 2768.23 |
| **8.** | **Control** | 86.00b | 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 |  |

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

**Peroxidase (PO) activity**

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

**Polyphenol oxidase (PPO) activity**

The maximum PPO activity was found in *P. fluorescens+ B. subtilis* (0.121 ∆A/mg/min) followed by *T. harzianum*(0.107 ∆A/mg/min) and *T. asperellum+ T. harzianum*(0.101 ∆A/mg/min) after third day of inoculation. Minimum PPO activity was observed in *B. maydis* treated and control with (0.039 ∆A/mg/min) and (0.058 ∆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.

**Phenylalanine ammonia lyase (PAL) activity**

The maximum PAL activity was found in *T. harzianum* (0.332 µmol cinnamic acid/mg/min) which was at par with *P. fluorescens+ B. subtilis* (0.325 µmol cinnamic acid/mg/min) and *T. asperellum* + *T. harzianum* (0.321 µmol cinnamic acid/mg/min) after fifth day of inoculation with pathogen. Minimal activity of PAL was found in *B. maydis* (0.127 µmol cinnamic acid/mg/min) and control (0.129 µmol 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.

**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 3rd 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).

**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 µg/gFW) was observed in *P. fluorescens+ B. subtilis* followed by *P. fluorescens* (354.3 µg/gFW) and minimum was observed in *B. maydis* treated plants (182 µg/gFW) followed by control (253 µg/gFW).

**Fig 2:** **Peroxidase activity (∆A/mg/min) in maize leaves after inoculation of *B. maydis* at various intervals**

**Fig 3: Polyphenol Oxidase activity (∆A/mg/min) in maize leaves after inoculation of *B. maydis* at various intervals**

**Fig 4: Phenylalanine Ammonia Lyase (µmol 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**

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

**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 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 enhance 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 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., Beneficial microbes such as plant growth-promoting fungi (PGPF) are known for inducing systemic resistance against broad plant diseases by different mechanism including defense enzymes (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.

Induced systemic resistance (ISR) in plants is similar to pathogen-induced systemic acquired resistance (SAR). ISR induces resistance in uninfected plant, covers broad pathogen range offering resistance in several plant species. Hence, the usage of PGPR is more efficient as biocontrol method to manage the disease and to improve cropping systems. Increased activities of PAL, POX, PPO, LOX, chitinase, and total phenol content have been thought to be essential components in the local and systemic resistance (Radjacommare et al. 2004 and Anupama et al. 2015). Antioxidant enzymes are secondary metabolites synthesized by plants. Increased antioxidant compounds were observed under different stress conditions, phenolics act as defense and signal compounds as well as protecting the plant from ultraviolet radiation and oxidising agents (Winkel-Shirley, 2002; Murthy *et al*., 2014). An increase in phenolics correlates to the increase in activity of enzymes involved in phenolic compounds metabolism. Induction of PAL was associated with increased biosynthesis of phenolic compounds like tannic, gallic, caffeic, chlorogenic and cinnamic acid (Rahman *et al*., 2012; Christopoulos and Tsantili, 2015).  Nasssimi and Taheri, 2017 also found the activation and accumulation of plant defence responses such as hydrogen peroxide (H2O2) 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. Decreased severity of the disease was associated with decreased levels of H2O2 and increased SOD activity.

**Conclusion:**

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

In the present study, PGPR-induced systemic resistance in seedlings raised from bioprimed 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 bioprimed seeds, followed by challenge inoculation. In addition, the concentration of phenolic compounds was found to be maximum even at the end of the evaluation period in bioprimed + challenge inoculated seedlings. In control and pathogen-inoculated seedlings, phenolics accumulation was observed to be lower and was constant throughout the experimental period when compared to other treatments. Therefore, seed biopriming can successfully be employed against chili anthracnose. Increased levels of defense-related enzymes and phenolics in bioprimed chili seedling under laboratory conditions were well correlated with the decreased incidence of chili anthracnose disease under greenhouse conditions. 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.

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