## Original Research Article

# Temporal Dynamics of Induced Defense Enzymes in Finger Millet: Unveiling the Role of Neem Leaf Extract and *Brevibacillus brevis* in Blast Disease Management


## ABSTRACT

**Aims:**To investigate the enzymatic responses of finger millet leaves, specifically β-1,3-glucanase, chitinase, and lipoxygenase (LOX), upon inoculation with the blast pathogen *Pyricularia grisea* and subsequent treatments with a bio-agent, botanical, fungicide, and salicylic acid. The study sought to determine the activity levels of these enzymes at different time intervals (24, 48, and 72 hours post-treatment) to understand their role in the plant's defense mechanism.

**Study design:** Completely Randomized Design (CRD)

**Place and duration of study:** Agricultural Research station, Perumallapalli, Acharya N G Ranga Agricultural university, between December 2023 to March 2024.

**Methodology:** Finger millet blast caused by *Pyricularia grisea* Sacc. was inoculated at twenty-one days old seedlings and immediately upon symptom development, the treatments were imposed. Treatments included seedling root dip and foliar spray with bacterial antagonist, *Brevibacillus brevis*; foliar spray with neem leaf extract (1500 ppm), carbendazim 12%+ mancozeb 63% (500 ppm) and salicylic acid (50 µM), challenged and unchallenged control. 24, 48 and 72 hours after treatment leaf samples were collected and examined spectrophotometrically for the analysis of enzymatic responses viz., β-1,3-glucanase, chitinase and lipoxygenase (LOX).

**Results:** The results revealed that the enzymatic response of β-1,3-glucanase exhibited its highest activity at 24 hours post-treatment in foliar spray with neem leaf extract, whereas chitinase activity reached its maximum at 72 hours post-treatment with neem leaf extract, with a value of 0.75 µg glucose min-1 $mg^{-1}$ protein. Lipoxygenase enzyme showed its peak activity

at 48 hours post-treatment with neem leaf extract, showing a significant increase from 264.72 to 474.62 µmol/HPO/min compared to other treatments.

**Conclusion:** We can create new tactics to manage blast disease by clarifying the plant's defense systems against *P. grisea* infection and the ways in which the pathogen harms the host.

**Key words:** *β-1,3-glucanase, Brevibacillus brevis, Chitinase, Induced defense enzymes, Lipoxygenase, Neem leaf extract, Pyricularia grisea.*

**Commented [SP1]:** Key word : Lipoxygenase, Neem leaf extract, Pyricularia grisea.

## 1. INTRODUCTION

Blast disease caused by *Pyricularia grisea* belongs to Asomycetes fungi, is one of the serious threats and most destructive disease that occurs widely in major millet growing regions of world. It is the major production constraint under natural conditions especially in finger millet cultivation causing considerable economic losses with varying degrees of damage. In India, the average yield loss due to finger millet blast in Tamilnadu reported to be 50- 100% (Sekhar et al*.,* 2018), Gujarat 35.78% (Prajapati et al *.,* 2013) and Karnataka 22.57% to 56.67% (Annual Progress Report of (ICAR)- (AICRP) on Small Millets., 2021).

Blast pathogen infect finger millet plant at all stages, however the young seedlings are more prone for the attack and show burnt appearance in nursery under severe infection (Singh *et al.,* 2010; Parthasarathy,2020). *P. grisea* attacks at different growth stages of the crop and leads to formation of typical symptoms like leaf blast, neck blast and finger blast (Nagaraja et al*.,* 2016; Palanna et al.,2021). Disease appears on leaf lamina with typical spindle shaped spots with grey or whitish center and brown to reddish brown margin, which enlarge, coalesce and give blasted appearance. Well-developed lesions may measure 0.5 x 2 cm. The pathogen also attacks culms, especially at the nodal region results in blackening of the infected area. However, the most damaging stage of the disease is when it attacks neck region. If the neck is infected the above parts of infected neck may become brownish resulting in total death of the plant (Sreenivasaprasad, 2004), ultimately causing yield losses of up to 100%. These losses pose economic challenges for farmers and can potentially lead to food crisis (Senthil et al., 2012; Prajapati et al., 2013; Negi et al., 2017).

In India, management of the blast disease is highly dependent on chemical fungicides and it has negative consequences on beneficial organisms in ecosystems and can seriously impact the health of agricultural workers, and rural communities. Additionally, the presence of fungicide residues raises food safety concerns for domestic consumers and creates obstacles for exporting crops. As plant protection chemicals pose hazards, there's a rising demand to investigate eco-friendly alternatives for disease management. A better understanding of the mechanisms involved in defense to *P. grisea* infection and responsible for damage to the host plant may provide new methods to control this disease.

Plants defend themselves against, the pathogen challenge by the activation of defense response pathways (de Wit, 2007). The systemic resistance induction process increases enzymatic activity of peroxidase (POX) and polyphenol oxidase (PPO) which are responsible for catalyzing lignin formation, and phenylalanine ammonia lyase (PAL), involved in the biosynthesis of phytoalexins and phenols. The pathogenesis-related proteins (PRPs), viz., β-1,3-glucanase (E.C.3.2.1.4) and chitinase (E.C. 3.2.1.14), enzymes that belong to PR-2 and PR-3 families, respectively (van Loon, Rep, & Pieterse, 2006) have been related more often to Systemic acquired resistance (SAR) and sometimes to Induced systemic resistance (ISR). All these enzymes have been shown to be involved in plant defense against pathogens in several pathosystem (Kini, Vasanthi, & Shetty, 2000). The activation and the expression levels of defense genes vary in different plant-pathogen interactions. Plants have also developed complex antioxidant defense systems that respond to biotic and abiotic stresses and mitigate the deleterious effects of reactive oxygen species (Panda, 2007).

Lipoxygenases are a family of dioxygenases, involved in the oxidation of polyunsaturated fatty acids to form hydroperoxides, which are highly reactive compounds that are toxic and initiate lipid peroxidation and cause damage to cell components. LOX derived fatty acid hydroperoxides can be further metabolized into volatile aldehydes and jasmonates and plays an important role in plant physiology. Little is known about the mechanisms of the defense enzymes upon pathogen attack. Keeping in view of this, studies were conducted under glasshouse condition to investigate the mechanism of Chitinase, β-1,3-glucanase and lipoxygenase enzymes response in finger millet plants under biotic stress condition.

## 2. MATERIAL AND METHODS

The experiment was conducted in glass house condition at Agricultural Research Station, Perumallapalle, Andhra pradesh, during *kharif* 2023-24.

Commented [SP2]: December 2023 to March 2024.

### 2.1 Plant material and inoculation

Plastic pots of 22×20 cm diameter were filled with sterilized soil, sand and FYM in 2:1:1 ratio and the seeds of Champavathi variety (susceptible variety for blast disease) were sown and kept in glass house at 30°C. To retain 10 plants per pot, seedlings were thinned at the one leaf stage.

Seedling root dip with *Brevibacillus brevis* ($1.5\times10^8$ $CFUml^{-1}$) was done as per procedure followed by Sekhar et al. (2018). The bacterial antagonist was inoculated into Nutrient broth (NB) and kept for 24 h incubation and this nutrient broth containing bacterial antagonist was used for seedling root dip and incubated for 1−2 h and planted.

From 10 days old culture of *Pyricularia grisea,* conidial suspension was prepared and was inoculated on 21 days old transplanted seedlings as foliar spray while water was sprayed on control treatment (Negi et al.,2017). The experiment was conducted with 7 treatments and each treatment was replicated thrice in a Completely Randomized Design (CRD). Immediately after the appearance of blast symptoms treatments were imposed.

Treatments:

$T_1$**:** Seedling root dip with *Brevibacillus brevis* (1 X $10^8$ $CFUml^{-1}$)

$T_2$: Foliar spray bacterial antagonist *Brevibacillus brevis* (1 X $10^8$ $CFUml^{-1}$)

$T_3$: Foliar spray with Neem leaf extract 1500 ppm

$T_4$: Foliar spray with Carbendazim 12%+ Mancozeb 63% WP at 500 ppm

$T_5$: Foliar spray with salicylic acid at 50µM

$T_6$: Challenged control (treated with pathogen)

$T_7$: Unchallenged control (sprayed with distilled water)

The leaf samples were collected at 24, 48 and 72 hrs after imposing treatments and the changes in activity of chitinase, β-1,3 glucanase and lipoxygenase enzymes were determined spectrophotometrically.

## 2.2 Biochemical assay for the enzymes

### 2.2.1 β-1,3 glucanase

Beta-1,3 glucanase enzyme activity was assayed as per procedure followed by Patil et al. (2016). Inoculated leaf samples were collected at 24, 48 and 72 hours post treatment. The leaf samples were ground to fine powder using liquid nitrogen and used for the assay. 0.1 g of ground leaf sample was extracted in 1.5 mL of 0.05 M sodium acetate buffer (pH 5.2), centrifuged at 15,000 rpm at 4 °C for 30 min and the supernatant stored at -20 °C. The extracted enzyme source was evaluated for β-1,3-glucanase (EC 3.2.1.4) activity. To assess this, 50 µL of the extract was incubated with 100 µL of 0.1% (w/v) laminarin in 0.05 M sodium acetate buffer (pH 5.2). The mixture was then incubated for 15 minutes at 37°C. Afterward, 500 µL of DNS reagent was added and the mixture was incubated at 100°C for 10 minutes. The resulting color change was measured at 540 nm.

### 2.2.2 Chitinase

Chitinase enzyme activity was assayed according to the method described by Patil et al. (2016). Leaf samples were collected at 24, 48, and 72 hours post-treatment after pathogen inoculation and stored at -20°C for further analysis. The frozen leaf samples were ground into a fine powder using liquid nitrogen for extraction. 0.1 g of ground leaf sample was extracted in 1.5 mL of 0.05 M sodium acetate buffer (pH 5.2), centrifuged at 15,000 rpm at 4°C for 30 minutes, and the supernatant was stored at -20°C. For the assay, 500 µL of the extract was mixed with 200 µL of colloidal chitin (10 mg/mL; w/v) and incubated at 37°C for 2 hours. Following this, 500 µL of DNS reagent was added, and the mixture was incubated in a boiling water bath. The resulting color change was measured at 570 nm.

### 2.2.3 Lipoxygenase

Lipoxygenase enzyme activity was assayed as per procedure followed by Kotapati et al., (2014). The leaves of the finger millet were blended into a fine powder and was suspended

in 0.05M Sodium phosphate buffer at pH 6.4 containing 1mM Phenyl methyl sulfonyl fluoride (PMSF). The homogenate was centrifuged for 10 min at 12000 rpm at 4 °C. The obtained supernatant was further assayed for lipoxygenase activity. The typical reaction mixture contained 2.8 ml of 50mM sodium phosphate buffer pH 6.4, the appropriate volume of the enzyme (10-100µl) and the reaction was initiated by addition of substrate to the reaction mixture and maintained to have 250µM for linoleic acid in the total volume.Activity was measured at 234nm spectrophotometrically by monitoring the increase in absorbance over a period of time

One unit of enzyme activity is defined as the amount of micro moles of hydroperoxide formed per minute. The lipoxygenase activity was calculated by using the following formula.

$$\text{Enzyme Activity} = \frac{\Delta \text{ difference} \times \text{Total volume of reaction mixture}}{e \times \text{volume of enzyme}}$$

e = 0.00275

**2.3 Statistical analysis**

The experimental data were statistically analyzed (Gomez and Gomez, 1984) using IBM SPSS statistics 2.0 package. The treatment impact was observed by analysis of variance (ANOVA) of Completely Randomized Design (CRD).

**3. RESULTS AND DISCUSSION**

In the present study, the aim was to understand the change in levels of major defense enzymes *viz.*, β-1-3 glucanase, Chitinase, Lipoxygenase and their accumulation in due course of time ie, at 24, 48 and 72 hrs after the treatment imposition.

**3.1 Activity of the defense enzymes**

**3.1.1 β-1-3 glucanase**

Beta-1,3-glucanase activity was measured using DNS reagent as the indicator of activity change compared to the blank. The highest enzyme activity was observed at 24 hours, followed by 48 hours after treatment. The lowest enzyme activity was recorded at 72 hours post-treatment. Among all the treatments the foliar spray with neem leaf extract at 1500 ppm showed highest enzyme activity at all the time intervals with the value of 0.79, 0.71 and 0.60 µg glucose $min^{-1}$ $mg^{-1}$ protein at 24, 48 and 72

hrs after imposing treatments respectively. And this was followed by seedling root dip with bacterial antagonist, *Brevibacillus brevis* with a reading of 0.67, 0.60 and 0.55 µg glucose $min^{-1}$ $mg^{-1}$ protein at 24, 48 and 72 hrs time interval respectively. The treatments $T_2$ (Foliar spray with *Brevibacillus brevis* at $1.5\times10^8$ $CFUml^{-1}$) showed the enzyme activity of 0.61, 0.59 and 0.50 µg glucose $min^{-1}$ $mg^{-1}$ protein at 24, 48 and 72 hrs time interval respectively and $T_5$ (Foliar spray with Salicylic acid 50 µM) showed the enzyme activity of 0.41, 0.35 and 0.31 µg glucose $min^{-1}$ $mg^{-1}$ protein at 24, 48 and 72 hrs time interval respectively .The unchallenged control ($T_7$) showed stable reading throughout the experiment (**Table 1**, **Figure. 1**).

**Table 1. β-1,3 glucanase enzyme activity (µg glucose $min^{-1}$ $mg^{-1}$ protein) at 24, 48 and 72 hours after treatment**

| Treatments | 24 HRS* | 48 HRS* | 72 HRS* |
|---|---|---|---|
| $T_1$:Seedling root dip with *Brevibacillus brevis* ($1\times 10^8$ $CFUml^{-1}$) | 0.67 | 0.60 | 0.55 |
| $T_2$: Foliar spray with *Brevibacillus brevis* ($1\times 10^8$ $CFUml^{-1}$) | 0.61 | 0.59 | 0.50 |
| $T_3$:Foliar spray with neem leaf extract at 1500 ppm | 0.79 | 0.71 | 0.60 |
| $T_4$:Foliar spray with Carbendazim12%+Mancozeb 63% WP at 500 ppm | 0.58 | 0.50 | 0.50 |
| $T_5$:Foliar spray with salicylic acid at 50µM | 0.41 | 0.35 | 0.31 |
| $T_6$:Challenged control | 0.30 | 0.25 | 0.20 |
| $T_7$:Unchallenged control | 0.18 | 0.18 | 0.18 |
| C.D | 0.02 | 0.01 | 0.01 |
| SE(d) | 0.01 | 0.01 | 0.01 |
| SE(m) | 0.01 | 0.01 | 0.01 |
| C.V | 2.5 | 1.8 | 1.86 |

* mean of three replications

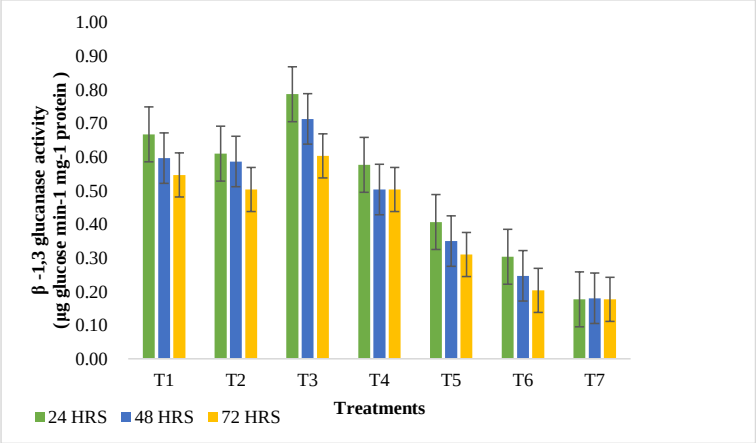


**Fig 1. Beta-1,3 glucanase enzyme activity in different treatments**

{The vertical bar in the figures indicates activity profile of defense related enzymes of control and different treatments after inoculating with *P. grisea* (infected) leaf Samples}.

Patil et al., (2016) reported in finger millet that PGPR enzymatic activity of β 1,3- glucanase was elevated at 24hpi. Radjacommare (2004) also reported maximum activity of β- 1,3- glucanase after pathogen inoculation at 24 hrs in finger millet. A study conducted by Qi and Yang (2002) on the quantification of fungal growth at various time intervals using quantitative PCR (qPCR) indicated that the formation of appressoria and the growth of hyphae in host plants are at their maximum at 24 hours post-inoculation (hpi).The current study demonstrates that at 24 hours post-inoculation (hpi), enzyme activity was markedly elevated, which correlates with the pathogen's penetration and colonization during the initial stages of hyphal development. This suggests that Beta-1,3- glucanase plays an important role in controlling the pathogen which was also reported in Rice blast by Anushree et al., 2016.

**3.1.2 Chitinase**

Chitinase activity was measured spectrophotometrically at 570 nm using DNS reagent as the indicator of activity. All the treatments showed superior enzyme activity over the control. The maximum chitinase enzyme activity was observed at 72 hrs after treatment followed by 48 hrs and least activity observed at 24 hrs after imposing treatments. Among those treatments ,T3

( foliar spray with neem leaf extract 1500 ppm) showed a profound increase in the enzyme activity at all the time intervals with a reading of 0.60, 0.65 and 0.78 µg glucose $min^{-1}$ $mg^{-1}$ protein at 24, 48 and 72 hrs after treatment imposition respectively. And this followed by $T_1$ (Seedling root dip with B1 $1.5\times10^8$ $CFUml^{-1}$) with a reading of 0,59, 0.61 and 0.68 µg glucose $min^{-1}$ $mg^{-1}$ protein at 24, 48 and 72 hrs after treatment imposition respectively. Whereas in the unchallenged control ($T_7$) chitinase activity remained more or less constant at all the time interval **(Table 2, Figure. 2)**.

**Table 2. Chitinase enzyme activity (µg glucose $min^{-1}$ $mg^{-1}$ protein) at 24, 48 and 72 hours after treatment**

| Treatments | 24 HRS* | 48 HRS* | 72 HRS* |
|---|---|---|---|
| $T_1$:Seedling root dip with *Brevibacillus brevis* ($1\times 10^8$ $CFUml^{-1}$) | 0.59 | 0.61 | 0.68 |
| $T_2$:Foliar spray with *Brevibacillus brevis* ($1\times 10^8$ $CFUml^{-1}$) | 0.54 | 0.57 | 0.66 |
| $T_3$:Foliar spray with neem leaf extract at 1500 ppm | 0.60 | 0.65 | 0.78 |
| $T_4$:Foliar spray with Carbendazim12%+Mancozeb 63% WP at 500 ppm | 0.41 | 0.51 | 0.63 |
| $T_5$:Foliar spray with salicylic acid at 50µM | 0.44 | 0.48 | 0.55 |
| $T_6$:Challenged control | 0.20 | 0.27 | 0.32 |
| $T_7$:Unchallenged control | 0.15 | 0.15 | 0.15 |
| C.D | 0.02 | 0.02 | 0.02 |
| SE(d) | 0.01 | 0.01 | 0.01 |
| SE(m) | 0.01 | 0.01 | 0.01 |
| C.V | 2.98 | 2.44 | 2.53 |

* mean of three replications

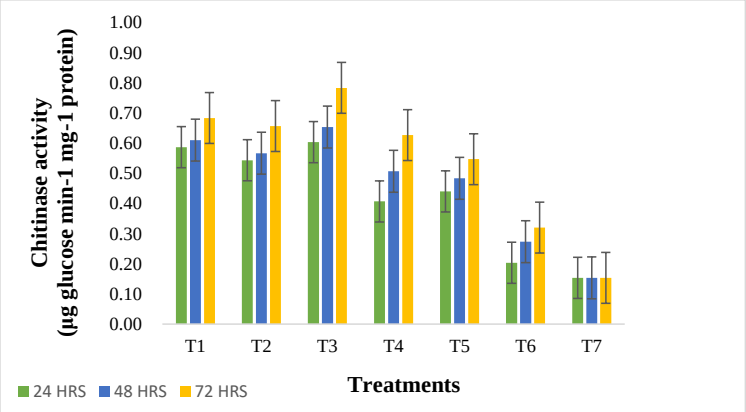


**Fig 2. Chitinase enzyme activity in different treatments**

{The vertical bar in the figures indicates activity profile of defense related enzymes of control and different treatments after inoculating with *P. grisea* (infected) leaf Samples}.

Time course studies on Chitinase accumulation in finger millet plants by Patil et al. (2016) demonstrated a significant increase in enzyme activity at post inoculation, while the similar trend was similarly observed in the current study. Anushree et al. (2016) also reported the increased levels of chitinase activity in *P. grisea* inoculated rice plants compared to control plants. Another study demonstrated that the chitinase enzyme can positively regulate the hypersensitive and defense responses of *Capsicum annuum* L. to infections caused by *Colletotrichum acutatum* (Ali, M et al. 2020).

**3.1.3 Lipoxygenases**

The LOX activity was measured at 234 nm spectrophotometrically at 24, 48 and 72 hrs after treatments. It was observed that LOX activity showed an increased trend from 24 hrs to 48 hrs, where it reached the peak activity and then started decreasing. The foliar spray with neem leaf extract at 1500 ppm showed a peak LOX reading at 48 hrs with a value of 474.62 µmol of HPO/min followed by seedling root dip with bacterial antagonist, *Brevibacillus brevis* (416.00 µmol of HPO/min). Whereas the unchallenged control showed a stable reading of 34.03 µmol of HPO/min during the course of experiment (**Table 3**, **Figure. 3**). A study conducted by Kotapati et al., (2014) reported an increase in the LOX activity at 48 hrs upon drought stress condition and decreased gradually after 48h in finger millet plants.

**Table 3. Lipoxygenase (LOX) enzyme activity (mol of HPO/min) at 24, 48 and 72 hours after treatment**

| Treatments | 24 HRS* | 48 HRS* | 72 HRS* |
|---|---|---|---|
| $T_1$:Seedling root dip with *Brevibacillus brevis* (1× $10^8$ CFUml−1) | 253.38 | 416.00 | 268.51 |
| $T_2$: Foliar spray with *Brevibacillus brevis* (1× $10^8$ $CFUml^{-1}$) | 217.46 | 385.74 | 264.73 |
| $T_3$:Foliar spray with neem leaf extract at 1500 ppm | 264.72 | 474.62 | 325.23 |
| $T_4$:Foliar spray with Carbendazim12%+Mancozeb 63% WP at 500 ppm | 149.38 | 245.82 | 166.40 |
| $T_5$:Foliar spray with salicylic acid at 50µM | 94.55 | 179.64 | 105.89 |
| $T_6$:Challenged control | 41.60 | 100.22 | 71.85 |
| $T_7$:Unchallenged control | 34.03 | 34.03 | 34.03 |
| C.D | 7.58 | 8.19 | 5.36 |
| SE(d) | 2.48 | 2.67 | 1.75 |
| SE(m) | 3.50 | 3.78 | 2.48 |
| C.V | 2.85 | 1.77 | 1.72 |

* mean of three replications

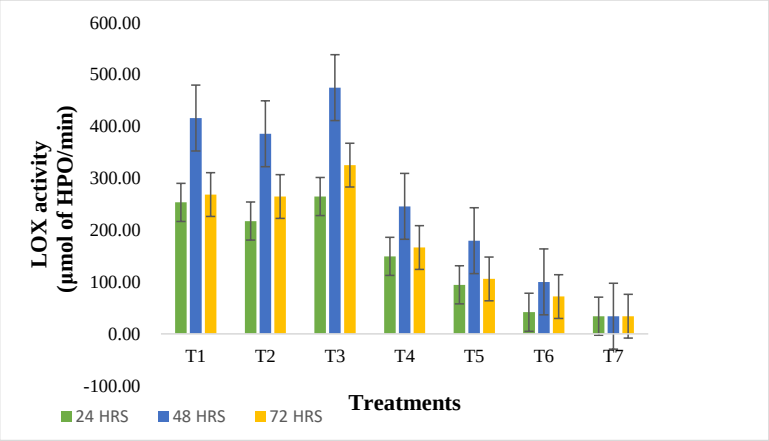


**Fig 3. Lipoxygenase enzyme activity in different treatments**

**{**The vertical bar in the figures indicates activity profile of defense related enzymes of control and different treatments after inoculating with *P. grisea* (infected) leaf Samples}.

Goel and Paul (2014) also reported the activities of LOX and POX upon treatment with neem extract in tomato plants against pathogen, *Pseudomonas syringae* pv. *tomato*, at 24 h intervals upto 5 days and disease severity was measured in the leaves emerging after 2 weeks of treatment. It was demonstrated that neem extract could significantly reduce disease severity in the treated plants by inducing activities of POX and LOX. Neem leaves are known to possess various bioactive compounds like azadirachtin, nimbin, and salannin. And these compounds can act as plant defense elicitors, which stimulate the plant's natural defense system. The neem compounds might activate signaling pathways within the plant, leading to the production of defense enzymes and other protective compounds.

Followed by the neem leaf extract, *Brevibacillus brevis,* a bacterial biocontrol agent reported the maximum enzyme activity. The various genera of Plant Growth-Promoting Rhizobacteria (PGPR) encompass species such as *Arthrobacter, Azospirillum, Bradyrhizobium, Bacillus, Pseudomonas, Paenibacillus, Achromobacter, Acinetobacter, Azotobacter, Herbaspirillum,* and *Gluconobacter* (Vacheron *et al.* 2013; Babu *et al.* 2015). PGPR promote disease resistance in plants by triggering specific physiological changes, such as the

accumulation of pathogenesis-related (PR) proteins like β-1,3-glucanase and chitinase, as well as the production of phytoalexins (Ramamoorthy et al. 2001).

Nehra et al. (2016) for the first time reported that *B. brevis* is a highly effective PGPR in cotton crops and encouraged for its widespread use to improve cotton growth and productivity. They reported phosphate solubilization, IAA production, acetylene reduction, and antifungal activity from *Brevibacillus brevis* SVC (II) 14 on cotton plants through various PGP traits.

SAR is effective against a wide variety of pathogens and relies on various plant hormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), or combinations of these (Thomma, 2001; Glazebrook, J. (2005); Grant, M 2006). The type of plant pathogen determines the level and effectiveness of both local and systemic resistance responses as well as the dependence on hormones (de Wit, 2007). SAR is closely linked with the production of pathogenesis-related (PR) proteins. When a plant is exposed to a pathogen infection, SAR is activated throughout the plant, leading to the enhanced production of PR proteins. They play a crucial role in plant defense mechanisms by inhibiting pathogen growth and spreading (Jones & Dangl, 2006). The induction of PR proteins is a key marker of SAR, providing long lasting protection against a broad spectrum of pathogens.

PR proteins include various enzymes such as chitinases, glucanases, and protease inhibitors, which degrade the cell walls of fungi and bacteria (Kini et al., 2000). During a fungal invasion, β-1,3 glucanases degrade the fungal cell wall, releasing oligomers known as β1,3/1,6-D-glucan. These oligomers act as elicitor oligosaccharides, triggering plant defense response and exhibiting direct antimicrobial activity (Finkina, 2017; Zribi, 2021). Plant β-1,3 glucanases in plant can work synergistically with chitinases, catalyzing the degradation of microorganisms' cell walls by hydrolyzing β-1,3 glucans and chitin, respectively and these enzymes, along with other hydrolases, are involved in breaking down cell membrane components, primarily in fungi (Perrot, 2022).

During pathogen–plant interactions, elicitor molecules are recognized, like β-1,3-glucanases, chitinases which can hydrolyze these elicitors, converting them into eliciting

oligosaccharides. The elicitors generated from chitin and β-1,3-glucanase activity can then activate a signaling network, leading to the activation of defense genes. These genes produce other PR proteins that accumulate and contribute to the degradation of pathogen cells (Finkina, 2017; Héloir, 2019; Jamiołkowska 2020; Ali Z, 2021).

Additionally, some PR proteins function as antimicrobial agents or signaling molecules to activate other defense pathways. Plants engineered to over express specific chitinase enzymes (family 190 from bean, tobacco, and rice exhibited enhanced resistance to fungal pathogens, indicates a direct link between high levels of chitinase production and fungal defense (Datta et al., 2001).

β-glucosidase an enzyme with a broad substrate specificity is a microbial cell wall degrading enzymes and have relationship to pathogenicity (Takeda et al., 2010). Plant β-glucosidase could play a crucial role in fungal defense by processing and releasing fungal glucan molecules that act as elicitors. These elicitors trigger a cascade of defense reactions within the plant, including the production of antifungal compounds like phytoalexins and phenylpropanoids. These compounds, generated by the breakdown of β-phenyl glucosides, can potentially restrict the spread of the blast fungus (P*. grisea*) in resistant varieties.

Yang, Jiang, Yan, and Zhu (2008) noted that microbial and fungal β-glucosidase are produced both extracellularly and intracellularly and are believed to be crucial in saccharifying cellulosic materials and obtaining nutrients by generating glucose. Plant β-glucosidase is involved in processing and releasing fungal glucan elicitors, which trigger a series of reactions in the host. These reactions include phytoalexin formation and the biosynthesis of phenylpropanoids and lignin-like phenol aglucones through the hydrolysis of β-phenyl glucosides. These aglucones are fundamentally fungitoxic and fungistatic, potentially limiting the spread of *P. grisea.*This induced resistance (IR) has the potential to manage pathogens or damaging factors, either entirely or to some extent (Chen et al., 2014).

Kumar et al. (2015) have reported the accumulation of β-1,3-glucanase, PAL, and chitinase enzymes in incompatible interactions of pearl millet. Filippi et al. (2011)

showed that stimulating the production of these β-glucosidase enzymes in rice plants through soil drenching with specific rhizobacteria led to the suppression of rice blast fungus *Magnaporthe oryzae*. Marla (2012) reported the role of LOX genes in plant development and during attack by blast pathogen *Magnaporthe grisea* in rice . The enhanced defense response of oxylipins was observed through PCR amplification of the OsLOX3 gene following inoculation with virulent strains of *M. grisea* and ectopic application of methyl jasmonate to injured leaf tissue in adult rice plants.

Plant growth-promoting rhizobacteria can enhance plant performance by triggering systemic defense responses, providing broad-spectrum resistance to plant pathogens. During pathogen attack, certain structural modifications occur that help to strengthen the cell wall against pathogen entry by depositing various compounds (Jankiewicz and Koltonowicz 2012). These modifications include the enhanced synthesis of secondary metabolites such as phytoalexins and phenolic compounds by *P. aeruginosa*, and enzymes like phenylalanine ammonia lyase, chitinase, β-1,3-glucanase, peroxidases, and polyphenol oxidase by *Bacillus pumilus* in different plant species against various pathogens (Jankiewicz and Koltonowicz 2012). Various beneficial microbe associated molecular patterns (MAMPs) are detected by the plant, leading to a mild yet effective activation of the plant's immune responses in systemic tissues (van Wees et al. 2008).

Cell wall degrading enzymes such as chitinases and glucanases and various types of metabolites produed by antagonistic bacteria that can limit the growth of other microorganisms (Shoda, 2000). Particularly, *B. subtilis* strains are known to secrete an array of low molecular weight antibiotic lipopeptides such as fengycin, surfactin and iturin with amphiphilic features. Fengycins, a type of plipastatin, and A, B, or C fengycin (Wang et al., 2015) are less hemolytic than surfactins and iturins but have strong antifungal activity and limit the growth of fungi and bacteria (Ongena and Jacques, 2008).

Ramanathan et al., (2002) conducted green house study to determine the induction of systemic defense enzymes in ragi plants following treatment with *P. fluorescens* and to study their role in imparting resistance against *Magnaporthe grisea*. The maximum activities were observed when plants were treated with PF 1 and such activities of chitinase and β- 1-3 glucanase were observed to be maximum at 4 days after treatment and thereafter the activities were found decreased . Similarly, maximum amount of polyphenol oxidase and peroxidase activities reached maximum at 2 days after treatment.

Krause et al. (2003) screened bacteria isolated from two composts for their capacity to elicit systemic protection against *Xanthomonas campestris* pv. a*rmoraciae.* Bargabus *et al.* ( 2002 & 2004) identified two strains of *B. pumilus* (strains 203-6 and 203-7) and one strain of *B. mycoides* (strain Bac J) that reduced the severity of Cercospora leaf spot in sugar beets, caused by *Cercospora beticola* Sacc.

## 4. CONCLUSIONS

The Defense enzymes such as β-1,3-glucanases, chitinases and lipoxygenases play a major role in combating the pathogen by the biosynthesis of phytoalexins and other defense related compounds, and also play a key role in the signaling pathway. In this study it is concluded that the maximum defense enzyme activity was observed in neem leaf extract treated plants. Studies on accumulation of these defense enzymes over time revealed that β-1,3-glucanase reached peak activity at 24 hours, chitinase reached maximum activity at 72 hours and lipoxygenase reached maximum enzyme activity at 48 hours after imposing treatments. And among the treatments, neem leaf extract and inoculation with *Brevibacillus brevis* recorded maximum acivity, indicating the role of PGPR bacteria in defense reaction. By elucidating the plant's defense mechanisms against *P. grisea* infection and the mechanisms by which the pathogen damages the host, we can develop novel strategies to control blast disease.

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