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# ANTAGONISTIC ROLE OF FUNGAL BIOCONTROL AGENTS FOR SUSTAINABLE MANAGEMENT OF RICE BLIGHT CAUSED BY *Curvularia lunata*

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## ABSTRACT

**Plant growth promoting fungi (PGPF) have received much attention in recent past due to their plant growth promoting ability and antagonistic activity against disease causing pathogens. They can suppress the pathogen directly by antagonistic mechanism or indirectly by inducing systemic resistance. The present study was aimed to assess the biocontrol efficacy of rhizospheric fungi, isolated from rice fields, against rice blight pathogen *Curvularia lunata.* Dual culture method was used to assess the antagonistic potential of isolates against the pathogen. Out of the 20 isolates, 14 isolates exerted positive effects against *C. lunata* in dual culture. SEM studies of interacting zone between the pathogen and isolates in dual culture revealed the mycoparasitic property of *Trichoderma aureoviride* TaN16 and *Aspergillus niger* AnD6 against the pathogenic fungus. These two isolates were found associated with HCN production. Three fungal isolates *viz., T. aureoviride* TaN16, *T. yunnanense* TaN17 and *Talaromyces purpureogenus* TpG11 triggered the induction of defense enzymes like phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase. The study revealed that the rhizospheric fungal isolates having biocontrol potential can serve as alternative to chemical fungicides under integrated disease management.**

**Keywords:** Antagonism, defense enzymes, hydrogen cyanide, plant growth promoting fungi, rhizosphere

## INTRODUCTION

There is increasing demand for rice production to meet the food supplication for worldwide escalating human populace. One of the strategies to achieve this target is the use of chemical fertilizers, fungicides, and pesticides. However, the chemical use has detrimental effect on the environment and living beings including plants, animals, and microbes (Habibah, 2011; Kalyabina *et al.*, 2021). Additionally, the long-term use of fungicides and pesticides generates resistance in pathogen (Aktar *et al.*, 2009; Lalève *et al*., 2014).

Plant growth promoting fungi (PGPF) is a group of fungi that can improve plant growth and productivity, in addition to inducing defense response against plant pathogen (Hossain *et al.*, 2007; Muslim *et al.*, 2019). These organisms reportedly muffle the plague of pathogens through several approaches. These approaches include i) induced systematic resistance (ISR) by which resistance level of host plant to pathogen is increased by PGPF in presence of jasmonic acid and ethylene; ii) mycoparasitism wherein the fungi directly exploit the pathogens as a prey. The mycoparasitism process involves the chemotropic growth of biocontrol agent, recognizing signals from the host trigger coiling of mycelium to trap the pathogen, emit extracellular enzymes for penetration and lysis of pathogenic hyphae (Moreno-Ruiz *et al.*, 2020). The process of competition involves

competition of biocontrol agents with phytopathogen for food and space for which there is a rapid bioagent growth to have control over the niche (Benítez *et al.,* 2004). The antibiosis where the antimicrobial compounds released by the biocontrol agents slow down or completely inhibit the growth of phytopathogens (Mendoza *et al.*, 2015). The PGPF also show antagonism against pathogens through cyanide production (Jogaiah *et al.,* 2013; Reithner *et al.,* 2011; Zhang *et al.*, 2018). Hydrogen cyanide (HCN), a volatile secondary metabolite, might cause the death of phytopathogen by disturbing the electron transport and interrupting energy supply to the cell (Abd El-Rahman *et al*., 2019). For their antagonistic properties, PGPF can be utilized as alternative to chemical fungicides in modern agricultural practices. Limlong *et al.* (2020) reported that yeasts *Torulaspora indica* DMKU-RP31 and *Wickerhamomyces anomalus* YE-42 could completely control rice seedling rot disease caused by *Curvularia lunata* and *Helminthosporium oryzae.* The present work was aimed to assess the role of some rhizospheric fungi against rice blight pathogen *C. lunata.*

## MATERIALS AND METHODS

### *Collection of rhizospheric soil and disease samples*

Rhizospheric soil from the roots of rice plants was collected in sterile polythene bags from different rice fields of Uttar Dinajpur, West Bengal (India). Besides, blighted rice grains were collected from the infected paddy fields of Raiganj (25.6329° N, 88.1319° E), Uttar Dinajpur and brought to the Mycology and Plant Pathology Laboratory, Raiganj University.

### *Isolation of fungal isolates*

The rhizospheric fungi was isolated following the serial dilution method of Johnson and Curl (1972). One milliliter of soil suspension of $10^{-4}$ and $10^{-5}$ dilution were poured aseptically into the Petri-dishes containing potato dextrose agar (PDA) amended with antibiotic Monocef (2.5 mg $mL^{-1}$), spread uniformly and sealed with parafilm. The Petri-dishes were incubated at $28^0$C for 3-7 days. The fungal colonies that appeared were picked up carefully and transferred into freshly prepared PDA slants/plates and incubated at $28\pm2^0$C for 7 days. The PDA slants were stored at $-4^0$C till further use. Twenty isolates were identified on their morphology characteristics and their identity got confirmed by Indian Type Culture Collection (ITCC), New Delhi (India) and National Fungal Culture Collection of India (NFCCI), Pune (India).

### *Isolation of pathogen*

Blighted rice seeds bearing conidia of the pathogen, viewed under microscope, were washed with distilled water, treated with 0.1% mercuric chloride for 2-3 min and rinsed with sterile distilled water. Samples were again treated with 70% ethanol for 1 min and washed four times with sterile distilled water. The diseased parts were aseptically air-dried for 5 min and cut into small bits. Diseased tissue bits were transferred to semi-solid PDA plates and incubated at 28±2°C until the desired growth of fungus was obtained. The fungal colonies that appeared on PDA plates were immediately transferred into freshly prepared PDA slants and incubated at 28±2°C for 7 days and stored at -4°C till further use. The pathogen was identified on the basis of morphology and submitted to Indian Type Culture Collection (ITCC) under Accession No 11,206.19.

### *Preparation of fungal inocula*

**Spore suspension**: The fungal isolates and pathogen were multiplied separately at 23±2°C for 10 days in 250 mL Erlenmeyer flasks containing 200 mL potato dextrose broth (PDB). After incubation, the culture broth was centrifuged at 10,000 rpm for 10 min and the pellets suspended in sterile distilled water and washed 3-4 times. Each washed fungal pellet was made into a turbid solution using sterile distilled water. The optical density of solution was adjusted to 0.45 at 610 nm to obtain $1x10^8$ cfu $mL^{-1}$ (Sudisha *et al.*, 2006).

**Formulation of maize granule inocula (MGI):** For the preparation of MGI of PGPF, maize grains (1 kg) were crushed in a mixer grinder for 2-3 min and then granules were semi-boiled for 5 min. The air-dried maize granules were amended with calcium carbonate (2%) and calcium sulphate (1%) at pH 6.48. The mixture was then poured in 500 mL Erlenmeyer flask (1/4), plugged with non-absorbent cotton, wrapped, and autoclaved. The autoclaved flasks were inoculated with mycelial discs (5 mm) taken from the actively growing margins of 7 days old cultures of fungal isolates. After 12-15 days incubation, the completely colonized maize granules were used as maize granules inoculum (MGI) and stored at 4°C prior to use.

***In vitro assay of antagonistic activity***

Dual culture plate method was performed to assay antagonistic activity of fungal isolates (Coşkuntuna and Özer, 2008). Fungal discs (5 mm) of pathogen and test organism were placed at opposite sides of PDA plate and incubated at 28±2°C for 7 days. All the 20 isolates were tested against the pathogen by dual culture technique. Percent inhibition of radial growth (PIRG) was calculated by using the formula of Fokkema (1976).

$$PIRG = (C - T)/C \times 100$$

Where, C and T are the growth diameter of pathogen in control and dual plate, respectively.

***Scanning electron microscopy (SEM)***

The zone of interaction between the antagonistic fungus and pathogen were examined under a scanning electron microscope (make JEOL, model JSM IT100).

***In vitro assay of hydrogen cyanide (HCN) production***

Fungal isolates were screened for HCN production as per Lorck (1948). Discs (5 mm) of each fungal isolate from 7 days old culture was inoculated on Petri-dishes having sterilized PDA amended with glycine (4.4 g $L^{-1}$). Whatman No. 1 filter paper previously soaked in a specific solution [0.5% picric acid and 2% sodium carbonate (w/v)] was properly placed under the lid of each Petri-dish. The plates were then sealed tightly with parafilm paper and incubated at 25±2°C for 7 days. After incubation, the appearance of orange to red colour on Whatman paper No.1 indicated the production of HCN. Three replicates were maintained for each fungal isolate.

***Application of PGPF and challenged inoculation to assay defense enzymes***

MGI of PGPF (25 g) were inoculated in the root zone of 15 days old rice plants grown in big pots. The lower leaves of rice plants were challenge-inoculated with the conidial suspension ($1x10^8$ cfu $mL^{-1}$) of *Curvularia lunata*. Two control sets (treated-uninoculated and untreated-uninoculated) were also maintained. The whole experiment was performed in a glass chamber to avoid any contamination. Untreated and uninoculated upper leaves of rice plants were sampled after 0, 2, 4, 6 and 8 days following the challenged inoculation of *C. lunata* for enzyme assay.

***Phenylalanine ammonia lyase (PAL) assay***

PAL activity was assayed by determining the conversion rate of L-phenylalanine to trans-cinnamic acid at 290 nm (Sadasivan and Manickam, 1996). For this, leaf tissue (1 g) was homogenized in 5 mL of 0.25 M borate buffer, at pH 8.7 containing 0.1 g insoluble polyvinylpyrrolidone (PVP) in ice-cold mortar and pestle. The homogenate was filtered through four-layered muslin cloth and centrifuged (12000 g) at 4°C for 15 min. The yellowish green supernatant was used as crude enzyme extract. The reaction mixture containing 0.2 mL distilled water and 1.0 mL 0.1M L-phenylalanine was incubated at 30°C for 30 min. The reaction was stopped by adding 0.5 mL of 1 M trichloroacetic acid. The absorbance was noted at 290 nm by using a UV-VIS spectrophotometer (LASANY, model LI 2700). The enzyme activity was expressed on fresh weight basis as μmol $min^{-1}$ $g^{-1}$ using trans-cinnamic acid as standard.

***Polyphenol oxidase (PPO) assay***

PPO activity was determined as per the method of Sadasivan and Manickam (1996). Rice leaves (1 g) were homogenized in 5.0 mL of 50 mM tris-HCl buffer, pH 7.2 containing 0.4 M sorbitol and

1.0 mM NaCl. The homogenate was centrifuged (12000 g) at 4°C for 10 min and supernatant used as crude source for polyphenol oxidase. In a cuvette, 2.5 mL of 0.1 M sodium phosphate buffer, pH 6.5 and 200 µL crude enzyme were mixed. The cuvette was placed in a UV-VIS spectrophotometer (LASANY; model LI 2700) and the initial reading was adjusted to zero at 495 nm. Then 300 µL of 0.01M catechol was added and the changes in absorbance were recorded at 1 min interval up to 5 min. The PPO enzyme activity was calculated as:

PPO enzyme activity = K x ($\Delta$A $min^{-1}$) µmol $min^{-1}$ $g^{-1}$ fresh weight tissue (K = 0.272 for PPO)

***Peroxidase assay***

The peroxidase activity was assayed as per Ray *et al.* (1998). Rice leaves (1 g) were extracted in 5.0 mL of 0.1M sodium phosphate buffer (pH 6.5) in a pre-chilled mortar and pestle at 4°C. The homogenate was filtered through muslin cloth and centrifuged (6000 g) at 4°C for 20 min. The supernatant was used as crude enzyme. In a cuvette, 1.5 mL of 0.05M guaiacol and 100 µL crude enzyme were mixed and placed in a UV-VIS spectrophotometer. The initial reading was adjusted to zero at 465 nm. Then, hydrogen peroxide (100 µL) was added and the changes in absorbance were noted for 5 min at 1 min interval. Enzyme activity was expressed as µmol of peroxidase $min^{-1}$ $g^{-1}$ on fresh weight basis.

***Statistical analysis***

The statistical analysis related to enzyme activity was performed using Student's t-test with the help of a statistical package SigmaPlot 12.0.

## RESULTS AND DISCUSSION

***Identification of fungal isolates***

The identification of 20 rhizospheric fungal isolates was confirmed by ITCC, New Delhi and NFCCI, Pune (India). The details of identified isolates with their Accession No. are given in Table 1.

**Table 1: Identification of fungal isolates from Indian Type Culture Collection (ITCC), New Delhi and National Fungal Culture Collection of India (NFCCI), Pune**

| Isolate | Accession No. | Specimen |
|---|---|---|
| AnK1 | NFCCI 4966 | *Aspergillus niger* |
| AnR2 | ITCC 11,205.19 | *Aspergillus niger* |
| AnH3 | NFCCI 4967 | *Aspergillus niger* |
| AnK4 | ITCC 11,204.19 | *Aspergillus niger* |
| AnD5 | ITCC 11,211.19 | *Aspergillus niger* |
| AnD6 | ITCC 11,204.19 | *Aspergillus niger* |
| TaK12 | ITCC 11,203.19 | *Trichoderma harzianum* |
| TaL13 | ITCC 11,209.19 | *Trichoderma asperellum* |
| TaM14 | ITCC 11,210.19 | *Trichoderma asperellum* |
| TaN15 | ITCC 11,212.19 | *Trichoderma asperellum* |
| TaN16 | NFCCI 4962 | *Trichoderma aureoviride* |
| TaN17 | NFCCI 4964 | *Trichoderma yunnanense* |
| TpG11 | NFCCI 4965 | *Talaromyces purpureogenus* |
| PcK7 | ITCC 11,201.19 | *Penicillium pinophilum* |
| PcR8 | ITCC 11,207.19 | *Penicillum purpurogenum* |
| PcK9 | NFCCI 4959 | *Penicillium citrinum* |
| PcK10 | NFCCI 4960 | *Penicillium citrinum* |
| PcK11 | NFCCI 4961 | *Penicillium citrinum* |
| Fm | ITCC 11,208.19 | *Fusarium moniliforme* |
| Fp | NFCCI 4963 | *Fusarium pseudoanthophilum* |

***Growth inhibition of pathogen and antagonistic activity of PGPF***

Of the 20 fungal isolates tested 14 isolates showed antagonism against *Curvularia lunata* (Fig. 1**)**. Different isolates belonging to *Aspergillus niger, Penicillium citrinum* and *P. purpurogenum* showed competition against *C. lunata* inciting radial growth inhibition of 53.33 to 93.33 (Table 2; Fig. 2). Two isolates *viz.*, *Trichoderma aureoviride* TaN16, and *A. niger* AnD6 showed mycoparasitism wherein the fungi engulfed the pathogen with the coiling of hyphae. The SEM of interacting zone between *T. aureoviride* TaN16 and *A. niger* AnD6 with the pathogen *C. lunata*

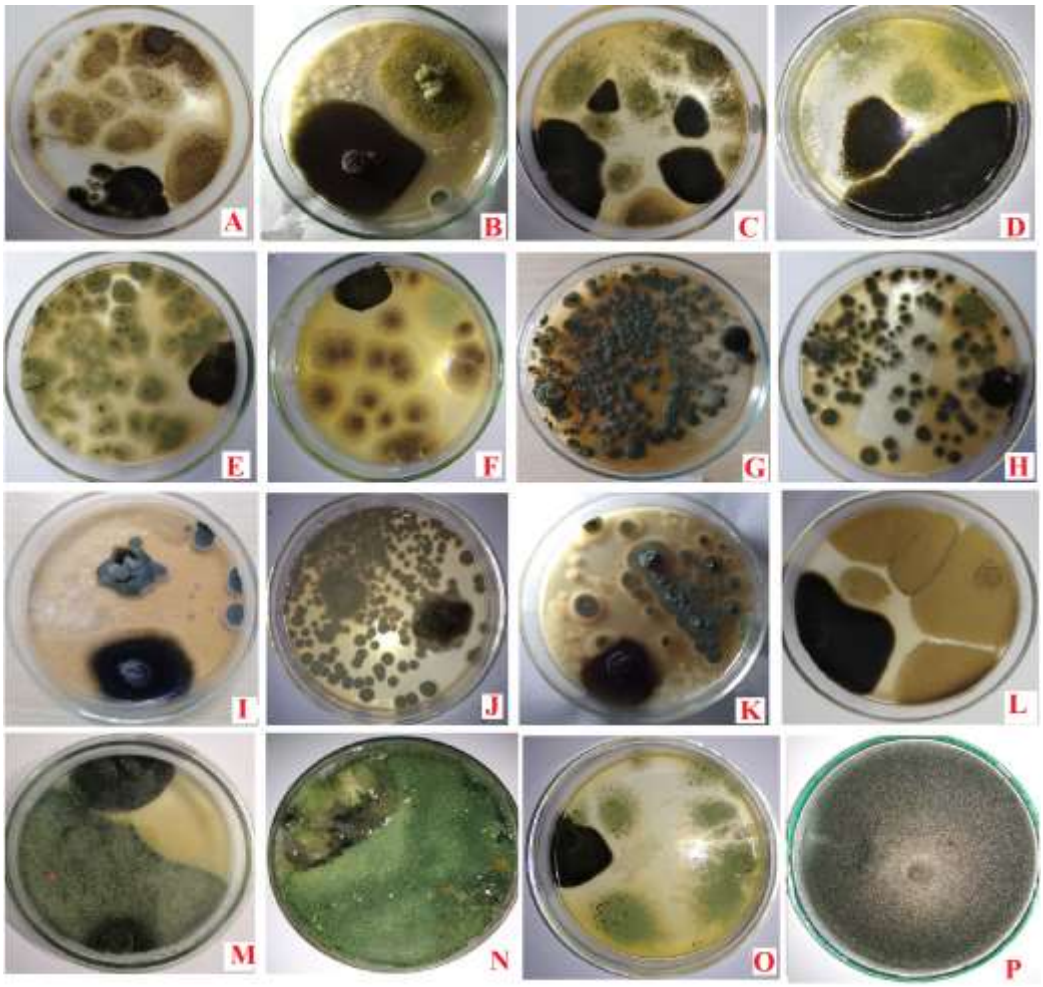


**Fig. 1: *In vitro* antagonistic activity of fungal isolates against *Curvularia lunata* inciting rice blight; A) *Aspergillus niger* AnK1, B) *A. niger* AnR2, C) *A. niger* AnH3, D) *A. niger* AnK4, E) *Trichoderma harzianum* TaK12, F) *A. niger* AnD6, G) *Penicillium pinophilum* PcK7, H) *P. citrinum* PcK9, I) *P. citrinum* PcK10, J) *P. citrinum* PcK11, K) *P. purpurogenum* PcR8, L) *Talaromyces purpureogenus* TpG11, M) *T. aureoviride* TaN16, N) *T. yunnanense* TaN17, O) *A. niger* AnD6, and P) *C. lunata* (control)**

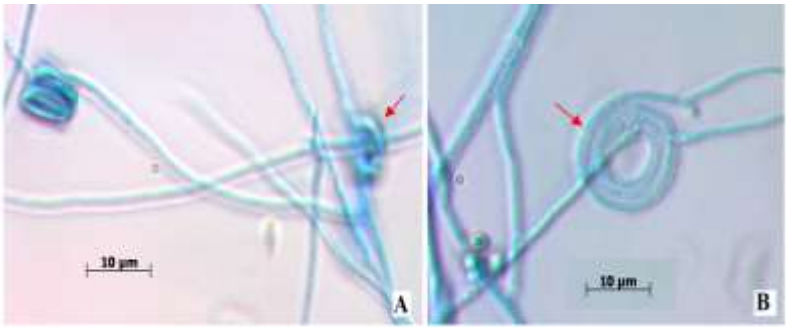


**Fig. 2: Mycoparasitism of fungal isolates by means of engulfing the pathogen *Curvularia lunata* with coiling hyphae (red arrow). A) *T. aureoviride* TaN16; B) *A. niger* AnD6**

confirmed mycoparasitic nature of these two isolates (Fig. 3). These findings are in line with Campos and Jacob (2021) who reported *A. niger* as a biocontrol agent against *Fusarium verticillioides.* Choi and Ahsan (2022) reported that *Aspergillus terreus* ANU-301 displayed efficacy against tomato Fusarium wilt and potato soft rot pathogens. *A. piperis* lysed the cell wall of phytopatho-genic fungi *Fusarium oxysporum* f. sp. *fabae* and *Sclerotinia sclerotiorum* (El-Debaiky and El-Badry, 2021). Three *Trichoderma* species *viz.*, *T. aureoviride* TaN16, *T. yunnanense* TaN17 and *T. harzianum* TaK12 exhibited antagonism

**Table 2: Antagonistic activity of fungal isolates against *C. lunata***

| Fungal isolates | Mean radial growth inhibition (%) | Antagonistic mechanism |
|---|---|---|
| *Aspergillus niger* AnK1 | 75.00 ± 5.8 | Competition |
| *A. niger* AnR2 | 53.33 ± 4.2 | Competition |
| *A. niger* AnH3 | 74.44 ± 6.2 | Competition |
| *A. niger* AnK4 | 62.22 ± 6.9 | Competition |
| *A. niger* AnD5 | 88.88 ± 7.1 | Competition |
| *A. niger* AnD6 | 91.11 ± 8.7 | Mycoparasitism |
| *Penicillium pinophilum* PcK7 | 93.33 ± 8.8 | Competition |
| *P. citrinum* PcK9 | 92.22 ± 8.6 | Competition |
| *P. citrinum* PcK10 | 83.33 ± 7.8 | Competition |
| *P. citrinum* PcK11 | 86.66 ± 7.6 | Competition |
| *P. purpurogenum* PcR8 | 88.88 ± 6.8 | Competition |
| *Trichoderma aureoviride* TaN16 | 75.55 ± 6.9 | Mycoparasitism |
| *T. yunnanense* TaN17 | 80.00 ± 8.2 | Competition |
| *Talaromyces purpureogenus* TpG11 | 86.66 ± 7.1 | Antibiosis |

± SE; Three replicas of each treatment was prepared

against the test pathogen in our study. Earlier reports support our findings on antagonistic activity of *Trichoderma* spp. against fungal pathogens (Benítez *et al*., 2004; Kumar and Khurana, 2021; Ramírez-Valdespino and Orrantia-Borunda, 2021). *T. harzianum* GT3-2 has been reported effective in reducing the severity of rice blast caused by *Pyricularia oryzae* (Elsharkawy *et al.*, 2015). *T. atroviride* BC0584 was observed to act as a promising biocontrol agent against the pathogens like *Fusarium avenaceum* and *F. culmorum* that cause damping-off in maize (Coninck *et al.*, 2020). *T. asperellum* also showed biocontrol efficacy against tomato bacterial wilt by *Ralstonia solanacearum* (Konappa *et al.*, 2018).

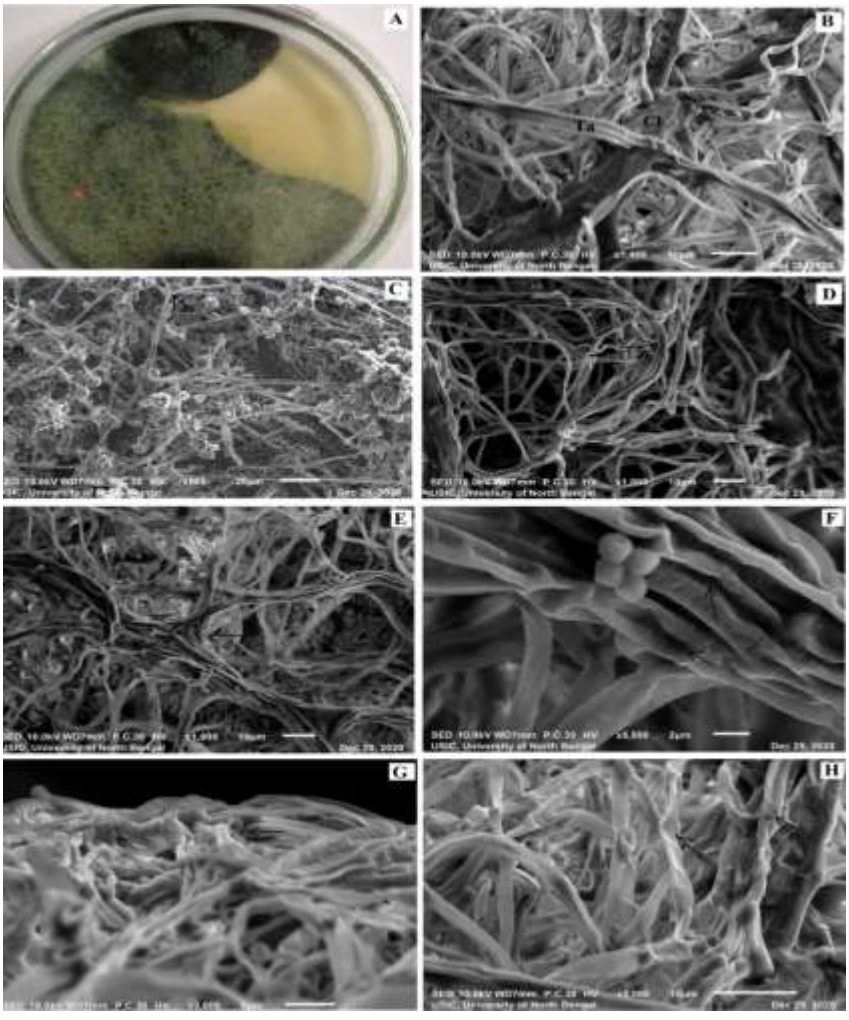


**Fig. 3: The scanning electron micrograph (SEM) of mycelial inhibition zone between *Curvularia lunata* and *Trichoderma aureoviride* TaN16 on dual culture exhibiting several abnormal features. A) Dual culture assay in Petri-plate; B) The mycelia of *C. lunata* and *T. aureoviride* TaN16; C-E) show mycoparasitism of PGPF on pathogen (in arrows); F) shows empty mycelium of pathogen; G) shows breakage of the mycelium of pathogen (in arrows); H) shows shriveling of pathogenic mycelium; Cl = *C. lunata*; Ta = *T. aureoviride* TaN16.**

***Hydrogen cyanide (HCN) production***

*Talaromyces purpureogenus* TpG11 showed highest HCN production, followed by *T. aureoviride* TaN16. A very low HCN production was observed in *Aspergillus niger* AnK1. Other isolates did not show any HCN production (Table 3; Fig. 4). HCN is recognized as a biocontrol product due to its toxicity against plant pathogens by disturbing the electron transport and interrupting energy supply to the cell (Abd El-Rahman *et al.*, 2019). This findings are in agreement with Potshangbam *et al.* (2017) who reported HCN production by several endophytic fungi of paddy and maize.

**Table 3: *In vitro* hydrogen cyanide (HCN) production by fungal isolates collected from rice rhizosphere**

| Fungal isolate | Degree of HCN production |
|---|---|
| *Penicillium pinophilum* PcK7 | - |
| *P. citrinum* PcK9 | - |
| *P. citrinum* PcK11 | - |
| *Aspergillus niger* AnK1 | + + |
| *Trichoderma aureoviride* TaN16 | +++ |
| *Talaromyces purpureogenus* TpG11 | + + + |
| Control | - |

– = No HCN production; + = Less HCN production; ++ = Moderate HCN production; +++ = High HCN production

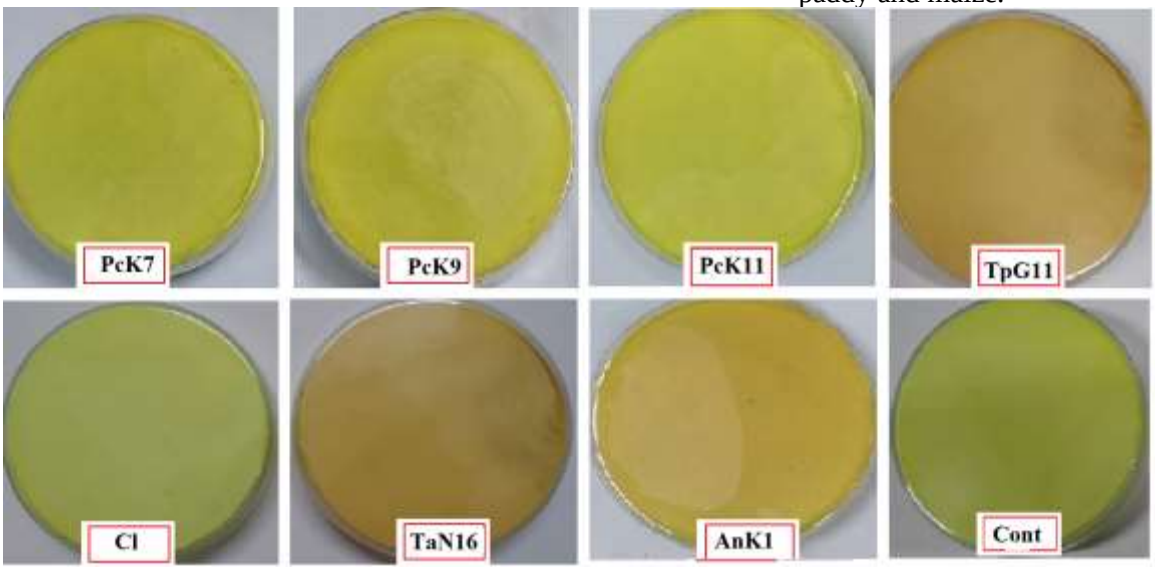


**Fig. 4: *In vitro* production of hydrogen cyanide (HCN) by fungal isolates collected from rice rhizosphere. PcK7-*Penicillium pinophilum*, PcK9- *Penicillium citrinum*, PcK11- *Penicillium citrinum*, TpG11- *Talaromyces purpureogenus*, Cl- *Curvularia lunata*, TaN16-*Trichoderma aureoviride*, AnK1- *Aspergillus niger*, Cont- Control set**

***Assays of defense enzymes***

About 3 to 4-fold increase in phenylalanine ammonia-lyase (PAL) activity was observed after 6 days of treatment in case of *T. aureoviride* TaN16 and *T. purpureogenus* TpG11 followed by challenged-inoculation with *C. lunata* (Table 4). In comparison to the treated-uninoculated plants, the treated plants challenge-inoculated with *C lunata* showed higher enzyme activity. Highest PAL induction was observed in the plants treated with *T. aureoviride* TaN16, followed by the plants treated with *T. purpureogenus* TpG11. Untreated plants inoculated with *C. lunata* did not show any significant increase in PAL activity after 4 days of inoculation. PAL activity increased gradually up to 6$^{th}$ day after challenge-inoculation with *C. lunata* and then declined. No change in enzyme activity was seen in untreated-uninoculated control sets.

Polyphenol oxidase (PPO) showed 3-4-fold surge in its activity after 6 days of treatment with *T. aureoviride* TaN16 and *T. purpureogenus* TpG11 followed by challenged inoculation with *C. lunata* (Table 5). Treated plants that were challenge-inoculated with *C. lunata* showed higher enzyme

**Table 4: Induction of phenylalanine ammonia lyase activity (µmol $min^{-1}$ $g^{-1}$ fresh tissue) of fungal isolates following the challenged inoculation with *Curvularia lunata* on rice plant**

| Treatments* | Days after inoculation | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| Control | $3.5 \pm 0.02^a$ | $3.6 \pm 0.00^a$ | $3.5 \pm 0.02^a$ | $3.6 \pm 0.02^a$ | $3.7 \pm 0.02^a$ |
| *C. lunata* (Cl) | $3.5 \pm 0.03^a$ | $4.4 \pm 0.02^b$ | $5.6 \pm 0.03^b$ | $4.2 \pm 0.03^b$ | $3.0 \pm 0.02^a$ |
| TaN16 | $3.6 \pm 0.02^a$ | $4.8 \pm 0.03^b$ | $5.2 \pm 0.01^b$ | $4.6 \pm 0.00^b$ | $4.5 \pm 0.02^b$ |
| TaN16 + Cl | $3.4 \pm 0.01^a$ | $4.2 \pm 0.02^b$ | $7.7 \pm 0.02^b$ | $13.6 \pm 0.03^b$ | $10.8 \pm 0.03^b$ |
| TpG11 | $3.4 \pm 0.02^a$ | $4.6 \pm 0.03^b$ | $4.8 \pm 0.02^b$ | $5.7 \pm 0.02^b$ | $4.7 \pm 0.02^b$ |
| TpG11 + Cl | $3.7 \pm 0.03^a$ | $4.7 \pm 0.02^b$ | $9.7 \pm 0.03^b$ | $12.8 \pm 0.03^b$ | $8.4 \pm 0.03^b$ |
| PcK9 | $3.4 \pm 0.02^a$ | $4.6 \pm 0.01^b$ | $4.8 \pm 0.02^b$ | $5.4 \pm 0.02^b$ | $5.1 \pm 0.02^b$ |
| PcK9+ Cl | $3.7 \pm 0.03^a$ | $4.2 \pm 0.02^b$ | $4.3 \pm 0.01^b$ | $5.2 \pm 0.02^b$ | $4.7 \pm 0.01^b$ |

*TaN16 = *Trichoderma aureoviridae* TaN16 TpG11= *Talaromyces purpurogenum* TpG11 PcK9 = *Penicillium citrinum* PcK9; The values are mean ± SE; The values superscripted with the same letters do not differ from each other at p = 0.05 (t-test)

activity as compared to the treated-uninoculated plants. Maximum PPO induction was found in plants treated with *T. purpureogenus* TpG11, followed by *T. aureoviride* TaN16. The untreated plants inoculated with *C. lunata* showed trivial rise in PPO activity after 4 days of inoculation. Steady increase in PPO activity was noticed till 6$^{th}$ day of inoculation with *C. lunata* and then dropped. No change in enzyme activity was recorded in untreated-uninoculated control.

**Table 5: Induction of polyphenol oxidase activity {K x ($\Delta A_{495}$ $min^{-1}$) µmol $min^{-1}$ $g^{-1}$ fresh tissue (K= 0.272 for PPO)] with different fungal isolates following challenged inoculation with *Curvularia lunata* on rice plant**

| Treatments* | Days after inoculation | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| Control | $3.2 \pm 0.02^a$ | $3.8 \pm 0.02^a$ | $3.1 \pm 0.02^a$ | $3.3 \pm 0.01^a$ | $3.4 \pm 0.02^a$ |
| *C. lunata* (Cl) | $3.7 \pm 0.01^a$ | $4.6 \pm 0.01^b$ | $5.5 \pm 0.01^b$ | $4.4 \pm 0.02^b$ | $3.4 \pm 0.01^a$ |
| TaN16 | $3.8 \pm 0.01^a$ | $4.4 \pm 0.01^b$ | $5.6 \pm 0.02^b$ | $6.8 \pm 0.03^b$ | $5.3 \pm 0.02^b$ |
| TaN16 + Cl | $3.9 \pm 0.02^a$ | $5.4 \pm 0.02^b$ | $8.6 \pm 0.02^b$ | $11.4 \pm 0.03^b$ | $9.8 \pm 0.03^b$ |
| TpG11 | $3.7 \pm 0.02^a$ | $4.8 \pm 0.01^b$ | $5.5 \pm 0.01^b$ | $6.4 \pm 0.01^b$ | $5.8 \pm 0.02^b$ |
| TpG11 + Cl | $3.7 \pm 0.01^a$ | $5.6 \pm 0.02^b$ | $8.9 \pm 0.03^b$ | $12.5 \pm 0.03^b$ | $8.5 \pm 0.01^b$ |
| PcK9 | $3.3 \pm 0.02^a$ | $4.3 \pm 0.02^b$ | $5.2 \pm 0.02^b$ | $5.5 \pm 0.02^b$ | $4.5 \pm 0.02^a$ |
| PcK9 + Cl | $3.7 \pm 0.01^a$ | $4.2 \pm 0.01^b$ | $5.3 \pm 0.01^b$ | $5.7 \pm 0.02^b$ | $6.5 \pm 0.01^b$ |

*TaN16 = *Trichoderma aureoviridae* TaN16 TpG11 = *Talaromyces purpurogenum* TpG11 PcK9 = *Penicillium citrinum* PcK9; The values are mean ± SE; The values superscripted with the same letters do not differ from each other at p = 0.05 (t-test)

About 2-fold hike was observed in peroxidase activity (POX) 4-6 days after treatment with *T. aureoviride* TaN16 and *T. purpureogenus* TpG11 followed by challenged inoculation with *C. lunata* (Table 6; Fig. 7). Treated plants challenge inoculated with *C. lunata* showed higher enzyme activity than treated-uninoculated plants. Maximum peroxidase induction was observed in plants treated with *T. purpureogenus* TpG11, followed by *T. aureoviride*. TaN16. Untreated plants inoculated with *C. lunata* showed slight increase in peroxidase activity after 4 days of inoculation. Like PAL and PPO, peroxidase activity was observed to increase gradually till 6$^{th}$ day followed by challenge-inoculation with *C. lunata* after which the activity was seen to decline. No change in enzyme activity was noted in untreated-uninoculated control sets.

PGPF play crucial role in the induction of defense enzymes such as PAL, PPO and POX against plant pathogens (Pieterse *et al.*, 2014). Numerous reports on induction of systemic resistance after the application of PGPF in agricultural practices are known (Bhandari *et al.*, 2021; Pieterse *et al.*, 2014; Pratap Singh *et al.*, 2021; Zin and Badaluddin, 2020).

**Table 6: Induction of peroxidase activity ($\Delta A_{465}$ min $^{-1}$ g $^{-1}$ fresh weight tissue; 1 unit = 0.001 absorbance) with different fungal isolates following challenged inoculation with *Curvularia lunata* on rice plant**

| Treatments* | Days after inoculation | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| Control | 72 ± 0.3$^a$ | 78 ± 0.2$^a$ | 74 ± 0.2$^a$ | 72 ± 0.2$^a$ | 76 ± 0.2$^a$ |
| *C. lunata* (Cl) | 71 ± 0.2$^a$ | 76 ± 0.2$^a$ | 85 ± 0.1$^b$ | 76 ± 0.1$^b$ | 76 ± 0.2$^a$ |
| TaN16 | 76 ± 0.1$^a$ | 85 ± 0.3$^b$ | 95 ± 0.2$^b$ | 87 ± 0.2$^b$ | 79 ± 0.1$^b$ |
| TaN16 + Cl | 75 ± 0.2$^a$ | 85 ± 0.2$^b$ | 135 ± 0.3$^b$ | 165 ± 0.3$^b$ | 143 ± 0.3$^b$ |
| TpG11 | 76 ± 0.2$^a$ | 83 ± 0.3$^b$ | 94 ± 0.2$^b$ | 83 ± 0.2$^b$ | 79 ± 0.2$^b$ |
| TpG11 + Cl | 76 ± 0.3$^a$ | 78 ± 0.2$^a$ | 125 ± 0.3$^b$ | 173 ± 0.3$^b$ | 162 ± 0.3$^b$ |
| PcK9 | 71 ± 0.2$^a$ | 77 ± 0.1$^a$ | 79 ± 0.2$^b$ | 75 ± 0.2$^b$ | 74 ± 0.2$^a$ |
| PcK9+ Cl | 73 ± 0.1$^a$ | 89 ± 0.2$^b$ | 99 ± 0.1$^b$ | 87 ± 0.1$^b$ | 86 ± 0.1$^b$ |

*TaN16 = *Trichoderma aureoviridae* TaN16 TpG11= *Talaromyces purpurogenum* TpG11 PcK9 = *Penicillium citrinum* PcK9; The values are mean ± SE; The values superscripted with the same letters do not differ from each other at p = 0.05 (t-test)

PAL acts as a key enzyme in phenyl-propanoid pathway for conversion of L-phenylalanine to trans-cinnamic acid in the first step in the synthesis of defense related compounds like lignin, phytoalexins such as isoflavonoids, and coumarins (Sohn *et al.*, 2021). PAL activity increases after external application of PGPF (Tiru, 2021). Recently Pratap Singh *et al.* (2021) reported that *Trichoderma* spp. facilitated defense response in brinjal against *Sclerotinia sclerotiorum.* PPO is one of the main defense-related enzymes in plant against phytopathogenic microbes that acts in phenol form on tannin and transform tannin to quinone form to impede pathogen (Mahadevan, 1996). POX is a stress-related defense enzyme which is induced in plants under environmental stress like heavy metals, salts, temperature (Mandal, 2019), and air pollution (Li, 2003). Gayoso *et al.* (2010) reported elevated activities of POX and PAL after interaction between tomato and *Verticillium dahlia*. Accumulation of PAL, PPO and POX mediated by *Streptomyces* was reported in rice against *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), causing rice bacterial leaf streak disease (Hata *et al*., 2021). Similar finding was also reported by Kagale *et al.* (2011) in interaction of rice plant with *Rhizoctonia solani*. Application of *Pseudomonas fluorescens*, *T. viride,* and *T. harzianum* as soil amendments to coconut plantation resulted in elevated levels of PAL, PPO and POX besides induced resistance against *Ganoderma* disease caused by *Ganoderma lucidum* (Lavanya *et al*., 2022). Recently, *Pseudomonas* spp. had been found to induce systemic resistance by enhanced production of PAL, PPO and POX in rice plants to *Rhizoctonia solani* that caused sheath blight disease (Elsharkawy *et al*., 2022).

**Conclusion:** PGPF play crucial role in plant protection acting against pathogenic microorganisms and can aid in sustainable crop production replacing the conventional harmful fungicides. The current study has led to the identification and exploration of PGPF which can prevent the pathogen as well as can trigger induced systemic resistance against rice blight pathogen *Curvularia lunata*. *Trichoderma aureoviride* TaN16 is found as most efficient isolate as it plays dual role of direct antagonism against the pathogen as well as induce resistance to plants. The isolated PGPFs being ecofriendly can be used in modern agricultural practices for integrated disease management to combat rice blight disease caused by *Curvularia lunata*.

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