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# Potential effects of plant growth promoting rhizobacteria (*Pseudomonas fluorescens)* on cowpea seedling health and damping off disease control

**S. Nandi[1,2]*#, A. Adhikari[1]#, S. Dutta[1], A. Chattopadhaya[1] and R. Nath[2]**

[1]All India Co-ordinated Research Project on Vegetable Crops, Directorate of research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India.
[2]Dept. of Plant Protection, PSB, Visva-Bharati, Sreeniketan, Bolpur, Birbhum, West Bengal, India.

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**Damping off caused by *Sclerotium rolfsii* on cowpea results in yield losses with serious socio-economic implication. Induction of defense responses by plant growth promoting rhizobacteria (PGPR) is largely associated with the production of defense enzyme phenyl ammonia lyase (PAL) and oxidative enzymes like peroxidases (PO) and poly phenol oxidase (PPO). In the present study, the effect of plant growth promoting rhizobacteria (*Pseudomonas fluorescence* (bv. V)) on both damping off development and growth parameters in cow pea seedlings were investigated. The best reduction in pre and post emergence damping off in cowpea seedlings was observed in BCPF 8-treated samples. Seed bacterization with BCPF 8 significantly increased peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) activities. The activation of these defense reactions by BCPF 8 was correlated with an enhanced resistance to damping-off caused by *S. rolfsii*. This study demonstrated the ability of the rhizobacteria BCPF 8 to induce systemic resistance in cowpea, suggesting that this legume is an Induced systemic resistance (ISR)-positive plant.**

**Key words:** Plant growth promoting rhizobacteria, vigour index, induction of systemic resistance, peroxidase, polyphenol oxidase, phenyl ammonia lyase, phenolics.

## INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is a food legume of significant economic importance worldwide. Cowpea diseases induced by species of pathogens belonging to various pathogenic groups (fungi, bacteria, viruses, nematodes, and parasitic flowering plants) constitute one of the most important constraints to profitable cowpea production in all agro-ecological zones where the crop is cultivated (Sendhilvel et al., 2005). Damping off of cowpea has been reported in many countries which can provoke 50 to 60% dry yield loss in new alluvial regions of West Bengal (Unpublished data, AICRP on Vegetable crops). *Sclerotium rolfsii* was by far the most common species isolated from all the agro-ecological zones and pathogenic on cowpea. Stress alleviation or disease control remains one of the most challenging issues to be addressed, which is especially true for cowpea considering the largely undefined area of cowpea self-defense mechanisms. Chemical fungicides application is the conventional strategy used for managing damping off for over 50 years. Though, fungicides have shown some promising results in controlling damping off, fungicide residues could lead to environmental pollution and human

*Corresponding author. E-mail: subhadipnandi87@gmail.com. Tel: +91 9433964327.

#Both authors contributed to this work.

**Table 1.** Seed treatments used in the study.

| Treatment number | Treatment name |
|---|---|
| **1** | Normal seeds in Non-infested soil (Control) |
| **2** | BCPF 7 in seed treatment+ *S. rolfsii* infested soil |
| **3** | BCPF 7 in seed treatment+ Non-infested soil |
| **4** | BCPF 8 in seed treatment+ *S. rolfsii* infested soil |
| **5** | BCPF 8 in seed treatment+ Non-infested soil |
| **6** | Carbendazim in seed treatment+ *S. rolfsii* infested soil |
| **7** | Carbendazim in seed treatment + Non-infested soil |
| **8** | Normal seeds in *S. rolfsii* infested soil |

health hazards. Biocontrol approaches may help to develop ecofriendly strategies for managing this disease in cowpea seedlings. Biological control represents both the oldest and youngest technology for the control of plant diseases and pest **(**Akinbode and Ikotun, 2008). Most people agree that agriculture could not have begun without the benefits of naturally occurring biological controls. Yet modern biological control achieved with introduced microorganisms is still in its infancy. The saprophytic pseudomonads associated with plants include *P. fluorescens*, *P. putida* and *P. aeruginosa*. The use of fluorescent pseudomonads is gaining importance for plant growth-promotion and biological control. Fluorescent pseudomonads could reduce disease severity in several crop plants through induced resistance phenomenon (Thahir Basha et al., 2012). Induced systemic resistance in crop plant is characterized by the induction of host-defense responses including, defense related enzymes synthesis and phenolics accumulation. In this context, we aimed to evaluate the biocontrol activity of some indigenous fluorescent pseudomonads against damping off disease in cowpea and to define the mechanisms implicated in this process.

## MATERIALS AND METHODS

### Fungal culture and inoculum preparation

Naturally infected cowpea plants as a source of *Sclerotium rolfsii* were collected from fields. Isolation was done by directly transferring mycelia and sclerotia of the fungus, found on the stem and collar of the infected plant to Potato Dextrose Agar (PDA- Hi-media) plates. All plates were kept at 28 ± 1°C in dark in an incubi-tor for 1 week. The inoculum for *S. rolfsii* was prepared by inoculating 50 g of sterile wheat grain medium in polyethylene bags with three 5 mm diameter fungal plugs and incubated at 24°C for 3 week. Colonized wheat grains were stored at 4°C until further use. Sterile wheat grains only were used as inoculums for the control treatment.

### Antagonistic activity of bacterial Isolates

Fluorescent pseudomonads designated as BCPF 7 and BCPF 8 were isolated from the soil collected from the rhizosphere of rice and chilli root respectively with King's medium B (KMB Hi-media) (King et al., 1954). The rhizobacterial isolates were characterized on the basis of their morphological (cell shape, cell arrangement, gram reaction), cultural (colony type, pigment production) and biochemical identification keys of Bossis (1995) for *Pseudomonas* sp. The antagonistic effects of the rhizobacterial isolates were assessed against *S. rolfsii* by a dual culture technique. The petri plates are poured with 20 ml PDA Hi-media (without antibiotic) and the fresh bacterial loopful culture was streaked linearly leaving 1 cm from the margin. The pathogens are placed as 5 mm disc from the 3 days old culture at the centre of each petri plate and plates were incubated at 28°C (±2°C) for 3 to 4 days. The distance between the fungal growth and the bacterial colonies was recorded as inhibition zone. For each treatment, three replications were used. Percent inhibition over control (inoculated with *S. rolfsii* disc, without bacterial streaking) was calculated by using the following formula:

$$I = \frac{C - T}{C} \times 100$$

Where, *I*, percent inhibition of mycelium; *C,* Growth of mycelium in control; *T*, Growth of mycelium in treatment. The rhizobacterial isolates were bio-assayed as vigor index for their ability to promote / inhibit seedling growth on cowpea using the method previously described by ISTA (1966).

### Seed treatment

Cowpea seeds (var. Kashi-kanchan) obtained from AICRP on Vegetable crop B.C.K.V, Kalyani, Nadia, West Bengal, India were surface sterilized for 30 s in 0.1% (w/v) mercuric chloride ($HgCl_2$), rinsed in 70% (v/v) ethanol for 3 min before rinsing three times in sterile distilled water. The efficacy of disinfection was tested by placing samples of the treated seeds on PDA (Hi-media) and Nutrient Agar (Hi-media) plates for any microbial growth (Table 1). Fluorescent pseudomonades were grown in Erlenmeyer flasks (250 ml) containing 100 ml of KMB broth (Hi-media) for 48 h on a rotary shaker at 28 ± 2°C. Cells were removed by centrifugation at 10 000 rmp for 10 min at 4°C and washed in sterile water. The pellet was resuspended in a small amount of sterile distilled water and then diluted with an adequate amount of sterile distilled water to obtain a bacterial suspension concentration of $10^8$ cfu $ml^{-1}$ ($OD_{595}$ = 0.3). For bacterization of seed, seeds of cowpea were surface sterilized with 0.1% (w/v) mercuric chloride ($HgCl_2$- Merk) for 30 s and rinsed in sterile distilled water and dried overnight under a sterile air stream. 10 ml of bacterial inoculums ($10^8$ cfu $ml^{-1}$) was put in a petri plate. To this, 100 mg of carboxymethylcellulose (CMC-Himedia) was added as adhesive agent. 1 g of seeds was soaked in 10 ml of bacterial suspension for 12 h and dried overnight in sterile petri plate. For fungicidal treatment, seeds were soaked in Carbendazim (0.1 $g.ml^{-1}$) for 30 min and the seeds soaked in sterile distilled water under aseptic conditions served as control.

**Table 2.** Determination of biovar and in-vitro antagonistic activity of bacterial isolates

| Bacterial isolate | Bio Var | Inhibition (%) | Zone of inhibition in cm (3 days after inoculation) | Vigour Index |
|---|---|---|---|---|
| BCPF 7 | III | 64.25 | 1.2 | 1938.25 |
| BCPF 8 | V | 70.05 | 1.7 | 2215.70 |

**Effect of different seed treatments on growth performance and disease incidence**

The experiment was carried out under glasshouse conditions with the daily temperature ranging from 28 to 30°C with 90% relative humidity (RH) during the study period. Planting trays with drain holes (39 × 28 × 11 cm), surface sterilized in 0.1% (w/v) mercuric chloride ($HgCl_2$) and rinsed with sterile distilled water were used to grow the plants. Each tray was filled with 2 kg of sterilized soil mixture (top soil: compost: sand = 3:2:1, v/v) amended with *S*. *rolfsii* inoculum (8 g $kg^{-1}$of soil). Infected soil was allowed to incubate for one week for the establishment of *S*. *rolfsii* in the soil. The soil moisture content was maintained at field capacity by daily watering. No supplementary fertilizer was added. The treatments were arranged in randomized block design with three replicates. The whole experiment was repeated twice. In Each treat-ment, 10 planting trays with 40 seeds per tray were used. Data on the seed germination was recorded on third day after sowing. Seedling length (cm), root length (cm), number of branches, leaves and nodules, fresh weights (g) were also recorded 25 days after sowing. Development of disease symptoms associated with *Sclero-tium rolfsii* damping off infection was observed and assessed based on the pre- (death of seedlings before they reached the surface of the soil) and post-emergence damping-off (wilting appearance) until seedling establishment. Infected seedlings were collected and the infection of *S. rolfsii* postulated by Koch's was confirmed. Disease development was expressed as disease incidence percent (DI, %) according to the formula: Disease incidence (%) = (Number of infected seedlings / Total number of seedlings assessed) × 100.

**Plant treatments for analysis of defense-related enzymes**

Another set of experiments was carried out under similar conditions to assess for a potential induction of defense related enzymes in cowpea plants. Seed treatment was carried out as mentioned above. Treated seeds were sown in plastic pots containing auto-claved mixture of peat-moss and soil. Treated seedlings (5 days old) were challenged inoculated by mycelial plug (0.2 g) method in collar region. Seedlings were planted at the rate of 5 transplants per pot and 10 pots per treatment were used. Sampling for induction of enzymatic activity was carried out at every 1 day after inoculation (DAI) for 10 days and the lesion length was recorded at different time intervals (days).

**Extraction and electrophoresis of different isoenzymes**

Tissue collected from collar region of cowpea seedlings was crushed with Sodium-Phosphate buffer (pH-7) for Peroxidase (PO), Sodium-Phosphate buffer (pH-6), for Poly-phenol oxidase (PPO) and Borate buffer (pH- 8.7), Phenyl ammonia lyase (PAL) and spectrophotometric assay respectively.

**Spectrophotometric assays**

Spectrophotometric assay of PO (EC 1.11.1.7), Poly Phenol Oxidase (PPO(EC 1.14.18.1)) was done by modifying the method of Malik and Singh (1980) and Hammerschmidt et al. (1982), respectively and expressed as changes in absorbance of fresh tissue per minute and phenyl ammonia lyase [PAL (EC 4.3.1.24 )] was done according to Dickerson et al. (1984) and enzyme activity was defined as µg cinnamic acid produced $min^{-1}$ $g^{-1}$ of tissue. Assay of phenol was done according to Malik and Singh (1980) and expressed as $mg^2$ per fresh $tissue^{-1}$. Each experiment was repeated three times.

**Extraction and electrophoresis of different isoenzymes**

Freshly harvested plant tissue was crushed with Na-P buffer (pH-7) for peroxidase (PO) isomer detection. Electrophoresis of PO was done in 10% polyacrylamide gel according to the method of Kahler and Allard, 1970.

**Staining of isoenzyme gel**

PO (EC 1.11.1.7) was stained, using orthodinisidin (1mg $ml^{-1}$ methanol) with 0.2 M hydrogen peroxide. The gel was incubated in dark until the brownish orange bands appeared (Malik and Singh, 1980).

**Statistical analysis**

The data collected during these investigations were subjected to appropriate statistical analysis using SPSS Statistical Tool 10.0.

## RESULTS

### *In vitro* antagonism

The present study revealed an efficient inhibition *of S. rolfsii growth* by fluorescent pseudomonades pretreatment in which two native isolates exhibited a strong antagonism effect against *S. rolfsii* in *in-vitro* assays (Table 2), illustrating an antifungal activity for both isolates. The antagonistic effect of BCPF-8 rhizobacteria was evidenced by the inhibition of the pathogen growth by 70.05% using dual culture method. The data depicted in Table 2 indicated that the vigour index based on germination percentage, root length and shoot length was also increased by treatments with BCPF-8 (2215.70) and PF-7 (1938.25). These two effective rhizobacterial antagonist isolates were selected for further characterization of biovar detection and used as inducer for development of systemic resistance in cowpea seedling against damping off disease incited by *S. rolfsii*. On the basis of phenotypical criteria (Bossis et al., 2000), BCPF 8 and BCPF 7 were identified as *P. fluorescens* biovar V and III, respectively (Tables 2 and 3).

**Table 3.** Biochemical characteristics for the identification of isolates (Bossis et al., 2000).

| Isolate | Fluorescence | Arginine | Oxidase | Tabac | Gelatine | Trehalose | Lev | Deni | L-ara | L(+)tart + |
|---|---|---|---|---|---|---|---|---|---|---|
| BCPF7 | + | + | + | - | + | - | - | + | NA | NA |
| BCPF8 | + | + | + | - | + | - | - | - | NA | NA |

L-ara, L-arabinose; Den, Denitrification; Lev, Levan; L(+) tart, L(+) tartrate, +, positive; –, negative; NA, Not Applicable.

### Effect of different seed treatments on growth performance and disease incidence

The effect of different seed treatments on plant growth parameters including germination percentage, shoot length, root length, fresh weight and numbers of nodules per root of cowpea seedlings in both infested and non-infested soil were recorded (Table 4). The highest germination rate was recorded in BCPF 8 treated seeds sowed in non-infested soil (94.7%) over untreated seeds. Among the infested soil BCPF 8 treated seeds show the highest germination rate (86%). In all infested soil, BCPF 8 treated seeds also shows the higher shoot length (21.33 cm), root length (7.33 cm), fresh weight (7.27 g). However, Carbendazim treated seeds shows the highest number of nodules per root (5.67) among all infested soils, which was statistically at par with that of BCPF 8 treated seeds sowed in infested soil (5.33).

In the present study, the efficacy of different seed treatment in controlling damping off disease of cowpea seedlings were also recorded and presented in Table 5. PGPR bio-formulations and fungicidal seed treatments were prepared individually and used in this study at different time intervals (days).

Disease incidence (%) was assessed at different time intervals (15 and 25 days after treatment). BCPF 8 treated seeds show the lowest disease incidence in post emergence (22.81) damping off of cowpea seedlings at 25 days after seed inoculation.

Our results show also that the BCPF 8 also recorded as the best seed treating bio-formulation as it controlled the disease incidence percentage in post emergence damping off of cowpea seedlings up to 37.31, over the untreated seeds sowed in *S. rolfsii* infested soil. Interestingly, in this present study, it is noteworthy that BCPF 8 bio-formulation followed by BCPF 7 bio-formulation was better than chemical fun-gicide Carbendazim in the control of damping off in cow-pea caused by deleterious pathogen *S. rolfsii*.

### Induction of defense mechanisms by different seed treatments

The induction of greater amount of defense related enzymes by PGPR bio-formulations treated plants are shown in Figure 1. Levels of peroxidase (PO) enzyme increased significantly within 5 days after inoculation and thereafter a sudden fall in activity was noteworthy in only pathogen challenged seedlings where as in seedlings challenged with pathogen and treated with BCPF 8 ex-presses an early and prolonged peroxidase activity up to 10 days after inoculation. The seedlings challenged with pathogen and treated with BCPF 8 expressed the highest (3.04 fold) increased activity over the untreated control (Figure 2). Similarly, the seedlings challenged with patho-gen and treated with BCPF 8 at 5 days after treatment had highest PPO activity (0.475) and a slow decrease in activity up to 0.368 at 10 days after inoculation. On the contrary, in only pathogen challenged seedlings the PPO activity reached highest (0.221) at 5 days after inocula-tion but the activity dropped down to 0.175 at 7 days after inoculation (Figure 3). Though the PAL activity increased at 5 days after inoculation to 0.024 and a quick fall of activity noticed at 7 days after inoculation in case of only pathogen challenged seedlings, the seedlings challen-ged with pathogen and treated with BCPF 8 possessed an early and 6.28 fold enhanced increased PAL activity at 3 days after inoculation (Figure 4). Peroxidase isoform PO 2, Rm = 0.38 (marked as white arrow) was noticed in seedlings challenged with pathogen and pre-treated with BCPF 8 (Figure 5). This isoform may be associated with the induction of systemic resistance in cowpea seedlings elicited by BCPF 8 and challenged by *S. rolfsii*.

## DISCUSSION

In this work we found that approximately 70% of *S. rolfsii* growth was inhibited by native *Pseudomonas* isolates in dual plate culture. Similarly, Tripathi and Johri (2002) observed *in vitro* inhibition of *Colletotrichum dermatium, Rhizoctonia solani* and *Sclerotium rolfsii* by fluorescent pseudomonads. The data presented in Table 2 indicates that the fluorescent pseudomonades biovar V isolates designated as BCPF-8 shared maximum vigour index evidenced by an enhancement of the germination rate, root length and shoot length, which confirm the findings of Rao et al. (1999) who observed positive effect of five isolates of fluorescent pseudomonades on growth of lentil by means of vigour index. The most possible exploration of *in-vitro* antagonism exhibited by the isolates BCPF8 and BCPF7, an attempt was made to develop effective biocontrol system management of damping off disease of cowpea under field conditions. Our results show that BCPF 8 is the most effective biocontrol agent against *S. rolfsii* by means of significant enhancement of the germination rate, shoot length, root length, fresh weight of the plant and number of nodule per root in infested as well as non-infested soil. Such enhancement of root nodulation

**Table 4.** Effect of seed treatments on seed germination percent after 3 days of sowing and seedling establishment of cowpea in *S. rolfsii* infested and non-infested soil mix after 25 days of sowing.

| Treatment | Germination % (after 3 DAS) | Shoot length (cm) | Root length (cm) | Fresh wt (g) | Number of nodules/root |
|---|---|---|---|---|---|
| Normal seeds in Non-infested soil (Control) | 70.3$^{b}$ | 14.67$^{b}$ | 6.17$^{b}$ | 5.70$^{b}$ | 4.67$^{ab}$ |
| BCPF 7 in seed treatment+ *S. rolfsii* infested soil | 82.3$^{e}$ | 18.33$^{c}$ | 7.07$^{cd}$ | 6.47$^{c}$ | 5.00$^{b}$ |
| BCPF 7 in seed treatment+ Non-infested soil | 94.7$^{g}$ | 21.60$^{c}$ | 8.23$^{e}$ | 8.37$^{e}$ | 7.33$^{d}$ |
| BCPF 8 in seed treatment+ *S. rolfsii* infested soil | 86$^{f}$ | 21.33$^{c}$ | 7.33$^{d}$ | 7.27$^{d}$ | 5.33$^{bc}$ |
| BCPF 8 in seed treatment+ Non-infested soil | 95.7$^{g}$ | 26.67$^{d}$ | 9.10$^{f}$ | 9.40$^{f}$ | 10.00$^{e}$ |
| Carbendazim in seed treatment+ *S. rolfsii* infested soil | 74.3$^{c}$ | 15.00$^{b}$ | 6.73$^{c}$ | 5.63$^{b}$ | 5.67$^{bc}$ |
| Carbendazim in seed treatment + Non-infested soil | 77.7$^{d}$ | 18.67$^{c}$ | 7.10$^{cd}$ | 6.27$^{c}$ | 6.33$^{cd}$ |
| Normal seeds in *S. rolfsii* infested soil | 64.3$^{a}$ | 9.33$^{a}$ | 5.33$^{a}$ | 4.97$^{a}$ | 3.67$^{a}$ |

Values are mean of three replications; DAS, Days after seed sowing; in a column, means followed by a common letter are not significantly different (p= 0.05) by DMRT.

by Pseudomonas sp. may be due to the production of plant growth-promoting substances (Shabayev et al., 1996). Similar finding also reported by Yeole and Dube (1997) where seed bacterization with rhizospheric *Pseu-domonas* isolates increased the germination rate, root length and shoot length of cotton, chilli, ground nut and soybean. In addition to beneficial effect of *Pseudomonas* sp. on the development of plants in pathogen infested soil, they also reflects improved plant growth in absence of pathogen, which strongly supported the finding of Avis et al. (2008). Inoculation of cowpea seeds with BCPF8 induced a faster and stronger reduction of pre and post emergence damping off disease in comparison with other bioformulations and fungicides tested in this work. *Pseudomonas* sp. has been broadly studied for their ability to reduce the development of various soil borne plant pathogens (Carisse et al., 2003).

Different modes of action for *Psedomonas* sp. have been reported, including the production of different antimicrobial compounds (Tharne et al., 2000), competition (Ellis et al., 1999) and induction of plant defense mechanisms (Sangeetha et al., 2010; Tonelli et al., 2011). Recent investigation on mechanisms of biological control by plant growth promoting rhizobacteria (PGPR) like fluorescent pseudomonads revealed that PGPR strains protects plants from pathogen attack by strengthening the epidermal and cortical walls with deposition of newly formed barriers beyond infection sites including callose, lignin and phenolics (M'Piga et al., 1997). Also PGPR could stimulate defense related genes expression (Chen et al., 2000) and the induction of enzymes responsible for phtoalexins synthesis (Maurhofer et al., 1994). The hyphae of the pathogen surrounded by phenolics substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Benhamou et al. (2000) reported that an endophytic bacterium, *Serratia plymuthica* induced the accumulation of phenolics in cucumber roots following infection by *P. ultimum.* In the present study, a higher accumulation of phenolics was recorded in cowpea seedlings inoculated with pathogens and pretreated with BCPF8 at 7 days after inoculation compared to other treatments. This increase in phenol content might indicate a possible involvement of such compounds in the enhanced resistance of cowpea seedlings to pathogen *S. rolfsii* by PGPR. This might have contributed to reduced infection by the *S. rolfsii* in cowpea seedlings. Peroxidase has been implicated in the last enzymatic step of lignin biosynthesis, that is, the oxidetion of hydroxyl cinnamyl alcohols into free radical inter mediates, which subsequently are coupled to lignin polymer (Gross, 1980).

Furthermore, peroxidase is involved in the production or modulation of active oxygen species which may play various roles directly or indirectly in reducing pathogen viability and spread (Lamb and Dixon, 1997). In this work, early and prolonged higher activity of PO from 3 days after inoculation may be correlated with the lowest patho-genicity of the pathogen in cowpea seedlings inoculated with *S. rolfsii* and pre-treated with BCPF8. Similarly, the higher PO activity was noticed in cucumber roots treated with *Pseudomonas corrugate* and challenged with

**Table 5.** Effect of seed treatments on post emergence damping off of cowpea in *S. rolfsii* infested and non-infested soil (page 1857).

| Treatments | 15 DAS post emergence damping off | 25 DAS post emergence damping off | Post emergence damping off (control %) |
|---|---|---|---|
| Normal seeds in non-infested soil (Control) | $0^{a}$ (0.0) | $0^{a}$ (0.0) | - |
| BCPF 7 in seed treatment + *S. rolfsii* infested soil | $19.16^{c}$ (10.77) | $25.33^{c}$ (18.33) | 30.39 |
| BCPF 7 in seed treatment + non-infested soil | $0^{a}$ (0.0) | $0^{a}$ (0.0) | - |
| BCPF 8 in seed treatment + *S. rolfsii* infested soil | $18.17^{b}$ (9.72) | $22.81^{b}$ (15.06) | 37.31 |
| BCPF 8 in seed treatment + non-infested soil | $0^{a}$ (0.0) | $0^{a}$ (0.0) | - |
| Carbendazim in seed treatment + *S. rolfsii* infested soil | $20.70^{d}$ (12.50) | $27.49^{d}$ (21.35) | 24.45 |
| Carbendazim in seed treatment + non-infested soil | $0^{a}$ (0.0) | $0^{a}$ (0.0) | - |
| Normal seeds in *S. rolfsii* infested soil | $25.33^{e}$ (18.3) | $36.39^{e}$ (35.21) | - |

DI– Days interval, DAS– days after seed sowing. Values are mean of three replications. Values in parentheses are % value. In a column, means followed by a common letter are not significantly different (p= 0.05) by DMRT.

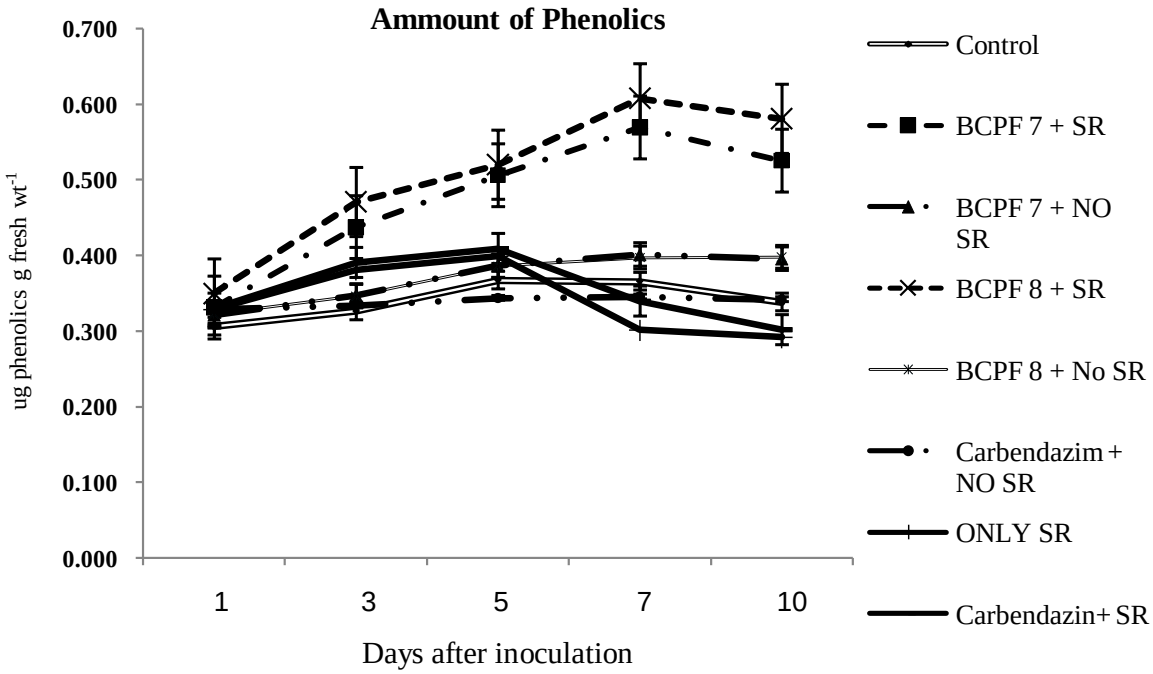


**Figure 1.** Changes of Phenolic compounds in different treatments at different hours after inoculation.

*Pythium aphanidermatum* (Chen et al., 2000). Biochemical analysis of rice plants raised from seeds treated with *P. fluorescens* show an early induction of PO (Nandakumar et al., 2001). Mishra (2006) reports an increased in the activity of PO in PGPR treated tea cuttings grown in pathogen infested soil. PPO catalyzes the last step in the biosynthesis of lignin and other oxidative phenols. The PPO activity was increased in cowpea seedlings inoculated with pathogen and pre-treated with BCPF 8 at 5 days after inoculation.

Similarly, induction of defense responses by PGPR is associated with the production of oxidative enzymes like PPO reported by Sangeetha et al. (2010). PO and PPO play a central role triggering the hypersensitive reaction (HR), in cross linking and lignifications of the cell wall and in transducing signals to adjacent non-challenged cells (Lamb and Dixon, 1997). PAL is an enzyme of the general phenylpropanoid metabolism and controls a key branch point in the biosynthetic pathways of flavonoid phytoalexins, which are antimicrobial compound (Bowles et al., 1990). The induction of PAL in cowpea seedlings pre-treated with PGPR (BCPF 8) and inoculated with pathogen *S. rolfsii* could indicate a possible involvement of phenyl propanoid metabolism in BCPF8-induced resistance to

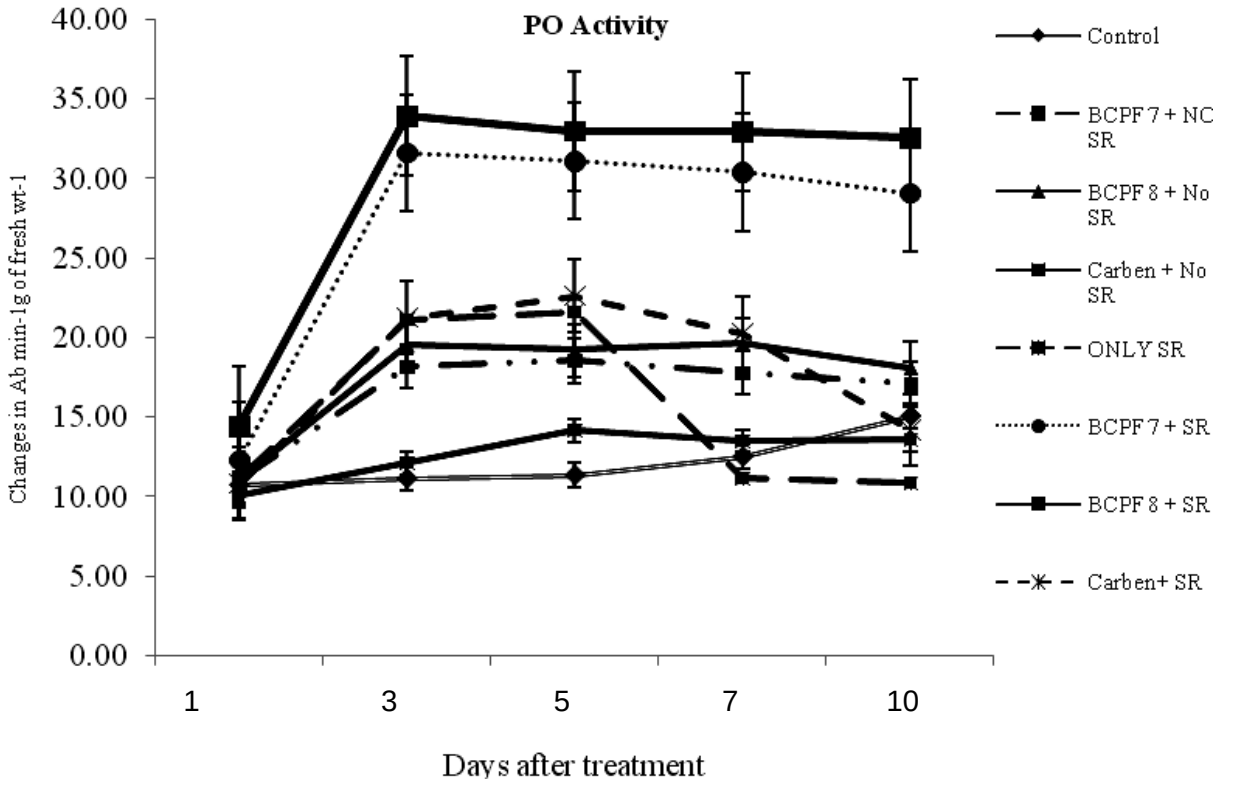


**Figure 2.** Changes of Peroxidase activity in different treatments at different hours after inoculation.

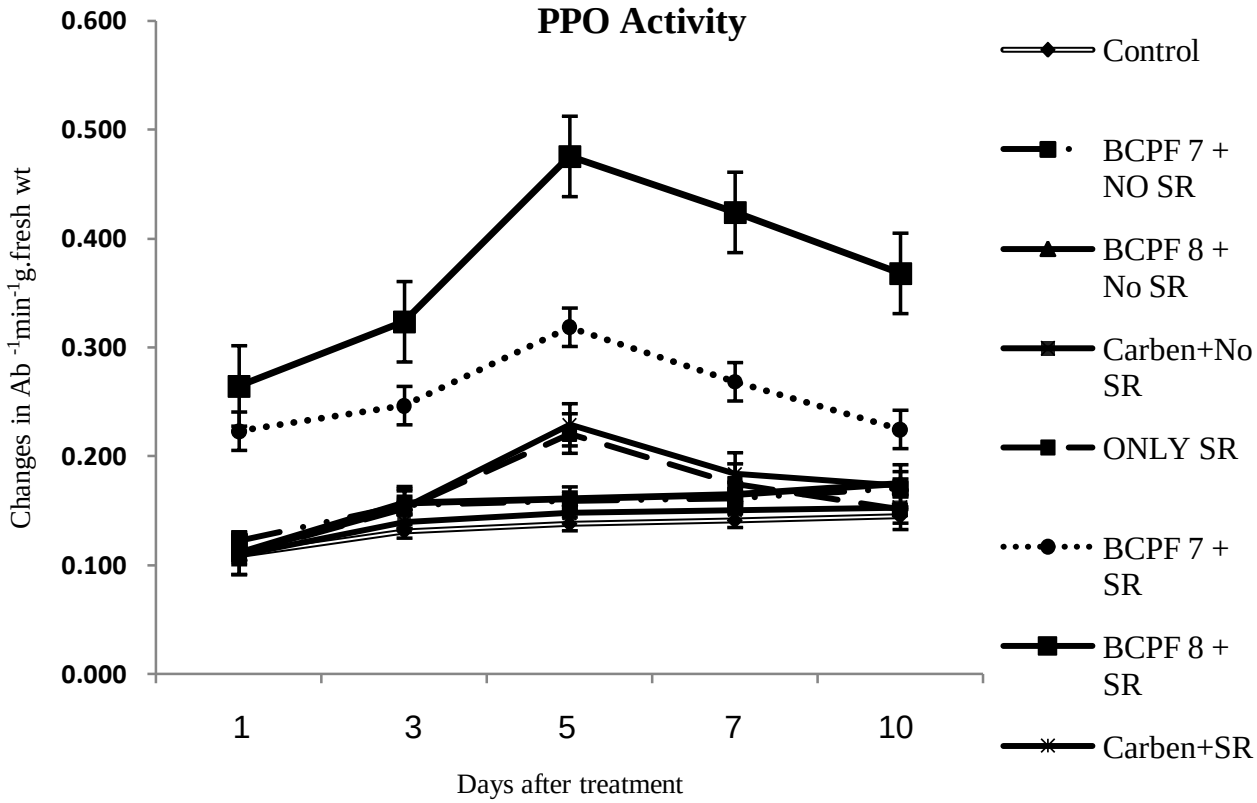


**Figure 3.** Changes of Polyphenol oxidase activity in different treatments at different hours after inoculation.

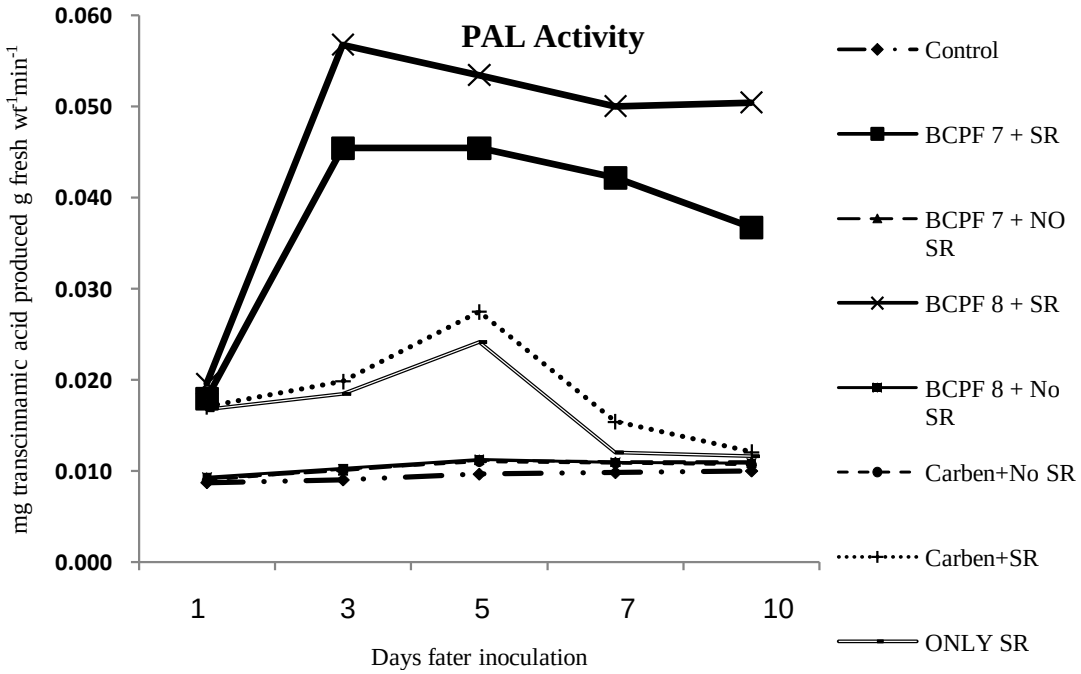


**Figure 4.** Changes of Phenyl ammonia lyase activity in different treatments at different hours after inoculation.

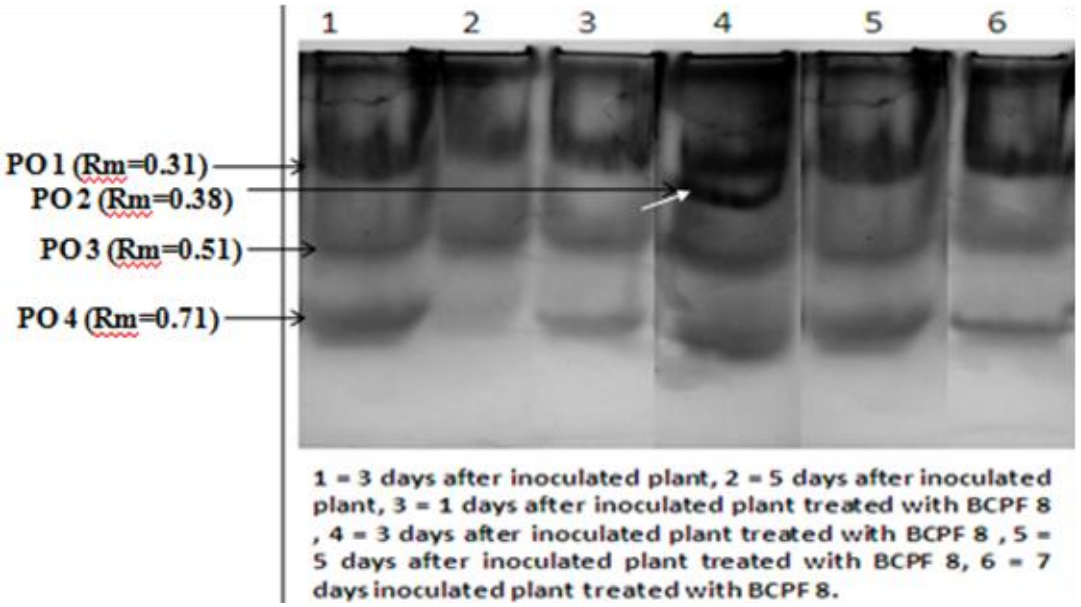


**Figure 5.** Changes of Peroxidase isomers in different treatments at different hours after inoculation.

damping off. Similarly, Meena et al. (2000) demonstrated that the increase in PAL activity correlated with disease incidence reduction when groundnut plants were sprayed with *P. fluorescens*. The rapid induction of PAL genes in incompatible plant-pathogen interactions might be due to the activation of a specific and appropriate signal transduction pathway.

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