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# Effect of different biocontrol agents and fungicides against stem rot of cowpea caused by *S. rolfsii* under pot condition

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**Abstract**
Stem rot is common disease of cowpea caused by *Sclerotium rolfsii*. Total nineteen treatments were selected for pot trials and biochemical studies. Among them, fungicides applied as a seed treatment and biocontrol agents applied as a soil application. In which, all the treatments significantly control the disease over control. Significantly minimum disease incidence (10.01%) was recorded in carbendazim 25% + mancozeb 50% WS at 2.5 g/kg of seed treatment. While, *T. koningii* which was at par with *T. virens* at 25 g/kg of soil application and propineb 70% WP at 2.5 g/kg of seed treatment, which were recorded 20.02, 24.85 and 26.59 percent disease incidence, respectively. Biochemical parameters except total sugar (total phenol and total protein) and defense related enzymes (CAT, PAL, POX, PPO and SOD) activities were increased in all the treatment as compared to healthy and *S. rolfsii* infected cowpea plant. While, total sugar content was decreased in cowpea plant after *S. rolfsii* infection as compared to healthy and seed treatment given by fungicides applied. Various biocontrol control agents applied as a soil application enhanced the total sugar content in plant.

**Keywords:** Cowpea, stem rot, *Sclerotium rolfsii*, biochemical parameters, defense related enzymes activity

## Introduction
The cowpea [*Vigna unguiculata* (L.) Walp] is a food legume belonging to the family *Fabaceae* or *Leguminosae* with chromosome number 2n = 22. It is an annual herbaceous legume from the genus *Vigna*. Cultivated cowpea commonly known as a black-eyed pea, southern pea, yard-long bean, catjang and crowder pea and also it known as "poor man's meat". Cowpea is originated from the Central Africa. It is an important food legume growing across the world many tropical and subtropical regions including tropical Africa, Asia and South America (Mahalakshmi *et al*. 2007) [20]. In India, cowpea is cultivated mainly in Gujarat, West Bengal, Tamil Nadu, Andhra Pradesh, Kerala and Orissa. Cowpea is considered to be nutrient dense food with low energy. Also, it provides a rich source of proteins and calories as well as minerals and vitamins (Goncalves *et al*. 2016) [10]. The cooked leaves contain protein, calcium, iron, phosphorus, riboflavin, niacin, ascorbic acid and beta-carotene. A seed can consists of 23-25% protein, 50-67% carbohydrate, 8-9% moisture and it has very low-fat content 3.99% (Rangel *et al*. 2003) [29]. Cowpea production is practiced under varying cropping systems including sole cropping, intercropping and mixed cropping system. It also suitable for green manuring, cover crop and catch crop which maintain productivity and fertility of soil. Biological nitrogen fixation (BNF) is one of the major benefits of cowpea production in cropping system. It has a unique ability to fix atmospheric nitrogen with its nodule association with soil dwelling bacteria. It can fix about 240 kg ha$^{-1}$ of atmospheric nitrogen and make available about 60-70 kg ha$^{-1}$ nitrogen available for succeeding crops grown in rotation with cowpea (Anonymous, 2006) [4]. Stem rot is common disease of cowpea caused by *Sclerotium rolfsii* and is epidemic which had been reported in warm moist climate condition worldwide. However, basal stem rot epidemics are dynamic and had been reported in warm moist climate condition (Nazerian and Piegham, 2021) [24]. Fery and Dukes (2002) [8] reported the southern blight of cowpea caused by *S. rolfsii* and might lead to plant mortality, reduced plant vigour and yield and dry seed yield loss up to 53.4 percent. Ramiah *et al*. (1976) [28] reported in India twenty percent mortality of cowpea plant has reported due to *S. rolfsii* only.
The hypersensitive reaction (HR) is one of the most efficient and visible parts of the defense mechanisms in plants against invading pathogens.

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It is associated with a coordinated and integrated set of metabolic alterations which are further pathogen ingress or alleviating stress. It includes a variety of novel proteins, phenols and secondary metabolites. After inoculation of *S. rolfsii* at collar region of cowpea plant, defense reaction occurs due to accumulation of PR proteins and different stress related enzymes. Although there are reports on role of biocontrol agents, fungicides and induced defense related enzymatic changes in plants. However, there is little information available on effect of biocontrol agent, fungicides and *S. rolfsii* to induce defense-related enzymes in cowpea plants. Therefore, the main objective of the present study was to determine the enzymatic activity and biochemical changes of cowpea plant under net house condition.

## Materials and Methods
### Isolation of the pathogen
Naturally stem rot infected cowpea plant samples were collected from the field of Pulse Research Station, J. A. U., Junagadh season of *Kharif*-2021. The pathogen was obtained by single hyphal tip method, further purified using single sclerotial body and maintained on PDA throughout the present study. Purified culture was maintained on PDA slants by storing it under refrigeration at 4 °C temperature (Rakh *et al*., 2011) [27].

### Mass multiplication of test pathogen and biocontrol agents
The test pathogen *S. rolfsii* and fungal biocontrol agents were mass multiplied on sterilized sorghum grains for pot culture studies. For this, healthy sorghum grains were washed thoroughly in tap water and half-boiled. After removing the excess water, grains were allowed to air dry and cooled at room temperature. Polyethylene bags were filled up with about 200 g of grains. Mouth of these bags were packed with piece of PVC pipe and plug with non-absorbent cotton. Then it was autoclaved at 121 °C and 15 lb psi (1.06 kg/cm$^2$) for 20 min for sterilization. The polyethylene bags containing the sterilized media (sorghum grain) were inoculated with mycelial disc of *S. rolfsii* (5 mm diameter) and biocontrol agents which further incubated at 28 ± 1 ºC for 10 days.

### Effect of biocontrol agents and fungicides against *S. rolfsii* under pot condition
According to Table-1 including control total twenty treatments were carried out for pot trials under net house of Department of Plant Pathology, Junagadh Agricultural University, Junagadh during summer 2022. Total 40 earthen pots (15 cm diameter) were filled with sterilized sandy loam soil and further inoculated with two weeks old culture of *S. rofsii* (prepared on sorghum grains medium) @ 25 g/kg pot soil and allowed to stabilize for three days according to Shirsole *et al*. (2019) [32]. Total thirteen fungicides were applied as a seed treatment and six biocontrol agents were applied as soil treatment during time of sowing. One set (two pots) was maintained without application of bioagents/fungicides and remaining all as a treated pot. Ten seeds per pot were sown in 15 cm diameter earthen pots. Two replications of each treatment were maintained. The disease infected plants were counted 10 days after sowing.

**Table 1:** Evaluation of biocontrol agents and different fungicides against *S. rolfsii* under pot condition

| Sr. No. | Treatments | Dosage (g or ml/kg or lit) |
|---|---|---|
| 1 | Seed treatment with mancozeb 75% WP | 2.5 |
| 2 | Seed treatment with thiram 75% WS | 2.5 |
| 3 | Seed treatment with propineb 70% WP | 2.5 |
| 4 | Seed treatment with zineb 75% WP | 2.5 |
| 5 | Seed treatment with tebuconazole 25.9% EC | 1 |
| 6 | Seed treatment with hexaconazole 5% EC | 1 |
| 7 | Seed treatment with azoxystrobin 23% SC | 1 |
| 8 | Seed treatment with tricyclazole 75% WP | 1 |
| 9 | Seed treatment with azoxystrobin 11% + tebuconazole 18.3% SC | 1 |
| 10 | Seed treatment with tebuconazole 10% + sulphur 65% WG | 1 |
| 11 | Seed treatment with carboxin 37.5%+ thiram 37.5% WS | 2 |
| 12 | Seed treatment with carbendazim 25% + mancozeb 50% WS | 2 |
| 13 | Seed treatment with fluxapyraxad 250 g/l + pyraclostrobin 250 g/l SC | 1 |
| 14 | Soil application with *Trichoderma koningii* ($2 \times 10^6$ cfu/g) | 25 |
| 15 | Soil application with *Trichoderma gliocladium* ($2 \times 10^6$ cfu/g) | 25 |
| 16 | Soil application with *Trichoderma virens* ($2 \times 10^6$ cfu/g) | 25 |
| 17 | Soil application with *Bacillus cereus* ($2 \times 10^8$ cfu/ml) | 25 |
| 18 | Soil application with *Pseudomonas fluorescens* ($2 \times 10^8$ cfu/ml) | 25 |
| 19 | Soil application with *Pseudomonas fluorescens* isolate-1 ($2 \times 10^8$ cfu/ml) | 25 |
| 20 | Control (*S. rolfsii*) | 25 |

### Total phenol
The total phenol content was estimated by the method described by Thimmaiah (1999) [33]. An aliquot of 0.2 ml was transferred in test tube and volume was made to 3 ml with distilled water and Folin-Ciocalteau reagent (0.5 ml). After three minutes, 2 ml of 20 percent sodium carbonate added. After cooling, the absorbance was recorded at 650 nm against a reagent blank. The amounts of phenols present in the sample was calculated as mg/g.

### True protein
The soluble protein content of the samples was assayed by using method given by
Lowry *et al*. (1951) [19]. One gram of shoot was macerated in mortar with 5 ml 0.1 M sodium phosphate buffer (pH 7.0). An aliquot of 0.1 ml supernatant was taken in test tube and the volume was made to 1 ml with distilled water followed by addition of 5 ml solution C mixed well and incubated at room temperature for ten minutes. A 0.5 milliliter of Folin-Ciocalteu's reagent (FC reagent) was diluted to 1 N, mixed

well and incubated at room temperature in dark for 30 minutes. The absorbance was recorded at 660 nm against blank. The amount of protein present in the sample was calculated as mg/g.

## Total sugar
The total sugar content was estimated by the method described by Dubois *et al*. (1956) [7]. The aliquot from the extract (0.1 ml) was pipetted into separate test tubes and the tubes were placed in a boiling water bath to evaporate the methanol. One ml of millipore water and 1 ml of 5 percent phenol was added in each test tube. Then 5 ml of sulphuric acid was added. The tubes were allowed to cool in ice-bath for 10-15 minutes. The intensity of colour was read at 490 nm on spectrophotometer. The amount of protein present in the sample was calculated as mg/g.

## Effect of defense enzyme activity in cowpea plant
To study the effect of biocontrol agents and fungicides on defense-related enzyme phenylalanine ammonia lyase (PAL) and various antioxidant enzymes such as catalase (CAT), peroxidase (POX), poly phenol oxidase (PPO) and superoxide dismutase (SOD) in cowpea plant against stem rot disease incited by *S. rolfsii*.

## Estimation of catalase (CAT) activity
The catalase (CAT) activity from the roots of the cowpea plant in all the treatments was assayed according to the protocol given by Aebi (1974). The activity of the enzyme was assayed by taking 2.6 ml of 0.1 M phosphate buffer (pH 6.4), 0.1 ml of enzyme extract and 0.1 ml of 1 percent $H_2O_2$. Absorbance was measured at 240 nm with help of spectrophotometer. The enzyme activity was expressed as $\Delta$ OD/min/g fresh weight.

## Estimation of peroxidase (POX) activity
The enzyme assay was performed as per the protocol given by Kar and Mishra (1976) [13]. Reaction mixture was prepared by adding 4.6 ml of 0.1M phosphate buffer (pH 6.4), 0.2 ml of pyrogallol (50 mM), and 0.1 ml of 50 µM $H_2O_2$ and 0.2 ml of enzyme extract. The mixture was incubated at 25 ℃ for 5 minutes. Then 0.5 ml of 5.0 percent $H_2SO_4$ was added to terminate the reaction. Absorbance was measured at 420 nm with the help of a spectrophotometer. The enzyme activity was expressed as $\Delta$ OD/min/g fresh weight.

## Estimation of phenylalanine ammonia lyase (PAL) activity
PAL activity was determined as the rate of conversion of L-phenyl alanine to trans cinnamic acid at 290 nm as per the method described by Ross and Sederoff (1992) [30]. Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The reaction was arrested by adding 0.5 ml of 1 M TCA and incubated at 37 °C for 5 min. The blank contains 0.4 ml of crude enzyme extract and 2.7 ml of 0.1 M borate buffer (pH 8.8) and absorbance was measured at 290 nm.

## Estimation of polyphenol oxidase activity (PPO)
The enzyme was assayed according to the protocol given by Kar and Mishra (1976) [13]. The reaction mixture was prepared by adding 4.5 ml of phosphate buffer, 0.2 ml of pyrogallol (50.0 µM) and 0.2 ml of enzyme extract. The mixture was incubated at 25 ℃ for 5 minutes. Then 0.5 ml of 5.0 percent

sulphuric acid was added to terminate the reaction. Absorbance was measured at 420 nm with the help of a spectrophotometer.

## Estimation of superoxide dismutase (SOD) activity
Superoxide dismutase (SOD) activity was assayed from the root samples of all the treatments of cowpea plants following the method described by Dhindsa *et al*. (1981) [6]. The sample (0.1 g) was ground with 1 ml of extraction buffer (0.1 M phosphate buffer, pH 7.5 containing 0.5 mM EDTA). Three ml of the reaction mixture containing 0.1 ml of 1.5 M sodium carbonate, 0.2 ml of 200 mM L-methionine, 0.1 ml of 2.25 mM NBT (Nitro-blue tetrazolium), 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml of distilled water and 0.1 ml of enzyme extract was taken in test tubes in duplicate from each enzyme sample. The reaction was started by adding 0.1 ml riboflavin (60 µM) and placing the tubes below a light source of two 15W fluorescent lamps for 15 minutes. Absorbance was recorded at 560 nm in a spectrophotometer.

## Statistical Analysis
Completely randomized block design was used for analyzing the data for all the chemical parameters and percent disease incidence. An experiment was caried out during *Kharif* season 2021. Percent disease incidence (PDI) was calculated by using the following formula given by Wheeler (1969) [35].

$$\text{Percent disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

## Results and Discussion
## Effect of biocontrol agents and fungicides against *S. rolfsii* under pot condition
The perusal data presented in Table-2 showed that all the treatments were significantly control the stem rot disease of cowpea over control. Significantly minimum disease incidence (10.01%) was recorded in carbendazim 25% + mancozeb 50% WS at 2.5 g/kg of seed treatment. While, *T. koningii* which was at par with *T. virens* at 25 g/kg of soil application and propineb 70% WP at 2.5 g/kg of seed treatment, which were recorded 20.02, 24.85 and 26.59 percent disease incidence, respectively. Also, carboxin 37.5% + thiram 37.5%WS (30.03%) at 2.5 g/kg of seed treatment gave minimum disease incidence of cowpea seedling. Maximum disease incidence was recorded in azoxystrobin 11% + sulphur 65% WG with 60.18 percent at 1 ml/kg of seed treatment.
The obtained result was agreement with Shirsole *et al*. (2019) [32] who reported seed treatment with carboxin 37.5% + thiram and carbendazim 12% + mancozeb 63% WP in chickpea gave 37.5 and 53.21 percent decrease collar rot over control, respectively. The maximum 95 percent germination recorded in *T. viride* applied as seed + soil application and disease incidence also reduced by Kumar *et al*. (2020) [16].

## Total phenol
The result present in Table-2 revealed that the total phenol content (mg/g of fresh weight) slightly increased in cowpea plant attacked by *S. rolfsii* along with various treatments under pot condition. The highest total phenol content with 4.98 mg/g of fresh weight was recorded in *T. virens* which was followed by *T. koningii* recorded with 4.63 mg/g of fresh

weight. The amount of total phenol was increased in stem rot infected tissues of cowpea as compared to healthy cowpea plant.

The phenolic compounds as constituents of lignin may contribute to enhance the mechanical strength of the host cell wall and may also inhibit fungal growth as they are fungi toxic in nature. The seed + soil application with *T. viride* enhanced phenol content followed by soil application after inoculation with *S. rolfsii* in cowpea Kumar *et al*. (2020) [16]. Adandonon *et al*. (2017) [1] recorded higher phenol content in the tolerant cultivars than the susceptible cultivars of cowpea and also it increased after seedlings infected with *S. rolfsii*. Higher amount of phenol was recorded in *S. rolfsii* infected plants compared to healthy one reported in cluster bean by Gahlot *et al* (2022) [9].

## Total protein
The change in total protein content as influenced by different treatments in cowpea plant (Table-2). The highest total protein content (10.06 mg/g of fresh weight) recorded in *T. koningii* which was at par with *T. virens* (9.82 mg/g of fresh weight) followed by *Bacillus cereus* which recorded total protein content with 9.25 mg/g fresh weight. The amount of total protein was increased in infected stem tissues of cowpea as compared to healthy. Also, total protein content slightly increased in fungicides treated plants as compared to healthy as well as *S. rolfsii* inoculated plant.

Vidyasekaran (2001) [34] reported that the increased synthesis of proteins during the infection may be due to activation of enzymes which are essential for the synthesis of various defense chemicals in plant. Result is agreement with Nandi *et al*. (2013) [23] who noticed that significantly increased in total protein content in cowpea plant after inoculation with *S. rolfsii*. The PR-proteins expression increasing after infection of *S. rolfsii* (Jogi *et al*. (2016) [12], Bosamia *et al*. (2020) [5].

**Table 2:** Effect of different biocontrol agents and fungicides on stem rot disease incidence and biochemical parameters in cowpea plant under pot condition

| Sr. No | Treatment | PDI | Total phenol | Total protein | Total sugar |
|---|---|---|---|---|---|
| 1 | Mancozeb 75% WP | 35.21 (33.24)* | 3.68 | 4.64 | 7.33 |
| 2 | Thiram 75% WP | 35.21 (33.24) | 3.57 | 5.32 | 7.18 |
| 3 | Propineb 70% WP | 31.04 (26.59) | 3.46 | 4.98 | 6.61 |
| 4 | Zineb 75% WP | 38.80 (39.27) | 3.45 | 4.95 | 6.88 |
| 5 | Tebuconazole 25.9% EC | 45.46 (50.81) | 3.49 | 5.04 | 7.06 |
| 6 | Hexaconazole 5% EC | 38.80 (39.27) | 3.44 | 5.47 | 6.67 |
| 7 | Azoxystrobin 23% SC | 40.48 (42.14) | 3.34 | 5.68 | 7.16 |
| 8 | Tricyclazole 75% WP | 45.46 (50.81) | 3.58 | 5.53 | 7.24 |
| 9 | Azoxystrobin 11% + tebuconazole 18.3% SC | 50.87 (60.18) | 3.30 | 5.17 | 6.57 |
| 10 | Tebuconazole 5% + sulphur 65% WG | 45.46 (50.81) | 3.59 | 5.57 | 6.63 |
| 11 | Carboxin 37.5% + thiram 37.5% WS | 33.23 (30.03) | 3.35 | 5.26 | 6.89 |
| 12 | Carbendazim 25% + mancozeb 50% WS | 18.44 (10.01) | 3.27 | 5.73 | 6.76 |
| 13 | Fluxapyraxad 250 G/L + pyraclostrobin 250 G/L SC | 47.91 (55.07) | 3.23 | 4.85 | 6.80 |
| 14 | *Trichoderma koningii* (2 x $10^6$ cfu/g) | 26.58 (20.02) | 4.63 | 10.06 | 9.88 |
| 15 | *Trichoderma gliocladium* (2 x $10^6$ cfu/g) | 33.23 (30.03) | 3.75 | 7.01 | 9.57 |
| 16 | *Trichoderma virens* (2 x $10^6$ cfu/g) | 29.90 (24.85) | 4.98 | 9.82 | 9.87 |
| 17 | *Bacillus cereus* (1 x $10^7$cfu/g) | 50.79 (60.04) | 3.93 | 9.25 | 10.23 |
| 18 | *Pseudomonas fluorescens* JAU-3 (1 x $10^7$ cfu/g) | 39.25 (40.04) | 4.03 | 8.99 | 9.10 |
| 19 | *Pseudomonas fluorescens* JAU-1 (1 x $10^7$ cfu/g) | 45.02 (50.04) | 3.97 | 6.28 | 10.50 |
| 20 | Control (*S. rolfsii*) | 90.00 (100.00) | 3.21 | 4.21 | 5.82 |
| 21 | Un inoculated | 0.00 (0.00) | 2.90 | 3.58 | 8.19 |
| | S.Em.± | 2.05 | 0.06 | 0.16 | 0.14 |
| | C.D. at 5% | 6.05 | 0.16 | 0.48 | 0.41 |
| | C.V. % | 6.84 | 2.16 | 3.77 | 2.48 |

*Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values. Note: Biochemical parameters were calculated as mg/g fresh weight of tissue

## Total sugar
The result presented in Table-2 revealed that the total sugar content was reduced in infected tissues of cowpea plant as well as fungicides treated plants as compared to healthy plant. The high amount of total sugar content recorded with *P. fluorescens* JAU isolate-1 (10.50 mg/g fresh weight) which was at par with *B. cereus* (10.23 mg/g fresh weight). The total sugar content was recorded in plant inoculated with *S. rolfsii* with 5.82 mg/g fresh weight.

The reduction in sugar content after infection may be due to rapid hydrolysis of sugars during pathogenesis through enzymes (hydrolases) secreted by pathogens and subsequent utilization by pathogens for their development (Poornima *et al*., 2016) [25]. The seed treatment with *T. harzianum* and *P. fluorescens* increased total soluble sugar in the stem tissue of the sunflower recorded by Lamba *et al*. (2008) [17]. Infection of some pathogens in plant bring changes in respiratory pathway and photosynthesis which are vital processes taking place inside the plant leading to wide fluctuation in sugars (Saraswathi and Reddy, 2012) [31].

## Estimation of catalase (CAT) activity
Data pertaining in Table-3 revealed that the catalase (CAT) activity (OD/min/g fresh weight) as influenced by different treatments in cowpea plant. The catalase (CAT) activity recorded in only *S. rolfsii* inoculated plant with 1.41 OD/min/g fresh weight. The highest catalase (CAT) activity 3.50 OD/min/g fresh weight recorded in *T. virens* which was at par with *T. gliocladium* recorded 3.41 OD/min/g fresh weight.

CAT may contribute in resistance by limiting the damage caused by free radicals and also act like antibiotic against invading pathogen. These results are supported by Amin and Jampala (2018) [3] recorded pyraclostrobin 13.3% + epoxiconazole 5% against *S. rolfsii* enhanced activity of catalase. The highest enzymatic activity (CAT, POX and PPO) in fungicides (carboxin + thiram and thiophanate methyl) followed by salicylic acid and *T. viride* compared to infected control were induced resistance in onion against *Sclerotium cepivorum* (Morsy *et al*., 2021) [22].

**Estimation of phenylalanine ammonia lyase (PAL) activity**
The result presented in Table -3 showed that the phenylalanine ammonia lyase activity recorded in *S. rolfsii* inoculated cowpea plant with 2.36 OD/min/g fresh weight. The highest phenylalanine ammonia lyase (PAL) activity 3.72 OD/min/g fresh weight was recorded in *P. fluorescens* JAU isloate-3 which was at par with *T. gliocladium* which recorded with 3.57 OD/min/g fresh weight followed by *T. konginii* (3.51 OD/min/g fresh weight).

The phenylalanine ammonia lyase (PAL) is a key enzyme in the biosynthesis of phenyl propane unit, which lead to the synthesis of compounds that have diverse defensive functions in plants such as cell wall strengthening, antimicrobials, phytoalexins, phenolics and signaling of molecules (salicylic acid) which induces gene expression related to biosynthesis and production of pathogenesis-related (PR) proteins in plants against biotic stress. An application with *P. fluorescens* at 30 DAS increased the activities of phenylalanine ammonia lyase (PAL) in leaves and stem tissue of sunflower (Lamba *et al*., 2008) [17]. Ragavi *et al*. (2017) [26] noticed that the soil application with *T. viride* + *B. subtilis* + *P. fluorescence* at 2.5 kg ha$^{-1}$ which increased the activity of phenylalanine ammonia lyase on the fourth day of inoculation in tuberose.

**Estimation of peroxidase (POX) activity**
The significantly higher peroxidase activity was recorded in *T. koningii* with 8.15 OD/min/g fresh weight (Table 3). While, *P. fluorescens* JAU isolate-3 was recorded 7.68 OD/min/g fresh weight of peroxidase activity which was at par with *B. cereus* and *T. virens* recorded with 7.61 and 7.53 OD/min/g fresh weight of peroxidase activity, respectively. The peroxidase activity recorded from 2.43 OD/min/g fresh weight in only *S. rolfsii* inoculated cowpea plant. Also, peroxidase activity slightly increased in fungicides treated plants as compared to healthy as well as *S. rolfsii* inoculated plant.

POX activity increased rigidity of plant cell wall by synthesizing cell-wall polymers (lignin and suberin) and elevation in POX further indicated its role as physical barriers against pathogen stress. Khalid (2014) [14] recorded biocontrol agents increased the activity of enzymes compared to untreated control and tested fungicide (vitavax-200) against *S. rolfsii* in beans and highest activity of peroxidase (POX) recorded in *B. subtilis* with 24.9 OD/min/g. The activities of

both polyphenol oxidase and peroxidase increased in chickpea plant after the treatment with *Trichoderma* sp. (Jayalakshmi *et al*., 2009) [11].

**Estimation of polyphenol oxidase (PPO) activity**
Data pertaining to polyphenol oxidase (PPO) activity (OD/min/g fresh weight) as influenced by different treatments in cowpea plant (Table 3). The polyphenol oxidase activity recorded from 2.45 OD/min/g fresh weight in cowpea plant inoculated with
*S. rolfsii* only. The highest polyphenol oxidase activity 6.68 OD/min/g fresh weight recorded in *T. koningii* which was followed by *P. fluorescens* JAU isolate-3 with 5.53 OD/min/g fresh weight.

The result supported with Mandal *et al*. (2009) [21] who reported that activity of peroxidase (POX) and polyphenol oxidase (PPO) increased after incorporation of bio fungicides are important in the defense mechanism against pathogens. The highest activity of polyphenol oxidase (PPO) in *T. viride* with 62.1 OD/min/gm in bean damping-off of disease caused by *S. rolfsii* (Khalid, 2014) [14]. PPO activity was found to be 4.0-fold higher in leaf of groundnut infected with *S. rolfsii* plant at 5 DAI as compared to uninfected plants (Khatediya *et al*., 2018) [15].

**Estimation of superoxide dismutase (SOD) activity**
The change in superoxide dismutase (SOD) activity (OD/min/g fresh weight) as influenced by different treatments in cowpea plant (Table 3). The superoxide dismutase activity recorded in *S. rolfsii* inoculated cowpea plant with 1.05 OD/min/g fresh weight. The highest superoxide dismutase activity 2.37 OD/min/g fresh weight was recorded in *P. fluorescens* JAU isolate-3 which was followed by *B. cereus* (2.13 OD/min/g fresh weight). The superoxide dismutase activity recorded slightly higher in fungicides treated plants as compared to healthy as well as *S. rolfsii* inoculated plant.

Oxidative stress due to result of relative oxygen species (ROS) production over than elimination. To reduce and repair the damage associated with ROS, plants have evolved excellent antioxidant systems. Among, plants antioxidant enzyme defense system, SOD plays a major role in catalyzing the disproportionation of $O_2^-$ and converting $O_2^-$ to $O_2$ and $H_2O_2$. So, it increased SOD activity for the elimination of excessive $O_2^-$ and oxidative damage induced by $H_2O_2$ (Wu *et al*., 2010). The result was agreement with Amin and Jampala (2018) [3] who recorded pyraclostrobin 13.3% + epoxiconazole 5% enhanced the activities of defense-related enzymes such as POX, CAT and SOD and PAL against *S. rolfsii* in groundnut, which were higher in treated plants as compared to untreated plants. The wheat seedlings treated with the difenoconazole showed significantly higher SOD activity than the controls. The increase in SOD activity could effectively avoid excessive accumulation of $O_2^-$ and $H_2O_2$ (Liu *et al*., 2021) [18].

**Table 3:** Effect of different biocontrol agents and fungicides on activity of defense related enzymes in cowpea plant under pot condition

| Sr. No. | Treatment | CAT | PAL | POX | PPO | SOD |
|---|---|---|---|---|---|---|
| 1 | Mancozeb 75% WP | 1.54 | 2.48 | 3.20 | 2.21 | 1.04 |
| 2 | Thiram 75% WP | 1.44 | 2.60 | 3.90 | 2.57 | 1.05 |
| 3 | Propineb 70% WP | 1.80 | 2.45 | 3.13 | 2.52 | 1.17 |
| 4 | Zineb 75% WP | 1.44 | 2.51 | 3.47 | 2.44 | 1.15 |
| 5 | Tebuconazole 25.9% EC | 1.58 | 2.43 | 2.55 | 2.51 | 1.10 |
| 6 | Hexaconazole 5% EC | 1.35 | 2.58 | 3.49 | 2.63 | 1.28 |
| 7 | Azoxystrobin 23% SC | 1.46 | 2.36 | 2.90 | 2.53 | 1.01 |
| 8 | Tricyclazole 75% WP | 1.37 | 2.50 | 3.73 | 2.26 | 0.99 |
| 9 | Azoxystrobin 11% + tebuconazole 18.3% SC | 1.44 | 2.53 | 2.69 | 2.22 | 1.06 |
| 10 | Tebuconazole 5% + sulphur 65% WG | 1.51 | 2.76 | 3.61 | 2.64 | 1.44 |
| 11 | Carboxin 37.5% + thiram 37.5% WS | 1.65 | 2.67 | 2.81 | 2.70 | 1.07 |
| 12 | Carbendazim 25% + mancozeb 50% WS | 1.55 | 2.82 | 4.09 | 2.64 | 1.06 |
| 13 | Fluxapyraxad 250 G/L + pyraclostrobin 250 G/L SC` | 1.67 | 2.51 | 1.83 | 2.20 | 0.98 |
| 14 | *Trichoderma koningii* ($2 \times 10^6$ cfu/g) | 2.70 | 3.51 | 8.15 | 6.68 | 2.05 |
| 15 | *Trichoderma gliocladium* ($2 \times 10^6$ cfu/g) | 3.41 | 3.57 | 6.84 | 5.39 | 1.90 |
| 16 | *Trichoderma virens* ($2 \times 10^6$ cfu/g) | 3.50 | 3.32 | 7.53 | 5.02 | 1.89 |
| 17 | *Bacillus cereus* ($1 \times 10^7$ cfu/g) | 2.04 | 2.80 | 7.61 | 5.29 | 2.13 |
| 18 | *Pseudomonas fluorescens* JAU-3 ($1 \times 10^7$ cfu/g) | 2.75 | 3.72 | 7.68 | 5.53 | 2.37 |
| 19 | *Pseudomonas fluorescens* JAU-1 ($1 \times 10^7$ cfu/g) | 1.78 | 2.90 | 6.11 | 3.73 | 1.37 |
| 20 | Control (*S. rolfsii*) | 1.41 | 2.36 | 3.12 | 2.45 | 1.05 |
| 21 | Un inoculated | 0.62 | 1.60 | 2.43 | 1.43 | 0.69 |
| | S.Em.± | 0.04 | 0.07 | 0.11 | 0.10 | 0.04 |
| | C.D. at 5 % | 0.11 | 0.20 | 0.33 | 0.29 | 0.12 |
| | C.V. % | 2.88 | 3.53 | 3.66 | 4.27 | 4.15 |

Note: Enzymes activity were calculated as OD/min/g fresh weight of tissue

## Conclusion
Seed treatment with carbendazim 25% + mancozeb 50% WS @ 2.5 g/kg of seed or soil application with *T. koningii* or *T. virens* @ 25 g/kg of soil reported significantly reduced the stem rot disease of cowpea.

It was found that the soil application with biocontrol agents and seed treatment with fungicides not only promoted plant growth but also systemically stimulated the synthesis of various plant defense enzymatic (CAT, POX, PAL, PPO and SOD) pathways which further impart host resistance against *S. rolfsii* through accumulation of various phenolic compounds. Also found to enhance biochemical parameters such as total sugar, total phenol and total protein contents compared with the infected and healthy cowpea plants.

## Acknowledgements

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