



Impact of Carbon Dioxide Levels on Biochemical Parameters Associated with Stem Rot Disease in Groundnut

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ABSTRACT

Background: *Sclerotium rolfsii* is a widespread soil-borne fungal pathogen recognized for causing disease in a wide variety of agricultural and horticultural crops globally. Despite the economic losses attributed to this pathogen, there are limited reports concerning the biochemical alterations in groundnut in response to increased carbon dioxide levels and pathogen interactions.

Methods: A pot culture experiment was carried out at ICRISAT during the *rabi* season 2022-23 in Open Top Chambers (OTC) to assess the biochemical responses of groundnut to climate change and pathogen interactions. The study was carried out at three different carbon dioxide levels (400 ppm, 550 ppm and 700 ppm) using the susceptible groundnut cultivar TMV 2 and the moderately resistant cultivar ICGV-14082.

Result: The levels of total phenols, oxalic acid, ascorbic acid, catalase, peroxidases, polyphenol oxidase, and chlorophyll content index were elevated in groundnut plants infected with stem rot due to increased carbon dioxide levels. The biochemical activity was high at its peak in inoculated plants on 4th and 6th day post-inoculation of the pathogen and gradually declined on 8th and 10th days.

Key words: Climate change, Defense enzymes, Open top chambers, *Sclerotium rolfsii*.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), commonly referred to as peanut, goober pea, monkey nut and jack nut is a significant oilseed crop grown in southern and northern India. Often regarded as the king of oilseeds, it has been cultivated since as early as 1000 B.C. (Wiess, 1983). India ranks second after China in terms of both area and production of groundnut, with primary cultivation occurring in Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu, and Rajasthan. The crop is cultivated over an area of 29.59 million hectares globally, with a total production of 48.75 million tonnes (FAOSTAT, 2019). In India, the crop covers 4.8 million hectares, yielding 9.2 million tonnes (INDIASTAT, 2019). In the state of Telangana, it is grown over 0.13 million hectares, producing 0.30 million tonnes, with a productivity of 2364 kg per hectare (Directorate of Economics and Statistics, 2019). They are high in mono-unsaturated fatty acids, primarily oleic acid. It aids in the reduction of LDL (bad cholesterol) and the increase of HDL (good cholesterol) levels within the blood (Vamshi *et al.*, 2024). Groundnut productivity is being affected by several abiotic and biotic stresses which include poor soil fertility, moisture stress, viral diseases, collar rot and stem rot (Babu and Deepika, 2022).

Soil-borne diseases are major constraints in groundnut production. *S. rolfsii* is a soil-borne plant pathogen inciting root rot, stem rot, collar rot, wilt and foot rot diseases in over 500 plant species of agricultural and horticultural crops worldwide (Aycock, 1966). Stem rot disease occurs under conditions of sufficient moisture and temperature that facilitate the growth and persistence of *Sclerotium rolfsii*. Groundnut plants are susceptible at all

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growth stages, including the seed germination stage which leads to pre-emergence rot, while young plants exhibits stem rot symptom. The duration for wilt occurrence ranging from 8 to 15 days. Younger plants are found to be more vulnerable, as the infection spread more quickly (Patil and Rane, 1983). The mycelium of the pathogen thrives best in sandy soils, whereas the sclerotia survive optimally in moist, aerobic conditions present at the soil surface (Punja, 1985).

Biotic and abiotic stresses trigger intricate biochemical and physiological alterations in plants. In many plant-pathogen interactions, these biochemical changes are marked by a swift increase in phenolic compounds and associated enzymes, often referred to as the hypersensitive response (Mondal *et al.*, 2012). Certain studies on biochemical modifications during pathogenesis indicate that specific defense biomolecules, such as phenols and sugars, as well as enzymes like peroxidase and polyphenol oxidase, are produced in higher quantities to enhance resistance against the pathogen (Jiang *et al.*, 2009). Such modifications in biochemical changes can be attributed to various defense mechanisms exhibited by the host during the process of pathogenesis (Jayaraj *et al.*, 2010).

Between 1960 and 2019, atmospheric CO₂ levels increased from 320 ppm to 412 ppm, with projections suggesting a 50% rise by 2050 if current trends continue. The average annual increase in CO₂ was 2.1 ppm from 2004-2013 as compared to 1.9 ppm from 1994-2003. Elevated CO₂ acts as a nutrient, enhancing agricultural productivity by boosting photosynthesis and water-use efficiency, while reducing transpiration due to decreased stomatal conductance. Most C3 crop plants are particularly responsive to atmospheric CO₂ levels, which significantly influence their photosynthesis, transpiration, biomass production and making current CO₂ levels a critical factor for crop performance.

In this context, the current study was investigated on the biochemical parameters in response to elevated CO₂ concentrations of 400 ppm, 550 ppm and 700 ppm.

MATERIALS AND METHODS

Treatment setup in open top chambers (OTC)

A pot culture experiment was carried out at ICRISAT during the *rabi* season 2022-23 in Open Top Chambers (OTC) to assess the biochemical responses of groundnut to climate change and pathogen interactions. The study was carried out at three different carbon dioxide levels (400 ppm, 550 ppm, and 700 ppm) using the susceptible groundnut cultivar TMV 2 and the moderately resistant cultivar ICGV-14082. Groundnut seedlings of 30-35 days old were inoculated by spreading the inoculum on sub surface of soil.

Assessment of biochemical changes in groundnut in response to climate change and pathogen interactions.

Spectrophotometric assay of total phenols, peroxidase, polyphenol oxidase, catalase, oxalic acid and ascorbic acid are explained here under the following headings.

Sample collection

Leaf and stem samples were collected from individual treatments to assess the induction of defense enzymes in response to pathogen attack in groundnut plants under Open Top Chambers (OTC). The samples were collected at 0, 2, 4, 6, 8, and 10 days post-inoculation (dpi) at two-days intervals. The collected samples were promptly

immersed in liquid nitrogen and stored at -80°C for later use.

Preparation of enzyme extract

Plant/stem quantity of (0.5 g) sample was homogenized with ten times of its volume of 80% ethanol. The residue was re-extracted with five times its volume of 80% ethanol, centrifuged, and the supernatant was combined. The supernatant was evaporated to dryness at 40°C for 4-5 hours using a speed vacuum evaporator and the residue was dissolved in known volume of distilled water (5 ml) for estimation of peroxidase, total phenols, polyphenol oxidase, and catalase assays.

Estimation of total phenols

A 10 ml aliquot of Folin-Ciocalteu reagent (FCR) was mixed in 1 ml of the alcohol extract in a test tube followed by 2 ml of 20% sodium carbonate solution. The mixture was heated in a boiling water bath for exactly one minute and then cooled. The solution was adjusted to a known volume with distilled water. The resulting blue complex was measured at 650 nm using a spectrophotometer. Catechol standards were used to calculate phenol concentration and expressed as mg/g fresh weight/mg protein (Malik and Singh, 1980).

Estimation of peroxidase

The reaction mixture comprised of 1.5 ml of 0.05 M pyrogallol and 0.5 ml of the enzyme extract. The reaction was initiated by adding 0.5 ml of 1% H₂O₂. The change in absorbance was recorded at 470 nm over a period of 3 minutes using a spectrophotometer. The boiled enzyme preparation was used as the blank. Enzyme activity was expressed as the change in absorbance at 470 nm per minute per gram of fresh weight of tissue per milligram of protein ($\Delta OD_{470} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ protein}$) (Hammerschmidt *et al.*, 1982).

Estimation of polyphenol oxidase

The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 0.5 ml of the enzyme extracts. To initiate the reaction, 0.5 ml of 0.5 M catechol was added and change in absorbance was recorded at 420 nm for 3 minutes using a spectrophotometer. The activity of polyphenol oxidase was expressed as the change in absorbance at 420 nm per minute per gram of fresh weight of tissue per milligram of protein ($DOD_{420} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ protein}$) (Mayer *et al.*, 1965).

Estimation of catalase

Catalase activity was measured spectrophotometrically following the method outlined by Chaparro-Giraldo *et al.* (2000). The assay consisted of 3 ml mixture with 100 mM potassium phosphate buffer (pH 7.5), 25 mM H₂O₂ (freshly prepared) and 100 µl of enzyme extract. H₂O₂ degradation was monitored at 240 nm over 3 minutes using a spectrophotometer with a blank containing no plant extract for reference. The reduction in optical density at this

wavelength was indicated by the decrease in H_2O_2 concentration. Enzyme activity was calculated using the extinction coefficient ($\epsilon_{240\text{ nm}} = 40\text{ M}^{-1}\text{ cm}^{-1}$) and expressed as μmol of H_2O_2 per minute per gram of fresh weight per milligram of protein ($\mu\text{mol H}_2\text{O}_2\text{ min}^{-1}\text{ g}^{-1}\text{ FW mg}^{-1}\text{ protein}$).

Estimation of oxalic acid

Groundnut stem and leaf samples (500 mg) from all treatments were extracted with 1.5 ml of 4N H_2SO_4 using a mortar and pestle. After adding 5 ml of diethyl ether, the mixture was heated for 6 hours at 60°C . Subsequently, 5 ml of 1N NaOH and 7 ml of distilled water were added, and the ether layer was evaporated. The remaining aqueous phase was combined with 4 ml of calcium acetate buffer (100 mM, pH 6.8) and centrifuged for 10 minutes at 3000 rpm. After washing the pellet, it was dissolved in 5 ml of 4N H_2SO_4 , heated and titrated with 0.02N KMnO_4 to a faint pink endpoint. The oxalic acid content was calculated based on the volume of KMnO_4 used (1 ml = 1.78 mg oxalic acid (Mahadevan and Sridhar, 1986).

Estimation of ascorbic acid

Ascorbic acid was quantified using the Dinitrophenyl hydrazine (DNPH) method (Sadasivam and Manickam, 1992). Groundnut leaf samples (500 mg) were extracted with 5 ml of 6% trichloroacetic acid (TCA) using mortar and pestle. The homogenate was centrifuged and the supernatant was collected. An aliquot (0.1 ml) was diluted to 1 ml with distilled water, then mixed with 2 ml of 2% DNPH reagent and two drops of 10% thiourea. After boiling at 80°C for 15 minutes, 5 ml of 80% H_2SO_4 was added and color intensity was measured at 540 nm using a spectrophotometer.

RESULTS AND DISCUSSION

The biochemical analysis of groundnut plants inoculated with *S. rolfsii* through soil application in OTC revealed higher

activity of enzymes like ascorbic acid, oxalic acid, peroxidases, catalase, total phenols and polyphenol oxidase.

Total phenols

The total phenol content analysis in groundnut plants inoculated with the virulent isolate of *S. rolfsii* (SrPWp) in OTC chamber through soil application indicated that among the sampling intervals, significantly highest quantity of phenols was expressed in inoculated plants on 4th and 6th day post inoculation of pathogen (dpi) and gradually decreased on 8th and 10th dpi (Fig 1).

In TMV-2 groundnut cultivar, highest mean phenol content of $0.33\text{ mg g}^{-1}\text{ FW mg}^{-1}\text{ protein}$ was recorded at 700 ppm of carbon dioxide which was followed by $0.29\text{ mg g}^{-1}\text{ FW mg}^{-1}\text{ protein}$ at 550 ppm and $0.24\text{ mg g}^{-1}\text{ FW mg}^{-1}\text{ protein}$ at 400 ppm concentration.

In ICGV-14482 cultivar, highest mean phenol content of $0.39\text{ mg g}^{-1}\text{ FW mg}^{-1}\text{ protein}$ was recorded at 700 ppm of concentration followed by $0.35\text{ mg g}^{-1}\text{ FW mg}^{-1}\text{ protein}$ at 550 ppm and $0.31\text{ mg g}^{-1}\text{ FW mg}^{-1}\text{ protein}$ at 400 ppm of concentration. Phenol content was increased by 29.16 per cent in infected groundnut cultivar ICGV-14082 as compared to infected cultivar TMV-2 at 400 ppm CO_2 level followed by 20.68 per cent and 18.18 per cent at 550 ppm and 700 ppm respectively. From the above results, phenol content was more in moderately resistant cultivar than susceptible cultivar and phenol content was increased as carbon dioxide levels increased.

Hasan and Meah (2019) reported that contents of ascorbic acid, total phenols, reducing sugars, total sugars and Ca-oxalate in the collar region were increased in three eggplant varieties (BAUBegun-1, BAUBegun-2, and Dohazari G) infected with *Sclerotium rolfsii*.

Tatmiya *et al.* (2020) observed that total phenol increased in *Sclerotium rolfsii* infected groundnut plants.

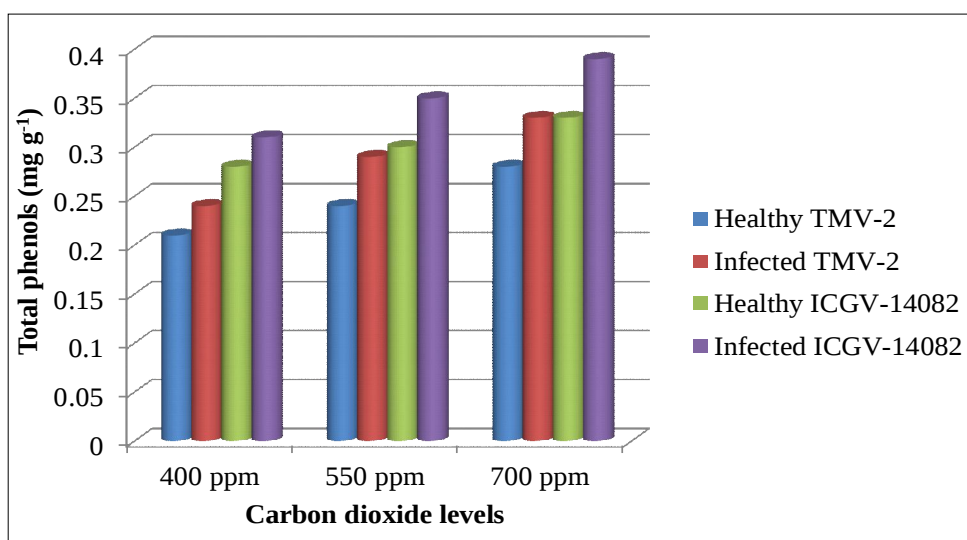


Fig 1: Effect of carbon dioxide levels on phenol activity (mg g^{-1}) in susceptible cultivar (TMV 2) and moderately resistant cultivar (ICGV-14082) groundnut plants.

Reddy and Sireesha (2014) found higher amount of total and ortho-di hydric phenols and protein content in stem rot infected tissues of susceptible variety of groundnut TMV2.

Parmar and Gohel (2024) reported that phenol amount was increased in resistant and susceptible diseased roots, but the higher increase of phenol was observed in resistant germplasm, while it was at low in susceptible germplasm.

Oxalic acid

The oxalic acid content analysis in groundnut plants inoculated with virulent isolate of *S. rolfii* (SrPWp) in OTC chamber through soil application indicated that among the sampling intervals, significantly highest amount of oxalic acid was expressed in inoculated plants on 4th and 6th day post inoculation of pathogen (dpi) and gradually decreased on 8th and 10th dpi (Fig 2).

In TMV-2 cultivar, highest mean oxalic acid content of 6.91 mg g⁻¹ FW was recorded at 700 ppm of carbon dioxide followed by 6.52 mg g⁻¹ FW at 550 ppm and 6.25 mg g⁻¹ FW at 400 ppm of concentration.

In ICGV-14482 cultivar, highest mean oxalic acid content of 7.51 mg g⁻¹ FW was recorded at 700 ppm of concentration followed by 7.18 mg g⁻¹ FW at 550 ppm and 7.00 mg g⁻¹ FW at 400 ppm of concentration.

Oxalic acid content was increased by 12 per cent in infected moderately resistant groundnut cultivar ICGV-14082 as compared to infected susceptible cultivar TMV-2 at 400 ppm CO₂ level followed by 10.12 per cent at 550 ppm and 8.68 per cent at 700 ppm. From the above results, it is observed that oxalic acid content was more in the moderately resistant cultivar (ICGV-14082) than the susceptible cultivar (TMV-2) and oxalic acid content was increased as carbon dioxide levels increased.

Pipaliya *et al.* (2017) was reported that the oxalic acid content increased by 95.68 per cent in *Sclerotium rolfii* infected groundnut plants.

Ascorbic acid

The ascorbic acid content in groundnut plants inoculated with virulent isolate of *S. rolfii* (SrPWp) in OTC chamber through soil application indicated that among the sampling intervals, significantly highest amount of ascorbic acid was expressed in inoculated plants on 4th and 6th day post inoculation of pathogen (dpi) and gradually decreased on 8th and 10th dpi (Fig 3). In TMV-2 cultivar, highest mean content of ascorbic acid of 0.31 mg g⁻¹ FW was recorded at 700 ppm of carbon dioxide followed by 0.28 mg g⁻¹ FW at 550 ppm and 0.24 mg g⁻¹ FW at 400 ppm of concentration. In ICGV-14482 cultivar, highest mean of ascorbic acid content of 0.38 mg g⁻¹ FW was recorded at 700 ppm of concentration followed by 0.36 mg g⁻¹ FW at 550 ppm and 0.31 mg g⁻¹ FW at 400 ppm of concentration. Ascorbic acid content increased by 29.16 per cent in infected moderately resistant cultivar ICGV-14082 as compared to infected susceptible cultivar TMV-2 at 400 ppm CO₂ level followed by 28.57 per cent and 22.58 per cent at 550 ppm and 700 ppm respectively. From the above results, we can note that ascorbic acid content was more in the moderately resistant cultivar than the susceptible cultivar and ascorbic acid content was increased as carbon dioxide levels increased.

Pipaliya *et al.* (2017) reported that the ascorbic acid was increased by 50.94 per cent in *Sclerotium rolfii* infected groundnut plants. Hasan and Meah (2019) reported that total phenols, ascorbic acid, total sugar, reducing sugar and Ca-oxalate contents of the collar region were increased in *Sclerotium rolfii* infected three egg plant varieties (BAUBegun-2, Dohazari G and BAUBegun-1).

Peroxidase

The peroxidase content analysis in groundnut plants inoculated with the virulent isolate of *S. rolfii* (SrPWp) in OTC chamber through soil application indicated that among the sampling intervals, significantly highest amount of

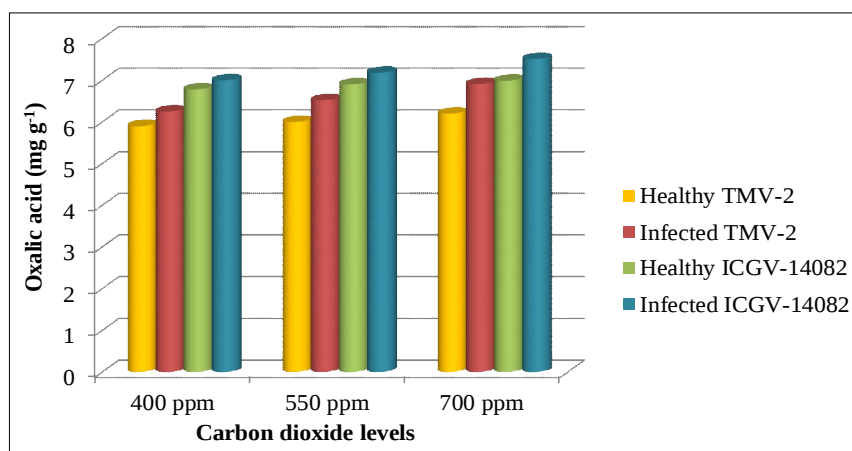


Fig 2: Effect of carbon dioxide levels on oxalic acid activity (mg g⁻¹) in susceptible cultivar (TMV 2) and moderately resistant cultivar (ICGV-14082) groundnut plants.

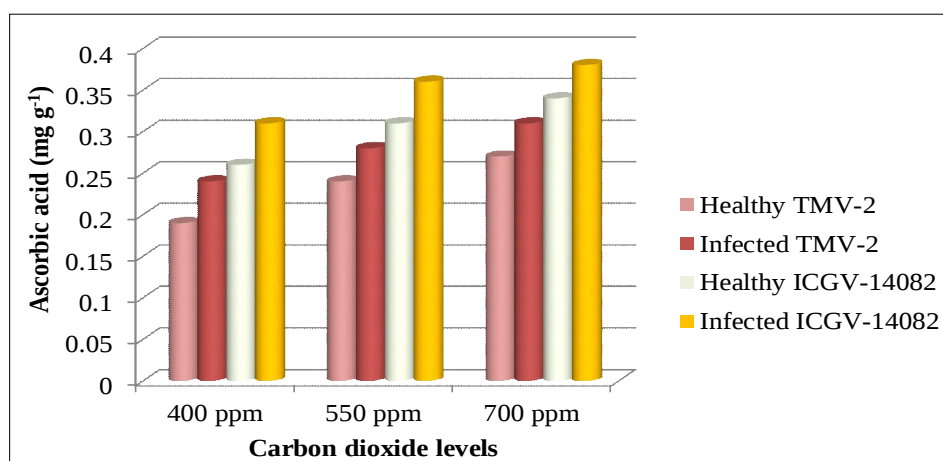


Fig 3: Effect of carbon dioxide levels on ascorbic acid activity (mg g^{-1}) in susceptible cultivar (TMV 2) and moderately resistant cultivar (ICGV-14082) groundnut plants.

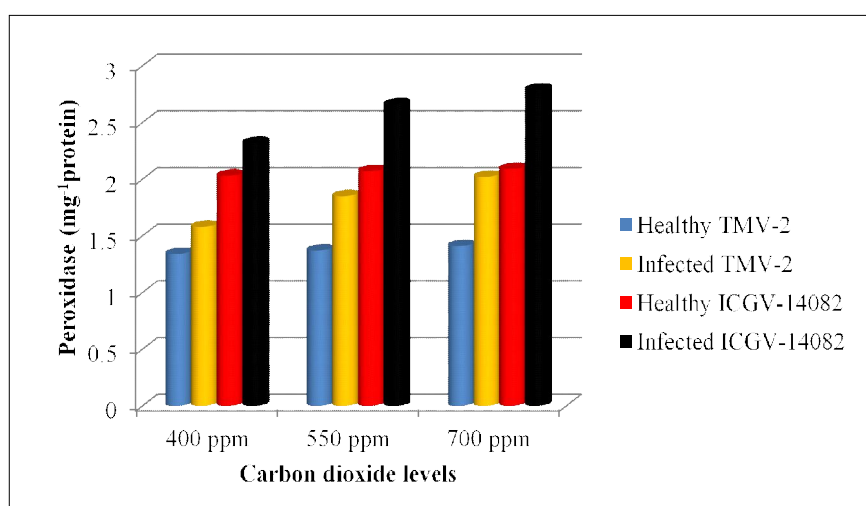


Fig 4: Effect of carbon dioxide levels on peroxidase (PO) activity ($\text{mg}^{-1} \text{protein}$) in susceptible cultivar (TMV 2) and moderately resistant cultivar (ICGV-14082) groundnut plants.

peroxidase was expressed in inoculated plants on 4th and 6th day post inoculation of pathogen (dpi) and gradually decreased on 8th and 10th dpi (Fig 4).

In TMV-2 cultivar, highest mean peroxidase content of $2.02 \Delta\text{OD}_{470} \text{ nm min}^{-1} \text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ was recorded at 700 ppm of carbon dioxide followed by $1.85 \Delta\text{OD}_{470} \text{ nm min}^{-1} \text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 550 ppm and $1.58 \Delta\text{OD}_{470} \text{ nm min}^{-1} \text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 400 ppm of concentration.

In ICGV-14482 cultivar, highest mean of peroxidase content was $2.79 \Delta\text{OD}_{470} \text{ nm min}^{-1} \text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ was recorded at 700 ppm of concentration followed by $2.66 \Delta\text{OD}_{470} \text{ nm min}^{-1} \text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 550 ppm and $2.32 \Delta\text{OD}_{470} \text{ nm min}^{-1} \text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 400 ppm of concentration.

Peroxidase content was increased by 46.83 per cent in the infected cultivar ICGV-14082 compared to the infected cultivar TMV-2 at 400 ppm CO_2 level which was followed by 43.78 per cent at 550 ppm and 38.11 per cent at 700 ppm.

It was observed that peroxidase levels were higher in the moderately resistant cultivar than the susceptible cultivar and peroxidase content increased as carbon dioxide levels increased.

Poornima *et al.* (2016) measured polyphenol oxidases (PPO), phenylalanine ammonia lyase (PAL) and peroxidases (POD) from healthy and *Sclerotium rolfsii*-inoculated stems of six genotypes of groundnut after 3 days of inoculation. They observed that the activity of PAL, PPO and POD began on the 3rd day after inoculation (DAI) and gradually increased until 6th DAI, after which it declined. Tatmiya *et al.* (2020) reported that catalase and peroxidase activity was found higher with significant correlation in *Sclerotium rolfsii* infected groundnut plant.

Singh *et al.* (2003) reported higher activity of β -1, 3-glucanase and peroxidase in the resistant cell line to *Fusarium oxysporum* f.sp. *ciceristhan* in the susceptible cultivars.

Pudjihartati *et al.* (2006) studied peroxidase activity of *S. rolfii* infected groundnut tissue and reported increased peroxidase activity and lignin content in the infected tissues. Regression analysis between POX and disease severity showed negative slope indicating the more resistance the genotype more peroxidase activity in tissues.

Catalase

The catalase content analysis in groundnut plants inoculated with SrPWp, the virulent isolate of *S. rolfii* in OTC chamber through soil application indicated that among the sampling intervals, significantly highest amount of catalase was expressed in inoculated plants on 4th and 6th day post inoculation of pathogen (dpi) and gradually decreased on 8th and 10th dpi (Fig 5).

In TMV-2 cultivar, highest mean catalase content of 1.05 $\mu\text{mole H}_2\text{O}_2 \text{ min}^{-1}\text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ was recorded at

700 ppm of carbon dioxide followed by 0.91 $\mu\text{mole H}_2\text{O}_2 \text{ min}^{-1}\text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 550 ppm and 0.78 $\mu\text{mole H}_2\text{O}_2 \text{ min}^{-1}\text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 400 ppm of concentration.

In ICGV-14482 cultivar, highest mean catalase content of 1.70 $\mu\text{mole H}_2\text{O}_2 \text{ min}^{-1}\text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ was recorded at 700 ppm of concentration followed by 1.63 $\mu\text{mole H}_2\text{O}_2 \text{ min}^{-1}\text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 550 ppm and 1.47 $\mu\text{mole H}_2\text{O}_2 \text{ min}^{-1}\text{g}^{-1} \text{FW mg}^{-1} \text{protein}$ at 400 ppm of concentration.

Catalase content was increased by 88.46 per cent in infected cultivar ICGV-14082 as compared to infected cultivar TMV-2 at 400 ppm CO_2 level followed by 79.12 per cent and 61.90 per cent at 550 ppm and 700 ppm respectively. From the above results, catalase content was more in moderately resistant cultivar than susceptible cultivar and catalase content was increased as carbon dioxide levels increased.

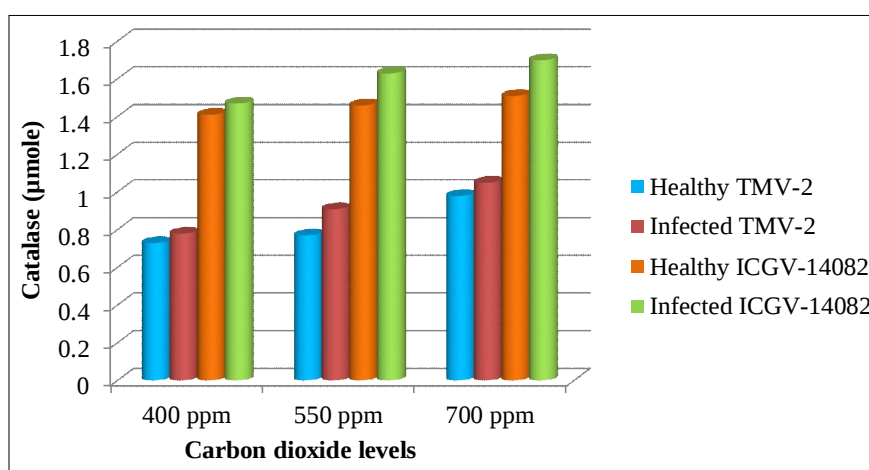


Fig 5: Effect of carbon dioxide levels on catalase activity (μmole) in susceptible cultivar (TMV 2) and moderately resistant cultivar (ICGV-14082) groundnut plants.

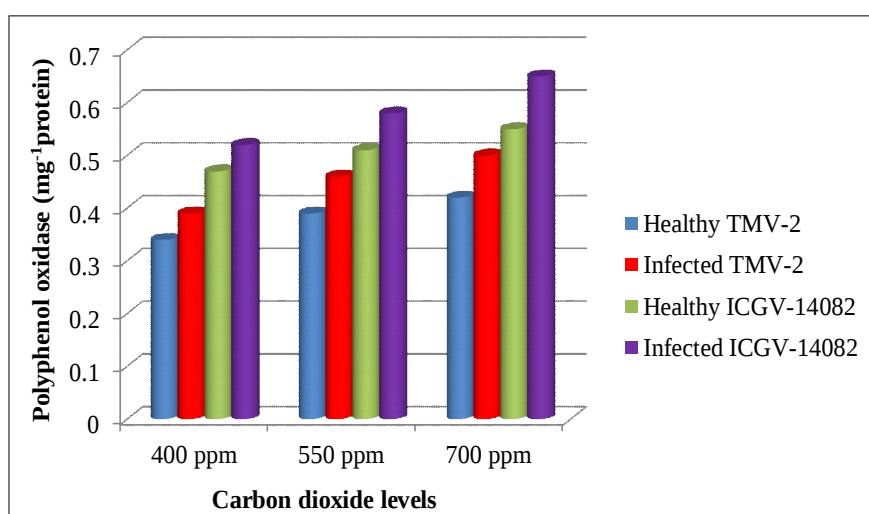


Fig 6: Effect of carbon dioxide levels on polyphenol oxidase activity ($\text{mg}^{-1}\text{protein}$) in susceptible cultivar (TMV 2) and moderately resistant cultivar (ICGV-14082) groundnut plants.

Tatmiya *et al.* (2020) reported that catalase and peroxidase activity was higher with significant correlation in *Sclerotium rolfsii* infected groundnut plant.

Garcia *et al.* (2002) reported that several antioxidant enzymes including catalase in roots and stems of resistant and susceptible chickpea cultivars inoculated with *Fusarium oxysporium* f. sp. *ciceri* the highly virulent race 5 of wilt showed catalase activities increased in infected roots.

Polyphenol oxidase

The polyphenol oxidase content analysis in groundnut plants inoculated with the virulent isolate of *S. rolfsii* (SrPWp) in OTC chamber through soil application indicated that among the sampling intervals, significantly highest amount of polyphenol oxidase was expressed in inoculated plants on 4th and 6th day post inoculation of pathogen (dpi) and gradually decreased on 8th and 10th dpi (Fig 6).

In TMV-2 cultivar, highest mean polyphenol oxidase content of 0.50 $\Delta OD_{420} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ protein}$ was recorded at 700 ppm of carbon dioxide followed by 0.46 $\Delta OD_{420} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ proteins}$ at 550 ppm and 0.39 $\Delta OD_{420} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ protein}$ at 400 ppm of concentration.

In ICGV-14482 cultivar, highest mean polyphenol oxidase content of 0.65 $\Delta OD_{420} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ protein}$ was recorded at 700 ppm concentration followed by 0.58 $\Delta OD_{420} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ protein}$ at 550 ppm and 0.52 $\Delta OD_{420} \text{ nm min}^{-1} \text{ g}^{-1} \text{ FW mg}^{-1} \text{ protein}$ at 400 ppm of concentration.

Polyphenol oxidase content was increased by 33.33% per cent in infected cultivar ICGV-14082 as compared to infected cultivar TMV-2 at 400 ppm CO_2 level followed by 26.08 per cent and 30 per cent at 550 ppm and 700 ppm, respectively. From the above results, we can note that polyphenol oxidase was more in moderately resistant cultivar than the susceptible cultivar and polyphenol oxidase content was increased as carbon dioxide levels increased.

Poomima *et al.* (2016) estimated peroxidases (POD), poly phenol oxidases (PPO) and phenyl alanine ammonia lyase (PAL) from healthy and *Sclerotium rolfsii* inoculated stems of 6 genotypes of groundnut after 3 days of inoculation and observed that the activity of PO, PPO and PAL began from 3rd DAI and gradually increased up to 6th DAI and thereafter declined.

Damodaran *et al.* (2009) found PAL and PPO activities were increased in roots of resistant banana hybrids over control and susceptible hybrids after infection with *F. oxysporum* f. sp. *cubense* race 1.

Saraswathi and Reddy (2012) found that the contents of Polyphenol oxidase (PPO), Peroxidase (POX) and Phenylalanine were increased throughout the sampling period of the stem rot disease as compared to healthy plants in groundnut.

The inactivation of pathogen pectolytic enzymes by the oxidized substrate of PPO is reported as a part of host resistance mechanism (Sarwar *et al.*, 2003).

CONCLUSION

In summary, biochemical defense serves as a robust mechanism in plants, aiding in their resistance to diseases. The accumulation of these compounds offers effective protection against pathogens. In groundnut plants infected with stem rot, levels of total phenols, oxalic acid, ascorbic acid, catalase, peroxidases and polyphenol oxidase rise as carbon dioxide levels increased. The heightened activities of these substances under elevated carbon dioxide conditions may be linked to the activation of antioxidant responses, which safeguard the plant from oxidative harm.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

- Aycock, R. (1966). Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of *Sclerotium* fungus after 70 years. Raleigh: North Carolina State University Technical Bulletin. p.174.
- Babu, G.N., Deepika, D.S. (2022). Survey for the Incidence of Stem Rot (*Sclerotium rolfsii* sacc) of Groundnut in Andhra Pradesh. Agricultural Science Digest. 42(5): 592-597. doi: 10.18805/ag.D-5507.
- Chaparro-Giraldo, A., Barata, R.M., Chabregas, S.M., Azevedo, R.A., Silva-Filho, M.C. (2000). Soybean leghemoglobin targeted to potato chloroplasts influences growth and development of transgenic plants. Plant Cell Reports. 19: 961-965.
- Damodaran, T., Kumar, N., Kavino, M. (2009). Breeding and evaluation of musa hybrids resistant to *Fusarium oxysporum* f. sp. *cubense* race 1. Journal of Fruits Science. 64: 3-12.
- Directorate of Economics and Statistics. (2019). In: Agricultural Statistics at a Glance. Directorate of Economics and Statistics. pp. 110.
- FAOSTAT. (2019). Food and Agricultural Organization statistics database (Faostat). <<http://faostat.fao.org/>>.
- Garcia-Limones, C., Hervas, A., Navas-Cortes, J.A., Mimenez-Diaz, R.M., Tena, M. (2002). Induction of antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interaction between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporium* f. sp. *ciceri*. Physiology and Molecular Plant Pathology. 61: 325-337.
- Hammerschmidt, R., Nuckles, E.M., Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiological Plant Pathology. 20: 73-82.
- Hasan, M.M., Meah, M.B. (2019). Anatomy and biochemical study of collar rot resistance in egg plant. International Journal of Pathogen Research. 2(2): 1-13.
- INDIASTAT. (2019). India statistics database (India stat). <https://www.indiastat.com>.
- Jayaraj, J., Ranganathan, B., Ramalingam, R., Muthukrishnan, S., Rethinasamy, V. (2010). Oxalic acid induced resistance to *Rhizoctonia solani* in rice is associated with induction of phenolics, peroxidase and pathogenesis-related proteins. Journal of Plant Interacation. 5: 147-157.

- Jiang, Y.F., Cheng, W.D., Qin, L.Q., Tan, X.J., Zhou, J.G., Xie, H.X., Chen, L. (2009). Physiological mechanism of exogenous salicylic acid induced resistance to sheath blight of maize (*Zea mays* L.). *Guang Agricultural Science*. 40: 242-245.
- Mahadevan, A., Sridhar, R. (1986). *Methods in Physiological Plant Pathology*. 3rd ed. Madras (India): Sivakami Publications. p. 178-179.
- Malik, C.P., Singh, M.B. (1980). *Plant Enzymology and Histo enzymology*. Kalyani Publishers, New Delhi.
- Mayer, A.M., Harel, E., Shaul, R.B. (1965). Assay of catechol oxidase: A critical comparison of methods. *Phytochemistry*. 5: 783-789.
- Mondal, A., Dutta, S., Nandi, S., Das, S., Chaudhuri, S. (2012). Changes in defence-related enzymes in rice responding to challenges by *Rhizoctonia solani*. *Archives of Phytopathology and Plant Protection*. 45: 1840-1851.
- Patil, M.B., Rane, M.S. (1983). Studies on host range effect of plant age on susceptibility and varietal reaction of groundnut to *Sclerotium rolfsii*. *Indian Journal of Mycology and Plant Pathology*. 13: 183-186.
- Pipaliya, H.R., Marviya, G.V., Ukani, P. K., Khunt, K.R., Movaliya, H. M., Bhuva, S.K. (2017). Biochemical and physiological changes in groundnut (*Arachis hypogaea* L.) in response to stem rot (*Sclerotium rolfsii*) infection. *International Journal of Chemical Studies*. 5(5): 983-991.
- Poornima, G.S., Sudini, H.K., Naik, M.K., (2016). Biochemical changes in groundnut (*Arachis hypogaea* L.) infected by stem and pod rot disease caused by *Sclerotium rolfsii* sacc. *Journal of Pure and Applied Microbiology*. 10(2): 1511-1517.
- Pudjihartati, E., Siswanto, I.S., Sudarsono, I. (2006). Chitinase activity in healthy and *Sclerotiumrolfsii* infected peanut. *Hayati Journal of Bioscience*. 2: 73-78.
- Punja, Z.K. (1985). The biology, ecology and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology*. 23: 97-127.
- Parmar, H.V., Gohel, N.M. (2024). Biochemical basis of resistance in chickpea (*Cicer arietinum* L.) against Wilt Complex. *Legume Research*. 47(11): 2011-2015. doi: 10.18805/LR-4795.
- Reddy, M.N., Sireesha, C.H. (2014). Role of oxidative enzymes and biochemical constituents in imparting resistance to groundnut (*Arachis hypogaea* L.) against stem rot disease caused by *Sclerotium rolfsii*. *Bioresearch*. 10(1): 36-41.
- Sadasivam, S., Manickam, A. (1992). *Biochemical Methods*. Wiley Eastern Ltd., New Delhi. 185-186.
- Saraswathi, M., Reddy, M.N. (2012). Phenolic acids associated with *Sclerotium rolfsii* in groundnut (*Arachis hypogaea* L.) during pathogenesis. *International Journal of Plant Pathology*. 3(2): 82-88.
- Sarwar, N., Sarwar, M., Jamil, F.F. (2003). Role of polyphenol oxidase and catalase in Ascochyta blight resistance in chickpea. *Pakistan Journal of Botany*. 35(1): 111-115.
- Singh, R., Sindhu, A., Singal, H.R., Singh, R. (2003). Biochemical basis of resistance in chickpea (*Cicer arietinum* L.) against *Fusarium* wilt. *Acta Phytopathologica Entomologica Hungarica*. 38(1): 13-19.
- Tatmiya, R.N., Padma, S.A., Rukam, S.T. (2020). Physiological and biochemical characterization of *Sclerotium rolfsii* with respect to its infection on groundnut. *Research Journal of Biotechnology*. 15(7): 83-88.
- Vamshi, J., Devi, G.U., Somraj, B., Maheswari, T.U., Supriya, K., Sudini, H.K. (2024). Biocontrol Efficacy of *Trichoderma* and *Bacillus* Isolates against *Sclerotium rolfsii* under *in vitro* Conditions. *Legume Research*. 1-9. doi: 10.18805/LR-5414.
- Wiess, E.A. (1983). *Oilseed Crops*: Longman Group Ltd.: London, UK.