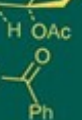
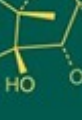
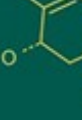
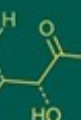
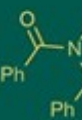


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## Biochemical profiling of groundnut plants infected with soil borne pathogens

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**ABSTRACT**

Groundnut (*Arachis hypogaea* L.), an important oilseed crop, is severely affected by soil borne fungal pathogens such as *Aspergillus niger*, *Agroathelia rolfsii* and *Macrophomina phaseolina*, resulting in significant yield losses. The present study was conducted during 2025 at the PG Laboratory, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh for evaluating the biochemical changes in healthy and soil borne infected groundnut plants. The infected plant samples showing typical symptoms of collar rot, stem rot and dry root rot were collected from Research Farm, Main Oilseeds Research Station, JAU, Junagadh where a research trail for biorational management of soil borne pathogens in groundnut was conducted with seven treatments. Results revealed that healthy plants recorded the highest levels of total sugar, reducing sugar, non-reducing sugar and total protein, with the lowest phenol content. Whereas, infected control plants showed reduced sugars and protein along with increased phenol accumulation. Among the treatments, the combined application of fluxapyroxad 333 g/l FS as seed treatment with soil application of *T. harzianum* and *P. fluorescens* - isolate 1 (T6) was most effective in restoring biochemical constituents and reducing phenol content, followed by T4 (fluxapyroxad 333 g/l FS + *T. harzianum*) and T1 (fluxapyroxad 333 g/l FS as seed treatment). Individual bioagent treatments showed moderate improvement as compared to the control.

**Keywords:** Groundnut, *A. niger*, *A. rolfsii*, *M. phaseolina*, Total sugars, Reducing and non-reducing sugars, Total protein, Total phenol, Biorational management

## 1. Introduction

Groundnut (*Arachis hypogaea* L.) is derived from two Greek words, *Arachis* meaning a legume and *hypogaea* meaning below ground, referring to the formation of pods below the soil surface. It is often called the “King of Oilseeds” because of its high oil content, wide adaptability and multipurpose uses (Hammons, 1982) [1]. It is believed to have originated in Brazil, Paraguay and Bolivia in South America (Upadhyaya et al., 2000) [2]. Groundnut kernels contain approximately 45 - 50% oil and 25 - 30% protein (Weiss, 1983) [3]. The oil primarily consists of triacylglycerols, rich in unsaturated fatty acids, particularly oleic acid (40 - 55%) and linoleic acid (25 - 35%). These unsaturated fatty acids are beneficial for human health as they help reduce cholesterol levels and the risk of cardiovascular diseases (Savage et al., 1994) [4]. However, its productivity is significantly affected by soil borne fungal diseases such as collar rot caused by *Aspergillus niger*, stem rot by *Agroathelia rolfsii* and dry root rot/charcoal rot by *Macrophomina phaseolina*.

Infection by these pathogens induces profound biochemical changes in host plants, particularly in primary metabolites such as total sugars, reducing and non-reducing sugars, proteins and secondary metabolites like phenols, which play a crucial role in plant defense mechanisms. Studies have shown that healthy groundnut plants generally possess higher carbohydrate content compared to infected tissues, whereas phenol and protein contents tend to increase in response to pathogen attack. Similarly, infection by *A. niger* and *M. phaseolina* has been reported to reduce carbohydrate reserves while altering protein composition in groundnut seeds (Mahatma et al., 2018) [5]. Therefore, comparative biochemical analysis of healthy and infected groundnut plants provides valuable insights into the physiological and defence responses against soil borne pathogens. Such studies are essential for understanding resistance mechanisms and developing effective disease management strategies.

## 2. Materials and methods

This experiment was conducted at PG Laboratory, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat during the year 2025 for estimation of non-structural carbohydrates i.e. total, reducing and non-reducing sugars, true protein and total phenol from the infected part of the plant from respective treatments. Infected samples of groundnut plants showing typical symptoms

of collar rot, stem rot and dry root rot were collected from Research Farm, Main Oilseeds Research Station, JAU, Junagadh, where the research trial for biorational management of soil borne pathogens in groundnut was laid out in Randomized Block Design with seven treatments and three repetitions.

The research trail comprised of seven treatments viz., fluxapyroxad 333 g/l FS as seed treatment (T<sub>1</sub>), seed treatment of *T. harzianum* (T<sub>2</sub>), seed treatment of *P. fluorescens* - isolate 1 (T<sub>3</sub>), fluxapyroxad 333 g/l FS as seed treatment + soil application of *T. harzianum* (T<sub>4</sub>), fluxapyroxad 333 g/l FS as seed treatment + soil application of *P. fluorescens* - isolate 1 (T<sub>5</sub>), fluxapyroxad 333 g/l FS as seed treatment + soil application of *T. harzianum* + soil application of *P. fluorescens* - isolate 1 (T<sub>6</sub>) and control (T<sub>7</sub>). The infected samples from all the seven treatments along with healthy plant were collected in morning hours and immediately kept in icebox and further processed for determination of all biochemical parameters under study.

## 2.1 Estimation of total sugars

Total sugar content was determined by the colorimetric method using anthrone reagent (Dubois *et al.*, 1956) [6].

### Reagents

1. Methanol 80%

$$\text{Total Sugar (mg/g)} = \frac{\text{Graph factor} \times \text{O.D.} \times \text{Total volume} \times 10^{-3}}{\text{Aliquote} \times \text{Sample weight}}$$

### 2.1.1 Reducing sugars

Reducing sugar content was measured following “Nelson’s modification of “Somogyi’s method” (Somogyi, 1952) [7] using arsenomolybdate colour forming reagent and two copper reagent “A” and “B”.

### Procedure

100 mg infected sample was taken and sugars were extracted twice with hot 80% alcohol and the supernatants were collected, evaporated on a water bath and the residue was dissolved in 10 ml of water. Aliquots of 0.1 or 0.2 ml of the alcohol-free extract were pipetted into

$$\text{Total Sugar (mg/g)} = \frac{\text{Graph factor} \times \text{O.D.} \times \text{Total volume} \times 10^{-3}}{\text{Aliquote} \times \text{Sample weight}}$$

### 2.1.2 Non-reducing sugar

The amount of non-reducing sugar was obtained by subtracting reducing sugar from the number of total sugars and multiplying the resultant with a constant factor 0.95 (Somogyi, 1952) [7].

## 2.2 Estimation of total phenols

The total phenol content was estimated by using Folin-ciocalteu reagent as per the method described by Thimmaiah (1999) [8].

### Reagents

- Ethanol 80%
- Folin-Ciocalteu reagent (FCR)
- 20% Sodium bicarbonate (Na<sub>2</sub>CO<sub>3</sub>)
- Standard (100 mg catechol in 100 ml of water). Diluted 10 times for a working standard.

### Procedure

One gram of infected tissue was ground with 10 ml of 80% ethanol using a mortar and pestle and the homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered and residue was re-extracted with five times the volume of 80% ethanol. The combined extract was cooled and evaporated to dryness on a water bath. The residue was then dissolved in 5 ml of distilled water and a

2. Phenol 5%

3. Sulphuric acid 96%

4. Standard (100 mg glucose in 100 ml of water). Diluted 10 times for a working standard.

### Procedure

0.5 g infected tissue was weighed and homogenized in 5 ml of 80% methanol using a mortar and pestle. Working standards (0.2-1.0 ml) were pipetted into a series of test tubes and 0.2 ml of the sample extract was taken in duplicate tubes such that the volume in all tubes was made up to 1 ml with distilled water and a blank was prepared with 1 ml distilled water. To each tube, 1 ml phenol and 5 ml of 96% sulfuric acid were added, mixed well and allowed to stand for 10 minutes, followed by incubation in a water bath at 25-30 °C for 20 minutes. The colour developed was measured at 490 nm using a spectrophotometer and total sugar content was calculated from the standard curve and expressed as mg/g fresh weight.

test tubes. Working standards (0.2-1.0 ml) were prepared in separate tubes and the volume in both sample and standard tubes was made up to 2 ml with distilled water, while a blank was prepared with 2 ml distilled water. Then, 1 ml of alkaline copper tartrate reagent was added to each tube, the tubes were heated in a boiling water bath for 10 minutes, cooled and 1 ml of arsenomolybdate acid reagent was added. The volume in each tube was made up to 10 ml with water and absorbance was measured at 620 nm using a spectrophotometer. The values were plotted against a glucose standard curve and the results were expressed as mg/g of fresh weight.

0.2 ml aliquot was taken in a test tube and the volume was made up to 3 ml with distilled water. To this, 0.5 ml of Folin-Ciocalteu reagent was added and after 3 minutes, 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were heated in a boiling water bath for 10 minutes and the absorbance was measured at 650 nm using a spectrophotometer. Total phenol content was calculated using a standard curve prepared with catechol (10-50 µg) and was expressed as mg/g fresh tissue weight.

## 2.3 Estimation of total protein

Extraction and estimation of total protein content was measured by Folin-Lowery’s method (Lowry *et al.*, 1951) [9].

### Reagents

- Solution A: 2 per cent Sodium carbonate (Anhydrous) in 0.1 N NaOH.
- Solution B: 0.5 per cent Copper sulphate (CuSO<sub>4</sub>.5H<sub>2</sub>O) in 1 per cent sodium potassium tartrate (prepare fresh).
- Solution C: Mix 50 ml of solution A with 1 ml of solution B just prior to use.
- Folin-Ciocalteu reagent (FCR).
- Stock standard protein solution: 50 mg of bovine serum albumin/50 ml of water.

- For working standard solution: Dilute 10 ml of the stock solution to 50 ml with water to obtain 200 µg protein/ml.

$$\text{Total Sugar (mg/g)} = \frac{\text{Graph factor} \times \text{O.D.} \times \text{Total volume} \times 10^{-3}}{\text{Aliquote} \times \text{Sample weight}}$$

### Procedure

One gram infected tissue was weighed and homogenized in 10 ml of 0.1 M sodium phosphate buffer, followed by centrifugation at 10,000 rpm for 20 minutes and the supernatant was collected for protein estimation. An aliquot of 0.2 ml of the extract was taken in a test tube and the volume was made up to 3 ml with distilled water. To this, 5 ml of alkaline copper solution (Solution C) was added and allowed to stand for 10 minutes at room temperature, followed by the addition of 0.5 ml of Folin–Ciocalteu reagent (1:1 v/v). The mixture was then incubated for 30 minutes at room temperature and the absorbance was measured at 660 nm using a spectrophotometer. The protein content was determined using a standard curve prepared with bovine serum albumin (50–300 µg) and expressed as mg/g of fresh weight.

### Statistical analysis

Statistical analysis was carried out as per standard methods as suggested by Panse *et al.* (1985) [10].

## 3. Results and discussion

The biochemical analysis of healthy and infected plants of groundnut with collar rot, stem rot and dry root rot with different treatments were undertaken in this experiment to determine changes in total, reducing and non-reducing sugars, true protein and total phenol. The results of biochemical properties are communicated here.

### 3.1 Biochemical characterization of healthy and *A. niger* infected plants

Table 3.1 shows that healthy plants recorded the highest values for total sugar (4.34 mg/g), reducing sugar (3.10 mg/g) and true protein (4.43 mg/g), respectively. In contrast, the control (infected plants)

$$\text{Total protein (mg/g)} = \frac{\text{Graph factor} \times \text{O.D.} \times \text{Total volume} \times 10^{-3}}{\text{Aliquote} \times \text{Sample weight}}$$

Among the treatments, the combined application (T<sub>6</sub>) (fluxapyroxad 333 g/l FS as seed treatment + soil application of *T. harzianum* + soil application of *P. fluorescens* - isolate 1) was proved most effective, recording higher total sugar (4.29 mg/g), reducing sugar (2.10 mg/g), non-reducing sugar (2.08 mg/g) and true protein (4.25 mg/g), closely approaching the healthy plant values. This was followed by T<sub>4</sub> (T<sub>1</sub> + soil application of *T. harzianum*), which also significantly improved total sugar (4.21 mg/g) and protein content (4.14 mg/g). Seed treatment with fluxapyroxad 333 g/l FS enhanced total sugar (4.05 mg/g) and true protein (4.08 mg/g) compared to control. Total phenol content was highest in the control (5.07 mg/g), whereas treated plants recorded comparatively lower phenol levels, particularly in the combined treatment (2.25 mg/g) and healthy plants (1.35 mg/g).

### 3.3 Biochemical characterization of healthy and *M. phaseolina* infected plants

Table 3.3 shows that healthy plants recorded the highest total sugar (4.34 mg/g), reducing sugar (3.10 mg/g), non-reducing sugar (1.17 mg/g) and true protein content (4.43 mg/g). In contrast, the control (infected plants) exhibited a marked decline in total sugar (2.27 mg/g), reducing sugar (1.46 mg/g) and true protein (2.07 mg/g),

showed a marked reduction in total sugar (2.13 mg/g), reducing sugar (1.68 mg/g), non-reducing sugar (0.42 mg/g) and protein content (2.13 mg/g), along with the highest phenol content (4.42 mg/g) due to *A. niger* infection.

Among the treatments, combined application (T<sub>6</sub>) (fluxapyroxad 333 g/l FS as seed treatment + soil application of *T. harzianum* + soil application of *P. fluorescens* - isolate 1) was found to be the most effective in improving biochemical parameters of infected plants, recording higher total sugar (3.83 mg/g), reducing sugar (2.40 mg/g) and true protein (4.04 mg/g) compared to individual treatments. This was followed by T<sub>4</sub> (T<sub>1</sub> + soil application of *T. harzianum*) (3.68 mg/g total sugar and 3.95 mg/g protein). Seed treatment with fluxapyroxad 333 g/l FS (T<sub>1</sub>) also enhanced total sugar (3.54 mg/g) and protein content (3.86 mg/g) over control. Total phenol content was highest in control plants (4.42 mg/g) and comparatively lower in treated plants, particularly in the combined treatment (1.76 mg/g) and healthy plants (1.35 mg/g).

### 3.2 Biochemical characterization of healthy and *A. rolfsii* infected plants

Table 3.2 shows that healthy plants recorded the highest values of total sugar (4.34 mg/g), reducing sugar (3.10 mg/g) and true protein (4.43 mg/g). In contrast, the control (infected plants) exhibited a substantial reduction in total sugar (1.61 mg/g), reducing sugar (1.48 mg/g), non-reducing sugar (0.12 mg/g) and true protein (2.22 mg/g), while showing the highest accumulation of total phenols (5.07 mg/g), due to *A. rolfsii* attack.

along with a sharp increase in total phenols (5.33 mg/g), due to *M. phaseolina* infection.

Among the treatments, the combined application (T<sub>6</sub>) (fluxapyroxad 333 g/l FS as seed treatment + soil application of *T. harzianum* + soil application of *P. fluorescens* - isolate 1) was found to be the most effective, registering higher total sugar (3.91 mg/g), reducing sugar (2.46 mg/g), non-reducing sugar (1.37 mg/g) and true protein (4.27 mg/g), approaching the levels observed in healthy plants. This was followed by T<sub>4</sub> (T<sub>1</sub> + soil application of *T. harzianum*), which recorded total sugar of 3.70 mg/g and protein content of 4.19 mg/g. Seed treatment with fluxapyroxad 333 g/l FS also improved biochemical constituents, particularly reducing sugar (2.40 mg/g) and protein (4.06 mg/g), compared to the untreated control. Total phenol content was highest in the untreated control (5.33 mg/g), whereas treated plants exhibited comparatively lower phenol levels, especially under combined treatment (2.30 mg/g) and in healthy plants (1.35 mg/g). Similar trends for biochemical characterization of healthy and infected groundnut plants with *A. niger*, *A. rolfsii* and *M. phaseolina* were also observed by Vijay Shree *et al.* (2022) [11], Nikita *et al.* (2021) [12] and Aiswarya *et al.* (2020) [13] which support the present findings.

**Table 3.1.** Biochemical characterization of healthy and *A. niger* infected plants

| Sr. No. | Treatment details                                                                        | Total sugar (mg/g) | Reducing sugar (mg/g) | Non reducing sugar (mg/g) | True protein (mg/g) | Total phenol (mg/g) |
|---------|------------------------------------------------------------------------------------------|--------------------|-----------------------|---------------------------|---------------------|---------------------|
| 1       | Fluxapyroxad 333 g/l FS as seed treatment (2-3 g/kg)                                     | 3.54               | 2.10                  | 1.36                      | 3.86                | 2.40                |
| 2       | Seed treatment of <i>Trichoderma harzianum</i> (8-10 g/kg)                               | 3.17               | 1.93                  | 1.17                      | 3.75                | 2.74                |
| 3       | Seed treatment of <i>Pseudomonas fluorescens</i> - isolate 1 (8-10 ml/kg)                | 3.00               | 1.87                  | 1.07                      | 3.68                | 2.81                |
| 4       | T <sub>1</sub> + soil application of <i>Trichoderma harzianum</i> (2.5 kg/ha)            | 3.68               | 2.23                  | 1.37                      | 3.95                | 2.24                |
| 5       | T <sub>1</sub> + soil application of <i>Pseudomonas fluorescens</i> - isolate 1 (3 l/ha) | 3.48               | 2.03                  | 1.37                      | 3.85                | 2.44                |
| 6       | T <sub>4</sub> + T <sub>5</sub>                                                          | 3.83               | 2.40                  | 1.35                      | 4.04                | 1.76                |
| 7       | Control                                                                                  | 2.13               | 1.68                  | 0.42                      | 2.13                | 4.42                |
| 8       | Healthy                                                                                  | 4.34               | 3.10                  | 1.17                      | 4.43                | 1.35                |
|         | S. Em. ±                                                                                 | 0.12               | 0.09                  | 0.10                      | 0.09                | 0.10                |
|         | C. D. at 5%                                                                              | 0.36               | 0.26                  | 0.37                      | 0.27                | 0.30                |
|         | C.V.%                                                                                    | 2.6                | 2.40                  | 2.58                      | 1.81                | 2.64                |

**Table 3.2.** Biochemical characterization of healthy and *A. rolfsii* infected plants

| Sr. No. | Treatment details                                                                        | Total sugar (mg/g) | Reducing sugar (mg/g) | Non reducing sugar (mg/g) | True protein (mg/g) | Total phenol (mg/g) |
|---------|------------------------------------------------------------------------------------------|--------------------|-----------------------|---------------------------|---------------------|---------------------|
| 1       | Fluxapyroxad 333 g/l FS as seed treatment (2-3 g/kg)                                     | 4.05               | 1.85                  | 2.09                      | 4.08                | 2.51                |
| 2       | Seed treatment of <i>Trichoderma harzianum</i> (8-10 g/kg)                               | 3.61               | 1.73                  | 1.78                      | 3.75                | 2.85                |
| 3       | Seed treatment of <i>Pseudomonas fluorescens</i> - isolate 1 (8-10 ml/kg)                | 3.54               | 1.71                  | 1.73                      | 3.62                | 2.98                |
| 4       | T <sub>1</sub> + soil application of <i>Trichoderma harzianum</i> (2.5 kg/ha)            | 4.21               | 2.03                  | 2.07                      | 4.14                | 2.43                |
| 5       | T <sub>1</sub> + soil application of <i>Pseudomonas fluorescens</i> - isolate 1 (3 l/ha) | 3.85               | 1.80                  | 1.94                      | 3.82                | 2.75                |
| 6       | T <sub>4</sub> + T <sub>5</sub>                                                          | 4.29               | 2.10                  | 2.08                      | 4.25                | 2.25                |
| 7       | Control                                                                                  | 1.61               | 1.48                  | 0.12                      | 2.22                | 5.07                |
| 8       | Healthy                                                                                  | 4.34               | 3.10                  | 1.17                      | 4.43                | 1.35                |
|         | S. Em. ±                                                                                 | 0.08               | 0.08                  | 0.23                      | 0.09                | 0.11                |
|         | C. D. at 5%                                                                              | 0.24               | 0.25                  | 0.83                      | 0.26                | 0.34                |
|         | C.V.%                                                                                    | 1.62               | 2.46                  | 2.94                      | 1.76                | 2.82                |

**Table 3.3.** Biochemical characterization of healthy and *M. phaseolina* infected plants

| Sr. No. | Treatment details                                                                        | Total sugar (mg/g) | Reducing sugar (mg/g) | Non reducing sugar (mg/g) | True protein (mg/g) | Total phenol (mg/g) |
|---------|------------------------------------------------------------------------------------------|--------------------|-----------------------|---------------------------|---------------------|---------------------|
| 1       | Fluxapyroxad 333 g/l FS as seed treatment (2-3 g/kg)                                     | 3.50               | 2.40                  | 1.04                      | 4.06                | 2.49                |
| 2       | Seed treatment of <i>Trichoderma harzianum</i> (8-10 g/kg)                               | 3.05               | 2.20                  | 0.80                      | 3.74                | 2.97                |
| 3       | Seed treatment of <i>Pseudomonas fluorescens</i> - isolate 1 (8-10 ml/kg)                | 3.07               | 2.07                  | 0.95                      | 3.48                | 3.32                |
| 4       | T <sub>1</sub> + soil application of <i>Trichoderma harzianum</i> (2.5 kg/ha)            | 3.70               | 2.42                  | 1.21                      | 4.19                | 2.45                |
| 5       | T <sub>1</sub> + soil application of <i>Pseudomonas fluorescens</i> - isolate 1 (3 l/ha) | 3.12               | 2.32                  | 0.76                      | 3.88                | 2.54                |
| 6       | T <sub>4</sub> + T <sub>5</sub>                                                          | 3.91               | 2.46                  | 1.37                      | 4.27                | 2.30                |
| 7       | Control                                                                                  | 2.27               | 1.46                  | 0.76                      | 2.07                | 5.33                |
| 8       | Healthy                                                                                  | 4.34               | 3.10                  | 1.17                      | 4.43                | 1.35                |
|         | S. Em. ±                                                                                 | 0.12               | 0.07                  | 0.08                      | 0.12                | 0.07                |
|         | C. D. at 5%                                                                              | 0.36               | 0.22                  | 0.29                      | 0.35                | 0.21                |

(continued on next page)

| Sr. No. | Treatment details | Total sugar (mg/g) | Reducing sugar (mg/g) | Non reducing sugar (mg/g) | True protein (mg/g) | Total phenol (mg/g) |
|---------|-------------------|--------------------|-----------------------|---------------------------|---------------------|---------------------|
|         | C.V.%             | 2.61               | 1.90                  | 2.28                      | 2.37                | 4.26                |

#### 4. Conclusion

Based on the present study, it can be concluded that healthy plants recorded the highest values for total sugar (4.34 mg/g), reducing sugar (3.10 mg/g), non-reducing sugar (up to 1.17 mg/g) and total protein (4.43 mg/g), with the lowest total phenol content (1.35 mg/g). Whereas, the untreated controls infected with *A. niger*, *A. rolfsii*, and *M. phaseolina* showed reductions in sugars and protein, followed by the highest phenol accumulation. Among the treatments, the combined application T<sub>6</sub> (fluxapyroxad 333 g/l FS as seed treatment + soil application of *T. harzianum* + soil application of *P. fluorescens* - isolate 1) was most effective in restoring biochemical constituents, with total sugar (3.83-4.29 mg/g), reducing sugar (2.10-2.46 mg/g), non-reducing sugar (1.37-2.08 mg/g), total protein (4.04-4.27 mg/g) and lowering phenol content (1.76-2.30 mg/g). This was followed by T<sub>4</sub> (fluxapyroxad 333 g/l FS as seed treatment + soil application of *T. harzianum*), which showed total sugar (3.68-4.21 mg/g) and protein content (3.95-4.19 mg/g), while T<sub>1</sub> (fluxapyroxad 333 g/l FS as seed treatment) showed moderate biochemical parameters. Individual applications of bioagents T<sub>2</sub> and T<sub>3</sub> showed moderate improvements in sugars and protein over the control.

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