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# Induction of defense enzymes in groundnut upon treatment with bioagents and botanicals

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**Abstract**
Groundnut is one of the most consumed food legumes of the world. Foliar diseases such as late leaf spot (*Phaeoisariopsis personata* Berk. and Curt.), early leaf spot (*Cercospora arachidicola* Hori.) and rust (*Puccinia arachidis* Spg.), poses major constraints in its production and productivity. Biological control offer better management opportunities than classical chemical control. Under present study, induction of defence enzymes (PAL (phenylalanine ammonia lyase), PO (peroxidase) and SOD (superoxide dismutase)) in groundnut treated with bioagents, botanicals and challenged with the foliar pathogens was studied. Highest PAL (3.161 U/mg protein) and POX (0.223 U/mg protein) activity was recorded in plants treated with sequential application of bioagents whereas, highest SOD activity was recorded in plants treated with *P. fluorescens* (9.492 U/mg protein). Untreated control has shown minimum defense enzyme activity. Results suggest the role of biocontrol agents in inducing higher defense enzyme activity.

**Keywords:** Defence, PAL, PO, SOD, Bioagents, Botanicals

## Introduction
Groundnut occupies an important position of being one of the most consumed food legumes of the world. Groundnut (*Arachis hypogaea* L.) falls under the family Leguminoseae and has chromosome no. 2n = 40 and is a native of South America. Primarily it is cultivated for oil, food and animal feed. It is an important monoecious legume and is an annual with respect to growth habit (Pande *et al.*, 2004) [15]. The crop is severely affected by several biotic factors (fungi, bacteria, viruses, nematodes, insect pests) and abiotic factors. Groundnut is comparatively more prone to diseases than most other crops. The biotic factors severely affecting the yield levels of groundnut are fungal foliar diseases like late leaf spot (*Phaeoisariopsis personata* Berk. and Curt.), early leaf spot (*Cercospora arachidicola* Hori.) and rust (*Puccinia arachidis* Speg.). These diseases wreak havoc throughout the growing regions globally. Grichar *et al.*, 1998; Nutsugah *et al.*, 2007, Subrahmanyam *et al.*, 1985 [8, 14]. The classical chemical control methods is most commonly used to manage the diseases but the use of fungicides frequently and indiscriminately causes environmental pollution and can leads to fungicide resistance development in pathogens (Harman *et al.*, 2004) [10]. Biological control using different biocontrol agents and botanicals seems to be a better alternative because these are known for both growth promotion and disease reduction (Chandrasekaran *et al.* 2015; Chandrasekaran and Chun 2016a, b; Ferraz *et al.*, 2015; Li *et al.*, 2015) [3, 4, 7, 13]. The Biocontrol agents induce physical, physiological and biochemical changes leading to biosynthesis of variety of defence related compunds and secondary metabolites. These defense compounds include PR proteins such as chitinase, β 1-3 glucanes, phenolics, phenylalanine ammonia lyase, superoxide dismutase, peroxidase and phytoalexins (Kloepper *et al.*, 1992) [11]. The defense enzymes is one of the most crucial and important factor in the host pathogen interaction. Phenylalanine ammonia-lyase (PAL) is the first enzyme that is produced in the phenylpropanoid pathway and leads to the biosynthesis of a variety of phenols. The activation of the phenylpropanoid metabolism is the initial disease resistance reactions of plants, leading to the synthesis of many defence-related compounds such as antimicrobial phytoalexins and lignin (Hahlbrock and Scheel, 1989) [9].

The increased activity of superoxide dismutase (SOD) was frequently reported in the plants in response to pathogen invasion. Increased activity of SOD is associated with the plant resistance. It catalyzes the dismutation reaction of the free radicals to hydrogen peroxide ($H_2O_2$) (Kobayashi *et al.*, 1995) [12]. Peroxidases (POX) are oxido-reductive enzymes and participate in the wall-building processes of host cells, such as oxidation of phenols, suberization and lignification of cells during the defense reaction against pathogenic agents (Chittoor *et al.*, 1999) [6]. Keeping in mind the importance of defence related enzymes and the necessity of adopting biocontrol practices under the face of growing environmental concern, the present study was planned to investigate the induction of defence enzymes, such as, PAL, SOD and PO in groundnut plants treated with bioagents, botanicals and challenged with the foliar pathogens.

## Material and Methods

Present study were carried out in the field during *kharif* 2017 at the bioresource farm of Institute of Organic Farming (IOF), the Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad. A field experiment was conducted in randomized block design at the bioresource farm of Institute of organic farming, University of Agricultural Sciences, Dharwad during *kharif* 2017. Highly susceptible variety JL-24 was taken for the study. Different bioagents and botanicals were evaluated for their efficacy to manage the three major fungal foliar diseases of groundnut by induction of defense enzymes.

The details of treatments for field evaluation are given below

| Treatment | Details of treatment | Concentration (%) |
|---|---|---|
| $T_1$ | *Trichoderma harzianum* (IOF strain) | 0.5 |
| $T_2$ | *Pseudomonas fluorescens* (IOF strain) | 0.5 |
| $T_3$ | *Bacillus subtilis* (IOF strain) | 0.5 |
| $T_4$ | *Lecanicillium lecanii* (IOF strain) | 0.5 |
| $T_5$ | *Trichoderma harzianum* - *Pseudomonas fluorescens* - *Bacillus subtilis* (consortia of bioagents) | 0.5 - 0.5 - 0.5 |
| $T_6$ | *Adhatoda vasica* (leaf extract) | 5 |
| $T_7$ | *Azadirachta indica* (oil) | 5 |
| $T_8$ | *Pongamia pinnata* (seed extract) | 5 |
| $T_9$ | *Adhatoda vasica* - *Azadirachta indica* - *Pongamia pinnata* (consortia of botanicals) | 5 - 5 – 5 |
| $T_{10}$ | Wettable sulphur (treated check) | 0.3 |
| $T_{11}$ | Only seed treatment with *Trichoderma harzianum* | 10 g/kg seed |
| $T_{12}$ | Untreated control | - |

Fresh leaf samples were collected six days after the treatment spray. Samples were crushed in liquid nitrogen and subjected to enzyme estimation using standard procedures and protocol.

**Phenylalanine Ammonia Lyase Activity:** PAL activity in fresh groundnut leaves was determined through the amount of tranc-cinnamic acid produced by taking absorbance reading in at 290 nm against blank, as described by Peltonen and Karjalainen (1995) [16]. Assay mixture consist of 0.5 ml enzyme extract (supernatant) and 2.5 ml phenylalanine 0.2 per cent (substrate prepared in buffer). Blank was run without phenylalanine 2.5 ml buffer instated of substrate (substrate blank) both the tube incubated at 40°C for half an hour and take the absorbance at 290 nm per 10 min difference up to half an hour against blank. The enzyme activity was calculated and expressed as units per mg enzyme.

**Peroxidase Activity:** Peroxidase (phenolic donor: hydrogen-peroxide oxidoreductase activity in the leaf tissue of groundnut plants was determined spectrophotometrically by measuring the increase in absorption as a result of formation of the oxidized product tetraguaiacol from guaiacol at 436 nm in the presence of $H_2O_2$ by the method of Chance and Maehly (1955) [2]. The reaction mixture (total 3.0 ml) which contained 2.88 ml of 0.1 M phosphate buffer pH 7.0, 0.05 ml of 20 mM guaiacol and 0.05 ml of 0.042 per cent $H_2O_2$ was monitored for three minutes at 436 nm using a substrate blank containing 2.98 ml of buffer (Substrate blank was used because the enzyme extract absorbed strongly at 436 nm. Hence, to nullify the absorbance of the extract, it was introduced in both the cuvettes). The reaction was then initiated by the addition of 0.02 ml of crude enzyme extract in both the cuvettes and the apparent increase of absorbance at 436 nm was recorded over 5 minutes. The procedure was standardized with pure peroxidase enzyme from horse radish (procured by Sigma Aldrich India) as per the above mentioned protocol. POX units were calculated using the molar absorptivity of 25.5 $M^{-1}cm^1$ for tetraguaiacol and expressed as units per mg enzyme.

**Superoxide Dismutase Activity:** The activity of superoxide dismutase (SOD) was assayed in the leaf tissue of groundnut leaves from the measurement of reduction of p-nitro blue tetrazolium chloride (NBT) spectrophotometrically at 560 nm according to the method of Beauchamp and Fridovich (1971) [1]. For the assay of each sample a set of three cuvettes was required which were referred as blank cell, control cell and sample cell respectively. Each cuvette was covered with aluminum foil. In a total 3.2 ml of the assay solution, the blank and control cell contained 2.95 ml reaction mixture, 180 µl phosphate buffer and 50µl riboflavin. Blank cell was incubated for 15 min completely in the dark (no irradiated) while; control cell was incubated completely in the light *i.e.* illuminated in a chamber with luminescent lamps (irradiated) for 15 min. The sample cell containing 2.95 ml of reaction mixture, 180 µl of phosphate buffer and 0.02 ml enzyme extract was incubated for 1 min and immediately after the incubation the reaction was initiated by the addition of 50 µl of riboflavin and illuminating the sample cuvette in a chamber with luminescent lamp for 15 min. For the blank and control 0.02 ml of enzyme was added just before taking the readings (The enzyme extract absorbed strongly at 560 nm. Hence to nullify the absorbance of the extract, it was introduced in both the cuvettes) and the extinction at 560 nm was read against blank. A no irradiated complete reaction mixture served as blank. A fifteen min irradiated complete reaction mixture without enzyme, which gave the maximal colour, served as control. The activity of SOD was calculated in terms of per cent inhibition of the photo reduction and expressed as units per mg enzyme.

## Results and Discussion

**Phenylalanine Ammonia Lyase (PAL):** Phenylalanine ammonia lyase activity in groundnut leaves differed significantly in treated plants and untreated control. Amongst all the treatments, maximum activity of PAL was recorded in sequential application of bioagents (3.161 U/mg protein) followed by *Pseudomonas fluorescens* (2.994 U/mg protein). Untreated control has recorded the least enzyme activity (1.885 U/mg protein).

**Peroxidase (POX):** Peroxidase (POX) activity differed

significantly in treated plants and untreated control. Amongst all the treatments, maximum activity of POX was recorded in sequential application of bioagents (0.223 U/mg protein) followed by *P. fluorescens* (0.185 U/mg protein). Untreated control has recorded the least enzyme activity (0.043 U/mg protein).

**Superoxide dismutase (SOD):** Superoxide dismutase activity differed significantly in treated plants and untreated control. Amongst all the treatments, maximum activity of SOD was recorded in *Pseudomonas fluorescens* (9.492 U/mg protein) followed by sequential application of bioagents (9.356 U/mg protein). Untreated control has recorded the least enzyme activity (3.862 U/mg protein).

The results are in accordance with the reports of earlier workers and confirmed the role of bioagents and botanicals in induction of the defense related enzymes and growth promotion. Sequential application of defense enzymes have shown highest enzyme levels and consequently better yield than other treatments. Results have clearly shown that the induction of defense enzymes contribute to lesser disease and higher yield. Chitra *et al.* (2006) reported that, the groundnut plants treated with *P. fluorescens* has recorded increased activity of Peroxidase and Polyphenol oxidase when challenge inoculated with *Alternaria alternata*. Similar results have been reported by Senthilraja *et al.* (2012), Sudhagar *et al.* (2000) in groundnut.

**Table 1:** Influence of ecofriendly disease management practices on enzymatic activity and yield

| Treatments | | Conc. (%) | Specific activity (Units per mg protein) | | | Yield (q/ha) |
|---|---|---|---|---|---|---|
| | | | Phenylalanine ammonia lyase (PAL) | Peroxidase (PO) | Superoxide dismutase (SOD) | |
| $T_1$ | *Trichoderma harzianum* | 0.5 | 2.861 | 0.137 | 9.128 | 34.99[b] |
| $T_2$ | *Pseudomonas fluorescens* | 0.5 | 2.994 | 0.185 | 9.492 | 35.96[b] |
| $T_3$ | *Bacillus subtilis* | 0.5 | 2.945 | 0.164 | 8.069 | 35.53[b] |
| $T_4$ | *Lecanicillium lecanii* | 0.5 | 2.454 | 0.107 | 6.955 | 28.55[c] |
| $T_5$ | *Trichoderma harzianum* – *Pseudomonas fluorescens* – *Bacillus subtilis* (sequential application) | 0.5-0.5-0.5 | 3.161 | 0.223 | 9.356 | 41.51[a] |
| $T_6$ | *Adhatoda vasica* | 5 | 2.155 | 0.072 | 5.767 | 28.16[c] |
| $T_7$ | *Azadirachta indica* | 5 | 2.356 | 0.108 | 6.706 | 31.64[b] |
| $T_8$ | *Pongamia pinnata* | 5 | 2.193 | 0.094 | 5.436 | 29.75[c] |
| $T_9$ | *Adhatoda vasica* – *Azadirachta indica* – *Pongamia pinnata* (sequential application) | 5-5-5 | 2.451 | 0.117 | 6.655 | 33.18[b] |
| $T_{10}$ | Wettable Sulphur (treated check) | 0.3 | 2.740 | 0.121 | 8.941 | 42.44[a] |
| $T_{11}$ | Only seed treatment with *T. harzianum* | - | 2.001 | 0.055 | 4.117 | 23.92[d] |
| $T_{112}$ | Untreated control | - | 1.885 | 0.043 | 3.862 | 22.45[d] |
| S.Em. ± | | | 0.08 | 0.002 | 0.13 | 1.90 |
| C.D. @ 5 % | | | 0.24 | 0.005 | 0.39 | 5.58 |
| C.V. (%) | | | 5.78 | 2.46 | 3.28 | 10.18 |

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