

# Role of Plant growth Regulator fungicide and Abscisic acid on the growth and biochemical properties of *Basella alba* as a model system

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## ABSTRACT

**Objective:** To study the effects of plant growth promoter fungicide & plant hormone Abscisic acid on the growth and biochemical properties of the *Basella alba* plant. In our study we have taken *Basella alba* (Malabar Spinach) as a model system for our study. **Methodology:** It was an experimental approach. Wines with 3-6 nodes were selected and planted separately in 20 pots. Difenconazole and ABA at a specific concentration were used. The plants were irrigated with tap water. The treatments were given on 25, 50 and 75 days after planting (DAP) by soil drenching. The plants were taken randomly on 45, 65 and 85 DAP for analyses and separated into root, stem and leaves used for determining growth and biochemical changes. **Results:** The total height of the plant increased with ABA treatment but decreased with Difenconazole whereas there was increase in root length in both the treatments. The total leaf area of the plant decreased in both Difenconazole and Abscisic acid treatment. We found significant increase in Chlorophyll, Carotenoid & Xanthophyll content of the plant against both the treatments. In the same way remarkable increase in the total protein content, amino acid content and phenol content was also observed after both the treatments. **Conclusion:** This study aims to reveal the efficacy of the growth regulating fungicide and Abscisic acid in the growth and biochemical properties of the plant. **Conclusion:** It was concluded that Difenconazole and Abscisic acid are effective in overall plant development though Difenconazole is least effective in increasing the total leaf area and height of the plant.

**Keywords:** *Basella*, *Abscisic acid*, *Difenconazole*, *Cytokinins*, *Fungicides*

## INTRODUCTION

Malabar spinach, (*Basella alba*) today is cultivated as a food plant throughout the warmer regions of the world *Basella alba* (Basellaceae family) or Malabar Spinach is a perennial vine native to tropical Asia where it is widely used as a leaf vegetable. There are 2 main species of Malabar spinach: *Basella alba*, which has green stem and thick fleshy leaves, and *Basella rubra* which has red stem. In Ayurveda, the leaves or aerial parts and stem of *Basella alba* have been used for the treatment of hemorrhoids, sexual weakness, anemia, constipation, ulcers and as a diuretic, laxative, Anti-cancer such as Melanoma, Leukemia. Its use has also been discovered as anticonvulsant, rubefacient, aperient, demulcent, anti-inflammatory, androgenic, antipyretic afflictions. Spoilage of green leafy vegetable is due to the activity of microorganisms, the condition favorable for their proliferation being moisture and warmth. The plant contains rich nutrients that shoots per 100 g edible portion contains water 93 g energy 79 kJ (19kal), Protein 1.8 g, Fat 0.3 g, Carbohydrate 3.4 g, Ca109 mg, P 52mg, Fe 1.2mg, Vitamin-A 8000 IU Thiamin 0.05 mg, Riboflavin 0.16mg, Niacin 0.50 mg, Folate 140 µg, and, Ascorbic acid 102 mg. The composition is comparable to other dark green leafy vegetables with high moisture and content The Abscisic acid (ABA) has role in plant response to a range of stresses including drought and salt stress. Major role of ABA has control over internal signaling mechanism enabling plants to survive adverse environmental conditions. Triazole compounds are generally used as fungicides, which have plant growth regulation properties. Effectiveness as fungicides or PGR is determined by the stereo chemical configuration of the substitutions on the carbon chain. Many workers have studied the growth regulating effect of triazole compounds on different plants. Growth substance like GA, Cytokinins, ABA and Ethylene are affected by the triazoles. The objectives of the present study were to determine the host pathogen interaction and to evaluate the effectiveness of PGR such as Difenconazole & ABA on growth and biochemical changes of *Basella alba* plant under field conditions.

## MATERIALS & METHODS

Food and medicinally important plant species, *Basella alba* was selected for the present investigation. Difenconazole a triazole group of fungicide having PGR properties is obtained from Syngenta, India Ltd., Mumbai (India). was used in this study. The plant growth regulators ABA (Abscisic acid) were purchased from Sigma Chemicals, Bangalore, was also used for the study.

### Treatments and samplings

Wines with 3-6 nodes were selected and planted separately in 20 pots. 20 mg/L Difenconazole and 15 mg/L ABA concentrations were used for the plant treatments. The plants were irrigated with tap water. The treatments were given on 25, 50 and 75 days after planting (DAP) by soil drenching. The plants were taken randomly on 45, 65 and 85 DAP for analyses and separated into root, stem and leaves used for determining growth and biochemical changes.

### Growth parameters

#### Height of the plant and roots length

The plant height was measured from the soil level to the tip of the shoot. The plant root length was measured from nodal initiation of the shoot to the tip of longest root.

#### Total chlorophyll content

Chlorophyll a and b, Carotenoids and Xanthophylls were extracted from leaves using standardized protocol. The Absorbance was measured at 645, 663, and 480 nm in Spectrophotometer using 80% acetone as blank. Chlorophyll content was calculated using the formula of Arnon.

#### Total leaf area

The total leaf area of the plants was measured using LICOR leaf electric area meter and expressed in cm per plant.

### Bio- chemical analysis

#### Amino acid

The plant tissue of 500 mg was taken and homogenized with 10 ml of 80 percent boiling ethanol. The extract was centrifuged at 800 rpm for 15min and supernatant was made up to 10 ml with 80 per cent ethanol used for the estimation of free amino acids. One ml of ethanol extract was taken in a 25 ml test tube and neutralized with 0.1 N NaOH using methyl red indicators. To this, 1 ml Ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 min, and then 5 ml of diluting reagent was added, cooled and made up to 25 ml with distilled water. The absorbance was read spectrophotometrically at 570 nm.

#### Total protein content

The protein content of *Basella alba* was estimated by grinding the plant tissue samples with 10 ml of 10 per cent TCA. The homogenate was centrifuged for 15 minutes at 800rpm. The supernatant was and to the pellet 5 ml of 0.1N NaOH was added to solubilize the protein and then centrifuged for 15 minutes. The supernatant was collected and made up to 10 ml with 0.1 N NaOH used as protein source. 0.1 ml of protein solution was taken to which 5 ml of Bradford reagent was added. The absorbance was measured after 2min against a blank reagent that contains as DW 0.1 ml of 0.1N NaOH and 5 ml of Bradford reagent.

#### Total phenol content

Five hundred milligrams of fresh plant tissue was ground in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved with 5ml of distilled water and used as extract. To 2 ml of the extract, 0.5 ml of Folin-Ciocalteu reagent was added. After 3 min, 2 ml of 20%  $\text{Na}_2\text{CO}_3$  solution was added and mixed thoroughly. The mixture was kept in boiling water for exactly one min and after cooling, the absorbance was read spectrophotometrically at 650 nm. The total phenols were determined using a standard curve prepared with different concentrations of gallic acid.

## RESULTS

### Effect of Difenoconazole and ABA on height of the plant

The total height of the plant increased with age in the control (Fig.1), ABA treated *Basella alba* plants, but it decreased under difenoconazole treatments, the increase was higher in ABA treated when compared to the Difenoconazole. Highest plant height was noted in 85 DAP under ABA treatments and it was nearly 132.41 per cent over the control. The plant height reduced under treatments with Difenoconazole in *Basella alba* plants. The ABA treatment increased the plant height (Fig.1).

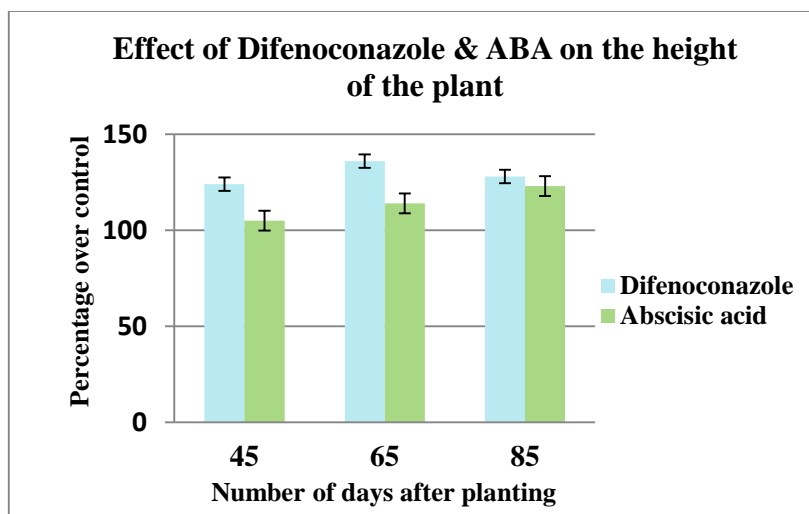


Fig 1: Graphical representation showing the effect of Difenoconazole and Abscisic acid on the height of plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

### Effect of Difenoconazole and Abscisic acid on root length

The total root length of the *Basella alba* plant increased with the age in control ABA and Difenoconazole treated plants, (Fig 2) but decrease in root length was observed in ABA treatment. The increase was higher in Difenoconazole treated on all the three analyses days DAP. The root length was increased with Difenoconazole treated plants to higher extent. On the other hand, ABA inhibited root growth in *Basella alba* (Fig.2).

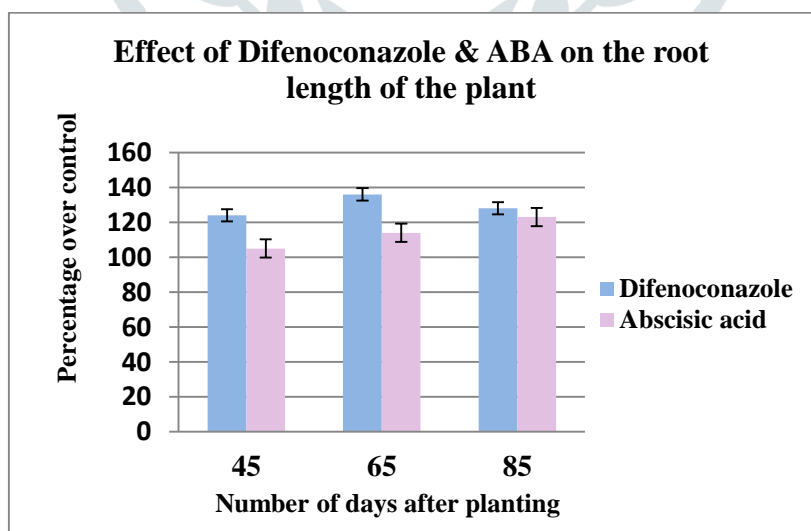


Fig 2: Graphical representation showing the effect of Difenoconazole and Abscisic acid on the root length of plant at different growth stages. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

### Effect of Difenconazole and Abscisic acid on total leaf area

The total leaf area of the plant decreased with the age in all the treatments (Fig. 3). The more decrease was prominent in ABA treated plants on 85 DAP when compared to Difenconazole treated plants. Difenconazole and ABA treatments were reducing the total leaf area when compared to control in *Basella alba* plants (Fig. 3).

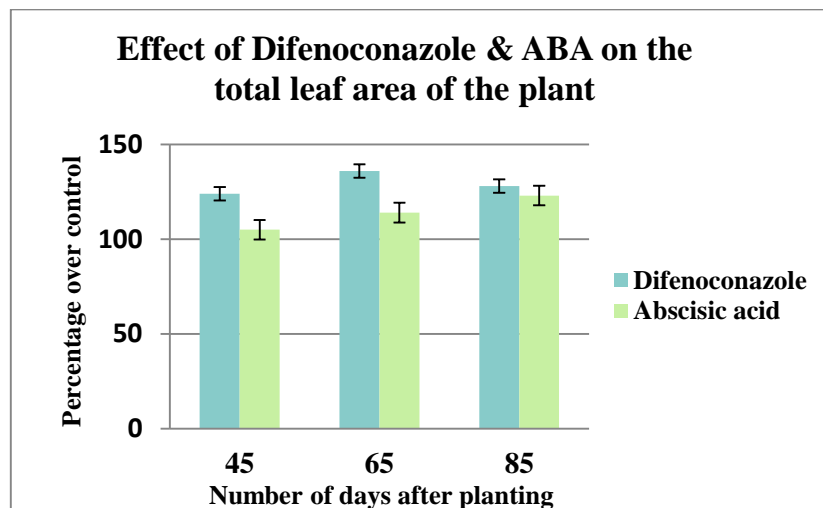


Fig 3: Graphical representation showing the effect of Difenconazole and Abscisic acid on the total leaf area of the plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

### Effect of Difenconazole and ABA on total chlorophyll content

The total chlorophyll content of leaves was increased with the age of plants (Fig. 4). The triazole treatments increased the total chlorophyll content to a higher level than the control and the increase being 145.00 per cent in Difenconazole treated *Basella alba* on 45 DAP in Difenconazole treatment. The total chlorophyll content of leaves of *Basella alba* increased with age of plant in Control, Difenconazole and ABA treatments (Fig. 4).

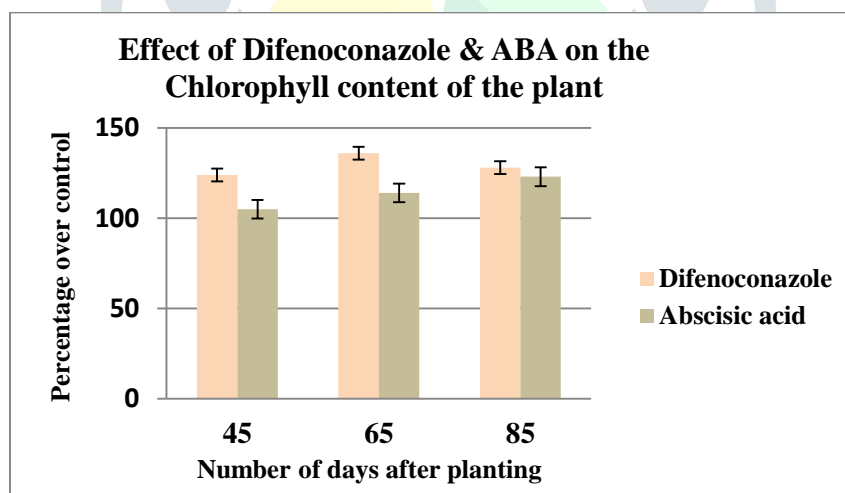


Fig 4: Graphical representation showing the effect of Difenconazole and Abscisic acid on the total Chlorophyll content of the plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

### Effect of Difenconazole and ABA on carotenoid content

The Carotenoid content increased with the age in control and treated plants on all sampling days the higher carotenoid contents were observed under ABA treatment on 85 DAP and it was 124.00 per cent over control (Fig.5). The treatment also increased the Carotenoid content on 65 DAP. The carotenoid content of the *Basella alba* leaves increased with age in control and treated plants (Fig. 5).

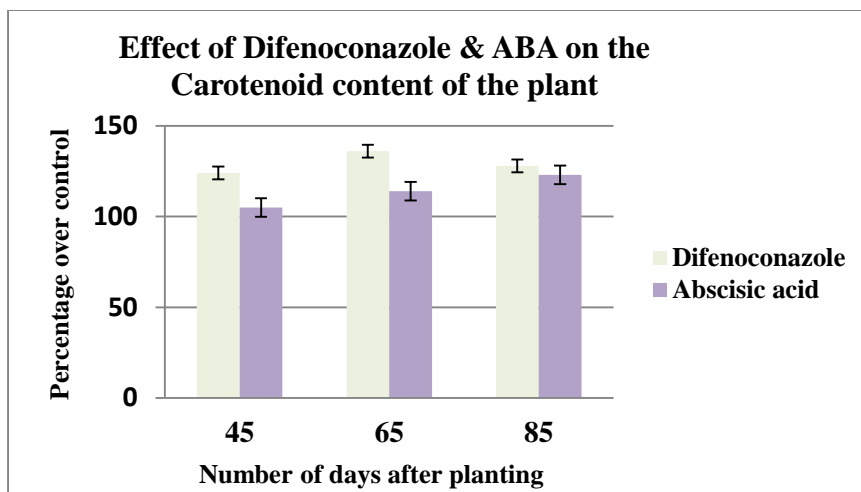


Fig 5: Graphical representation showing the effect of Difenoconazole and Abscisic acid on the total Carotenoid content of the plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

#### Effect of Difenoconazole and ABA on xanthophyll content

The Xanthophyll content of *Basella alba* leaves increased with age in the control and treatment plant. (Fig. 6). Difenoconazole treatment caused an increase in Xanthophyll content at all stages of growth when compared to control and it was 132.00 per cent over the control in *Basella alba*. The ABA treatment also increased the Xanthophylls content 110.22 per cent over the control in *Basella alba* on 85 DAP The Xanthophyll content of the *Basella alba* leaves increased with age in control and treated plants (Fig.6).

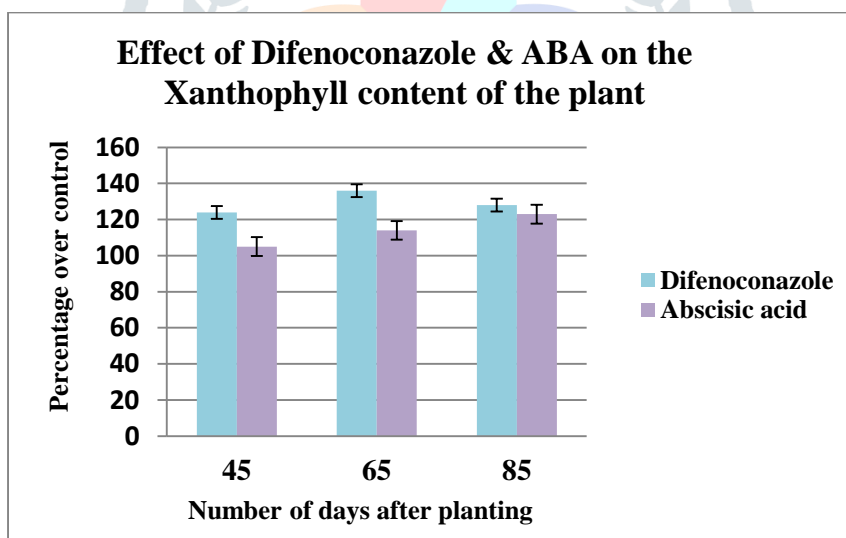


Fig 6: Graphical representation showing the effect of Difenoconazole and Abscisic acid on the total Xanthophyll content of the plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

#### Effect of Difenoconazole and ABA on total protein content of the plant

The *Basella alba* protein content increased with the age in control and treatments in all stages. In plant tissues like stem, root, there was a gradual increase in protein content under Difenoconazole treatments when compared to control (Fig.7). A maximum increase was noted to on 85 DAP in treatments it was 134.22 over control in leaf. The protein content increased high extent in all the parts of the plants with age of the *Basella alba* plants with treatments. Among the treatment, Difenoconazole caused higher level of protein accumulation in all parts of plants of *Basella alba* (Fig. 7).

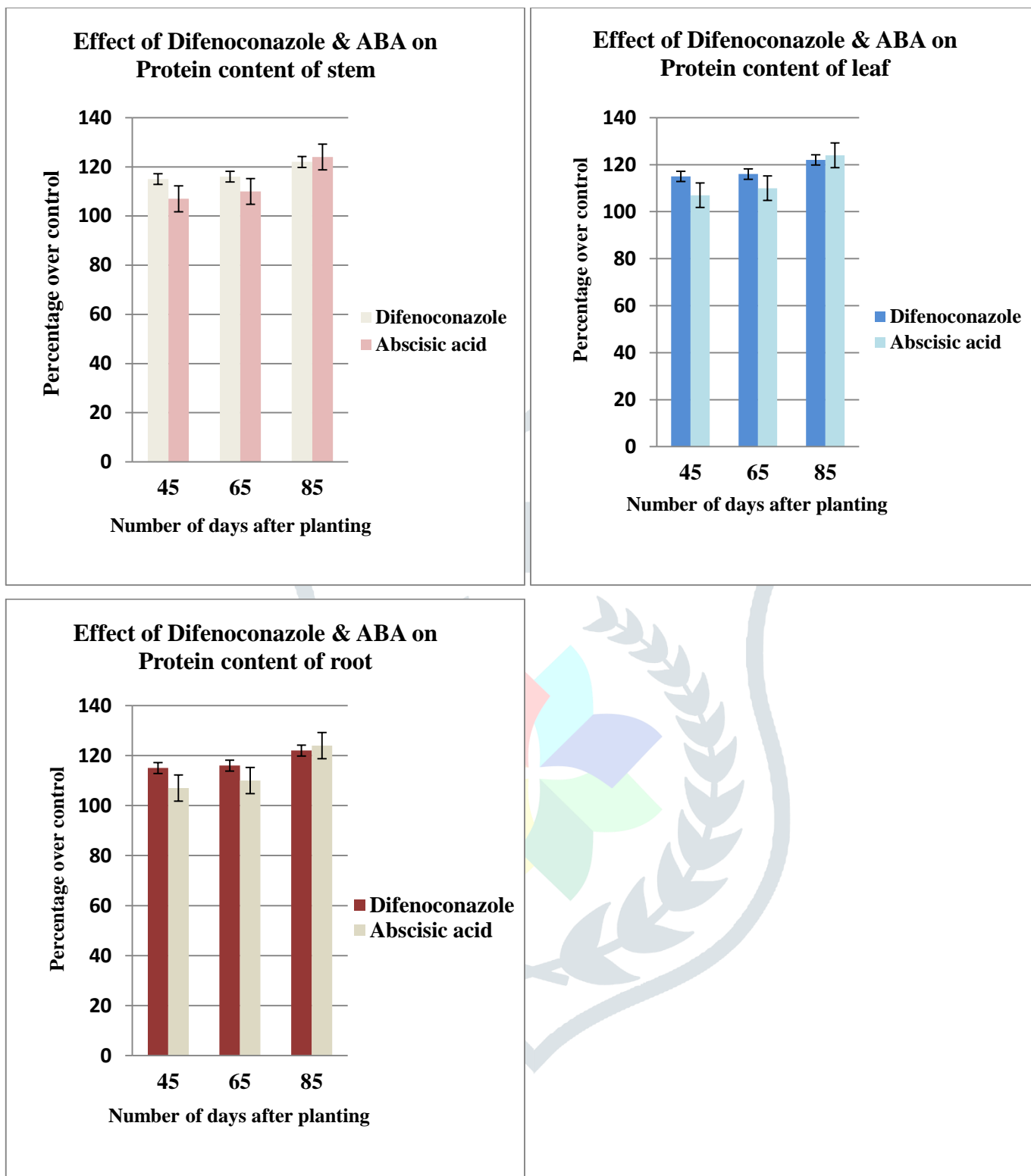


Fig 7: Graphical representation showing the effect of Difenoconazole and Absciscic acid on the total protein content of content in various parts of the plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

**Effect of Difenoconazole and ABA on amino acid content of the plant**

In *Basella alba* the amino acid content increased with the age in control and treated plants in all growth stages (Fig.8). In leaf maximum increase was 128.45 per cent over control was on 85 DAP in Difenoconazole treatment when compared to ABA treatment. The amino acid content increased a higher extent in leaf, stem and root parts of the plants with treatments. Among the



treatments Difenoconazole caused higher level of amino acid among the organs analysed. Leaf tissues accumulated high level of amino acid than stem and root tissues (Fig. 8).

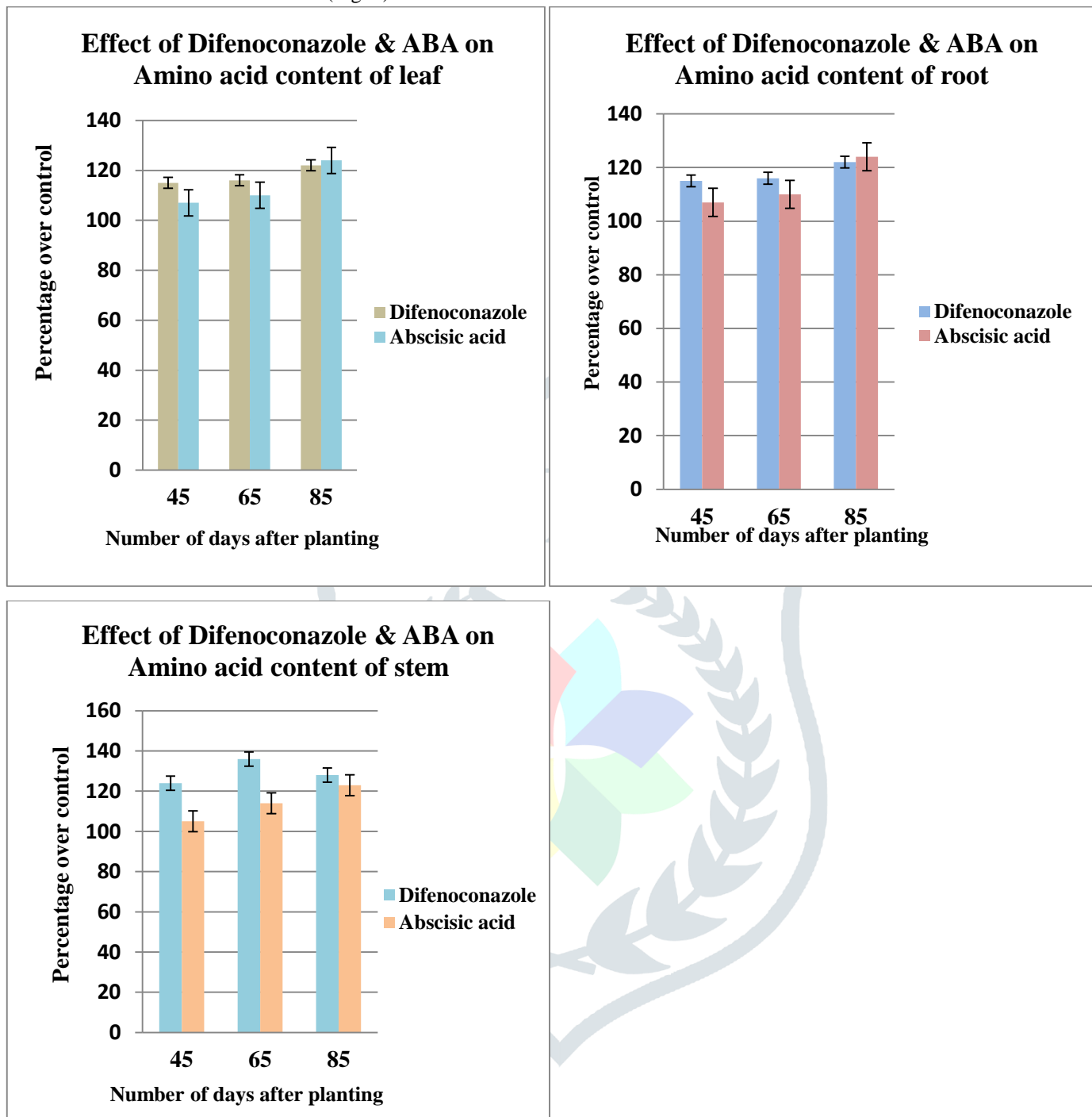


Fig 8: Graphical representation showing the effect of Difenoconazole and Abscisic acid on the Amino acid content in various parts of the plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

**Effect of Difenoconazole and ABA on total phenol content of the plant**

Total phenol content increased with the age in control and treatments in all stages of growth (Fig. 9). In plant tissues like root, stem and leaves there was a gradual increase in total phenol content under Difenoconazole and ABA treatments when compared to control. A maximum increase was noted on 85 DAP in both treatments and it was 122.20 over control in leaves. But in the stem and root the total phenol content were reduced in Difenoconazole and ABA treatments when compared to leaf (Fig. 9).

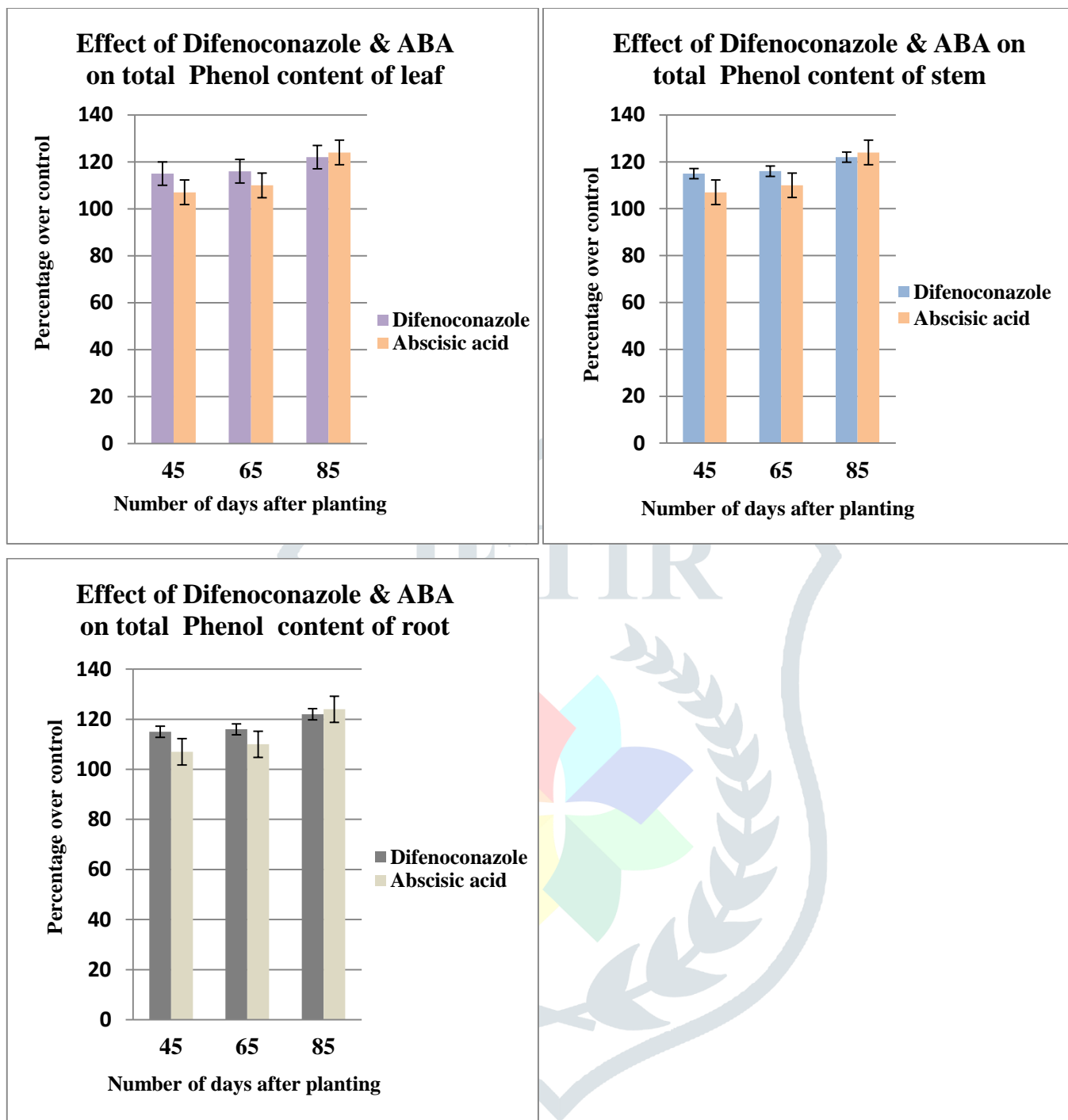


Fig 9: Graphical representation showing the effect of Difenonazole and Abscisic acid on the total Phenol content in various parts of the plant. The percentage was calculated over the control in successive number of days after planting.  $P < 0.0001$  which is considered to be extremely statistically significant. There were significant changes of the biochemical parameters in this three different no of days after planting (DAP) as analyzed by One- way ANOVA.

### Conclusions & Discussions

These results demonstrate the effect of Abscisic acid and Difenonazole which is a plant growth promoting fungicide on *Basella alba*. Difenonazole is a systemic triazole fungicide used in a number of plant diseases of fruits, vegetables and ornamental plants caused by different types of fungi. It affects biosynthesis of Ergosterol in the fungi by inhibiting C-14 demethylation of sterols resulting in morphological and functional changes in the fungal cell membrane. ABA functions in many plant developmental processes, including seed and bud dormancy, the control of organ size and stomatal closure. It is especially important for plants in the response to environmental stresses, including drought, soil salinity, cold tolerance, freezing tolerance, heat stress and heavy metal ion tolerance. It has been found that there are remarkable effects of Difenonazole and Abscisic acid on the physical parameters and biochemical properties of the plant. It has been found that the height of the plant



increased with Abscisic acid (ABA) treatments but it was markedly decreased with Difenconazole treatment which showed that Abscisic acid plays an important role in the development of the plant. It has been reported that Triazole treatments reduced stem elongation and plant height in *Plectranthus forskholii*, in Cassava, and *Catharanthus roseus*. The growth retarding effect of triazole is caused by the inhibition of plant hormone Gibberellin. It was showed that the total root length of the *Basella alba* plant increased with the age in control plants with treatment of both Difenconazole and Abscisic acid but there was marked decrease in root length in ABA treatment. On the other hand, ABA inhibited root growth in *Basella alba*. Triadimefon treatment increased the root growth in Mungbean. An increase in root length was reported in paclobutrazol and triadimefon treated in *Catharanthus roseus*. Paclobutrazol increased the root length and enhanced the lateral roots in tomato plants. The total leaf area of the plant decreased in both ABA & Difenconazole treatment. Paclobutrazole reduced the leaf area in tomato and barley. The leaf area is reduced in *Catharanthus roseus* plants under PBZ treatment. Uniconazole reduced the leaf number in *Pyreantha* species. Application of ABA at room temperature results in reduced leaf production in many plants straw berry and soybean by triazole treatment. A decreased leaf size was observed in the triazole treated *Beta vulgaris*. There was significant reduction of total leaf area in *Barassica napus* with Paclobutrazole treatment. Difenconazole and Abscisic acid treatment also increased the fresh and dry weight of the plant (data not shown). Triazole compounds inhibited gibberellins biosynthesis, Cytokinin and Abscisic acid stimulated synthesis in response treatment and by counter acting gibberellin action might be the cause for increase root growth. ABA plays a critical role in regulating plants water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance through signaling mechanism. The total Chlorophyll, Xanthophyll and Carotenoid content increased in both ABA & Difenconazole treatment plants in successive number of days. Paclobutrazole treatment increased the chlorophyll a, b and carotenoid pigments in the leaf of tomato, wheat and barley seedling. Paclobutrazole treatment increased the xanthophyll content in flower of *Catharanthus* plants. Triazole treatment increased the Carotenoid content in *Catharanthus* plants. Triadimefon treatment increased the carotenoid content to higher level in Cucumber. An increase in carotenoid was reported in maize plants treated with ABA. The *Basella alba* protein content increased with the age in control and treatments in all stages. In plant tissues like stem, root, there was a gradual increase in protein content under PCZ treatments. Triadimefon treatment parts of increased the protein content in *Raphanus sativus*, Cow pea and Cucumber seedling and in Paclobutrazole treated wheat seedling and *Brassica carinata*. Triadimefon treatments also increased the protein content in the roots of *Catharanthus*. There was sharp increase in the total Phenol content of the plant under Difenconazole and Abscisic acid application. The increase in Phenol content was observed in every parts of the plant i.e root, stem and leaves. In the same way Difenconazole and ABA caused increase in the amino acid content of the plant and maximum increase was observed in the leaf over 85 days after planting. It was also found that the leaf tissues accumulates higher amino acid than the stem and leaf tissues. There were reports that Triadimefon increased the amino acid content in radish and soybean and similar results has been observed in Uniconazole treated *Phaseolus vulgaris*. Paclobutrazole induced a moderate increase in amino acid content and *Catharanthus*. Thus from this analysis it has been concluded that the Plant growth promoting fungicide Difenconazole and phytohormone Abscisic acid has important role in the physical and biochemical development of the plant though genetic and molecular analysis requires further investigation.

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