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Effect of Paclobutrazol and ABA on Total Phenol Contents in Different Parts of Holy Basil (*Ocimum sanctum*)

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Abstract: The present investigation was conducted to determine the effect of PBZ and ABA on total phenol contents in different parts of *Ocimum sanctum.* Paclobutrazol (PBZ) a triazole fungicide, having plant growth regulator (PGR) properties, is reported to inhibit gibberellic acid biosynthesis and increase in abscisic acid and cytokinin contents. So, it necessitates the need to investigate the efficacy of this compound in the enhancement of phytochemical content of *Ocimum santum* in order to increase the medicinal properties, thereby make it an economically valuable medicinal plant. The phytochemical component like total phenol was increased considerably under PBZ and ABA treatments in *Ocimum sanctum* plants when compared to control.

Key words: *Ocimum sanctum,* Phytochemicals, Total phenol contents

INTRODUCTION

The naturally occurring plant growth substances include auxin, gibberellin, cytokinin, abscisic acid and ethylene. The term plant growth regulators are not only restricted to synthetic compounds but also include the naturally occurring hormones [1-3]. Hence, the plant growth regulator can be defined as either natural or synthetic compounds that modify the plant growth and development pattern exerting profound influence on many physiological processes [4-6].

Abscisic acid commonly considered as a stress hormone has been implicated on plant acclimatization and protection against various biotic and abiotic stresses such as disease infection, heat, chilling, drought and flooding [3-7]. Elevated ABA levels have been correlated with increased tolerance to cold stress [7-9]. ABA is also involved in many physiological processes, such as photosynthesis. It has been demonstrated that ABA plays important roles in stomatal movements, the regulation of photosynthetic enzyme activities, the stability of photosynthetic apparatus and the gene expressions involved in chloroplasts [4-7].

Triazole compounds are the chemicals belongs to a class of compounds known as ergosterol biosynthesis inhibitors and are used as fungicides as wells as plant growth regulators [7-9]. Triazole compounds are characterized by a ring structure containing 3 nitrogen atoms, a chlorophenyl and a carbon side chain [8-11]. The inhibition of Gibberellin biosynthesis is the main reason behind the PGR properties of triazoles. Growth substance like ethylene, cytokinins and ABA are also affected by the triazoles [12-14]. Triazoles induce a variety of morphological, physiological and biochemical responses in plants, including a reduction in shoot elongation, stimulation of rooting, reducing gibberellin biosynthesis increased chlorophyll content, altered carbohydrate status, increased stress tolerance, delayed senescence, increased cytokinin synthesis and a transient rise in ABA synthesis and alterations in secondary metabolite contents [4-11].

Medicinally important plant species, *Ocimum santum* L. belonging to the family Lamiaceae was selected for the present investigation. It is of high traditional medicinal value as it is one of the important constituents of Ayurveda, Homeopathy and Siddha systems of medicine. It is commonly used for curing skin diseases, hepatic

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disorders, cold, cough, malarial fever and an antidote for snakebite. This plant contains many aromatic and volatile oils and main constituent is Euginol. Several recent investigations using these extracts have indicated that *Ocimum* possess significant anti-stress [15] and anti-carcinogenic properties [16]. The active principles of *Ocimum* comprising of phenols and flavonoids have been shown to have significant anti-inflammatory activity both *invivo* and *invitro* [17].

The work on the effect of these triazole compounds and PGR in the *Ocimum sanctum* is scanty. Therefore the present investigation was undertaken to study the effect of paclobutrazol and ABA on the Phytochemical constituents like total phenol contents.

MATERIALS AND METHODS

Medicinally important plant species, *Ocimum santum* L. belonging to the family Lameaceae was selected for the present investigation. The seeds of two varieties were collected from the Horticulture Department, Faculty of Agriculture, Annamalai University, Tamil Nadu.

Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-trizol-1-yl)-pentan-3-ol], a triazolic group of fungicide having PGR properties, is obtained as cultar 25% W/V paclobutrazol from Zeneca ICI Agrochemical Ltd., Mumbai, India and used for the study. ABA was obtained from Sigma Chemicals, Bangalore.

The experimental part of this work was carried out in Botanical Garden and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu. During the study, average temperature was ±32/26°C and relative humidity (RH) varied between 60-75 per cent.

MATERIALS AND METHODS

The plants were raised in Botanical Garden, during the months of January – May, 2007. The seeds were sown separately in raised seed beds by broadcasting method and covered with paddy straw to ensure proper germination. The nursery beds were watered twice a day and weeded regularly in order to ensure healthy growth of the seedlings.

The seedlings were transplanted in 12 plants in each vials to 9 cement vials having 1m diameters, in the vials filled with red soil, sand and FYM in 1:1:1 ratio. The vials were irrigated immediately after transplantation and subsequent irrigation was done twice in a week to keep the optimum moisture level required in the soil.

PBZ and ABA Treatments: In the preliminary experiments, 2, 5, 10, 15 and 20 mg L^{-1} paclobutrazol and 2.5, 5, 7.5 and 10 μM L^{-1} ABA were used for treatments to determine the optimum concentration of paclobutrazol and ABA. Among the treatments, 15 mg L^{-1} paclobutrazol and 7.5 μM L^{-1} ABA concentrations increased at 50 percent of dry weight significantly and higher concentration slightly decreased the growth and dry weight. In the lower concentrations, there was no change in dry weight and growth of the plant. Hence, 15 mg L^{-1} paclobutrazol and 7.5 μM L^{-1} ABA concentration was used to study the effect on the *Ocimum santum* plants.

Three vials each were taken for treatments with PBZ and ABA respectively and 3 vials were kept untreated and served as control. 15 mg L^{-1} paclobutrazol and 7.5 μM L^{-1} ABA were given to each plant by soil drenching. The treatment was given on 50, 70 and 90 DAP.

The plants were uprooted randomly on 60, 80 and 100 DAP and separated into root, stem, leaves and flowers used for determining growth, biochemical, antioxidant potential and phenol contents. phytochemical analysis

Estimation of Total Phenols: Total phenol was estimated by the method of Malick and Singh [18].

Extraction: 500 mg 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 5 ml of distilled water and used as extract.

Estimation: To 2 ml of the extract, 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% Na_2CO_3 solution was mixed thoroughly. The mixture was kept in boiling water for exactly one minute. and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentration of gallic acid. The results were expressed in milligrams per gram fresh weight.

RESULTS

Total Phenol Contents (Table 1; Fig. 1)

Root: Total phenol increased in both PBZ and ABA treated plants with increase in age. PBZ treatment significantly increased the total phenol and it was 102.46 per cent and 101.11 per cent in PBZ and ABA treated plants respectively when compared with control on 100 DAP.

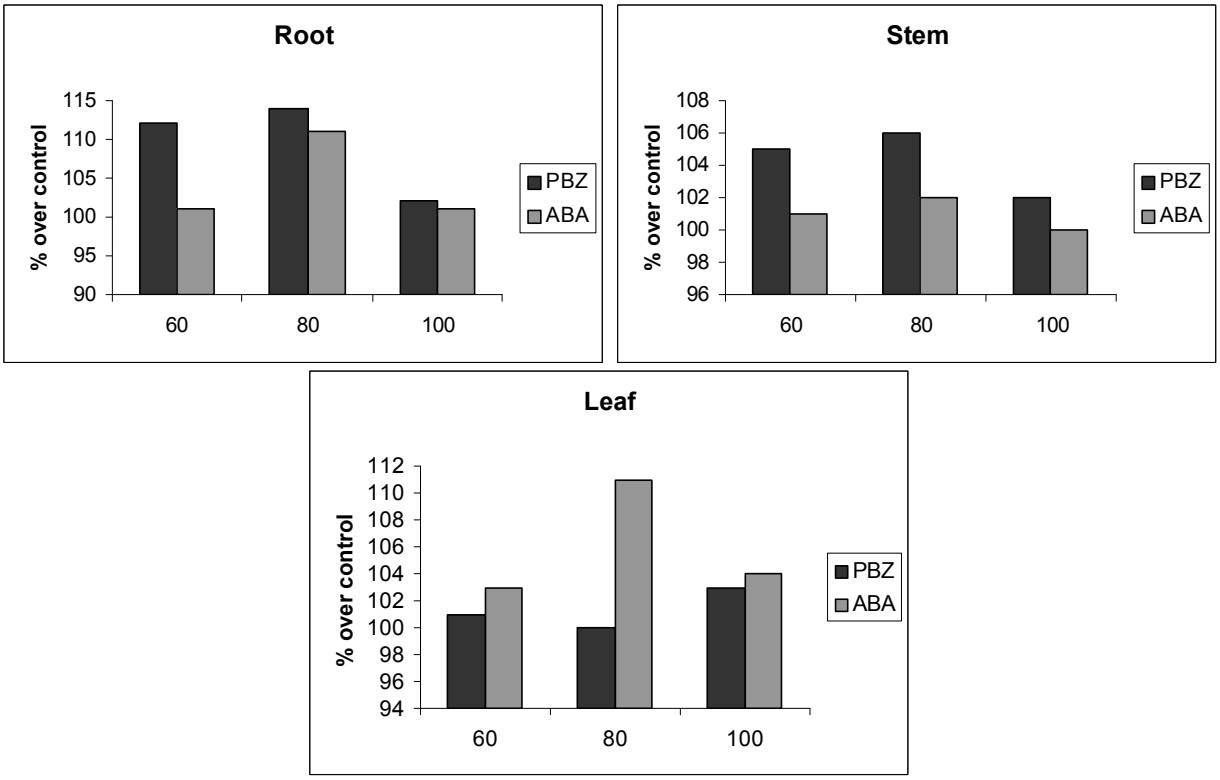

Fig. 1: Effect of paclobutrazol and abscisic acid on percentage increase or decrease (when compared to untreated control) of phenol content in *Ocimum sanctum*

Table 1: Effect of paclobutrazol and ABA on total phenol content (mg g-1 FW) of Ocimum sanctum (values are mean±S.D of 3 samples)

Growth stages (DAS)	Control	Paclobutrazol	ABA
Root			
60	0.70±0.028	1.24±0.045	3.26±0.125
80	3.90±0.088	2.28±0.061	6.84±0.099
100	11.78±0.440	12.07±0.447	11.91±0.441
Stem			
60	0.13±0.005	0.29±0.012	0.26±0.002
80	4.24±0.095	6.31±0.098	7.22±0.102
100	11.81±0.412	12.05±0.415	11.90±0.425
Leaf			
60	0.76±0.029	1.32±0.047	1.08±0.037
80	4.54±0.089	8.27±0.120	7.24±0.123
100	11.72±0.420	11.92±0.448	11.89±0.440

Stem: PBZ treatment increased the total phenol in stem when compared with control plants. The highest activity was recorded with PBZ and followed by ABA treatments. PBZ treatment increased the total phenol and it was 102.03 per cent and 100.76 per cent in ABA treated plants when compared with control on 100 DAP.

Leaf: The total phenol in leaves increased with PBZ treatment at all stages of growth and the increase was 103.71 per cent over control on 100 DAP. The total phenol was increased to a larger extent by the ABA treatments and it was 104.45 percent over control on 100 DAP when compared to the control plants.

DISCUSSION

The present investigation was conducted to determine the effect of PBZ and ABA alters the growth and antioxidant status of *Ocimum sanctum*. The results on total phenol contents in different parts of plants are discussed hereunder. Plants form the earliest companion of mankind, not only by providing food and shelter but also by serving the humanity in the cure of different ailments. Drugs from plants can be placed into two broad categories. Firstly, they are included in complex mixtures containing a wide variety of compounds and secondly they are used as pure, chemically defined active principles. Most of the plant-derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous people and some of these

could not be substituted, despite the enormous advancement in synthetic chemistry. India is a gold mine of treasures with traditional and practical knowledge of herbal medicines. Globally a positive trend has blossomed in favours of traditional and integrative health sciences both in research and practices.

In the present study, the total phenol content significantly increased with PBZ and ABA treatments in *Ocimum sanctum* at all stages of growth. Total phenol content in the leaves of mango seedlings registered siginificant increase as a result of PBZ treatments, the increase being more marked at higher dosage of PBZ [4-6].

It has been suggested that peroxidase could act as efficient H_2O_2 scavenging system in plant vacuoles in the presence of phenolics and reduced ascorbate [7-9]. There proposed a hypothesize that a cycle where H_2O_2 is scavenged by phenolic compounds. Phenolics are oxidized to phenoxyl radicals. This phenoxyl radicals reduces the ascorbic acid into mono dehydro- ascorbate. Thus phenols act as an intermediatory ROS acceptor in the vacuoles. This increase of phenols by triazoles may be further enhancing the antioxidant capacity of *Ocimum sanctum* along with other antioxidants.

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