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Difenoconozole and Propiconazole's Effects on Antioxidant Potentials of *Gloriosa superba* Linn

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Abstracts: The effect of different triazole compounds, viz. difenoconozole (DIZ) and propiconazole (PPZ) treatments on the antioxidant potentials of *Gloriosa superba*. Plants were treated with DIZ at 10mg L^{-1} and PPZ at 10 mg L^{-1} separately by soil drenching on 35, 50, 65 and 80 day after planting (DAP). The plants were harvested randomly on 45, 60, 75 and 90 DAP for determining the effects of both triazoles on enzymatic antioxidant contents like peroxidase (POX), polyphenol oxidase (PPO), Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) activites. All the analyses were done in the tubers, stem and leaf of both control and treated plants. It was found that both these triazole treatments have profound effects on the antioxidant metabolism and caused enhancement in enzymatic antioxidant potentials under treatments. These results suggested that the application of triazoles may be a useful tool to increase the antioxidant production and alkaloid production in medicinal plants.

Key words: *Gloriosa superba* · Difenoconozole · Propiconazole · Antioxidant enzymes

INTRODUCTION

Over the past few years several triazole derivatives, collectively described as sterol biosynthesis inhibitors, have been developed for use either as plant growth regulators or as fungicides. Examples which have become commercially available include triadimefon (Bayleton) and propiconazole (Banner) developed as fungicides and, paclobutrazol (Bonzi) and uniconazole (Sumagic) developed as growth retardants. However, all of these products can exhibit both fungicidal and growth regulating properties to varying degrees [1]. Triadimefon has inhibited growth of broad bean at or above rates therapeutic for rust [2], while myclobutanil (Systhane) and other triazole fungicides have retarded growth of New Guinea impatiens [3]. Propiconazole [Banner] reduced leaf area of pecan leaves [4] and retarded early seedling growth of redroot pigweed [5]. Plant growth regulators (PGRs) are commonly used during greenhouse and nursery production of flowering bedding plants. Fungicides are also likely to be applied to prevent or control diseases during production. The purpose of this study was to investigate possible PGR/fungicide additive or interactive effects resulting from applications of these two groups of chemicals.

Triazole compounds used for their fungi toxicity also have plant growth regulating properties and thus modulate the balance of important plant hormones including GA, ABA and cytokinins [6, 7]. Triazoles inhibit gibberellins and ergosterol biosynthesis in plants [8] and induce a variety of morphological and biochemical responses in plants, including inhibited shoot elongation, stimulated root growth and increased cytokinins and ABA and altered ergosterol biosynthesis [7]. Triadimefon and hexaconazole are triazole compounds with fungicidal and plant growth regulating properties [9, 10]. The application of these triazole compounds can alter the metabolic equilibrium, result in stress-like symptoms in plants [11] but simultaneously, they can protect plants from apparently unrelated abiotic stresses like NaCl stress [12]. Triazole compounds have been shown to improve the yield of many root crops such as carrot, radish, sugar beet and potato [7]. Some of the previous works carried out in our lab revealed the morphological and physiological changes associated with triazole treatment in various plants, include the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, alkaloid production and enhancement in carbohydrate metabolism in *Manihot esculenta* [13].

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Difenoconozole (DIZ) and Propiconazole (PPZ) are triazole group of fungicides, having plant growth regulator (PGR) properties, are reported to inhibit gibberellic acid biosynthesis and increase in abscisic acid and cytokinin contents [7].

Gloriosa superba L. is a medicinal plant belonging to the family Liliaceae. Seeds and tubers contain important alkaloids such as colchicine and colchicoside, which are used to treat gout and rheumatism [14]. In the Indian systems of medicine, the tubers are used as tonic, antiperiodic, antihelmenthic and also against snake bites [15]. The plant is known as 'Kalihari' in Hindi, 'Manthori khizangu' in Malayalam and 'Kazhappai kizhangu' in Tamil. The purpose of the present study is to understand the effect of difenoconozole and propiconazole on the enzymatic antioxidant potentials i.e. peroxidase (POX), polyphenol oxidase (PPO), Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) activities in *G.Superba.*

MATERIALS AND METHODS

Medicinally important plant species, *Gloriosa superba* L. (Family: Liliaceae) was selected for the present investigation. The tubers obtained from Department of Horticulture, Faculty of Agriculture, Annamalai University, Tamil nadu, India. The triazole compound Difenoconozole and propiconozol was obtained from Syngenta, India Ltd. Mumbai. During the study, average temperature was 32/26°C (maximum/minimum) and relative humidity (RH) varied between 60-75 per cent. The experimental part of this work was carried out in Stress Physiology Lab, Department of Botany, Annamalai University, Tamilnadu. The methodologies adopted are described below.

Cultivation Methods: The plants were raised in field condition in Udaiyarpalayam village (Ariyalur Dist) Tamilnadu, India. The tubers were sown during September 2010 to January 2011 in a completely randomized block design (CRBD) with three replications. The experimental area was tilled and planting furrows (30 cm deep) made at a distance of 1.5 m, 20 days before planting. Plotting mixture (red earth, sand, vermicompost and coir compost in 1:1:1:1 ratio) was applied in the furrows to ensure nutrient supply to the young plants. Each plot consisted of three 5 m long rows with inter and intra row spacing of 150 cm and 30 cm respectively. The plots were irrigated at weekly intervals. Recommended agronomic and plant protection practices were adopted.

Treatments: Three plots were selected by completely randomized block design (CRBD). 10mg L^{-1} PPZ and DIZ, these concentrations were used for the treatment plants and control plants, irrigated with tap water. The treatments were given on 35, 50, 65 and 80 days after planting (DAP) by soil drenching. The plants were taken randomly on 45, 60, 75 and 90 DAP and separated into tuber, stem and leaves and used for determining enzymatic antioxidant contents.

Enzymatic Antioxidant Estimations and Assays

Peroxidase [POX, EC 1.11.1.7]: Peroxidase was assayed by the method of [16]. Assay mixture of peroxidase contained 2ml of 0.1M phosphate buffer (pH 6.8), 1ml of 0.01M pyrogallol, 1ml of 0 .005M H_2O_2 and 0.5 ml of enzyme extract. The solution was incubated for 5min at 25°C after which the reaction was terminated by adding 1ml of 2.5N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420nm against a blank prepared by adding the extract after the addition of 2.5N H_2SO_4 at zero time. The activity was expressed in unit mg^{-1} protein. One unit is defined as the change in the absorbance by 0.1 min^{-1} mg^{-1} protein.

Ascorbate Peroxidase [APX, EC 1.11.1.1]: The activity of APX was determined by the method of [17]. The reaction mixture (1 ml) contained 50mM potassium phosphate buffer (pH 7.0), 0.5mM ascorbic acid, 0.1mM H_2O_2 and 200il of enzyme extract.The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient2.9mM^{-1} cm^{-1}). The enzyme activity was expressed in Umg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

Superoxide Dismutase [SOD, EC 1.15.1.1]: The activity of SOD was assayed as described by [18] The reaction mixture contained1.17×10-6Mriboflavin,0.1M methionine, 2×10-5M KCN and 5.6×10-5M nitroblue tetrazolium (NBT) salt dissolved in 3ml of 0.05M sodium phosphate buffer (pH 7.8). Three millilitres of the reaction medium was added to 1ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30 ?C for 1 h. identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity is expressed in Umg^{-1} protein (U = change in 0.1 absorbance h^{-1} mg^{-1} protein).

Catalase [CAT, 1.11.1.6]: The activity of CAT was measured according the method of [19] with small modification. The assay mixture contained 2.6 ml of 50mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition ofH_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in Umg^{-1} protein (U=1mM of H_2O_2 reduction min^{-1} mg^{-1} protein). For all the enzymatic calculations protein was determined by the method of Bradford, using bovine serum albumin (BSA, Sigma, USA) as the standard

Polyphenol Oxidase [PPO, EC 1.10.3.1]: The assay of PPO was carried out by the method of [16]. Assay mixture for PPO contained 2ml of 0.1M phosphate buffer (pH 6.0), 1ml of 0.1M catechol and 0.5ml of enzyme extract. This was incubated for 5 min at 25°C, after which adding 1ml of 2.5N H_2SO_4 stopped the reaction. The absorbency of the purpurogallin formed was read at 495 nm. To the blank 2.5 N H_2SO_4 was added of the zero time of same assay mixture. PPO activity is expressed in U mg^{-1} protein (U=Change in 0.1 absorbance min^{-1} mg^{-1} protein). For all the enzymatic calculations protein was determined by the method of Bradford [1976], using bovine serum albumin (BSA, Sigma, USA) as the standard.

Statistics: Statistical analysis was performed using one way analysis of variance [ANOVA] followed by Duncan's Multiple Range Test [DMRT]. The values are mean ± SD for seven samples in each group. *P* values = 0.05 were considered as significant.

RESULTS AND DISCUSSION

Antioxidant Enzymes: In case of control plants, the POX-activity remains low, the addition of DIZ and PPZ enhanced the POX-activity and it was highly marked over untreated plants (Table 1). Table 2 illustrates APX activity in the Glory lily plants under cultivation with or without DIZ and PPZ treatments. It is clear that the APX activity was increased due to DIZ and PPZ treatments in all parts of the plant (Table 2). The SOD- activity in leaf, stem and tuber increased to a great extent in DIZ- and PPZ-treated plants when compared to control. CAT- activity was highly influenced in all parts of the plants due to DIZ and PPZ treatments. PPO- activity also increased under DIZ and PPZ treatment in white *G.superba* when compared to control plants (Tables 3 - 5).

Treatment with DIZ and PPZ increased the POX activity in all the parts of *Gloriosa superba* when compared to control. *Radix astragali* plants under water deficit stress showed an enhancement in POX activity [20]. Water deficit stress increased the POX activity in soybean [21] and which is further increased by uniconazole treatment. Increased peroxidase activity is a common response to oxidative and abiotic stresses. Therefore peroxidase could be part of the enzymatic system connected with the increase in ethylene formation in plants like spinach [22]. The ascorbate peroxidase activity increased under different treatments in *Gloriosa superba*. Triazoles increased the level of APX activity in *Solenostemon rotundifolius* [23]. Paclobutrazol increased the APX activity in peanut plants under drought stress [24]; similar results were observed in *vigna* plants under propiconazole treatments [25]. The treatments DIZ and PPZ significantly increased the SOD activity in the leaves, stem and tuber of *Gloriosa superba*. An increase in SOD activity was reported in tridimefon treatment in banana [26]. Similar results were observed in uniconazole treatment in wheat [27] and cassia seedlings [28]. The PPO activity of the plants increased with the age in control and treated in *Gloriosa superba* plants. An increased in PPO activity was found in grape vines under paclobutrazol treatment. [29].Tomato plants showed higher PPO activity when subjected to salt stress in combination with paclobutrazol treatments [30]. The activity of catalase increased with DIZ and PPZ treatments in *Gloriosa superba*. An increase in catalase activity was noted in

Fig. 1: Shows the morphologically increased growths of Gloriosa superba tuber on 75 DAP.

Table 1: Difenoconozole and propiconazole - induced changes in the POX content (U mg $^{-1}$ protein) of *Gloriosa superba* (values are mean ± S.D of three samples).

Growth stages	Control	Difenoconozole	Propiconazole
Tuber			
45	0.058 ^{a} ±0.0073	0.064 ^{a} ±0.0095	0.065 ^{a}±0.0045
60	0.071 ^{a} ±0.0032	0.078 ^{b} ±0.0026	0.081 ^{b}±0.0036
75	0.079 ^{a} ±0.0010	0.105^{bc} ±0.0032	0.109 ^{c}±0.0043
90	0.113 ^{a} ±0.0032	0.126 ^{b} ±0.0020	0.130 ^{b}±0.0043
Stem			
45	0.044 ^{a} ±0.0042	0.046 ^{a} ±0.0053	0.046 ^{a} ±0.0025
60	0.088 ^{a} ±0.0021	0.105 ^{c} ±0.0015	0.097 ^{b} ±0.0026
75	0.101 ^{a} ±0.0020	0.118 ^{b} ±0.0015	0.144 ^{c} ±0.0015
90	0.117 ^{a} ±0.0020	0.143 ^{b} ±0.0040	0.166 ^{c} ±0.0021
Leaf			
45	0.097 ^{a} ±0.0036	0.100 ^{a} ±0.0025	0.103 ^{a}±0.0021
60	0.145 ^{a} ±0.0025	0.157 ^{b} ±0.0025	0.159 ^{b}±0.0070
75	0.234 ^{a} ±0.0043	0.251 ^{b} ±0.0038	0.245 ^{b}±0.0050
90	0.263 ^{a} ±0.0237	0.311 ^{a} ±0.0110	0.315 ^{a} ±0.0086

Values are given as mean ± SD of six experiments in each group. Values that are not sharing a common superscript [a,b,c] differ significantly at $P \leq 0.05$ [DMRT].

Table 2: Difenoconozole and propiconazole - induced changes in the APX content (U mg $^{-1}$ protein) of *Gloriosa superba* (values are mean ± S.D of three samples).

Growth stages	Control	Difenoconozole	Propiconazole
Tuber			
45	0.072^{a} ±0.0083	0.0751 ^{a} ±0.0035	0.075 ^{a} ±0.0044
60	0.093^{a} ±0.0056	0.122 ^{b} ±0.0049	0.126 ^{c}±0.0052
75	0.106^{a} ±0.0041	0.122 ^{b} ±0.0070	0.125 ^{b} ±0.0045
90	0.129^{a}±0.0020	0.206 ^{b}±0.0041	0.233^{b} ±0.0026
Stem			
45	0.087 ^{a} ±0.0015	0.099 ^{b} ±0.0050	0.098 ^{b} ±0.0015
60	0.118 ^{a} ±0.0031	0.125 ^{b}± 0.0025	0.124 ^{a} ±0.0025
75	0.098 ^{a}±0.0015	0.110 ^{b} ±0.0031	0.124 ^{c}±0.0390
90	0.151^{a}±0.0031	0.197^{b}±0.0076	0.208 ^{c}±0.0041
Leaf			
45	0.098 ^{a} ±0.0015	0.114 ^{b}±0.004	0.116 ^{b} ±0.0036
60	0.205 ^{a} ±0.0035	0.251 ^{b} ±0.0312	0.251 ^{b} ±0.0312
75	0.226 ^{a} ±0.0025	0.325 ^{b} ±0.0061	0.355 ^{c} ±0.0021
90	0.296 ^{a} ±0.0061	0.331 ^{b} ±0.0084	0.387 ^{c} ±0.0025

Values are given as mean ± SD of six experiments in each group. Values that are not sharing a common superscript [a,b,c] differ significantly at $P \leq 0.05$ [DMRT].

Table 3: Difenoconozole and propiconazole - induced changes in the SOD content (U mg^{-1} protein) of *Gloriosa superba* (values are mean ± S.D of three samples).

Growth stages	Control	Difenoconozole	Propiconazole
Tuber			
45	0.108 ^{a} ±0.0021	0.121^{b} ±0.0076	0.124^{b} ±0.0015
60	0.195 ^{a} ±0.0015	0.242 ^{b} ±0.0026	0.248 ^{c} ±0.001
75	0.218 ^{a} ±0.0052	0.286 ^{b} ±0.0025	0.283 ^{b} ±0.0022
90	0.212 ^{a} ±0.0025	0.286 ^{b} ±0.0025	0.305 ^{c} ±0.0095
Stem			
45	0.047 ^{a} ±0.0025	0.051 ^{a} ±0.0038	0.057 ^{b} ±0.0026
60	0.102 ^{a} ±0.0025	0.111 ^{b} ±0.0035	0.134 ^{c} ±0.0021
75	0.108 ^{a} ±0.0015	0.125 ^{b} ±0.0035	0.121 ^{b} ±0.0038
90	0.120 ^{a} ±0.0010	0.130 ^{b} ±0.0015	0.132 ^{b} ±0.0045

Table 3: Continue

Growth stages	Control	Difenoconozole	Propiconazole
Leaf			
45	0.066 ^{a} ±0.0031	0.075 ^{b}±0.0025	0.081 ^{c} ±0.002
60	0.088 ^{a} ±0.0015	0.096 ^{b} ±0.0030	0.099 ^{b} ±0.0031
75	0.0713 ^{a}±0.0025	0.078 ^{a} ±0.0021	0.091 ^{b} ±0.0075
90	0.073 ^{a} ±0.0020	0.081 ^{b} ±0.0020	0.088 ^{c} ±0.0021

Values are given as mean ± SD of six experiments in each group. Values that are not sharing a common superscript [a,b,c] differ significantly at $P \leq 0.05$ [DMRT].

Table 4: Difenoconozole and propiconazole - induced changes in the CAT content (U mg^{-1} protein) of *Gloriosa superba* (values are mean ± S.D of three samples).

Growth stages	Control	Difenoconozole	Propiconazole
Tuber			
45	0.019 ^{a}±0.002	0.019 ^{a} ±0.001	0.021 ^{a}±0.0015
60	0.046 ^{a} ±0.0025	0.050^{ab} ±0.0015	0.054 ^{b} ±0.0035
75	0.030 ^{a} ±0.0025	0.0361^{b} ±0.0029	0.033^{ab} ±0.0025
90	0.055 ^{a} ±0.0021	0.061^{b} ±0.0011	0.065 ^{c} ±0.0015
Stem			
45	0.012 ^{a} ±0.0025	0.015 ^{a} ±0.0039	0.016 ^{a}±0.0031
60	0.030 ^{a} ±0.0011	0.031 ^{a} ±0.0025	0.046 ^{b}±0.0040
75	0.035 ^{a} ±0.0022	0.044 ^{b} ±0.0025	0.046±0.0011
90	0.057 ^{a} ±0.0021	0.067 ^{c} ±0.0004	0.061 ^{b}±0.001
Leaf			
45	0.024 ^{a} ±0.0015	0.027 ^{a} ±0.0029	0.026 ^{a} ±0.0030
60	0.034 ^{a} ±0.0012	0.038 ^{a} ±0.0031	0.036 ^{a}±0.0053
75	0.057 ^{a} ±0.0020	0.073 ^{b} ±0.0027	0.092 ^{c} ±0.0031
90	0.063 ^{a} ±0.0027	0.072 ^{b}±0.0076	0.073 ^{b} ±0.0046

Values are given as mean ± SD of six experiments in each group. Values that are not sharing a common superscript [a,b,c] differ significantly at $P \leq 0.05$ [DMRT].

Table 5: Difenoconozole and propiconazole - induced changes in the PPO content (U mg $^{-1}$ protein) of *Gloriosa superba* (values are mean ± S.D of three samples).

Growth stages	Control	Difenoconozole	Propiconazole
Tuber			
45	0.056 ^{a} ±0.0021	0.065 ^{b}±0.0021	0.060 ^{a} ±0.0020
60	0.069 ^{a} ±0.0015	0.079 ^{b} ±0.0020	0.078 ^{b}±0.0037
75	0.078 ^{a} ±0.0026	0.089 ^{b} ±0.0025	0.085 ^{b} ±0.0051
90	0.096 ^{a}±0.049	0.121^{b}±0.0189	0.117 ^{b} ±0.0087
Stem			
45	0.017 ^{a} ±0.0025	0.022 ^{b}±0.0015	0.0224 ^{b}±0.009
60	0.050 ^{a}±0.001	0.056 ^{b} ±0.0026	0.062 ^{c} ±0.0025
75	0.054 ^{a} ±0.0025	0.060 ^{a} ±0.0015	0.0603^{ab}±0.002
90	0.095 ^{a}±0.0046	0.112 ^{b} ±0.0070	0.1074 ^{b}±0.0032
Leaf			
45	0.038 ^{a} ±0.0031	0.045 ^{ab}±0.003	0.052 ^{b}±0.007
60	0.072 ^{a} ±0.0017	0.089 ^{b}±0.002	0.099 ^{c} ±0.0047
75	0.085 ^{a}±0.002	0.096 ^{b} ±0.0031	0.103^{bc} ±0.0061
90	0.103 ^{a} ±0.0020	0.127 ^{b} ±0.0020	0.133 ^{c}±0.0025

Values are given as mean ± SD of six experiments in each group. Values that are not sharing a common superscript [a,b,c] differ significantly at $P \leq 0.05$ [DMRT].

barley leaves and there by effective scavenging of H_2O_2 to provide antioxidant defense mechanism [31]. The H_2O_2 scavenging system represented by APX and CAT are more important in imparting tolerance that SOD as reported in oxidative stress in wheat [32, 33 and 34]. An increase in catalase activity was noted in triazole treatment in *Catharanthus* [35, 36].

CONCLUSION

From the results of this investigation, it is clear that, DIZ and PPZ treatments increased the enzymatic antioxidants in *Gloriosa superba*. Hence, it can be concluded that the application of DIZ [15 mg L^{-1} $plant^{-1}$] and PPZ [10 mg L^{-1} $plant^{-1}$] could well be used as a antioxidant potential tool to manipulate antioxidant metabolism and also to increase the medicinal properties of the Glory lily plant.

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