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Studies on Effect of Antioxidant Enzymes Salicylic Acid and Jasmonic Acid Treated Plants of Acalypha indica.L

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ABSTRACT

*Acalypha indica.*L one of the traditional medicinal small annual shrubs belongs to the Euphorbiaceous Family, it grows up to 30-75 cm in height and is distributed in wet, temperate and tropical areas. It is available in gardens, road sides and throughout India. In the present work, we try to assess the antioxidant enzymes activity of SA & JA treated and control plants of Acalypha. Plants were treated with alone and different concentration combinations of SA & JA. After 55 days treated plants leaves were used for extractions. For extracting antioxidant enzymes, fresh leaves (0.5 gr) were ground using a tissue grinder in 5 m L of 50 mM cooled phosphate buffer (ph 7.8) placed in an ice bath. The homogenate was centrifuged at 15000 rpm for 20 min at 4 ^{0}C. The supernatant was used for determining the activities of enzymes. In this we find the antioxidant enzymes are Proline, superoxide dismutase (SOD), Glutathione reductase (GR), Ascorbic peroxidase (APX), Glycine betaine (GB). Hightest antioxidant enzyme activity was observed at the combination of T8 (3mM SA + 2μM JA) and lowest in T6 (1 mM SA+2μM JA). All alone and combination of SA & JA treated plants were contains high antioxidant enzyme activity, when compared to control one.

Keywords: *Acalypha indica*. L, Plant growth regulators (SA & JA), Antioxidant enzymes.

I. INTRODUCTION

Acalypha indica Linn. (Euphorbiaceae) is an annual erect herb and it is cosmopolitan distributed common weed plant (Ramachandran, 2008) [1]. It is used in treating pneumonia, asthma, rheumatism and also an emetic emmenagogue and anthelmintic (Chopra and Nayar, 1956) [2]. The juice of this plant used to treat a number of skin disorders and also been reported to possess contraceptive activity (Bourdy and Walker, 1992) [3]. In India, *Acalypha indica* has been extensively used in Ayurvedic system of medicine for various ailments like hepato protective, anti-inflammatory, antitissive, antifungal, wound healing and also antibacterial agent (Gupta*, et,. al* 2010) [4]. The plant based medicine was prominently used in

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India and China for curing diseases (Duraipandyan *et al.*, 2007) [5]. In India, the plant derived traditional used in various methods like Ayurveda, Siddha, Unani and homeopathy. Because, India has an abundant source of plant flora throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods by Ayurveda and Homeopathy (Srinivasan *et al.,* 2007) [6]. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effect of the phytochemicals in them (Adesokan *et al.,* 2008) [7]. The Antioxidant activity of the extracts was analyzed by evaluating superoxide and hydroxyl radical scavenging activity and effect on lipid peroxidation. The ethanol extract showed significant antioxidant activity in all the free radical scavenging tests (Ranganathan *et al.,* 2013) [8]. Salicylic acid is an important and well-studied endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in plant defense in addition to their impact on plant growth and development (Vicent,; Lu, 2009; drees *et al.*,2011) [9]. Jasmonic acid (JA) is an endogenous plant growth regulator widely distributed in higher plants (Meyer *et.al* 1984) [10]. Salicylic acid is well known for systemic acquired resistance, induce in plant response to many pathogens, and can also elicit the production of secondary metabolites in plants (Hayat *et al.,* 2010) [11], (Pieterse *et al.,* 1999) [12]. The present effort has been made objective of this current study was to evaluate the influence of Jasmonic acid (JA) and Salicylic acid (SA) on antioxidant activity of *Acalypha indica*.L

II. METHODS AND MATERIAL

Process of plant treatment with SA and JA:

A.indica. L plants were treated with Salicylic acid (SA) and Jasmonic acid (JA) individually and in combination with different concentrations for every 15 days of interval up to 55 days. SA and JA were applied to the plants as foliar spray.T1 (Control), T2 (1.0mM SA), T3 (3.0mM SA), T4 (200 μM JA), T5 (400 μM JA), T6 (1mM SA + 200 μM JA), T7 (1mM SA+ 400 μM JA), T8 (3mM SA+200 μM JA) and T9 (3mM SA + 400 μM JA).

Preparation of Extracts: For extracting antioxidant enzymes, fresh leaves (0.5 g) were ground using a tissue grinder in 5 mL of 50 mM cooled phosphate buffer (pH7.8) placed in an ice bath. The homogenate was centrifuged at 15000 rpm for 20 min at 4°C. The supernatant was used for determining the activities of the enzymes.

Antioxidant enzyme analysis of treated and control plants of Acalypha indica.L:

Proline determination:

The proline from the third leaf from top was estimated according to the method of (Bates *et al.*. 1973) [13a]. A sample of 0.5 g fresh leaf tissue was homogenized in 10 ml of 3 % Sulfosalicylic acid and the homogenate was filtered through Whatman No. 2 filter paper. Then 2.0 ml of the filtrate were mixed with 2.0 ml acid Ninhydrin, 20 ml 6 M Orthophosphoric acid, and 2 ml of Glacial acetic acid in a test tube. This mixture was incubated at 100 o C for 60 minutes and then cooled in an ice bath. Finally, 4.0 ml of Toluene were added to the solution and mixed vigorously by passing a continuous stream of air for 1-2 min. The chromophore containing Toluene was aspirated from the aqueous phase, warmed at room temperature and the absorbance was read at 520 nm using Toluene as a blank. The proline concentration was determined from a standard curve and calculated on fresh weight basis as follows:- 1μmole proline g -1 fresh weight = (μg proline ml -1 x ml of toluene/115.5)/ (g of sample).

Superoxide dismutase (SOD):

The activity of SOD was determined by measuring its ability to inhibit the photo reduction of Nitroblue tetrazolium (NBT) following the method of

(Giannopolitis and Ries 1977) [14]. The reaction solution (3 ml) contained 50 μM NBT, 1.3 μM Riboflavin, 13 mM Methionine, 75 mM EDTA, 50 mM Phosphate buffer (pH 7.8), and 20 to 50 μl of enzyme extract. The test tubes containing the reaction solution were irradiated under light (15 fluorescent lamps) at 78 μmol m - 2 s -1 for 15 min. The absorbance of the irradiated solution at 560 nm was read using a spectrophotometer (IRMECO, U2020). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photo reduction.

Glutathione reductase estimation(GR):

The glutathione reductase activity was determined by the method (Mavis and Stellwagen, 1968) [15]. Prepared the glutathione standards simultaneously with the samples for they assayed together. Each sample, including unknown and standard are in triplicate. In a 96-well plate, add 25 μL of the 1X Glutathione Reductase solutions to each well to be tested. Add 25 μL of the 1X NADPH solutions to each well to be tested. Add 100 μL of the prepared glutathione standards or samples to each well to be tested. Mix thoroughly. Ensure that the plate reader is prepared for a kinetic assay and is set to read at 405 nm. Add 50 μL of the 1X Chromogen and mix briefly. Immediately begin recording the absorbance at 405 nm at 1 minute intervals for 10 minutes. If using all the wells within the plate at one time, then it may be necessary to record the absorbance at 2 minute intervals. Then calculate the concentration of standards and samples.

Glycine betaine determination (GB):

The glycine betaine was determined following the (Grieve and Grattan; 1983) [16] method. The dry leaf material (1.0 g) was ground in 10 ml of distilled water and filtered. After filtration, 1 ml of the extract was mixed with 1 ml of 2M HCl. Then 0.5 ml of this mixture was taken in a glass tube and 0.2 ml of Potassium tri-iodide solution was added to it. The contents were shaken and cooled in an ice bath for 90 min with occasional shaking. Then 2.0 ml of ice cooled distilled water and 20 ml of 1-2 Dichloromethane (cooled at -10 o C) were added to the mixture. The two layers formed in the mixture were mixed by passing a continuous stream of air for 1-2 min while tubes were still in the ice bath (4 o C). The upper aqueous layer was discarded and optical density of the organic layer was measured at 365 nm. The concentrations of the betaine were calculated against the standard curve.

Ascorbate peroxidase estimation (APX):

The activity of ascorbate peroxidase (APX) was measured using the method of (Nakano and Asada 1981) [17a]. The reaction cocktail (3 ml) contains a mixture of 50 mM phosphate buffer (pH 7.0), 0.2 mM EDTA, 2% H2O2, 0.5 mM ascorbic acid and 0.1 ml of enzyme extract. The decrease in ascorbate concentration was followed by a decline in the optical density at 290 nm, and activity was calculated using the extinction coefficient (2.8 mM -1 cm -1 at 290 nm) for ascorbate.

III. RESULTS AND DISCUSSION

Estimation of proline:

The estimation of proline is carried out by the method of (Bates et al., 1973) [18b] 25, 40 and 55 days of SA&JA treated plants contained high proline along with increasing hormonal concentrations. Highest proline content was observed at the combination of T8 (3mMSA+2μM JA) (24.8μmol/g) and lowest Proline content in T6 (1mM SA + 2μMJA) (22.0 μmol/g). In the alone concentrations of SA & amp; JA treated plants, highest Proline content was present in T3 (3.0mMSA) (19.04 μmol/g). All alone and combination of SA & amp; JA treated plants contained high proline content, when compared to control (15.06μmol/g) Acalypha indica.L plants.

Superoxide dismutase (SOD):

The estimation of superoxide dismutase (SOD) is carried out by the method of (Giannopolitis and Ries 1977) [19] 25,40 and 55 days of SA& amp; JA treated plants contained high SOD along with increasing hormonal concentrations. Highest SOD content was observed at the combination of T8 (3mMSA+2µMJA) (0.87µmol/g) and lowest SOD content in T6 (1mMSA + 2µM JA) (0.64 µmol/g). In the alone concentrations of SA & JA treated plants, highest SOD content was present in T5 (4.0µM JA) (0.62µmol/g). All alone and combination of SA & amp; JA treated plants contained high SOD content, when compared to control (0.25µmol/g) Acalypha indica.L plants.

Glutathione reductase (GR):

The estimation of Glutathione reductase (GR) is carried out by the method (Mavis and Stell wagon 1968) [20]. 25, 40, and 55 days of SA& amp; JA treated plants contained high GR along with increasing hormonal concentrations. Highest GR content was observed at the combination of T8 (3mM SA+2µM JA) (2.80µmol/g) and lowest GR content in T6 (1mM SA + 2µM JA) (1.95 µ mol/g). In the alone concentrations of SA& amp; JA and combination of SA& amp; JA treated plants contained high GR constant, when compared to control (0.45µmol/g) Acalypha indica.L.

Ascorbic peroxidase (APX):

The estimation of ascorbic peroxidase (APX) is carried out by the method of (Nakano and Asada 1981) [21b]. 25,40and 55 days of SA& amp; JA treated plants contained high APX along with increasing hormonal concentrations. Highest APX content was observed at the combination of T8 (3mM SA+2µM JA) (16.72µmol/g) and lowest APX content in T6 (1mM SA + 2µM JA) (15.97 µ mol/g). In the alone concentrations of SA& amp; JA treated plants, highest APX content was present in T3 (3.0mM SA) (14.12µmol/g). All alone and combination of SA& amp; JA treated plants contained high APX constant, when compared to control (6.57µmol/g) Acalypha indica.L.

Glycine betaine:

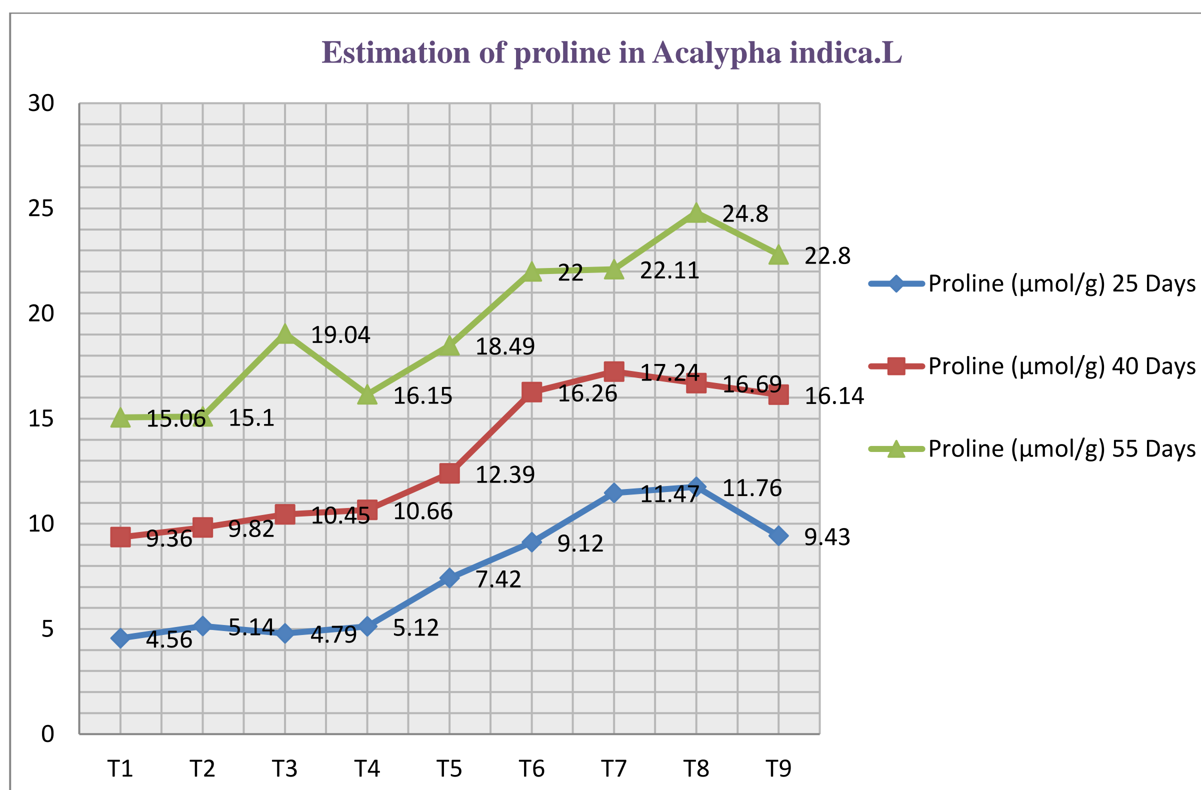
The estimation of Glycine betaine is carried out by the method of (Grieve et. al 1983) [22]. 25, 40 and 55 days of SA& amp; JA treated plants contained high GB along with increasing hormonal concentrations. Highest GB content was observed at the combination of T7 (1mM SA+4µM JA), (7.63µmol/g) and lowest GB content in T6 (1mM SA + 2µM JA) (6.51 µ Mol/g). In the alone concentrations of SA& amp; JA treated plants, highest GB content was present in T3 (3.0mM SA), (5.16 µ Mol/g). All alone and combination of SA& amp; JA treated plants contained high GB constant, when compared to control (1.57µmol/g) Acalypha indica.L.

Statistical analysis:

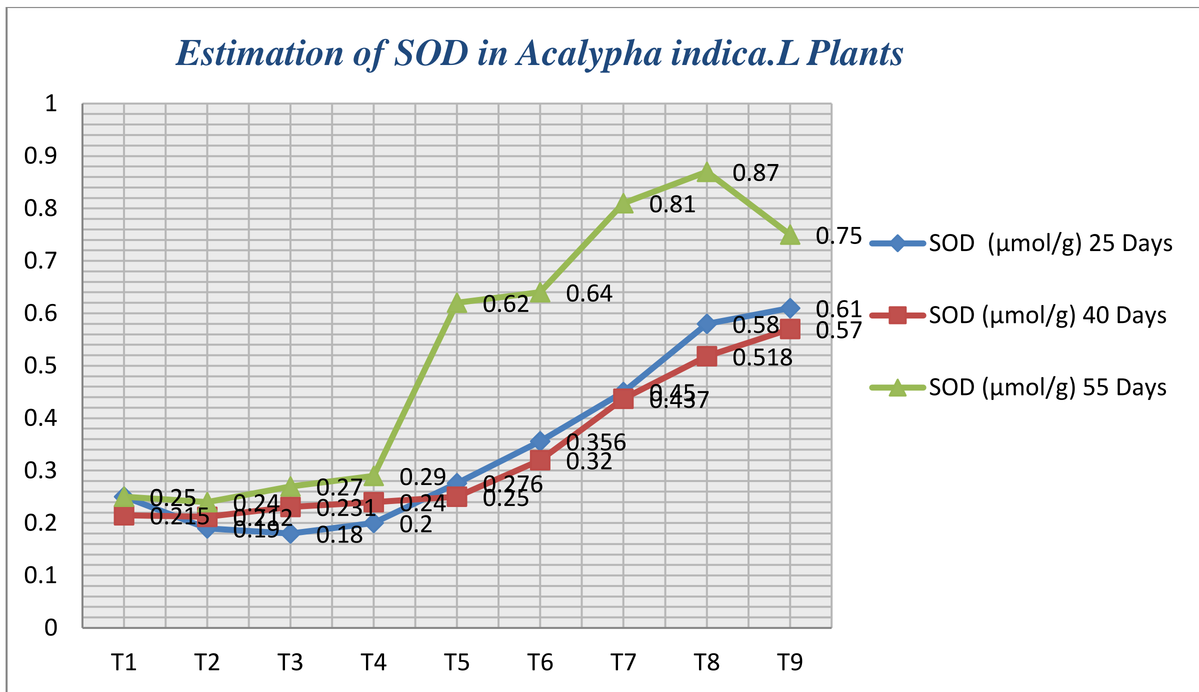
Four factor completely randomized design (Analysis of variance technique) of the data was computed for all attributes by using the MSTAT Computer Program (MSTAT Development Team, 1989). Four factors were five area samples, salt treatments, growth stages and different levels of glycine betaine. The bar graph using mean ± S.E values was drawn by using Microsoft Excel software. The Duncan's New Multiple Range test at 5% level of probability was used to test the differences among mean values following (Torrie et., al 1986)[23].

Table.4 Antioxidant enzyme analysis of 25, 40 and 55 days hormone (SA& JA) treated and control plants of A. indica.L

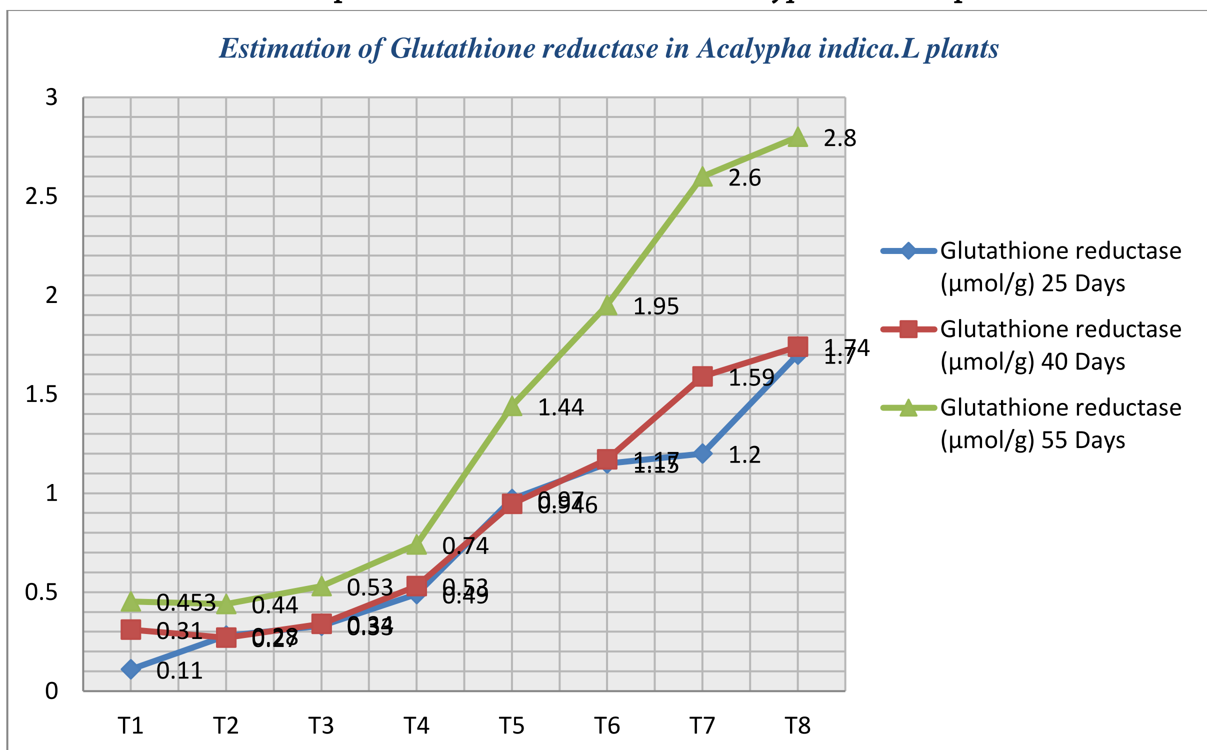
	Proline (µmol/g)			SOD (µmol/g)			Glutathione reductase (µmol/g)			Ascorbic peroxidase			Glycine Betaine (µmol/g)		
	25Days	40 Days	55 Days	25 Days	40 Days	55 Days	25 Days	40 Days	55 Days	25 Days	40 Days	55 Days	25 Days	40 Days	55 Days
T1	4.56±0.75	9.36±0.63	15.06±0.56	0.25±0.74	0.215±0.26	0.25±0.15	0.111±0.11	0.31±0.23	0.453±0.18	0.55±0.52	3.79±0.39	6.57±0.86	0.55±0.47	1.57±0.28	1.57±0.54
T2	5.14±0.21	9.82±0.446	15.1±0.48	0.19±0.52	0.18±0.89	0.24±0.27	0.28±0.17	0.27±0.16	0.44±0.17	1.65±0.14	3.47±0.82	6.51±0.37	1.65±0.69	2.1±0.18	2.1±0.27
T3	4.79±0.47	10.45±0.38	19.04±0.27	0.20±0.72	0.212±0.26	0.27±0.25	0.33±0.09	0.34±0.26	0.53±0.24	1.45±0.33	5.82±0.69	14.12±0.95	1.45±0.71	2.16±0.63	5.16±0.87
T4	5.12±0.58	10.66±0.29	16.15±0.62	0.24±0.57	0.231±0.83	0.29±0.17	0.49±0.17	0.53±0.30	0.74±0.22	1.67±0.87	6.27±0.26	8.10±0.49	1.67±0.57	3.47±0.47	3.47±0.18
T5	7.42±0.61	12.39±0.19	18.49±0.38	0.25±0.29	0.276±0.37	0.62±0.27	0.97±0.19	0.946±0.22	1.44±0.18	1.98±0.38	10.48±0.76	12.01±0.26	1.98±0.72	4.29±0.85	4.29±0.25
T6	9.12±0.28	16.26±0.37	22±0.15	0.32±0.48	0.356±0.44	0.64±0.19	1.15±0.20	1.17±0.24	1.95±0.33	1.85±0.65	12.32±0.99	15.1±0.76	1.85±0.85	6.51±0.23	6.51±0.82
T7	11.47±0.36	17.24±0.41	24.11±0.42	0.45±0.82	0.437±0.32	0.81±0.21	1.2±0.15	1.59±0.37	2.60±0.23	2±0.29	12.75±0.21	15.97±0.35	2.00±0.29	7.63±0.77	7.63±0.14
T8	11.76±0.16	16.69±0.38	24.8±0.59	0.58±0.39	0.518±0.64	0.87±0.16	1.7±0.22	1.74±0.28	2.80±0.25	2.1±0.35	12.58±0.62	16.72±0.73	2.10±0.58	4.17±0.19	7.47±0.86
T9	9.43±0.67	16.14±0.32	22.8±0.47	0.61±0.73	0.57±0.88	0.75±0.11	1.87±0.29	1.92±0.23	2.00±0.14	3.87±0.67	12.47±0.54	15.49±0.98	3.87±0.69	7.15±0.91	7.15±0.27



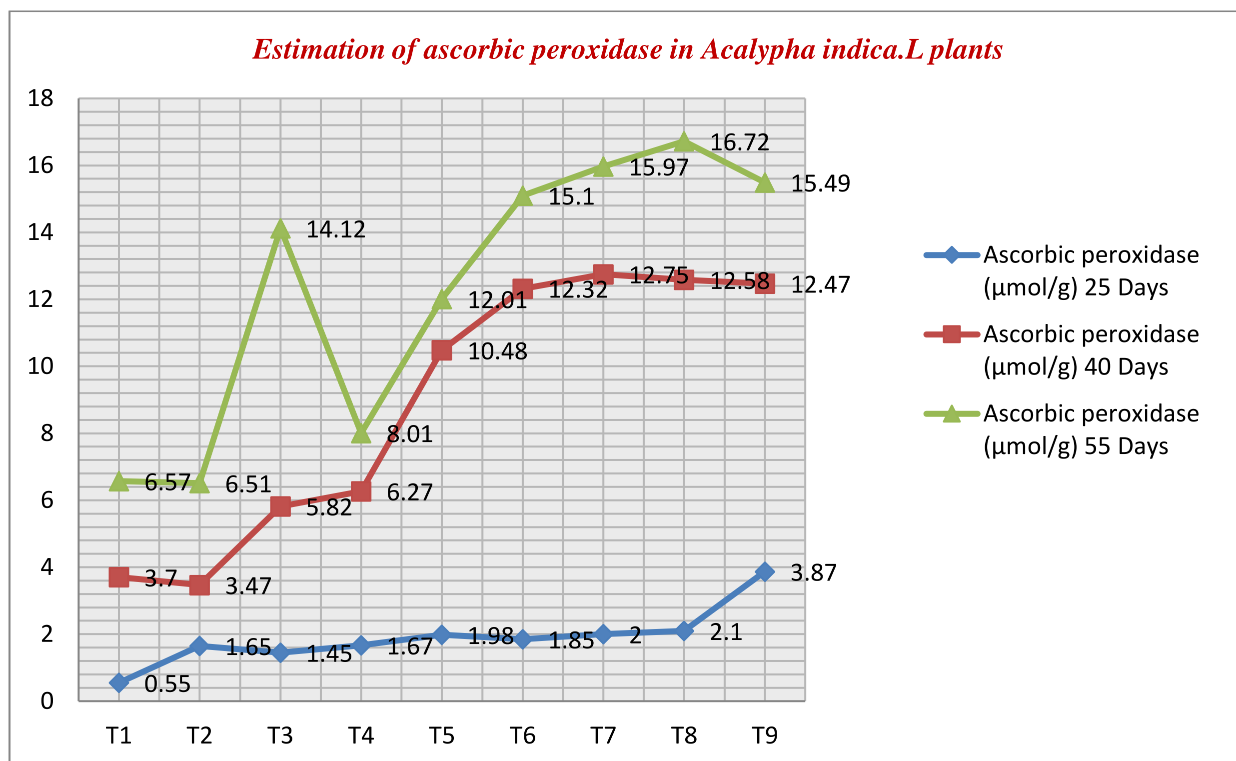
Effect on Proline content in *Acalypha indica* L. plants



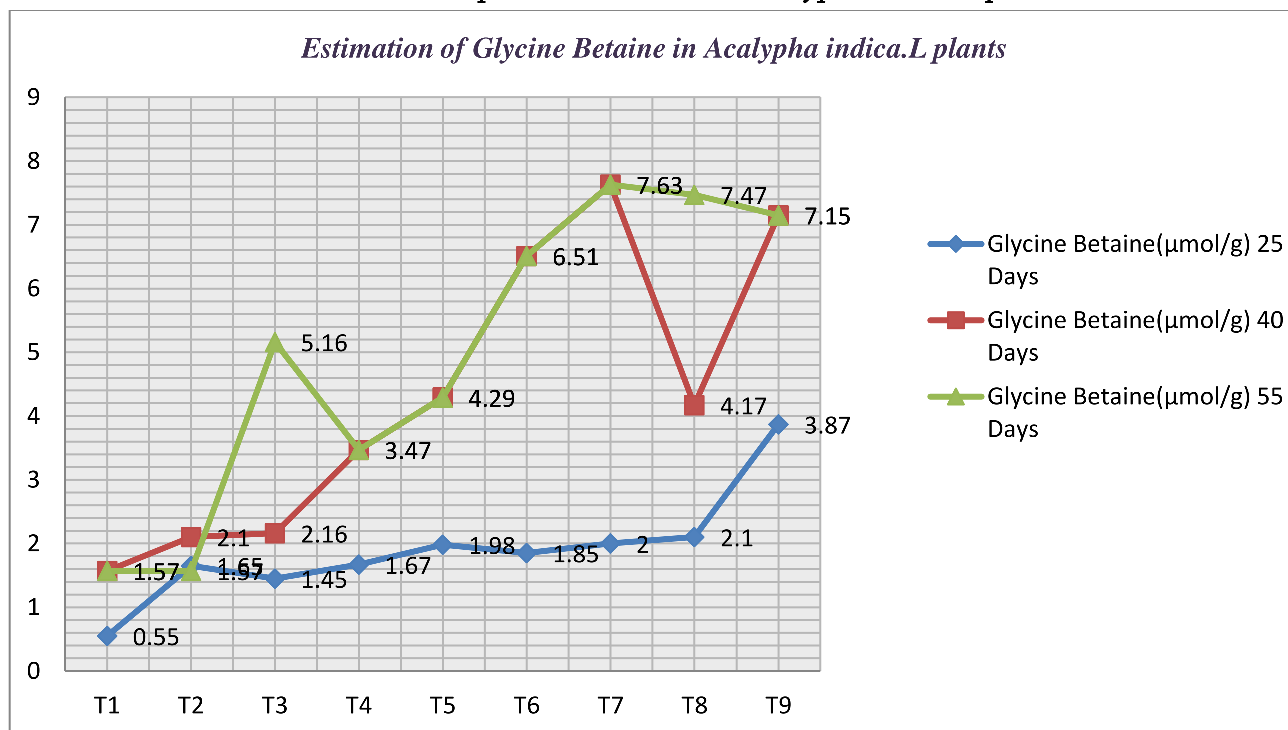
Effect on superoxide dismutase content in *Acalypha indica* L. plants.



Effect on glutathione reductase content in *Acalypha indica* L. plants



Effect on ascorbic peroxidase content in *Acalypha indica* L. plants



Effect on glycine betaine content in *Acalypha indica* L. plants

IV. CONCLUSION

In the present work, the data indicates alone and different combinations of SA and JA treated plants exhibit the more antioxidant enzyme activity. In this we find the antioxidant enzymes are Proline, superoxide dismutase (SOD), Glutathione reductase (GR), ascorbic peroxidase (APX), and Glycine betaine (GB). Hightest antioxidant enzyme activity was observed at the combination of T8 (3mM SA + 2µM

JA) and lowest in T6 (1 mM SA+2µM JA). All alone and combination of SA & JA treated plants were contains high antioxidant enzyme activity, when compared to control one.

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