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Physiological Differences Between SA&JA Treated and Control Plants of *Acalypha indica*.L Estimation Chlorophyll-a, b, Total chlorophyll and Carotenoids.

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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, temperature and tropical areas. It is available in gardens, road side and throughout India. The plant has close connection with conventional medicine such as Ayurveda, Unani, Siddha. It has been used to cure Pneumoniae, asthma, rheumatism and other diseases. SA and JA are a group of growth agents in many crops and medicinal plants. In the present work, the focus was laid on the recent studies of SA & JA effects on plant physiological differences between treated and control plants estimation of chlorophyll-a, b, total chlorophyll, Carotenoids. The plants were treated with SA & JA individually and in combination. T2 (1mM SA), T3 (3mM SA), T4 (200µM JA), T5 (400µM JA) were individual and T6 (1mM SA + 200µM JA), T7 (1mM SA+ 400µM JA), T8 (3mMSA+200µM JA) and T9 (3mM SA + 400µM JA) were in combination of the above growth agents. In the present work we observed physiological variations ,chlorophyll-a, chlorophyll-b, total chlorophyll, Carotenoids in *acalypha* leaves with increasing dose dependent manner in the treated plants when compared to the controlled one (T1). In the present study, we observed all the combinations of SA&JA treated plants exhibit more chlorophyll content T7 (0.81µg/g), T8 (0.87µg/g) and T9 (0.97 µg/g) including control plants T1(0.738 µg/g) when compared to alone concentration of SA&JA treated plants. Highest Carotenoids were present in combinations of SA&JA treated plants T7 (0.620µg/g), T8 (0.621µg/g) and T9 (0.623µg/g).

Key words: *Acalypha indica*.L, medicinal plants, Salicylic Acid (SA) & Jasmonic Acid (JA), chlorophylls, Carotenoids.

I. INTTRODUCTION

Acalypha indica .L belongs to Euphorbiaceae family, it is commonly called "Indian copperleaf" the plant grows along with crops and other wet lands throughout the year. It is morphologically small erect herb and growing up to more than 60 cm. The branches are ascending angled and velvet-hairy. The leaves are triangular ovate shaped and leaf stalks are longer than 3-5 cm Flowers are stalk less, borne on erect axillary spikes longer than the leaves. Male flowers are crowded and minute, female flowers are attached with the inflorescence axis, each subtended by a conspicuous semi copula leaf-like toothed green bract nearly 7 mm long. Capsule is bristly, 1 mm broad. This plant mainly grows in Andhra Pradesh, West Bengal, Kerala, and Tamil Nadu (Manisha M *et al.*, 2011). It is a yearly upright aromatic plant 30-75 cm in height. The leaves of the plant contain to be report toward seize the superiority of contraceptive action (Vaishnav and Gupta 1996). The extract of the origin is to be able to condensed the blood sugar stage via 30% (Vaishnava et *al*,.1993). Leaves possess anti-periodic with laxative properties and the leaf extract can be applied to insect bites and contraceptive activity (Bourdy & Walter, 1992a). It has been reported to be useful in treating pneumonia, asthma, rheumatism and several other ailments (Chopra et al., 1956). The leaves of the plant can be used in the diagnosis of jaundice, piles and also externally skin eruptions, ring worms. The seed is slightly sweet and also possess laxative, carminative, cooling improves the appetite (Chopra et al., 2006). In the present work, we were focused mainly on the action of the commonly used methyl jasmonate (MJA), Jasmonate and Salicylic acid (SA) effect on *Acalypha indica* plants. According to the World health organization (WHO), the medicinal plants would be a very significant source for a variety of drugs and most of the population in the world depends on traditional medicine. Jones *et al*., (2006) reported that a scientific study is needed to determine bioactive compounds from the plants comparatively with plant benefits *Acalypha indica*.L plant has close connection with conventional medicine such as Ayurveda, Unani and Siddha. It is implemented by Indian old generations (Sivasankari, 2014). It has been widely using in the traditional medicinal system of India. Gupta (2010) reported to have hepatoprotective, anti-inflammatory, antitussive, antifungal, antibacterial and wound healing activity. The root of this plant is treated as a stimulant, harsh and tough purgative (Silberbush et al,.2010). The leaves extract of the plant reduces mutagenicity in E. coli. The leaves are laxative while a moisturizer for the diagnosis of facial paralysis (Li H *et al*,.2012). The leaves of the plant can be used in the diagnosis of jaundice, piles and also externally skin eruptions, ring worms. Leaves possess anti-periodic with laxative properties and the leaf extract can be applied to insect bites (Bourdy G and Walter A 1992b). Root is of use during agitation, heart disease plus retains excretions. The 50 % ethanol extracts of pods reveal the antifertility activity in female albino rats. My present investigation was done for estimation of chlorophyll-a, b and Carotenoids in *Acalypha indica. L*. The Foliar spray of SA at 10-4 Mol/L significantly enhanced the physiological characteristics such as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids

contents. (Nadeem et al 2012). Jasmonic acid also helps in chlorophyll and carotenoid accumulation and neutralizes the toxic effect of Cu2+ on seedlings. It's a first report of JA effect on photosynthetic pigment (Sharma et al., 2013).

II. MATERIALS AND METHODS

A. indica. L Seeds sowing and transplantation:

A. *indica*.L seeds were washed with tap water several times and sown in the seed bed prepared in the field for the seed germination. Seed germination occurred within seven days after sowing. 9 individual plots (1 for controlled, 4 for 2 individual hormones with 2 different concentrations and 4 for combination of hormones with 4 different concentrations) each of 5X5m length and width size were prepared in the field. The seedlings were transplanted from seedbed to individual 9 plots marked with treatment status. 30 seedlings were planted in each plot.

B. with 5 rows each containing 6 plants. The field was maintained for up to 55 days for plant growth.

Collection of plant hormones:

The plant hormones Salicylic Acid (SA) and Jasmonic Acid (JA) were procured from Sigma Aldrich, Hyderabad.

Preparation of plant hormones for the treatment:

The hormone concentrations were selected based on preliminary experiments. Such as, 1mM, 3mM and 200µM and 400µM SA and JA were selected. 0.069g of SA was dissolved in 500ml of double distilled water for the concentration of 1m M and 0.207g SA was dissolved in 500 ml of distilled water for 3mM concentrations and taken 20 and 40 ml of 10 mM concentrated Jasmonic acid make up with 1L distilled water for 200 and 400µM concentrations respectively.

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), T9 (3mM SA + 400 µM JA).

Physiological differences between SA&JA treated and control plants of *Acalypha* indica.L

Chlorophyll and carotenoid estimation (mg/g):

Chlorophyll and carotenoid content of experimental material was estimated by the method of Hiscox and Israelstam (1979). The fully matured third leaf (Three leaf lets) of each plant at the time of anthesis after the removal of midrib was taken for analysis of Chlorophyll a, Chlorophyll b and Carotenoids. The freshly cut leaf samples of 25 mg were dissolved in 5 ml of Dimethyl Sulphoxide (DMSO) and the tubes were incubated overnight in the dark. The resultant chlorophyll containing solution was measured at 645 nm and 663 nm by spectrophotometer. The amount of chlorophyll present in the leaves was calculated in terms of milligrams of chlorophyll per gram of leaf tissue as follows.

Chlorophyll- a (mg /g) = (12.7 × O.D. 663) – (2.69 × O.D. 645) × V / 1000 × w

Chlorophyll -b (mg /g) = (22.9 × O.D. 645) – (4.68 × O.D. 663) × V /1000 × w

Total chlorophyll = Chlorophyll 'a' + Chlorophyll 'b'

The extract thus obtained was measured at 480 nm and 510 nm for estimating Carotenoids. The amount of Carotenoids present in the acalypha leaves was expressed as of milligram of chlorophyll per gram of leaf tissue as follows,

Carotenoids in mg/g of fresh leaf tissue = 7.0 (A 480) – 1.47 (A 510) ×V ×W × 1000

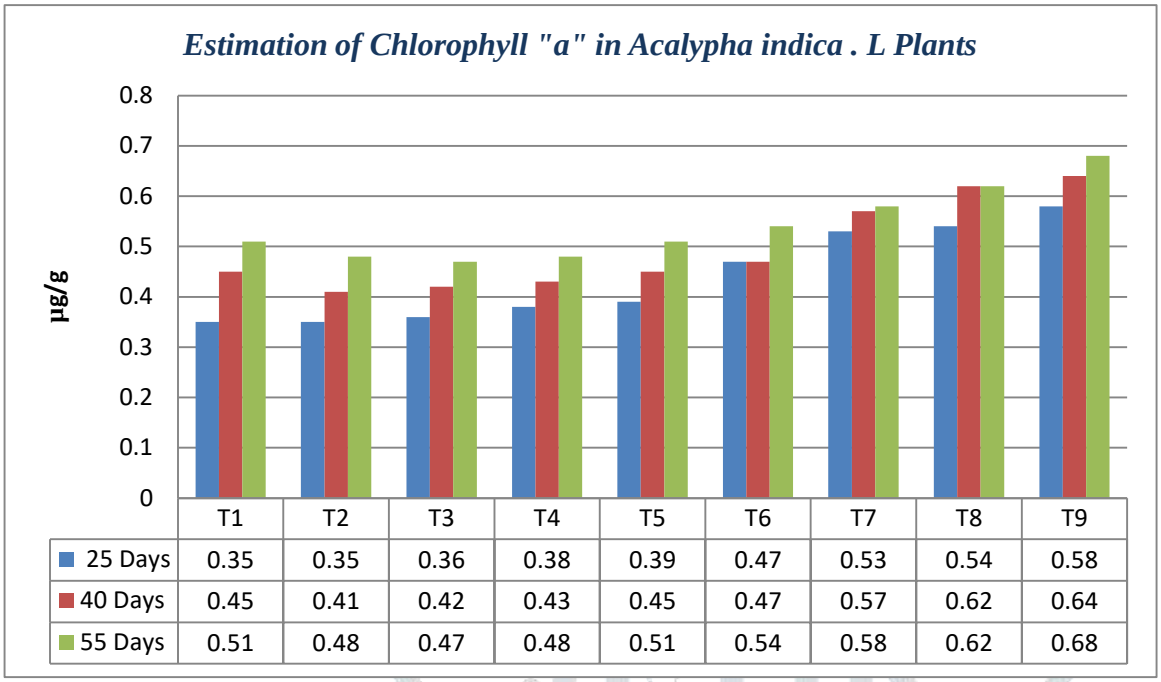
A= Absorbance of the extract at the specified wavelength V= Final volume of the solution (ml) W= Weight of the sample (g).

III. RESULTS & DISCUSION

Estimation of chlorophyll content in SA&JA treated and terrestrial plants:

The chlorophyll "a" concentration was measured in both SA&JA treated and control plants every interval of 15 days. All the alone and combination SA&JA treated plants contained high chlorophyll-a when compared to control plants. Maximum chlorophyll-a content was observed at the combination of SA&JA T9 (3mM SA + 4µM JA) (0.685µg/g) and minimum chlorophyll a contains alone combination of T2 (1.0mM JA) (0.482 µg/g) at all

stages of plant growth like 25 40 and 55days respectively (Fig.1).



	T1	T2	T3	T4	T5	T6	T7	T8	T9
25 Days	0.35	0.35	0.36	0.38	0.39	0.47	0.53	0.54	0.58
40 Days	0.45	0.41	0.42	0.43	0.45	0.47	0.57	0.62	0.64
55 Days	0.51	0.48	0.47	0.48	0.51	0.54	0.58	0.62	0.68

Fig.1. Estimation of chlorophyll "a" in SA & JA treated and control plants of *Acalypha indica*.

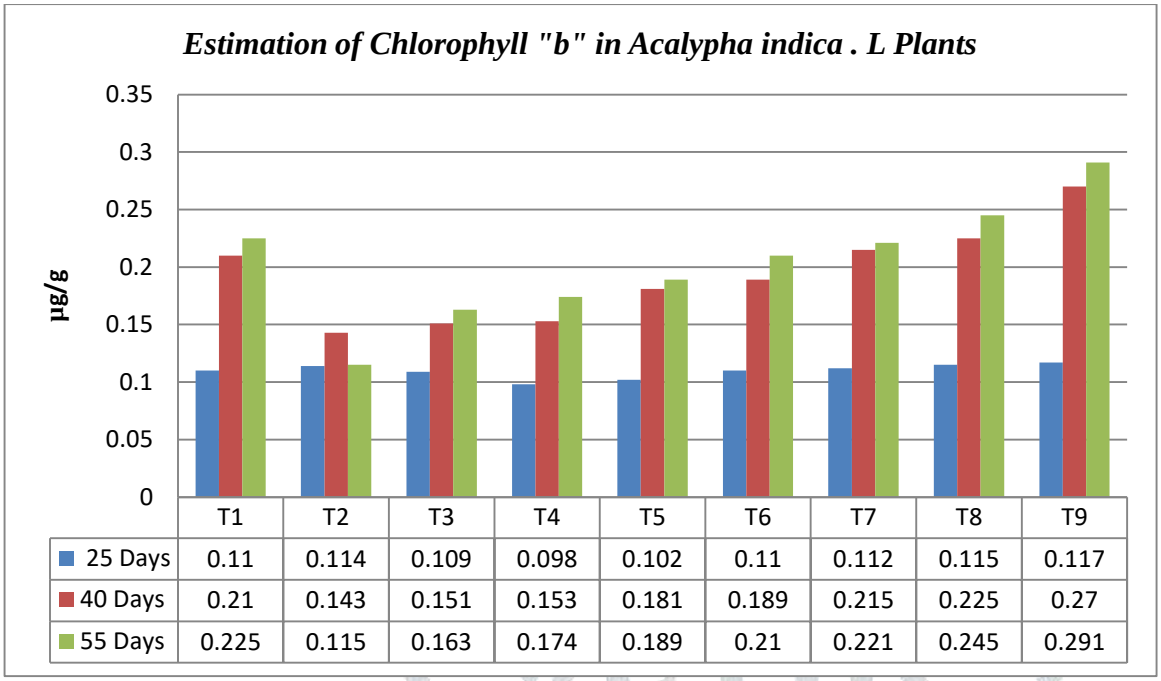
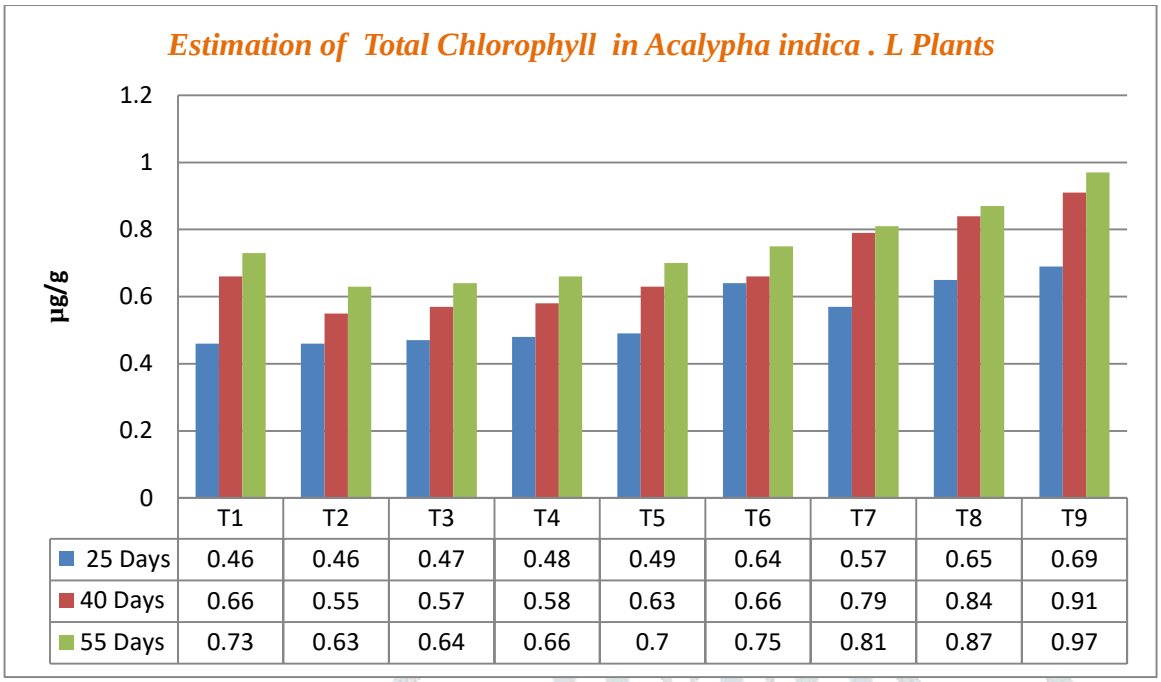


Fig.2. Estimation of chlorophyll "b" in SA & JA treated and control plants of *Acalypha indica*.L

The chlorophyll "b" concentration was measured in both SA&JA treated and control plants every interval of 15 days. All the alone and combination SA&JA treated plants contained high chlorophyll-b when compared to control plants. Maximum chlorophyll -b content was observed at the combination of SA&JA T9 (3mM SA + 4µM JA) (0.291µg/g) and minimum chlorophyll-b contains alone combination of T2 (1.0mM JA) (0.115 µg/g) at all stages of plant growth like 25, 40 and 55 days respectively (Fig.2). Alone concentrations of SA&JA treated plants T2 (0.155µg/g), T3 (0.163µg/g), T4 (0.174µg/g) and T5 (0.189µg/g) contains low chlorophyll -b, when compared to control plants (0.225 µg/g) after 55 days of growth. The combination of SA&JA treated plants with the highest chlorophyll b was observed T7 (0.221µg/g), T8 (0.245µg/g) and T9 (0.291 µg/g). Based on the hormonal concentration and combinations chlorophyll-b content was increased.

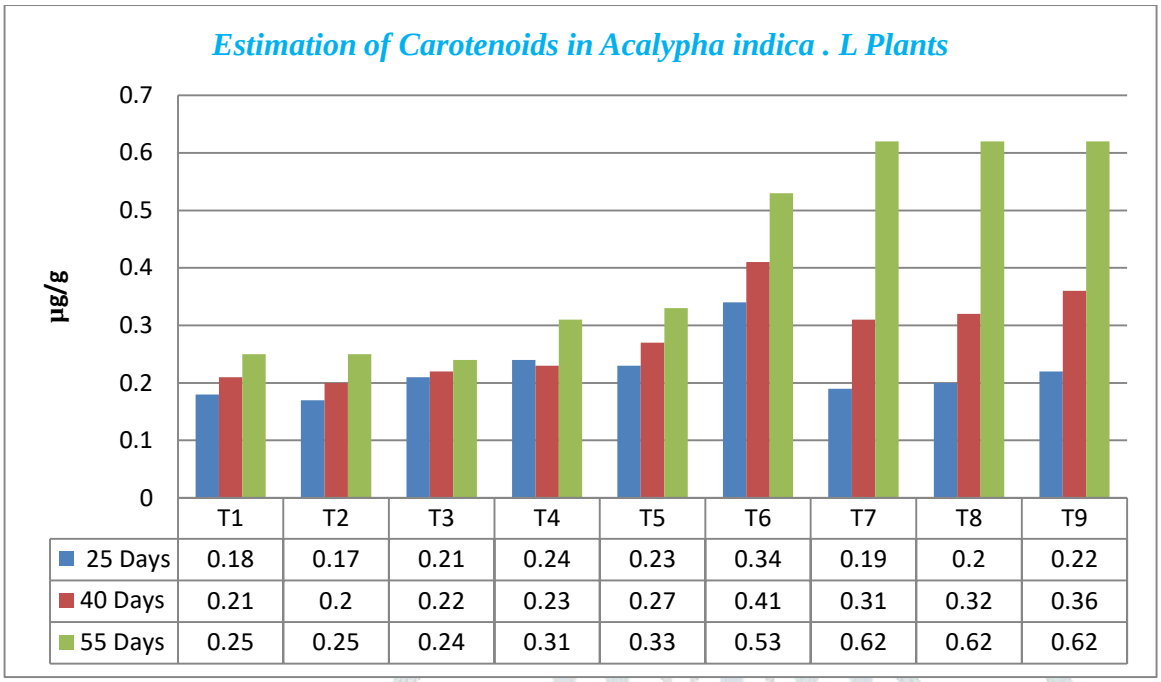


	T1	T2	T3	T4	T5	T6	T7	T8	T9
25 Days	0.46	0.46	0.47	0.48	0.49	0.64	0.57	0.65	0.69
40 Days	0.66	0.55	0.57	0.58	0.63	0.66	0.79	0.84	0.91
55 Days	0.73	0.63	0.64	0.66	0.7	0.75	0.81	0.87	0.97

Fig. 3: Estimation of total chlorophyll in SA & JA treated and control plants of *Acalypha indica*.L

Total chlorophyll concentration was measured in both SA&JA treated and control plants every interval of 15 days. All the alone and combination SA&JA treated plants contained high total chlorophyll when compared to control plants. Maximum total chlorophyll content was observed at the combination of SA&JA T9 (3mM SA + 4µM JA) (0.97µg/g) and minimum total chlorophyll contains alone combination of T2 (1.0mM JA) (0.63 µg/g) at all stages of plant growth like 25, 40 and 55 days respectively (Fig.3). Total chlorophyll concentration plays a vital role in plant growth. In the present study, we observed all the combinations of SA&JA treated plants exhibit more chlorophyll content T7 (0.81µg/g), T8 (0.87µg/g) and T9 (0.97 µg/g) including control plants T1 (0.738 µg/g) when compared to alone concentration of SA&JA treated plants (Fig.3).

Highest Carotenoids were present in combinations of SA&JA treated plants T7(0.620µg/g), T8 (0.621µg/g) and T9 (0.623µg/g) we observed all the alone concentration of SA&JA treated plants exhibit more Carotenoids content T2(0.25µg/g), T3 (0.24µg/g) and T4 (0.31 µg/g) ,T5 (0.33µg/g),T6 (0.53 µg/g) including control plants T1(0.25 µg/g).(Fig.4).



	T1	T2	T3	T4	T5	T6	T7	T8	T9
25 Days	0.18	0.17	0.21	0.24	0.23	0.34	0.19	0.2	0.22
40 Days	0.21	0.2	0.22	0.23	0.27	0.41	0.31	0.32	0.36
55 Days	0.25	0.25	0.24	0.31	0.33	0.53	0.62	0.62	0.62

Fig.4: Estimation of Carotenoids in SA & JA treated and control plants of *Acalypha indica*.L

IV.CONCLUSION

A. indica.L plants treated with different concentrations of SA & JA either individually or in combination exhibited considerable increase in physiological parameters chlorophyll-a, chlorophyll-b, total chlorophyll, Carotenoids. After 55 days of plant growth, SA & JA treated plants showed effective pigments concentrations. In which of treated plants, The T7, T8 and T9 treated plants showed enhanced Pigments compared to other treated plants. All the treated plants were more active than treated alone concentration plants. In control plants compare to the treated alone plants with the control chlorophyll-a, chlorophyll-b enhanced. The combined usage of SA & JA hormones exhibited maximum positive effective on *A. indica.L* plant growth development and physiological variations.

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