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Advantages of Exogenous Supply of Plant growth Regulators through Foliar Spray on Physio-Chemical Properties of Robusta Coffee Var. C x R during Pre Monsoon

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ABSTRACT

In a study on influence of foliar application of PGRs on physio-chemical properties of Robusta coffee during pre monsoon conducted at Coffee Research Sub Station, Chettalli results indicated that plant growth regulators were on par with each other but significantly superior to control and water sprayed plants. Higher relative water content (11.13 %), epicuticular wax (65.07 %), transpiration rate (86.93%), stomatal conductance (43.09%), Instantaneous WUE (-20 %), intrinsic WUE (1.82 %), net photosynthesis (44.32 %), internal CO_2 concentration (63.28 %), mesophyll efficiency (15.4 %), carboxylation efficiency (77 %), chlorophyll A (44.09 %), Chlorophyll B (41.45 %) and Total chlorophyll (43.33 %), Chlorophyll ratio (-0.07 %) and nitrate reductase activity (57 %) was found in PGR treated plants during pre monsoon. The increased photosynthetic activities and parameters resulted in higher total carbohydrates (46.33 %), starch (45.16 %), soluble proteins (39.45 %), phenols (53.91 %) and proline (62.62 %) contents in coffee.

Key Words: PGRs, Robusta Coffee, RWC, Net Photosynthesis, Proline, Coffee Physiology

INTRODUCTION

Coffee is grown commonly in hilly tracts of Western Ghat regions of Karnataka. Being perennial plant, it suffers either by dry condition during pre monsoon or excessive water logged conditions in monsoon. Among these two situations, pre monsoon condition is critical period during which the success of coffee crop depends on flowering. Improper and inadequate summer rainfall results in floral abnormalities in these cultivars. Irrigation is an important management practice to overcome the adverse effects of dry spells although excessive irrigation can adversely affect the yield. Early irrigation before the full maturity of flower buds can lead to floral abnormalities and running blossom which results in poor fruit set. Irrigation is an expensive operation and water resources are limited in many of the coffee estates, hence irrigating the plants at the correct time based on the needs of the plants is essential to increase the yield. Inadequate and uneven distribution of rainfall causes drought conditions in coffee, which affects vegetative growth, induces floral abnormalities, results in poor fruit set and prolonged drought after fruit set increases production of more pea berries and 'B' grade beans and ultimately loss in crop yield.

Many physiological attributes like relative water content, epicuticular wax, photosynthetic efficiency, net photosynthesis, stomatal conductance, instantaneous and intrinsic water use efficiency, carboxylation and mesophyll efficiency, chlorophyll content etc get altered during the adverse and normal climatic conditions and also many chemical compositions of plants like major nutrients, minor and micro nutrients, carbohydrates, starch, phenols, proline, soluble proteins etc vary with climatic conditions to overcome adverse climatic conditions. These can be achieved during climatic variations by external supply of plant growth regulators. Exogenous application of plant growth regulators (PGRs) found useful to improve the physiological attributes (D'Souza *et al.*,1992) in coffee besides coffee yield. The foliar application of plant growth regulators also reported to be useful in improving the crop yield, vegetative growth besides quality. proline accumulation has been extensively employed in screening genotypes for drought tolerance besides physiological efficiency. Besides proline, a range of solutes accumulate during osmotic adjustment in both fully expanded and growing tissues. The solutes include inorganic ions, soluble carbohydrates and amino acids (Munns *et al.,* 1979; Ravens *et al.,* 1979; Jones *et al.,* 1980). There are also reports that nitrate reductase activity is adversely affected by moisture stress. Sairam and Dube (1984) have observed that stability of enzyme activity during moisture stress might be associated with drought tolerance. An attempt has been made in a study to evaluate the epicuticular wax (ECW) as one of the parameters for screening the cultivars for drought tolerance in coffee. The higher content of ECW with less decrease of Relative Water Content (RWC) during soil moisture stress period in coffee plants was observed (Anonymous, 2002). Increase of epicuticular wax content (ECW) during summer month and decrease during monsoon period in both arabica and robusta plants were reported and considered that this trait could be an important trait to assess drought tolerance ability in coffee plants (Anonymous, 2005; Divya, 2008).

The role of endogenous level of hormones directly or indirectly involved in physiological processes by exogenous application of plant growth regulators invites great attention in coffee. Under the circumstances of climatic variations, coffee undergoes many physio-chemical changes to overcome the variations of climate. In this regard, external plant growth regulators play important role during pre monsoon in managing the physiological properties and chemical composition of plants to get consistent higher yields and quality. As many growth regulators used in coffee were of chemical in nature, a study on the effect of foliar spray of natural plant growth regulators in comparison with chemical PGRs on physio-chemical properties of coffee was studied.

MATERIAL AND METHODS

Treatments: The study was conducted at Coffee Research sub Station, Chettalli during 2016 to 2018 for two seasons to confirm the results. The study was taken up in 20 year old Robusta coffee variety CxR. Seven treatments were finalized as given below including the standard recommended PGR.

- T1: No spray (control)
- T2: Water spray (Control)
- T3: Plant extract (*Gliricidia sepia* & *Lantana camara*) 1%
- T4: Arka Microbial Consortium 1 % (ICAR)
- T5: *Lantana camera* + Dimethyl *Sulfoxide* (DMSO) + $CuSo_4$ 1 %
- T6: Salicylic acid 0.025 %
- T7: Alpha (α) Napthyl Acetic Acid 0.025 % (Standard Recommendation in coffee)

Plant Material: Trial was conducted using Robusta cv. C *x* R (*Coffea canephora Pierre ex froehner*)**:** This variety is being cultivated commonly in the coffee growing zones of India. It is a selected cultivar known for large bush size with robust growth. The leaves are broad and oblong. It is a semi drooping type with large number of secondary and tertiary branches. The long internodes of 5 to 7 cm with large clusters of fruits vary from 25 to 50 per cropping nodes. This variety found to have high root biomass, good water use efficiency, vigorous growth and resistance to major pest and diseases. The cultivar possesses high carbon exchange rates. The fruits are bolder in size with around 70 % `AB' grades. The fruits are reddish to dark red in color. A crop yield of 1800 to 2000 Kg/ha Clean Coffee could be expected under well cultivation practices.

Arka Microbial Consortia: Arka Microbial Consortium, developed by ICAR is a carrier based product which contains N fixing, P & Zn solubilizing and plant growth promoting microbes in a single formulation. 10 ml formulation was mixed in one liter water and used for spraying.

Glyiricidia and Lantana Extraction**:** These being found in all tropical areas of coffee growing regions are available in plenty. Hence, were used to prepare extraction. Leaves of both *Glyricidia sepia* and *Lantana camara* were collected fresh. 2 Kg leaves of both were chopped into small pieces and immersed in 10 litre of boiled water and kept for 24 hours. Then the solution was filtered using a cloth and the filtrate was mixed with 200 litres of water. The extract so prepared was sprayed to the plants covering the lower surface of the leaves.

Dimethyl Sulfoxide (DMSO): Among the more important properties of DMSO, its ability to readily penetrate biological membranes to increase the uptake of essential plant nutrients and to influence the growth habit of crops is very important. Besides, Dimethyl Sulfoxide (DMSO) is a widely used solvent for the extraction of chlorophylls from leaves of higher plants. The method is preferred because the time-consuming steps of grinding and centrifuging are not required and the extracts are stable for a long time period (Dimosthenis Nikolopoulos *et al* 2008). Hence, in this study, to increase the efficacy and solubility of Lantana camera extract (1 %), DMSO and $CuSO_4$ were used in combination with these extracts as one of the treatments.

Salicylic Acid: Salicylic acid (SA) is one of the potential plant growth regulators (PGRs) that regulate plant growth and development by triggering many physiological and metabolic processes. Being less studied chemical in coffee, this was used as one of the treatments in the present study in comparison with the standard recommendation.

αNAA - Alpha Napthyl Acetic Acid *:* This is tested and recommended as standard in coffee by CCRI (Anon, 2014) mostly used only for inducing flowering and enhancing yield of coffee. Hence, this is included as one of the treatments for standard comparison.

Treatment Imposition: In this study, in order to understand the physio-chemical changes that occur in coffee due to foliar spray of plant growth regulators, the treatments were imposed twice. Once during post blossom and other one in pre monsoon. Observations were recorded both during pre monsoon in summer

Observations and Data Analysis*:* Observations were made one month after the second spray on various physio-chemical properties using standard prescribed methodologies. The observations recorded were analyzed by Randomized Complete Block Design and significance was tested 5 % probability.

RESULTS

Relative Water Content and Epicuticular Wax: In the study of two years as shown in Table 1, PGR treated plants had significantly higher relative water content than control and water sprayed plants. The mean of two seasons indicated that *Glyricidia* + *Lantana* plants had the highest Relative Water Content of 84 % followed by 83.8 % with Microbial consortium, 83.7 % with DMSO, 83.5 % with NAA and 83.4 % with Salicylic acid which were 10.77 to 11.5 % increase over the Relative Water Content of control (75.3 %) and water sprayed plants (74.5 %). The average epicuticular wax over two years was highest with NAA (25.6 µg/Cm^2) followed by Salicylic acid (25.4 µg/Cm^2), DMSO and *Glyricidia* + *Lantana* (25.3 µg/Cm^2) and Microbial consortium (25.2 µg/Cm^2) which were 64.02 to 66.85 % higher as compared to epicuticular wax of plants in control (15.4 µg/Cm^2) and water spray (15.5 µg/Cm^2) treatments.

Table 1. Relative water content and epicuticular wax as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Relative Water Content			Variation (%)	Epicuticular Wax (µg/Cm^2)			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	82.8	67.8	75.3		14.0	16.7	15.4	
T2	81.6	67.3	74.5		13.6	17.4	15.5	
T3	87.3	80.7	84.0	**11.50**	22.4	28.2	25.3	**64.67**
T4	87.4	80.3	83.8	**11.32**	22.4	28.0	25.2	**64.02**
T5	87.4	80.1	83.7	**11.17**	22.1	28.4	25.3	**64.54**
T6	87.5	79.4	83.4	**10.77**	22.6	28.2	25.4	**65.26**
T7	87.6	79.5	83.5	**10.89**	23.2	28.0	25.6	**66.85**
F Test 5 %	55.533*	112.547*	Mean	11.13	165.719*	441.039*	Mean	65.07
CD 5%	0.839	1.41			0.813	0.635		

Transpiration Rate (E) and Stomatal Conductance (gs): Transpiration rate was found to be significantly higher in all PGR treated plants over control and water sprayed plants in both the years of study. On an average of two years, Transpiration Rate was highest with NAA (1.7 m moles m^{-2} s^{-1}) followed by Salicylic acid (1.65 m moles m^{-2} s^{-1}), Microbial consortium (1.62 m moles m^{-2} s^{-1}), *Glyricidia* + *Lantana* (1.6 m moles m^{-2} s^{-1}) and DMSO (1.52 m moles m^{-2} s^{-1}) which were 76.3 to 95.95 % increase over Transpiration Rate in control (0.87 m moles m^{-2} s^{-1}) and water sprayed (0.85 m moles m^{-2} s^{-1}) plants (Table 2). The mean Stomatal Conductance was highest 0.082 moles m^{-2} s^{-1} with Microbial consortium followed by *Glyricidia* + *Lantana* (0.079 moles m^{-2} s^{-1}), 0.078 moles m^{-2} s^{-1} Stomatal Conductance in DMSO and NAA, 0.077 moles m^{-2} s^{-1} in Salicylic acid which were 40 to 49.09 % increase over the Stomatal Conductance of 0.055 moles m^{-2} s^{-1} in control and 0.058 moles m^{-2} s^{-1} in water sprayed plants.

Table 2. Transpiration rate and stomatal conductance as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Transpiration Rate (E) (m moles m^{-2} s^{-1})			Variation (%)	Stomatal Conductance (gs) (moles m^{-2} s^{-1})			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	1.13	0.6	0.87		0.055	0.055	0.055	
T2	1.10	0.6	0.85		0.058	0.057	0.058	
T3	2.09	1.1	1.60	**84.39**	0.074	0.083	0.079	**42.73**
T4	2.14	1.1	1.62	**87.28**	0.082	0.082	0.082	**49.09**
T5	2.15	0.9	1.52	**76.30**	0.080	0.076	0.078	**41.82**
T6	2.20	1.1	1.65	**90.75**	0.079	0.075	0.077	**40.00**
T7	2.19	1.2	1.70	**95.95**	0.081	0.075	0.078	**41.82**
F Test 5 %	131.329*	44.936*	Mean	86.93	25.021*	31.412*	Mean	43.09
CD 5%	0.109	0.09			0.005	0.005		

Net Photosynthesis and Internal CO_2 Concentration: The mean Net Photosynthesis was highest 8.84 µ moles m^{-2} s^{-1} in DMSO treated plants followed by 8.77, 8.72, 8.66 and 8.65 µ moles m^{-2} s^{-1} in NAA, Microbial consortium, *Glyricidia* + *Lantana* and Salicylic acid respectively which were 43.01 to 46.24 % increase over the Net Photosynthesis of 6.05 and 6.48 µ moles m^{-2} s^{-1} in control and water treated plants respectively. Statistically and significantly higher Internal CO_2 Concentration was found in the plants of PGR treatments in both first and second year as compared to control and water sprayed plants but were on par within the treatments. The mean Internal CO_2 Concentration of two years indicated that Internal CO_2 Concentration was highest in Salicylic acid (241.1 **µl l^{-1}**), followed by Microbial consortium (238.4 **µl l^{-1}**), DMSO (238.2 **µl l^{-1}**), NAA (235.4 **µl l^{-1}**) and *Glyricidia* + *Lantana* (235 **µl l^{-1}**) which were 61.44 to 65.66 % increase over the same Internal CO_2 Concentration in control (145.5 **µl l^{-1}**) and water sprayed plants (145.4 **µl l^{-1}**) treated plants. Data is given in Table 3.

Table 3. Net photosynthesis and internal Co_2 concentration as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Net Photosynthesis (µ moles m^{-2} s^{-1})			Variation (%)	Internal CO_2 Conc **(µl l^{-1})**			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	6.53	5.56	6.05		151.1	140.0	145.5	
T2	6.17	6.78	6.48		151.1	139.8	145.4	**-**
T3	9.57	7.74	8.66	**43.18**	253.7	216.2	235.0	**61.44**
T4	9.45	7.98	8.72	**44.17**	243.1	233.8	238.4	**63.84**
T5	9.49	8.19	8.84	**46.24**	250.7	225.8	238.2	**63.70**
T6	9.48	7.81	8.65	**43.01**	256.3	225.9	241.1	**65.66**
T7	9.55	7.98	8.77	**45.00**	251.8	219.1	235.4	**61.77**
F Test 5 %	163.636*	30.372*	Mean	44.32	176.497*	63.306*	Mean	63.28
CD 5%	0.297	0.42			9.039	12.792		

Instantaneous Water Use Efficiency and Intrinsic Water Use Efficiency: From the Table 4, it is observed that **t**he mean Instantaneous Water Use Efficiency was 7.6 and 8.6 µ moles $mmol^{-1}$ in control and water sprayed treated plants respectively, which were 11.07 to 26.02 % higher compared to the Instantaneous Water Use Efficiency of DMSO (6.8 µ moles $mmol^{-1}$), Microbial consortium (6.1 µ moles $mmol^{-1}$), *Glyricidia* + *Lantana* (6.1 µ moles $mmol^{-1}$), Salicylic acid (6.0 µ moles $mmol^{-1}$) and NAA (5.6 µ moles $mmol^{-1}$),. Over a period of two years study, mean Intrinsic Water Use Efficiency was highest in DMSO (116.3 **µmol mol^{-1}**) followed by 115.6 **µmol mol^{-1}** in NAA, 114.8 **µmol mol^{-1}** in Salicylic acid, 114.2 **µmol mol^{-1}** in *Glyricidia* + *Lantana*, 108.6 **µmol mol^{-1}** in Microbial consortium treated plants which were 2.11 to 3.93 % increase over Intrinsic Water Use Efficiency of control (111.9 **µmol mol^{-1}**) and water sprayed (114.4 **µmol mol^{-1}**) plants.

Table 4. Instantaneous and intrinsic water use efficiency as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Instantaneous WUE **(IWUE)** **(µ moles $mmol^{-1}$)**			Variation (%)	Intrinsic WUE **(Pn/gs)** **(µmol mol^{-1})**			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	5.9	9.4	7.6		120.5	103.2	111.9	

T2	5.8	11.5	8.6		107.8	121.0	114.4	
T3	4.6	7.5	6.1	**-20.51**	132.4	96.1	114.2	**2.11**
T4	4.5	7.7	6.1	**-20.58**	117.8	99.4	108.6	**-2.91**
T5	4.5	9.1	6.8	**-11.07**	121.7	110.9	116.3	**3.93**
T6	4.3	7.6	6.0	**-21.82**	123.1	106.5	114.8	**2.62**
T7	4.4	6.9	5.6	**-26.02**	121.9	109.4	115.6	**3.34**
F Test 5 %	10.55*	14.205*	Mean	-20.00	3.39*	4.416*	Mean	1.82
CD 5%	0.515	1.028			9.717	9.598		

Mesophyll Efficiency and Carboxylation Efficiency: The average Mesophyll Efficiency was highest 3205.3 in Salicylic acid followed by 3133.6 in DMSO, 3115.3 in NAA, 3107.6 in *Glyricidia* + *Lantana* and 2985.9 in Microbial consortium treated plants. The control and water sprayed plants had 2694.6 and 2574.6 Mesophyll Efficiency respectively which were 10.81 to 18.95 % less over PGR treated plants (Table 5). The mean of two seasons indicated that Carboxylation efficiency was 0.0355 $\mu molm^{-2}s^{-1}(\mu l\ l^{-1})^{-1}$ in both T7 and T4 followed by 0.036 $\mu molm^{-2}s^{-1}(\mu l\ l^{-1})^{-1}$ in DMSO, 0.035 $\mu molm^{-2}s^{-1}(\mu l\ l^{-1})^{-1}$ in both *Glyricidia* + *Lantana* & Salicylic acid which were 75 to 80 % increase over Carboxylation efficiency of control (0.02 $\mu molm^{-2}s^{-1}(\mu l\ l^{-1})^{-1}$) and water sprayed (0.02 $\mu molm^{-2}s^{-1}(\mu l\ l^{-1})^{-1}$) plants. Table 16 shows information on Carboxylation efficiency.

Nitrate Reductase Activity: Nitrate Reductase Activity was statistically and significantly higher in all PGR treated plants as compared to control and water sprayed plants during the study of two years. Over the two years, average Nitrate Reductase Activity was 0.40 µmoles/Hour/g in both control and water sprayed plants which was 55 to 62.5 % less compared to Nitrate Reductase Activity of 0.65 µmoles/Hour/g in Microbial consortium, 0.63 µmoles/Hour/g in *Glyricidia* + *Lantana*, 0.62 µmoles/Hour/g in DMSO, Salicylic acid and NAA treated plants. Data on nitrate reductase activity is presented in Table 6

Table 5. Mesophyll and carboxylation efficiency as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Mesophyll Efficiency **(gm)** **Ci/gs**			Variation (%)	Carboxylation efficiency ($\mu molm^{-2}s^{-1}(\mu l\ l^{-1})^{-1}$)			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	2800.4	2588.7	2694.6		0.020	0.015	0.0200	
T2	2649.7	2499.6	2574.6		0.020	0.015	0.0175	
T3	3520.5	2694.8	3107.6	**15.33**	0.030	0.040	0.0350	**75.00**
T4	3042.6	2929.3	2985.9	**10.81**	0.030	0.041	0.0355	**77.50**
T5	3216.2	3051.1	3133.6	**16.29**	0.030	0.042	0.0360	**80.00**
T6	3317.2	3093.4	3205.3	**18.95**	0.030	0.040	0.0350	**75.00**
T7	3217.0	3013.5	3115.3	**15.61**	0.030	0.041	0.0355	**77.50**
F Test 5 %	6.136*	4.29*	Mean	15.40	47.48*	149.35*	Mean	77
CD 5%	298.758	284.712			0.002	0.002		

Table 6. Nitrate reductase activity as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Nitrate Reductase Activity (µmoles/Hour/g of Fresh wt)			Variation (%)
	Year 1	Year 2	Mean	
T1	0.35	0.45	0.40	
T2	0.36	0.44	0.40	
T3	0.49	0.77	0.63	**57.50**
T4	0.50	0.80	0.65	**62.50**
T5	0.50	0.73	0.62	**55.00**
T6	0.52	0.72	0.62	**55.00**
T7	0.51	0.72	0.62	**55.00**
F Test 5 %	27.902*	38.5*	Mean	57
CD 5%	0.035	0.061		

Chlorophyll 'a' and Chlorophyll 'b': Chlorophyll being the most important physiological factor for production, during the period of study, Chlorophyll 'a' and Chlorophyll 'b' were influenced greatly by PGR treatments (Table 7). Over the period of two years study, the average Chlorophyll 'a' was highest in Salicylic acid (2.53 mg/gram) followed by Microbial consortium (2.49 mg/gram), DMSO (2.44 mg/gram), *Glyricidia + Lantana* & NAA with 2.43 mg/gram which were 42.11 to 47.95 % increase over Chlorophyll 'a' content of control (1.71 mg/gram) and water sprayed (1.72 mg/gram) plants. Chlorophyll 'b' content of PGR treated plants was significantly high compared to that of control and water treated plants during the study of two years. On an average, Chlorophyll 'b' content was 0.99 mg/gram in all PGR treated plants except Microbial consortium (0.92 mg/gram) which was 33.33 to 43.48 % increase over control (0.69 mg/gram) and water sprayed (0.71 mg/gram) plants.

Table 7. Chlorophyll 'a' and 'b' as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Chlorophyll 'a' mg/gram			Variation (%)	Chlorophyll 'b' mg/gram			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	1.69	1.72	1.71		0.57	0.81	0.69	
T2	1.72	1.72	1.72		0.59	0.82	0.71	
T3	2.44	2.41	2.43	**42.11**	1.08	0.89	0.99	**43.48**
T4	2.51	2.47	2.49	**45.61**	0.94	0.89	0.92	**33.33**
T5	2.51	2.37	2.44	**42.69**	1.10	0.87	0.99	**43.48**
T6	2.57	2.49	2.53	**47.95**	1.08	0.90	0.99	**43.48**
T7	2.53	2.33	2.43	**42.11**	1.09	0.88	0.99	**43.48**
F Test 5 %	102.242*	82.489*	Mean	44.09	19.654*	7.215*	Mean	41.45
CD 5%	0.095	0.092			0.132	0.033		

Total Chlorophyll and Chlorophyll a/b Ratio: The mean total chlorophyll was highest in Salicylic acid (3.52), 3.43 in DMSO, 3.42 in *Glyricidia + Lantana* & NAA, 3.41 in Microbial consortium which were 42.08 to 46.67 % increase over the total chlorophyll content of control (2.4) and water sprayed (2.42) plants. Data on total chlorophyll is shown in Table 8. In case of Chlorophyll a/b Ratio, all PGR treated plants had significantly and statistically lower ratio as compared to control and water sprayed plants during the first year of the study. While the results were reversed in the second year and all PGR treated plants had significantly higher ratio compared to control and water sprayed treatments. The mean Chlorophyll a/b Ratio of two years indicated that Microbial consortium resulted in the highest 2.82 Chlorophyll a/b Ratio followed by 2.65 in Salicylic acid which were 6.82 and 0.38 % increase over control. Both in *Glyricidia + Lantana* & DMSO had 2.58 and the least 2.56 Chlorophyll a/b Ratio in NAA treated plants which were 2.27 and to 3.03 % less Chlorophyll a/b Ratio compared to control (2.64) and water treated plants (2.62). Data is shown in Table 6.19.

Table 8. Total chlorophyll and chlorophyll a/b ratio as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon

Treatments	Total Chlorophyll			Variation (%)	Chlorophyll a/b Ratio			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	2.26	2.54	2.40		3.14	2.14	2.64	
T2	2.31	2.53	2.42		3.13	2.11	2.62	
T3	3.53	3.30	3.42	**42.50**	2.42	2.73	2.58	**-2.27**
T4	3.45	3.36	3.41	**42.08**	2.84	2.79	2.82	**6.82**
T5	3.61	3.24	3.43	**42.92**	2.42	2.73	2.58	**-2.27**
T6	3.65	3.39	3.52	**46.67**	2.53	2.76	2.65	**0.38**
T7	3.62	3.21	3.42	**42.50**	2.47	2.65	2.56	**-3.03**
F Test 5 %	85.717*	69.527*		43.33	3.461*	41.833*		-0.07
CD 5%	0.166	0.111			0.428	0.114		

Total Carbohydrates and Starch Content: Over two years, mean Total Carbohydrates was 7.65 in T5, 7.63 in T7 and 7.62 in T6, 7.52 % in T4 and 7.48 in T3 which were 44.4 to 47.68 % increase over Total Carbohydrates content in control (5.18 %) and water sprayed (5.22 %) plants. Starch content of plants was high during the first year and less in second year (Table 9). But in both the years, PGR treated plants had significantly higher starch content compared to control and water sprayed plants. Over two years, mean starch content was highest 4.6 in Microbial consortium treated plants followed by 4.5 in DMSO, Salicylic acid and NAA treated plants, 4.4 % in *Glyricidia + Lantana* treated plants which were 41.94 to 48.39 % increase over control (3.1 %) and 3 % starch in water treated plants.

Table 9. Total carbohydrates and starch content as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon								
Treatments	Total Carbohydrates (%)			Variation (%)	Starch (%)			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	5.0	5.3	5.18		4.0	2.1	3.1	
T2	5.0	5.4	5.22		3.9	2.1	3.0	
T3	7.1	7.9	7.48	**44.40**	5.9	3.0	4.4	**41.94**
T4	7.0	8.1	7.52	**45.17**	6.1	3.0	4.6	**48.39**
T5	7.2	8.1	7.65	**47.68**	5.9	3.0	4.5	**45.16**
T6	7.2	8.0	7.62	**47.10**	6.1	3.0	4.5	**45.16**
T7	7.0	8.3	7.63	**47.30**	6.0	3.0	4.5	**45.16**
F Test 5 %	32.863*	111.755*	Mean	46.33	72.758*	272.921*	Mean	45.16
CD 5%	0.428	0.309			0.291	0.064		

Proline and Phenol content: Like all other factors, proline content was also statistically and significantly higher in PGR treated plants compared to control and water sprayed in both the years. The mean proline content was highest 2.16 µmoles/g with DMSO followed by 2.12 µmoles/g with *Glyricidia + Lantana* & Salicylic acid, 2.11 µmoles/g in Microbial consortium and 2.0 µmoles/g 6 with NAA which were 58.46 to 63.08 % increase over proline content of control (1.3 µmoles/g) and water sprayed (1.27) plants. Data on proline is presented in Table 10. The average phenol content of plants was highest 0.28 in *Glyricidia + Lantana* & Microbial consortium treated plants followed by 0.27 % in plants of DMSO, Salicylic acid and NAA which were 56.52 to 52.17 % increase over phenol contents of control (0.21 %) and water sprayed (0.20 %) plants. In both the years, phenol content was statistically higher compared to control and water treated plants but were on par with each other.

Soluble Protein: Soluble protein data is given in Table 11. Statistical analysis indicated that soluble protein was found significantly high in PGR treated plants compared to control and water sprayed plants but were on par among themselves in both the years of the study. The mean soluble protein was highest 27.47 mg/g in NAA treated plants followed by 27.32 mg/g in DMSO, 27.14 mg/g in *Glyricidia + Lantana*, 27.11 mg/g in Salicylic acid and 27.06 mg/g in Microbial consortium which were 38.63 to 40.73 % increase over soluble protein of plants under control (19.52 mg/g) and water spray (19.34 mg/g) treatments.

Table 10. Proline and Phenol contents as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon								
Treatments	Proline (µmoles/g of Dry Wt)			Variation (%)	Phenol (%)			Variation (%)
	Year 1	Year 2	Mean		Year 1	Year 2	Mean	
T1	1.20	1.39	1.30		0.21	0.25	0.23	
T2	1.17	1.36	1.27		0.20	0.25	0.23	
T3	2.01	2.22	2.12	**63.08**	0.28	0.42	0.35	**52.17**
T4	1.97	2.25	2.11	**62.31**	0.28	0.43	0.36	**56.52**
T5	2.07	2.24	2.16	**66.15**	0.27	0.44	0.36	**56.52**
T6	2.07	2.17	2.12	**63.08**	0.27	0.43	0.35	**52.17**
T7	1.94	2.17	2.06	**58.46**	0.27	0.42	0.35	**52.17**
F Test 5 %	94.504*	115.491*	Mean	62.62	49.132*	55.304*	Mean	53.91
CD 5%	0.102	0.094			0.012	0.028		

Table 11. Soluble protein as affected by Growth Regulators in yielding plants of Robusta Variety CxR in pre monsoon				
Treatments	Soluble Protein (mg/g Fresh Wt)			Variation (%)
	Year 1	Year 2	Mean	
T1	18.0	21.0	19.52	
T2	17.8	20.9	19.34	
T3	31.0	23.3	27.14	**39.04**
T4	30.9	23.2	27.06	**38.63**
T5	31.0	23.6	27.32	**39.96**
T6	30.9	23.3	27.11	**38.88**
T7	31.1	23.8	27.47	**40.73**

F Test 5 %	2696.7*	79.715*	Mean	39.45
CD 5%	0.302	0.342		

DISCUSSION AND CONCLUSION

From the results it can be seen that all plant growth regulators enhanced most of the physiological and chemical properties of Robusta in pre-monsoon significantly compared to control and water sprayed plants. And all the PGRs influenced almost on par with each other, where as both control and water sprayed plants had almost equal or on par physiological properties significantly lower to PGRs. Lower relative water content in control and water sprayed plants (11.13% lower) could be attributed to lower epicuticular wax (65.07 % less) as compared to PGR treated plants. The higher relative water content in PGR treated plants could be attributed to higher transpiration rate (86.93 % higher). Because of higher transpiration rate, PGR treated plants had extracted more water from roots and thereby resulted in higher turgidity of leaves. Higher internal CO_2 concentration (63.28 % higher) in PGR treated plants could be attributed to higher stomatal conductance (43.09 % high), which enhanced the exchange of gases across the atmosphere and leaf tissue. This in turn resulted in higher net photosynthesis in PGR treated plants (44.32 % high) compared to control and water sprayed plants. The increased CO_2 concentration has lead to increased mesophyll efficiency (15.4 %) and carboxylation efficiency (77 %) in PGR treated plants.

Higher net photosynthesis could be attributed to increased chlorophyll ‘a’ (44.09 %) and ‘b’ (41.45 %) and total chlorophyll (43.33 % high) contents that were resulted in PGR treated plants as compared to control and water sprayed plants. There was an increase of 57 % increase in nitrate reductase activity in PGR treated plants. The increased photosynthetic parameters in turn resulted in increased total carbohydrates (46.33 %) and starch (45.16 %). As indicator of plant resistance, in pre monsoon, increased phenol (53.91 %) and soluble proteins (39.45 %). As an indicator to stress increased proline (62.62 %) was also found in PGR treated plants compared to control and water sprayed plants. Mallikarjun *et al.*,.(2000) reported maintenance of higher physiological water use efficiency and carboxylation efficiency in drought tolerant cultivars at water stress conditions Studies conducted for two seasons using thirteen coffee genotypes indicated significantly higher net photosynthesis (pn) in Sln. 9, Sln. 1 R and Sln. 12 compared to rest of the cultivars. Under stressful conditions, these phenolics are drastically accumulated in the plant for survival (Lattanzio, 2013; Sharma et al., 2019). Phenolic compounds, namely esters, flavonoids, lignin, and tannins, act as antioxidants and fight against these abiotic stress conditions in the plant cells (Selmar, 2008). It was inferred in a study that proline accumulation is one of the major mechanisms of drought tolerance in plants (Rao et al., 2015; Yadav et al., 2019). The results can be supported by the earlier work of D Souza *et al.*, (2009) on biochemical and physiological changes in drought conditions which indicated that Soluble protein, proline and epicuticular wax increase significantly during drought and all other gas exchange parameters such as net photosynthesis, stomatal conductance, transpiration rate, carboxylation efficiency and instantaneous water use efficiency reduced significantly during the drought and returned to normal after relieving from stress. Chandragiri was found moderately drought tolerant compared to Sln. 9.

Overall study on use of plant growth regulators indicated that foliar application of plant growth regulators trigger the production of stress hormones and chemicals in coffee plants to mitigate drought situations in pre monsoon season resulting in better performance. Application of Glyricidia+Lantana, Lantana+DMSO, Microbial consortium, Salicylic acid and NAA had equal effects to manage physio-chemical properties of Robusta coffee in pre monsoon conditions.

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