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Exogenous Foliar Application of Phenolic Acids on Quality Constituents of Tea

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Abstract: Exogenous foliar application of phenolic acid could alter the biochemical composition and enhance the antioxidant potential there by protect the living organisms from oxidative injuries. Though the reports on foliar application of phenolics in relation to stress are available their role on quality constituents are lacking in crop plants and in particular, the beverage crop, tea. A phenolic acids like salicylic acid (SA), cinnamic acid (CA) and p-coumaric acid (PCA) were applied individually at various concentrations (100, 250 and 500 ppm) in order to document the changes in the quality constituents of tea crop. When compared to control all the treatments, significantly increased the contents of green leaf polyphenols and flavanoids and corresponding enzymes under field conditions.
Keywords: Phenolic acid, Antioxidant, Catechin

I. INTRODUCTION

Recent studies have demonstrated that tea is more than a mere stimulant beverage because of its medicinal properties particularly the polyphenols present in higher levels [1]. Quality of the made tea depends on the tender crop shoots with exceptional biochemical make up which enriched with polyphenolic constituents [2]. Polyphenolic compounds are a large group of secondary metabolites widely distributed in plants which can be divided into two major subgroups, flavonols and phenolic acids [3]. Tea contains major proportion of polyphenolic compounds which accounts to 30% on dry matter basis. Two third of polyphenols are flavanols, commonly known as catechins. Higher amount of catechins in tea leaves imparts characteristic astringency, bitterness and impart quality to made tea [4]. Primarily, catechins are comprised of several fractions like simple catechin (+C), (-) epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC), and (-) epigallocatechin gallate (EGCG) [5]. Salicylic acid (SA) protects the plants from soil moisture stress. There are reports available performing to the impact of SA on Brassica nigra [6], Pisum sativum [7] and Arabidopsis [8], [9] in relation to drought. SA has a direct physiological effect through the alteration of antioxidant enzyme activities [10], [11] . And hence exogenous application of SA can improved the yield and yield related traits[12],[13][14]. It has been reported that salicylic acid induced systemic acquired resistance (SAR) in controlling blister blight disease of tea[15]. Potential role for cinnamic acid (CA) as a regulator of the expression of phenylpropanoid biosynthetic genes. It should be noted that the induction of a transferase involved in the formation of chlorogenic acid appears to be mediated by increases in endogenous CA pools [16]. The present study was designed to document the alterations in biochemical constituents as influenced by varying levels of exogenous application of phenolic acids and results are discussed.

II. MATERIALS AND METHOD

A. Experimental Design

A randomised block design experiment was conducted during 2010 (January – May coinciding soil moisture stress) at UPASI Tea Research Experimental Farm Valparai Talk, 1050 meters above MSL. Tea bushes of three years from pruning representing, high yielding and moderate tea clone, UPASI-8 was selected for the study. Randomized block design experiment was conducted with ten treatments and replicated four times. Each experimental plot was consisted by 40 bushes (~40 m^2). Three different phenolics (SA, CA and PCA) foliar applied at three different concentrations (100,250 and 500 ppm) besides the untreated control. All the chemicals were purchased from M/&. Merck, Mumbai, India. Foliar application was executed, individually, a day after plucking. Required quantity of chemicals were dissolved in water, and applied on the foliage using with hand operated knapsack sprayer at spray volume of 200 L/ha. During the whole experimental period, four rounds of foliar applications were carried out at monthly interval. After second round of foliar application crop shoots from each replicate were collected regularly and subjected to biochemical quantification. All the cultural operators were carried out as per the recommendations of[17]. Foliar application of NK was not carried out in the experimental block during the experimental period which imparts drought tolerance [18].

B. Quantification of Biochemical Constituents and Enzyme Assay

For all the biochemical quantification/ enzyme assays freshly collected crop shoots were used except for catechin fractions. Quantification of total polyphenols, catechins and amino acid was carried out adopting the methods reported by [19], [20] [21], respectively. Results were expressed as per cent dry matter recovery. Catechin fractions were quantified using the HPLC adopting the ISO procedure[22] and computation of data was carried out according to [23] and catechin index was worked out according to [24]. Peroxidase activity was determined using pyrogallol as substrate and expressed as unit mg^{-1} protein[25]. Polyphenol oxidase was assayed was following the method described by [26] and expressed as U/mg protein while phenyl alanine ammonia lyase was documented as per the method reported by [27] One unit of enzyme activity is expressed as µmole of cinnamic acid formed per minute per mg protein. Protein was quantified spectrophotometrically with bovine serum albumin as a standard [28].

C. Statistical Analysis

Data generated were subjected to statistical analysis wherever possible and presented with standard error mean, critical difference and co-efficient of variation.

III. RESULTS AND DISCUSSION

A. Biochemical Constituents in Green Shoots

When compared to the control, polyphenol content significantly increased in SA application at 250 ppm concentration. However concentration (100 ppm) of SA enhanced polyphenol content marginally while 500 ppm inhibited polyphenol accumulation. There was no significant difference observed with foliar application of CA and PCA. Catechin content was significantly increased in response to foliar application of phenolics, except CA 100 ppm. There was a definite trend in enhancement of catechins: where it attained its peak against 250 ppm phenolic application and declined at 500 ppm. Among the phenolics, SA and CA at 250 ppm exhibited significantly higher values of catechin then that of PCA. Amino acids have been reported as the primary factor in the taste of green tea, which is responsible for colour and aroma of black tea brew [29]. When compared with tremendous increase in amino acids was observed with increasing concentrations of foliar applied phenolics.

B. Relative Distribution of Flavanols

Earlier studies demonstrated that catechin levels could be related to black tea quality. Total catechins are directly related with individual flavanols. Individual catechins are grouped as gallated and non- gallated catechin and or trihydroxylated and dihydroxylated catechins on the basis of chemical structure. SA-250 ppm recorded maximum quantum of flavanols followed by CA-250 ppm and PCA-500 ppm (Table2). The catechin index varied significantly in accordance with relative distribution of catechin fractions. Among the flavanols, EGCG accounted the higher amount followed by EGC/ ECG and EC/ (+) catechins, irrespective of the total application of phenolic acids and their varying degree of concentration. Even though total catechin content was higher in SA-250 ppm the values of catechin index was highest in PCA-250 ppm followed by PCA-100 ppm and SA-250 ppm. Computation analysis of various forms of catechins exhibited differential pattern of their distribution in response to foliar application of phenolic acids (Table 3).It is interesting note that GA (sum of EGCG and ECG) was higher in SA treated samples when compared to that CA and PCA. Similar trend was noticed with respect to THG (sum of EGC and EGCG) as well. NG (sum of EGC,+CAT and EC) and DHC (sum of +CAT, EC and ECG) revealed almost equal values but when compared with untreated control the values are higher. Interestingly GA/ NG and THC/ DHC ratio values are higher in the case of untreated control. While the foliar application of phenolic acids fited the composition of catechin fractions by the GA/ NG and THC/ DHC values. SA-2 treatment registered higher values of (15.33 %) of gallated catechins followed by PCA-250 ppm (15.09%) and CA-500 ppm (14.80%). Both the gallated and trihydroxylated catechins accounted greater than 13% each while non-gallated and dihydroxylated catechins registered around 3 to 4% each, individually. Results indicated that the individual fraction improved by foliar application of phenolic acids which played magnificent role in catechin conformation.

C. Quality Related Enzyme

Tea polyphenols are responsible for the astringency and briskness of tea brew. During the processing, the polyphenols undergo enzymatic oxidation and transformed into theaflavins and thearubigins which contributes to the quality of black tea brew [24]. PPO and POX is the major enzyme involved in tea oxidation of polyphenols, it catalyses the initial reaction during oxidation. Orthodiphenols are oxidised to form their corresponding quinones which are then spontaneously transformed to more complex oxidation products. In the context of part her well of processing of tea leaves, enzymes gained important role. Both SA and CA at

optima concentration of 250 ppm enhanced PPO, POX and Pal activity (Table 4). Lower concentrations of SA and CA increased all the three oxidative enzymes, however, higher concentrations showed a sharp decline. PAL plays a vital role in the biosynthesis of flavanols, the prime substrate for polyphenol oxidase and is intrinsically involved with tea quality [30]. Foliar studies have shown an increase in endogenous antioxidant enzyme levels, quality and yield as a result of exogenous application of SA [31],[32],[33].On the basis of the results obtained in the present study that foliar application of phenolic acids improves the quality constituents of crop shoots. Results indicated that SA 250 ppm, CA 250 ppm and PCA 100 ppm significantly improves the quality constituents of crop shoots as for as enzyme activities are concerned. Among the three phenolics tried, PCA accelerated PAL activity at higher rate than thee other two (SA and CA). In general the enzyme activity ranged in the following order. PAL> PPO> POX. On the other hand PCA declined the antioxidant enzymes rapidly. Concentration of foliar applied phenolics had a negative relationship with PPO, POX and PAL. However, the enzymatic activity in response to foliar application was significantly enhanced when compared to the treated control.

Computational values on substrate to enzyme ratio demonstrated antioxant enzymes level enhanced then that of substrate (Table 5). On the other hand flavonols and PPO ratio showed different trend where foliar application of phenolics enhanced the devisable and medically valuable catechin content rather then total polyphenols. Polyphenols to Pox and polyphenols to PAL ratios severs also similar trend but influence of PCA application was significant in the case of CAT: PAL. Computational improvisation revealed that foliar application of phenolic acids enhanced the overall quality attributes and specifically the flavanols.

IV. CONCLUSION

The study demonstrated that foliar application of phenolic acids not only impart the drought / stress tolerance. They also involved in metabolic activities there by enhancing the quality attributes in tea. As far so studied report is available on quality enhancement in response to phenolic acid applications. This results of the study opened new avenue for extensive studies.

V. ACKNOWLEDGEMENT

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Table 1 Biochemical constituents in green shoots

	Polyphenol (%)	Catechin(%)	Aminoacid (%)
Control	31.83	17.21	3.75
SA- 1	32.33	19.50	5.59
SA-2	32.58	20.88	5.71
SA- 3	32.22	20.05	6.36
CA -1	32.14	17.60	5.67
CA –2	32.36	20.77	5.60
CA –3	32.06	19.78	5.96
PCA –1	31.76	19.86	4.55
PCA – 2	31.75	19.95	5.37
PCA –3	31.78	20.09	6.01
CD at p 0.05	0.50	0.44	0.31
CV	1.05	1.26	4.12
SE	0.29	0.21	0.15
SA- Salicylic acid , CA- Cinnamic acid , PCA – p- Coumaric acid 1- 100 ppm, 2- 250 ppm, 3- 500 ppm			

Table 2 Relative distribution of various forms of catechin (% dry weight)

	EGC	CAT	EC	EGCG	ECG	Catechin Index
Control	7.15	4.13	5.34	75.77	7.60	0.18
SA-1	7.83	5.44	5.49	73.47	7.77	0.18
SA-2	6.99	5.68	7.67	71.13	8.53	0.23
SA-3	8.48	5.42	6.64	71.13	8.32	0.21
CA-1	8.61	4.15	8.39	71.56	7.29	0.22
CA-2	7.72	6.10	6.19	67.85	8.04	0.20
CA-3	9.22	9.38	7.45	71.78	8.52	0.24
PCA-1	7.87	3.99	10.34	70.02	7.78	0.26
PCA-2	8.54	3.39	10.01	68.55	9.51	0.28
PCA-3	8.86	7.70	4.55	69.56	9.33	0.20
SA- Salicylic acid , CA- Cinnamic acid , PCA – p- Coumaric acid 100 ppm, 2- 250 ppm, 3- 500 ppm. EGC, (-)-epigallocatechin; CAT, (+)-catechin; EGCG, (-)-Epigallocatechin gallate; EC, (-)epicatechin; ECG, (-)epicatechin gallate; CI- Catechin index.						

Table 3 Distribution pattern of flavanols groups (% dry weight)

	GA	**NG**	**THC**	**DHC**	**GA/ NG**	**THC/DHC**
Control	14.32	2.86	14.24	2.93	5.01	4.86
SA-1	15.03	3.47	15.04	3.46	4.33	4.35
SA-2	15.33	3.91	15.03	4.21	3.92	3.57
SA-3	15.05	3.89	15.08	3.86	3.87	3.91
CA-1	14.33	3.84	14.57	3.60	3.73	4.04
CA-2	14.54	3.84	14.48	3.90	3.79	3.72
CA-3	14.80	4.80	14.93	4.67	3.08	3.20
PCA-1	14.74	4.20	14.76	4.19	3.51	3.52
PCA-2	14.82	4.16	14.63	4.35	3.56	3.36
PCA-3	15.09	4.04	15.00	4.13	3.74	3.63
GA- gallated, NG- non gallated, THC- trihydroxy catechin, DHC- dihydroxy catechin						

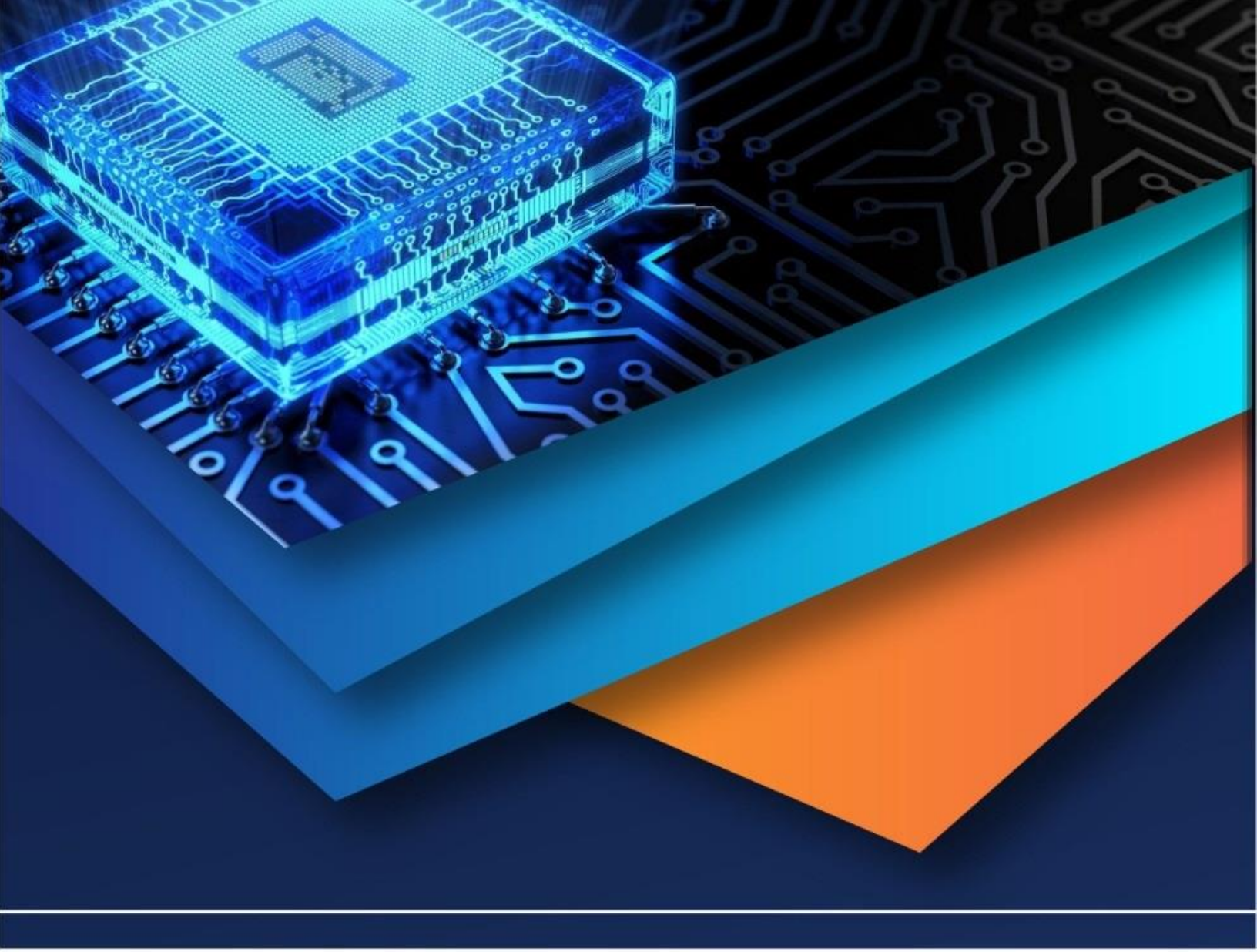
Table 4 Quality related enzymes

	POX(µ moles product formed /mg protein)	PPO (U/ mg protein)	PAL (µ mole cinnamic acid formed / mg protein)
Control	58.31	527.36	0.670
SA-1	70.21	560.88	0.908
SA-2	71.47	586.33	1.067
SA-3	69.55	558.62	0.682
CA -1	63.58	549.75	0.674
CA -2	79.45	564.46	0.804
CA -3	78.92	563.16	0.703
PCA -1	81.43	588.37	1.446

PCA -2	61.63	555.04	1.337
PCA -3	58.70	547.02	1.170
CD at p 0.05	2.32	16.45	0.01
CV	1.95	1.71	0.76
SE	1.11	7.83	0.006
SA- Salicylic acid , CA- Cinnamic acid , PCA – p- Coumaric acid 1- 100 ppm, 2- 250 ppm, 3- 500 ppm			

Table 5 Enzyme substrate ratio

	PP;PPO	CAT:PPO	PP:POX	CAT:Pox	PP:PAL	CAT:PAL
Control	0.060	0.033	0.546	0.295	47.53	25.69
SA-1	0.057	0.035	0.458	0.278	35.41	21.48
SA-2	0.056	0.036	0.468	0.300	30.54	19.57
SA-3	0.051	0.036	0.396	0.280	41.52	29.39
CA -1	0.058	0.032	0.506	0.277	47.71	26.13
CA -2	0.057	0.037	0.407	0.261	40.25	25.83
CA -3	0.057	0.035	0.406	0.251	45.61	28.14
PCA -1	0.054	0.034	0.390	0.244	21.97	13.74
PCA -2	0.057	0.036	0.515	0.324	23.75	14.92
PCA -3	0.058	0.037	0.541	0.342	27.17	17.17
SA- Salicylic acid , CA- Cinnamic acid , PCA – p- Coumaric acid 1- 100 ppm, 2- 250 ppm, 3- 500 ppm						



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