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Role of Biologically Active Amino Acid Formulations on Quality and Crop Productivity of Tea (*Camellia* sp.)

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Abstract: Present study deals with foliar application of active amino acids based commercial formulations with particular reference to physiological attributes and their synergism with crop productivity. Application of Aminolforte resulted in momentous improvement in stomatal conductance and SPAD values while it reduced the diffusion resistance. Identical results were obtained in Humiforte 20, Fosnutren 20R and Kadostim 20 with varying degree of responses. Biochemical analysis revealed a significant increase in the content of total polyphenols and amino acids due to application of increasing concentrations of Aminolforte 20. Even though catechins increased marginally with application of Fosnutren 20R, positive improvement in the amino acid content was also noticed. Quality attributes like theaflavins, thearubigins, total liquor colour, colour index, briskness index and caffeine of made tea samples had a significant improvement in response to the foliar application. Significant improvement in productivity, physiological attributes, biochemical constituents, quality parameters of the tea was evident with reference to the foliar application of bioformulations. Responses of the individual active amino acid formulations are presented and discussed in detail.

Key words: Amino acid formulations, *Camellia*, foliar application, physiological attributes, tea

INTRODUCTION

Growth is a multifactor dependant rhythmic phenomenon. Besides the availability of nutrients, plants require a minute quantum of growth regulators for their growth and development and these are available/synthesized endogenously in the plant system. Under favourable conditions, the levels of endogenous growth promoting substances are higher than that of inhibiting substances which indeed, accelerate growth and development of the plants (Gensheng, 1991). At certain circumstances, levels of inhibitors increase thereby tilt the promoter: inhibitor ratio which arrests the plant growth or the plant enters in to a dormant stage (Kumar and Manivel, 1998) leading to crop loss. Under these circumstances, foliar applications of growth regulators are needed to tilt the ratio towards promoters and to enhance the growth and development in crop plants. All the regulators available in the market are either synthetic compounds or natural products extracted from plants, microbes, seaweeds, etc. Mostly they are applied as foliar spray although soil applications of granules are also suggested in certain cases. Commonly applied growth promoters are amino acid

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formulations, mixtures of nutrients, hydrolyzed proteins, triacontanol, humic acids, sea weed extracts and brasinolides (Mandal *et al*., 2007). Khan *et al*. (2002) reported foliar application of chitosan for enhanced physiological efficiency of plants. Mustafa *et al*. (2009) studied the effect of Plant Growth Regulators (PGR) for improvement of ornamental plants. There are several reports on the effect of growth promoters on crop plants with particular reference to increased vegetative growth characters, yield and quality (Hampt *et al*., 1994). The comparative effects of GA_3, KN, BA, ethylene, 24-epibrassinolide, triacontanol and polyamines alone or in combinations, on germination and early seedling growth under high temperature conditions of barley and radish seeds were studied by Kursat and Kabar (2007). The influence of gibberellic acid and kinetin in alleviating salinity stress of *Allenrolfea occidenta* was reported by Bilquees and Khan (2008).

Tea is a foliage crop where shoots are harvested periodically and hence stress is imposed physically and physiologically. As a result, plants undergo dormancy, a physiological malfunctioning that will result in the temporary cessation of growth. During this period, tea plants produce more number of dormant buds called banji to evade the stress. In recent years, implementation of shears for harvesting has forced the tea plants to produce dormant buds rapidly for a longer generation. Under favourable conditions, dormant bud resume its growth and unfold six to nine leaves in a series before enter into second phase of dormancy if the shoots are plucked properly (Ramaswamy, 1986). Under continuous shear harvesting system, redemption of axillary bud growth ended up in a dormant one, even before attaining a two leaves stage.

Earlier reports on commercial PGR formulations based on triacontanol, sea weed extracts and complex of amino acids revealed that foliar application of these chemicals enhanced the productivity and reduced the banji content in the harvest without affecting the quality of final produce (Mandal *et al*., 2007). Similar studies were carried out in cotton to increase yield (Biles and Cothren, 2001). Even though the applications of all generation of plant growth promoters were reported in tea, no attempts were made to document the bio-efficay of active amino acid formulations. The present investigation was aimed to document the influence of foliar applied active formulations of amino acids on growth, physiology and biochemical make up of green leaves and made tea.

MATERIALS AND METHODS

Materials

Experiments were carried out in randomized block using the clone, UPASI-9 during the agricultural year, 2007. Each experimental block contained forty bushes, replicated three times and the data were statistically analyzed. Four active bioformulations of amino acid mixtures (Humiforte 20, Kadostim 20, Fosnutren 20R and Aminolforte 20) supplied by M/s. Inagrosa Industries Agro Biologicals, Madrid, Spain were used and the details of the formulations are furnished in the Table 1.

All the agricultural practices were carried out in the field adopting the recommendations of Muraleedharan *et al*. (2007). Foliar applications of nutrients were not carried out in the

Table 1: Details of the formulations used in the study

Brand name	Active ingredient	Formulation type
Humiforte 20	Amino acids and oligopeptides	Bio complex providing NPK
Kadostim 20	Amino acids mixture	Potassium leaf nutrient complex
Fosnutren 20R	Amino acids and oligopeptides	Active amino acids
Aminolforte 20	Amino acids and oligopeptides	Synthesized nutrients

experimental block during the period of study. Pretreatment yield of the experiment at blocks was monitored for three months before the start of the experiment. Two foliar applications of the formulations were carried out during the months April/May and September/October coinciding with the high cropping seasons. The PGR was mixed with running stream water, filtered and applied on the foliage using hand operated knapsack sprayer with a spray volume of 200 L ha^{-1}. Foliar application of PGRs was carried out one day after harvest and unsprayed plots served as control.

Details of Study

Harvesting of the crop was carried out at 15 to 18 days interval using hand held shears. Yield and yield attributes, particularly the percent banji in the harvest, were monitored at every plucking round, for a period of one year. Productivity Index (PI) in response to foliar applied growth promoters was calculated according to Sharma and Satyanarayana (1990) where PI = Yield of the foliar applied plot/mean yield of the field (unit area). Physiological components such as stomatal conductance (Gs) was monitored with steady state porometer (Delta T, Li-COR, USA) and diffusion resistance (r) and total chlorophyll were computed from Gs and SPAD values, respectively. Gs of the fully expanded leaves representing each treatment with replicates were measured at photosynthetically active radiation with intensity of 900-950 μmol/m^2/sec.

Green Leaves and Made Tea Analyses for Biochemical and Quality Constituents

Green leaf constituents like polyphenols (Dev Choudhary and Goswami, 1983), total catechins (Swain and Hillis, 1959) and total free amino acids (Moore and Stein, 1948) were analyzed with the samples collected during the 2nd harvest after 2nd spray in each high cropping seasons (May and October/November). Black CTC tea was manufactured with crop shoots comprising of three leaves and a bud or soft banji's collected during first plucking after second and fourth spray to study the quality attributes. Quality attributes such as Theaflavins (TF), Thearubigins (TR), Total Liquour Colour (TLC), Highly Polymerized Substances (HPS), Caffeine, Water Extract (WE), Colour Index (CI) and Briskness Index (BI) were studied adopting the standard procedures of Thanaraj and Seshadari (1990) and Ramaswamy (1986). A portion of made tea samples were sent to professional tea tasters' for organoleptic evaluation (Infusion, Colour, Strength and Briskness); which relates with market value realization of the produce.

Statistical Analysis

Data obtained in all aspects were subjected to Analysis of Variance (ANOVA) and mean values of the replicates were presented with critical difference (CD) at five per cent probability and co-efficient of variations (CV in percent) wherever applicable (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Yield and Yield Attributes

Marginal yield improvement was noticed when Aminolforte 20 applied at 300 mL ha^{-1} while no prominent difference in reduction of banji shoots was observed due to foliar application of Aminolforte 20 (Table 2). Significant improvement in yield was evident in response to foliar application of Humiforte 20 at 150, 200 and 300 mL ha^{-1} with a concentration dependent decrease in the percentage of banji shoots in the harvest. Yield

Table 2: Yield and yield attributes in response to foliar application of active amino acid formulations

Treatments	Yield, kg made tea ha^{-1} (Apr-Dec'07)	PI*	Banji content (%)
CA1. Untreated control	2745.00	0.99	79.00
A1. Aminolforte 20 (150 mL ha^{-1})	2614.00	0.95	81.00
A2. Aminolforte 20 (200 mL ha^{-1})	2646.00	0.96	77.00
A3. Aminolforte 20 (300 mL ha^{-1})	2634.00	0.96	73.00
CD at p = 0.05	160.00		5.75
CV (%)	18.43		10.49
CF1. Untreated control	3006.00	0.95	75.00
F1. Fosnutren 20R (150 mL ha^{-1})	2851.00	0.90	54.00
F2. Fosnutren 20R (200 mL ha^{-1})	2929.00	0.92	60.00
F3. Fosnutren 20R (300 mL ha^{-1})	3598.00	1.13	59.00
CD at p = 0.05	162.00		6.89
CV (%)	14.60		14.50
CH1. Untreated control	2847.00	0.88	75.00
H1. Humiforte 20 (150 mL ha^{-1})	3115.00	0.96	56.00
H2. Humiforte 20 (200 mL ha^{-1})	3173.00	0.98	55.00
H3. Humiforte 20 (300 mL ha^{-1})	3921.00	1.21	53.00
CD at p = 0.05	177.00		3.42
CV (%)	15.17		7.32
CK1. Untreated control	2747.00	0.85	80.00
K1. Kadostim 20 (150 mL ha^{-1})	2958.00	0.92	67.00
K2. Kadostim 20 (200 mL ha^{-1})	3038.00	0.94	64.00
K3. Kadostim 20 (300 mL ha^{-1})	3679.00	1.14	59.00
CD at p = 0.05	201.00		2.88
CV (%)	18.23		5.74

*PI: Productivity index, CD: Critical difference at 5% probability, CV: Coefficient of variations

improvement was noticed in response to foliar application of Fosnutren 20R only at 300 mL ha^{-1}. However, reduction in banji shoots was observed even at 150 and 200 mL ha^{-1} treatments when compared to untreated control. Foliar application of Kadostim 20 at 150, 200 and 300 mL ha^{-1} contributed to improvement in yield and significant reduction in banji content when compared to control. Phenomenal increase in yield with significantly lower proportion of banji content was evident when Kadostim 20 was applied at 300 mL ha^{-1}. Out of the four amino acid formulations tested, application of Aminolforte 20Resulted in low values of productivity index compared to that of untreated control. Other formulations showed concentration dependant increase in PI when compared to control (Table 2). There are several reports available on phytomass productivity in tea due to foliar application of PGRs (Manivel *et al*., 1994; Kumar *et al*., 1999; Mandal *et al*., 2007). However, all these reports showed a yield improvement of less than 19%. Whereas, the present study documented higher improvement in yield when compared to untreated control. The present data on PI positively correlated with the yield of tea plants. Earlier studies reported by Asad *et al*. (2002), Fraser and Percival (2003), Sabirov *et al*. (2003) and Yildirim (2007) related the effect of foliar application of nutrients/biostimulants on productivity, quality and growth (vigor) of vegetables, tree species and fodder crops.

Physiological Components

Foliar application of active amino acid formulations significantly enhanced the physiological attributes of tea (Table 3). Application of Aminolforte resulted in improvement in the values of stomatal conductance, diffusion resistance and SPAD values. Humiforte 20 at 200 and 300 mL ha^{-1} registered major improvement for all the physiological parameters. Considerable reduction in the diffusion resistance was noticed due to application of Humiforte 20. Similarly, Fosnutren 20 exerted a significant improvement in all the physiological attributes. Concentration dependant reduction in diffusion resistance was noticed due to foliar application of active amino acid formulations. Physiological attributes

Table 3: Impact of foliar application of active amino acid compounds on physiological attributes of clonal teas

Treatments	Stomatal conductance (cm sec^{-1})	Diffusion resistance (sec cm^{-1})	Chlorophyll (mg g^{-1}) (fr. wt.)
CA1. Untreated control	0.29	3.51	3.40
A1. Aminolforte 20 (150 mL ha^{-1})	0.45	2.22	3.50
A2. Aminolforte 20 (200 mL ha^{-1})	0.45	2.24	3.68
A3. Aminolforte 20 (300 mL ha^{-1})	0.43	2.32	3.66
CD at p = 0.05	0.02	0.15	0.08
CV (%)	6.91	7.82	2.42
CF1. Untreated control	0.29	3.51	3.40
F1. Fosnutren 20R (150 mL ha^{-1})	0.42	2.38	3.54
F2. Fosnutren 20R (200 mL ha^{-1})	0.44	2.26	3.64
F3. Fosnutren 20R (300 mL ha^{-1})	0.47	2.14	3.19
CD at p = 0.05	0.02	0.16	0.09
CV (%)	6.69	8.14	2.91
CH1. Untreated control	0.29	3.51	3.40
H1. Humiforte 20 (150 mL ha^{-1})	0.36	2.78	3.46
H2. Humiforte 20 (200 mL ha^{-1})	0.43	2.36	3.56
H3. Humiforte 20 (300 mL ha^{-1})	0.38	2.66	3.60
CD at p = 0.05	0.05	0.16	0.11
CV (%)	9.25	8.43	3.38
CK1. Untreated control	0.29	3.51	3.40
K1. Kadostim 20 (150 mL ha^{-1})	0.31	3.27	3.45
K2. Kadostim 20 (200 mL ha^{-1})	0.34	2.98	3.45
K3. Kadostim 20 (300 mL ha^{-1})	0.40	2.53	3.75
CD at p = 0.05	0.02	0.22	0.08
CV (%)	8.79	9.31	2.47

CD: Critical difference at 5% probability, CV: Coefficient of variation in percent

were enhanced due to foliar application of Kadostim 20 and the values were increased with increasing concentrations of the foliar applied formulations. Li *et al*. (2000) reported an improvement in chlorophyll content (SPAD) which may be due to the higher biological activity that encouraged the availability of nutrients to produce high energy.

Manivel *et al*. (1994) reported that foliar application of biostimulants enhanced the photosynthetic carbon dioxide assimilation in tea. Here again, irrespective of the commercial formulations, stomatal conductance of the plants increased noticeably. It is evident that photosynthetic carbon dioxide assimilation and stomatal conductance had a direct positive correlation with each other (Kumar and Thomas, 2004). However, stomatal conductance had a significant negative correlation with diffusion resistance. Chlorophyll content of the tea leaves treated with growth regulators enhanced appreciably. This in turn was corroborated by the increased values of physiological attributes. In spite of enhanced physiological activity, a decline in the PI revealed unfavourable partitioning of assimilates and not the economic yield in the case of Aminolforte 20. Improvement in chlorophyll content and chlorophyll fluorescence emissions due to the foliar application of commercial biostimulants had been reported earlier by Fraser and Percival (2003).

Biochemical Constituents

Biochemical analysis revealed a significant increase in total polyphenols and amino acids in response to foliar application of Aminolforte 20; increasing concentrations of Aminolforte 20 enhanced the amino acids and polyphenols linearly. On the other hand, improvement in catechin content was noticed only at 150 mL ha^{-1}. Noticeable increase in polyphenols is observed in all the treatments of Humiforte 20. However, the contents of catechins and amino acids did not exhibit considerable improvement in any of the treatments of Humiforte 20. Among the biochemical constituents analyzed, total polyphenols revealed a noteworthy increase when Fosnutren 20R was applied at 300 mL ha^{-1}. Even though catechins increased marginally, positive improvement in the amino acid content was noticed

Table 4: Influence of foliar applied active amino acid formulations on biochemical constituents of tea crop shoots

Treatments	Polyphenols (%)	Catechins (%)	Amino acids (%)
CA1. Untreated control	21.79	16.59	4.01
A1. Aminolforte 20 (150 mL ha^{-1})	24.38	17.24	4.30
A2. Aminolforte 20 (200 mL ha^{-1})	23.13	16.49	4.20
A3. Aminolforte 20 (300 mL ha^{-1})	23.29	16.40	4.43
CD at p = 0.05	1.00	0.55	0.15
CV (%)	5.85	4.39	4.69
CF1. Untreated control	20.02	14.77	3.39
F1. Fosnutren 20R (150 mL ha^{-1})	20.16	14.97	4.22
F2. Fosnutren 20R (200 mL ha^{-1})	20.17	14.94	3.59
F3. Fosnutren 20R (300 mL ha^{-1})	20.66	15.44	3.38
CD at p = 0.05	0.60	0.69	0.23
CV (%)	3.94	6.18	8.80
CH1. Untreated control	21.79	16.59	3.84
H1. Humiforte 20 (150 mL ha^{-1})	22.37	15.99	3.62
H2. Humiforte 20 (200 mL ha^{-1})	23.17	15.93	3.41
H3. Humiforte 20 (300 mL ha^{-1})	22.72	16.68	3.92
CD at p = 0.05	0.44	1.19	0.19
CV (%)	2.63	9.85	6.83
CK1. Untreated control	20.01	14.77	3.36
K1. Kadostim 20 (150 mL ha^{-1})	19.93	14.31	3.50
K2. Kadostim 20 (200 mL ha^{-1})	21.68	14.23	3.53
K3. Kadostim 20 (300 mL ha^{-1})	22.83	15.68	3.54
CD at p = 0.05	1.10	0.63	0.06
CV (%)	6.95	5.67	2.19

CD: Critical difference at 5% probability, CV: Coefficient of variation in percent

when Fosnutren 20R was applied at 150 mL ha^{-1}. There was an increase in total polyphenols, catechins and amino acids with increasing concentration of Kadostim 20 (Table 4). Increased values of biochemical constituents strengthened the role of biologically active amino acids in the metabolism of tea plants as it was reported earlier by Vogtmann *et al*. (1993), where the organic supplements used to produce nutritionally enriched quality vegetables. Results obtained in the present study substantiate the earlier work by Kumar *et al*. (1999) on foliar applied amino acid formulations in tea. Mandal *et al*. (2007) in their review highlighted the enhancement of biochemical attributes in response to foliar application of growth regulators in tea.

Quality Attributes and Organoleptic Evaluation

Foliar application of Aminolforte 20 had a positive impact on certain quality parameters like TF, TR, CI and BI of made tea samples (Table 5). Application of Aminolforte at 150 mL ha^{-1} enhanced the TR values resulting in enhancement in the ratio of TR to TF. But other quality parameters such as HPS and TLC did not have any significant improvement in any of the concentrations tested with Aminolforte. Overall quality parameters of made tea samples had a significant improvement in response to the foliar application of Humiforte 20 at 300 mL ha^{-1}. Quality parameters such as TF, TR, HPS, TLC of made tea samples had noticeable improvement in response to the foliar application of Fosnutren 20R. At all concentrations tested, this biostimulant enhanced the values of colour index and briskness index. There was reduction in the caffeine content due to the foliar application of Fosnutren. Overall, quality of made tea had remarkable improvement in response to foliar application of Kadostim 20. There was no variation in caffeine content among the different treatments of Kadostim 20 when compared to untreated control. Colour index was low in the Kadostim applied samples (Table 5).

Professional tasters' awarded higher marks for infusion and strength for the samples applied with Aminolforte. Overall tasters' score indicated that there was good

Table 5: Influence of Kadostim 20, Aminolforte 20, Fosnutren 20R and Humiforte 20 on quality attributes of made tea and liquor characteristics

	Made tea characteristics								Liquor characteristics				
Treat	TF	TR	TR:TF	HPS	TLC	CI	BI	Caf	Infusion	Colour	Strength	Briskness	Total score
CA1	1.13	11.70	10.4	10.70	4.13	5.04	26.67	3.10	4.00	5.00	4.50	4.00	17.50
A1	1.05	12.03	11.45	10.52	3.95	4.66	27.21	2.81	5.50	5.00	5.00	4.50	20.00
A2	1.18	12.29	10.43	9.97	4.00	5.30	31.39	2.58	5.25	5.00	5.00	4.25	19.50
A3	1.38	12.72	9.25	9.43	4.15	6.22	35.81	2.47	5.25	5.00	5.00	4.50	19.75
CD	0.03	0.23	0.17	0.37	0.12	0.18	0.81	0.10	0.28	0.19	0.31	0.28	0.93
CV	3.30	2.63	2.29	4.70	3.74	4.64	3.68	4.75	7.53	5.16	8.51	8.83	6.50
CF1	1.13	11.70	10.40	10.70	4.13	5.04	26.67	3.10	4.00	5.00	4.50	4.00	17.50
F1	1.15	11.36	9.91	10.25	4.15	5.30	29.55	2.73	5.75	5.25	5.50	5.00	21.50
F2	1.18	11.63	9.94	10.53	3.88	5.34	34.78	2.26	6.00	6.00	6.00	5.50	23.50
F3	1.17	11.70	10.05	10.30	3.95	5.32	34.63	2.26	5.25	5.00	5.00	4.25	19.50
CD	0.03	0.36	0.29	0.29	0.26	0.13	2.42	0.23	0.28	0.16	0.30	0.25	0.85
CV	3.78	4.18	4.00	3.56	8.37	3.32	10.69	11.4	7.28	4.02	7.90	7.32	5.68
CH1	1.13	11.10	10.40	10.70	4.13	5.04	26.67	3.44	4.00	5.00	4.50	4.00	17.50
H1	1.22	11.49	9.57	10.43	4.05	5.55	37.87	2.67	4.75	4.50	4.75	4.25	18.25
H2	1.16	10.91	9.45	10.17	4.19	5.49	32.11	3.03	5.00	4.75	4.50	4.25	18.50
H3	1.26	11.38	9.03	10.72	4.59	5.72	31.91	3.32	5.50	5.25	5.00	4.25	20.00
CD	0.06	0.19	0.20	0.16	0.14	0.19	1.17	0.19	0.44	0.15	0.29	0.22	0.98
CV	6.46	4.67	2.75	2.02	4.42	4.82	5.08	7.00	12.03	4.22	8.15	6.94	7.07
CK1	1.13	11.70	10.40	10.70	4.13	5.04	26.67	3.10	4.00	5.00	4.50	4.00	17.50
K1	1.15	12.11	10.60	11.08	4.61	4.96	26.02	3.27	5.50	5.25	5.50	5.00	21.25
K2	1.25	12.77	10.17	12.53	4.86	4.94	28.90	3.13	5.75	5.25	6.00	5.00	22.00
K3	1.13	13.67	12.18	11.55	4.91	4.47	28.99	2.77	5.75	5.25	5.75	5.00	21.75
C.D.	0.04	0.64	0.51	0.46	0.13	0.22	1.75	0.19	0.28	0.49	0.30	0.23	0.81
C.V.	4.36	7.07	6.61	5.35	3.78	5.92	8.44	8.43	7.28	14.39	7.57	6.90	5.38

Treat: Treatment, Made tea characteristics, TF: Theaflavins (%), TR: Thearubigins (%) HPS: High polymerised substances (%), TLC: Total liquor colour, CI: Colour index, BI: Briskness index and Caf. caffiene (%); CD: Critical difference at 5% probability, CV: Coefficient of variation in percent

improvement in liquor characteristics due to foliar application of Aminolforte 20 at 150 mL ha^{-1} treatment. Tasters' gave high scores for infusion, colour, strength and briskness for the samples applied with Humiforte 20 at 300 mL ha^{-1}. There was improvement in infusion, colour, strength and briskness which in turn reflected on the total score for Fosnutren 20R applied samples. Overall score indicated a considerable improvement in liquor characteristics due to foliar application of Fosnutren 20R both at 150 and 200 mL ha^{-1}. Scores for infusion, colour, strength and briskness were higher for the teas manufactured with leaves harvested from Kadostim 20 applied bushes (Table 5).

Since the commercial formulations tested are biologically originated amino acids, deterioration of quality attributes is not expected. Applied concentrations of individual formulations altered the scale of synthesis of green leaf quality attributes which influenced the made tea characteristics. However, concentration dependent factor has not reflected on the organoleptic evaluation where the scores varied marginally.

CONCLUSION

Among the different formulations tested, no improvement in the yield was noticed due to Aminolforte 20 application. But, overall improvement in the physiological, biochemical and quality characteristics was noticed due to the application of Aminolforte 20 at 300 mL ha^{-1}. Humiforte 20 at 300 mL ha^{-1} was found to increase the yield to about thirty 37% and there was significant improvement in physiological attributes, quality constituents and liquour characteristics. Application of Fosnutren 20R at 300 mL ha^{-1} enhanced the yield significantly and lowest concentration itself resulted in a noticeable improvement in physiological attributes. Even though, the quality constituents did not show an increase, the liquor characteristics were improved in all Fosnutren 20 concentrations. Comprehensive yield improvement and biochemical constituents with an overall increase in quality parameters of made tea was noticed due to foliar application of Kadostim 20 at 300 mL ha^{-1}.

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