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BANGLADESH TEA RESEARCH INSTITUTE

SRIMANGAL-3210, MOULVIBAZAR

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Preface

Tea culture is an art, a science and an epigraph of agro-industrial technology. An awareness towards scientific cultivation of tea must be generated among the tea planters so that an effective technology-infusion is made in all stages of tea management.

I am very much pleased to inform that Bangladesh Tea Research Institute (BTRI) is going to publish the 49th volume of the 'Tea Journal of Bangladesh', a research based prime publication. Basic and applied research in all aspects of tea culture will ensure high yield and quality tea production. This journal will help the tea planters, tea scientists, technocrats and entrepreneurs who are associated with tea industry of Bangladesh to have a cognizable understanding some new basic concepts of tea technology.

The articles of this volume cover a wide range of topics, methods, and geography in the field of tea science. It is also a matter of great pride that in order to increase field production and improve the quality of tea, this journal has concentrated an eco-friendly combination of method for our tea community. At last, I thank the all authors, reviewers, editorial board members and advisory committee members for providing their valuable efforts, suggestions and knowledge in ensuring high quality articles emerge through the double-blind peer review process. Despite all efforts, unintentional errors in various forms may appear in this journal. We ardently request our valuable readers to pardon us for such unintentional mistakes and shall consider ourselves rewarded to receive any evocative criticism. Finally, I wish the future success of the tea industry of Bangladesh.



Major General Md. Ashrafur Islam, *ndc, psc*
Chief Advisor
Tea Journal of Bangladesh

Editorial

The 49th volume of Tea Journal of Bangladesh contains eight research articles. The first article is about yield and quality performances of four vegetative propagated test clones of tea namely Ph/9/68A, Sh/D/11/18, A/8/61 and SC/12/28 with control BTRI released very popular clone named BT2. It was found that among these test clones, Sh/D/11/18 and SC/12/28 both test clones possess excellent (90-95%) rooting ability. In case of yield performance at mature stage, test clone SC/12/28 appeared superior (3341.75 kg ha⁻¹) to other test clones which was followed by A/8/61 (3320.37 kg ha⁻¹) and Sh/D/11/18 (3304.15 kg ha⁻¹), production of both A/8/61 and Sh/D/11/18 test clones were statistically similar. However, in quality parameters, Sh/D/11/18 test clone has been found to be 'Excellent' cup quality. On the other hand, SC/12/28 has been observed to be highly tolerant to drought while Sh/D/11/18 was found to be drought hardy than other test clones. Considering the morphological, yield, quality and drought parameters, test clone Sh/D/11/18 were released as 'Improved Quality Clone' as 'BT22' while SC/12/28 were released as 'Drought Tolerant and Flavoury Standard Clone' as 'BT23' in the BT-series to the industry in 2021.

The second article titled 'Validation of different IPM techniques for sustainable tea production in Bangladesh'. The article emphasized on different IPM techniques for sustainable tea production developed by BTRI and which was validated by Project Development Unit (PDU). The validation trial was conducted at Baraora Tea Estate, Sreemangal, Moulvibazar; Amtali Tea Estate, Bahubal, Habiganj and Six small tea gardens, Panchagarh Sadar, Panchagarh. Result revealed that the solar power light trap & yellow sticky trap captured greater number of thrips (112.32/trap) and (434.84/trap) followed by moths of looper caterpillar (34.64/trap) and (17.56/trap), respectively. The parasitism of *Bracon hebetor* was found very effective and the maximum mortality (98.42%) of looper caterpillar by the larval parasitoid of *B. hebetor* was found at 21 days after release in the field condition. Therefore, different IPM techniques in tea such as solar power light traps, yellow sticky traps and *B. hebetor* as larval parasitoid against major pests of tea have been validated and found to be satisfactory. The managers, planters, small growers will be benefitted by using these ecofriendly IPM techniques for sustainable and safe tea production.

The third article titled 'Effects of different types of compost on yield and yield related parameters of young tea [*Camellia sinensis* (L.) O. Kuntze]'. In this experiment, three different types of compost viz. Vermicompost (T₁), Farm yard compost (T₂), Water hyacinth compost (T₃) as treatments and Decomposed cowdung as the Control (T₄) along with recommended chemical fertilizers were used. Growth, development and yield related different parameters were observed and recorded during the first five years after planting. At the end of 5th year after planting, highest number of branches found in T₄ (21) and found statistically similar with T₁ (18) and T₃ (18) whereas the lowest was found in T₂ (16). Similarly, base diameter was maximum in T₄ (33.0 mm) which was statistically similar with T₃ (32.9 mm) and T₁ (32.5 mm) but different with T₂ (28.9 mm).

The fourth article is about to find the residue levels of commonly used two organophosphate insecticides, namely Chlorpyrifos and Quinalphos in green leaf and black tea, at various plucking intervals after spraying during 2017-2018. The initial (0 days) pesticide residue of chlorpyrifos was 7.822 mg kg⁻¹ in green leaf and 0.893 mg kg⁻¹ in black tea, respectively. These were reduced to an undetectable level in the green leaf on the 10th day and 0.013 mg kg⁻¹ in the black tea on the 7th day. On the other hand, quinalphos residue levels were initially (0 day) detected in green leaf and black tea at 4.973 and 0.193 mg kg⁻¹, respectively. It decreased to undetectable levels on the 5 and 10 days in both cases. The result showed that pesticide residue decreased considerably in the process stage. Chlorpyrifos and quinalphos had pesticide residue degradation rates ranges from 70.70-92.07% and 94.21-96.12%, respectively.

Fifth article of this journal is on assessing biochemical and sensory changes of tea during standardization of a 'modern green tea processing unit' for manufacturing high quality green tea. Different parameters were changed during processing according to the treatments and the product green tea samples were analyzed biochemically for the determination of quality. From the results, it can be suggested that 1 hour Withering → Fixation at 270°C → 55 min Rolling → Roll Drying at 200°C → Final Drying at 120°C can be set as a common procedure for manufacturing quality green tea using the newly established green tea factory of Bangladesh Tea Research Institute.

The sixth article is related to the assessment of occurrence of plant parasitic nematodes (PPNs) and their relationship with soil properties from July 2015 to December 2015 at the Lackatoorah, Malnicherra, and Ali Bahar tea estates in the north Sylhet region of Bangladesh. The result revealed that out of 60 collected soil samples from different locations of selected tea estates, 47 were infested with PPNs with frequencies of 78.33%. The highest frequency of PPNs was observed in the soil samples of the secondary nursery bed of selected tea gardens, with a frequency of 93.00%. The maximum frequency of occurrence (80.00%) was observed in the root samples of the secondary nursery bed, and that was the minimum (26.67%) in the second-year plantation. Maximum PPN was found in the sand, silt, and clay within the range of 70-80%, 8-13%, and 9-18%, respectively. The population of PPNs was linearly and positively correlated with soil pH and organic matter (%) in all three tea estates.

The seventh article is about to assess the difference of made tea volume and made tea quality due to different green leaf quality. The volume of made tea varies with the green leaf quality. Tea volume of individual each grade (FBOP, BOP, GBOP, OF and Dust) was lowest in GL_{80%} while highest in GL_{30%} leaf quality. Total polyphenols (16.23%), theaflavin (0.89%), brightness (27.77%) and caffeine (3.17%) found highest in green leaf with 80% good leaf. Best ratio of TF:TR (1:10.39) was found in green leaf with 80% while the low quality of made tea with 1:12.47 ratio was found in low quality of green leaf (30% good leaf). Correlation Coefficient (r) were +0.88*** and +0.80***, respectively for caffeine and organoleptic score against good leaf (%) also explaining the presence of strong positive relationship between them.

The eighth article is related to the assessment of the best time of Light Pruning (LP) in the Chattogram Hill Tracts of Bangladesh. Starting from 1st November, seven different dates were tested maintaining about 20 days interval. Results showed that significantly highest number of plucking points/bush/round (42.33) and yield (2400 kg ha⁻¹) was obtained when LP was done on 10th December, followed by 30th December and 20th November. It was observed that no mortality was observed when pruning was done on 1st November, 20th November, 10th December and 30th December. From the findings, 10th December was identified as the ideal time of LP for the tea plantations in the Chattogram hill tracts of Bangladesh for higher productivity.

All these research articles would have direct impact on the technological thrust needed for the Bangladesh Tea Industry. The main objective of this journal is to disseminate our new research findings to the tea industry for the improvement of tea culture, production and management. We sincerely express our heartiest gratitude to our respected Chairman, Bangladesh Tea Board for providing all sorts of support to materialize this journal. I also express my heartfelt thanks to all the BTRI scientists who have worked so hard to make this journal possible by their valuable research findings.



(Dr. Md. Ismail Hossain)
Chief Editor



COMPARATIVE STUDY ON THE PERFORMANCE OF FOUR TEST CLONES OF TEA

Md. Ismail Hossain*

Abstract

The present experiment was carried out to study the performances of four vegetatively propagated test clones of tea namely Ph/9/68A, Sh/D/11/18, A/8/61 and SC/12/28 with very popular clone named BT2 as control. Morphologically test clone Sh/D/11/18 is the light-leaved Assam agrotype which is key indicator for better quality while test clone SC/12/28 is China Monipuri Hybrid agrotype is for more drought tolerance. It was also found that, Sh/D/11/18 and SC/12/28 both test clones possess excellent (90-95%) rooting ability. In case of yield performance at mature stage, test clone SC/12/28 appeared superior (3341.75 kg ha⁻¹) to other test clones which was followed by A/8/61 (3320.37 kg ha⁻¹) and Sh/D/11/18 (3304.15 kg ha⁻¹), production of both A/8/61 and Sh/D/11/18 test clones were statistically similar. However, in quality parameters, Sh/D/11/18 test clone has been found to be 'Excellent' cup quality. On the other hand, SC/12/28 has been observed to be highly tolerant to drought while Sh/D/11/18 was found to be drought hardy than other test clones. Considering the morphological, yield, quality and drought parameters, test clone Sh/D/11/18 were released as 'Improved Quality Clone' as 'BT22' while SC/12/28 were released as 'Drought Tolerant Standard Clone' as 'BT23' in the BT-series to the industry in 2021.

Keywords: Tea, Yield performance, Cup quality, Test clones

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Introduction

The tea plant, *Camellia sinensis* (L.) O. Kuntze, is a woody plant that is indigenous to China and belongs to the genus *Camellia* of the family Theaceae (Chang & Bartholomew, 1984; Taniguchi et al., 2012). Due to its therapeutic benefits, tea has become one of the most popular non-alcoholic beverages in the world. Its appeal as a "health drink" has increased significantly day-by-day. The tea sector in Bangladesh contributes 1% of the country's GDP, making it one of the main sources of revenue for the national treasury (Raza, 2019).

With a grant area of 285139.39 acres and a tea area of 152256.61 acres, there are currently 166 tea estates in Bangladesh (PDU, 2020). In 1947, there were 28,734 ha of tea cultivation, producing 18.36 million kg, with a yield of 656 kg per hectare. By 2020, however, there were 62,168.46 ha of tea cultivation, producing 86.39 million kg, with a yield of 1561 kg per hectare (BTB, 2021). In contrast to the global situation, tea exports and production in Bangladesh is negatively correlated. About 30.98 million kg of tea were exported in 1980; by 2020, that number had dropped to approximately 2.17 million kg, a 92.99% decline. This point of view implies the need to increase production with improved export-quality liquor and to investigate alternate strategies for boosting tea export and cultivation profits. Our tea industry also facing problem of low production due to prolonged drought period during last couple of years. In order to address consumer demand and maximize tea growers' profit margins, it is urgently necessary to concentrate on varietal development in Bangladesh that combines high production, better quality and severe drought tolerance.

Since the beginnings of BTRI, it has put emphasis on clonal selection and hybridization programs with the goal of developing planting materials that have the potential to be high-yielding, high-quality and drought resistant. The program for clonal selection has begun in 1959 while hybridization in 1965 (Rashid & Alam, 1990). As a result of these efforts, the institution has so far provided the industry with twenty-three vegetative clones of BT-series. In the current investigation, four vegetatively propagated test clones of tea were compared to a control BT2 to examine the long-term yield, quality and drought performances.

Materials and Methods

The experiment was carried out with four test clones of tea in the experimental field of BTRI, during the period from 2007 to 2020. During rooting trial, rooting ability of particular each test clones were calculated by the equation; rooting ability = (Cuttings survived)/ (Total cutting planted) X 100. After rooting trial in the nursery, the selected test clones, namely Ph/9/68A, Sh/D/11/18, A/8/61 and SC/12/28 were put to long term yield and quality trial during 2007 at BTRI Farm. The experiment was laid out in a 5 m x 5 m Randomized Complete Block Design (RCBD) with 105 cm x 60 cm spacing of plot size and each plot had a total of 25 plants. For the purpose of comparing yield, quality and other parameters, BTRI released standard variety BT2 has been used as control. In a rain-fed environment, the experiment was carried out. Application of the fertilizer mixture was followed as BTRI instructions (BTRI, 1994; BTRI, 1998). As per BTRI guidelines, young and mature tea pruning was practiced for young tea: Decentre-Prune-Skiff-Prune-Skiff (BTRI, 1986) and for mature tea: Light prune-Deep skiff-Medium skiff-Light skiff (BTRI, 2002). Throughout the experimental period, the green leaves of per plot was plucked at weekly intervals from mid-March to mid-December. The amount of made tea produced (kg ha^{-1}) was estimated using a 23% recovery rate from green leaves. The clonal category (yield or standard or quality clone) of a particular tea clone was done on the basis of 'yield' and 'cup quality' as follow:

Table 1. The categories of tea clones as yield, standard and quality clones.

Yield (made tea kg ha^{-1})	>4000 kg ha^{-1}	3000-4000 kg ha^{-1}	2500-3000 kg ha^{-1}
Cup Quality	AA* or A*	AA*	E*
Category of clones	Yield clone	Standard clone	Quality clone

* Quality score: E = Excellent (>34 out of 50)

AA = above average (32 to <34 out of 50)

A = average (30 to < out of 50)

BA = Below Average (<30 out of 50).

The traditional organoleptic tasting procedure was used to evaluate the organoleptic quality of manufactured teas of each test clones and scoring was done on the basis of liquor characteristics (BTRI, 2009; BTRI, 2021). In order to make the liquor, boiling water was poured into a mug with a 142 ml (0.25 pint) capacity which contained 2.5 g of tea. The liquor was then poured into a bowl after 5 minutes of brewing as well as the infused leaf had to be taken from the mug into the inverted lid, which was then placed on top of the mug. Both the infused leaf and the liquor were then tasted by taster panel. Infused leaf appearance and liquor characteristics were assessed by scoring numerically out of 50 points as below:

- Infusion (10)
- Liquor characteristics (40 points): Liquor colour (10 points), Briskness (10 points), Strength (10 points), Creaming down (10 points)

Five plant samples from each test clone with each replication were taken during the third year of the experiment to measure the average depth of root and root shoot ratio in order to evaluate the drought performance. Lychor was used to measure leaf water potential, rate of photosynthesis, transpiration loss and water usage efficiency. Leaf samples were extracted using 3% aqueous sulfosalicylic acid to determine their proline concentration. Two milliliters of the filtered solution were pipetted into the test tube, along with two milliliters of acid ninhydrin and glacial acetic acid. A UV spectrophotometer was used to quantify the separated toluene at 520 nm in comparison to a reagent blank after being incubated at hot bathing and ice bathing. By using a leaf and diluted methanolic extract, total chlorophyll (sum of chlorophyll a & chlorophyll b) was computed and measured at 470, 653 and 666 nm using a UV-Visible spectrophotometer with methanol used as a blank. The relative leaf water content was determined using the Barr & Weatherley (1962), and the Chlorophyll stability index was computed using the following equation generated by Kaloyereas (1958). Chlorophyll Stability Index (%) = (Optical density value of heated sample / Optical density value of unheated sample) X 100. All data were compiled and analyzed statistically in MSTAT program in a microcomputer. The Duncan's Multiple Range Test (DMRT) was used to compare means after the analysis of variance was calculated.

Results and Discussion

General and morphological characteristics

General characteristics of four test clones (Ph/9/68A, Sh/D/11/18, A/8/61 and SC/12/28) and control BT2 is given in Table 02. From Table 02, it was observed that, test clone Sh/D/11/18 is the light-leaved Assam agrotype while test clone SC/12/28 is China Monipuri Hybrid agrotype. Wellensiek (1947) observed that a better tea quality was found in light green leaves of Assam agrotypes while Manipuri types was for drought tolerance (Greenway, 1945). Pruning recovery of all the test clones were high. It was also found that Sh/D/11/18 and SC/12/28 both test clones possess excellent rooting ability (Figure 1 of Appendix I).

Table 2. General characteristics of four test clones and BT2 as control.

Test clones				
Ph/9/68A	Plago-orthotropic, compact bush, densely branched with heavy girth and quite satisfactory spread.	Prominent long apex, uniformly serrated, leaf blade is quite embossed and slightly wavy.	High	80-85%
Sh/D/11/18	The plant falls under light-leaved Assam agrotype, semi-orthotropic, plucking shoots are soft, medium sized, dense and evenly distributed on the plucking table.	Leaves are light green, broad in size, glossy and smooth with semi-erect leaf pose. Leaf apex is prominent with uniformly serrated margin.	High	90-95%
A/8/61	Ortho-plagiotropic, compact plucking table, fairly dense plucking points, heavy girth with good branching behaviour	Leaves are light green, broad in size, texture is thick, soft and leathery, and apex is less prominent, leaf pose semi horizontal.	High	80-85%
SC/12/28	China Monipuri Hybrid agrotype with heavy girth and quite satisfactory spread and profuse branching. The plant has medium bush with orthotropic growth habit.	Leaves are dark green, medium to broad in size, glossy and smooth with semi-erect leaf pose. Leaf apex is prominent with uniformly serrated margin.	High	90-95%
BT2	Orthotropic, densely branched, comparatively loose frame, but effective branches, very well and uniform flushing behaviour, moderately floriferous.	Leaf texture fairly thick and soft, semi-dark green. Apex less pointed, lamina considerably equal width in mid region, serration uniform and semi-erect.	High	85-90%

Note: Rooting ability: Excellent = >90%, Good = >80% - <90%, Medium = <70% - 80%

Pruning recovery: High, very good, good, fair/moderate

Some morphological characteristics are provided in Table 3. It was observed that 100 fresh shoot weight, shoot length, leaf area, lamina length, serration/leaf, pruning sticks/bush at FFP-2 and plucking point /bush/year were significantly differs within the test clones. Test clone Sh/D/11/18, A/8/61 and SC/12/28 gave higher results in most cases than the control BT2.

Table 3. Morphological characteristics of four test clones and BT2 as control.

Characteristics	Ph/9/68A	Sh/D/11/18	A/8/61	SC/12/28	BT2	LSD at 5% level of significance
100 fresh shoot weight (g) (2L+B)	83.45b	91.35a	91.34a	91.75a	87.55ab	3.56
Shoot dry matter (%)	21.32	22.41	22.11	22.63	22.25	NS
Shoot length (cm)- (2L+B)	7.11b	7.98a	7.15b	7.85ab	7.13b	0.43
Mature leaf area (cm ²)	41.43b	45.39a	42.34ab	42.14ab	35.25c	2.11
Leaf lamina length (cm)	11.34c	14.75a	11.33c	13.62b	12.15ab	1.21
Leaf lamina breadth (cm)	6.31	6.28	6.38	6.45	5.39	NS
No. of bullation/leaf	12.33	12.36	11.37	12.41	11.43	NS
No. of serration/leaf	67.53c	75.47b	69.65bc	79.65a	78.22ab	2.31
No. of pruning sticks/bush at FFP-2	15.69bc	17.26ab	17.31ab	18.31a	16.35b	1.02
Wt (kg) of pruning litter/bush at FFP-2	1.17	1.43	1.13	1.28	1.21	NS
Number of plucking point /bush/year	461b	487ab	489ab	512a	494ab	5.85

Within row values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

NS= Not Significant

Yield performance

The made tea production (kg ha⁻¹) are presented in Table 4 for immature stage. From Table 4 it reveals that at initial stage of growth, control BT2 (1544.75 kg ha⁻¹) showed significant higher average yield trend than rest of the test clones. Made tea production of test clone SC/12/28 (1259.89 kg ha⁻¹) and A/8/61 (1259.89 kg ha⁻¹) were statistically similar and followed by Sh/D/11/18 (1172.66 kg ha⁻¹). Lowest production was observed by test clone Ph/9/68A (989.43 kg ha⁻¹).

Table 4. Made tea production (kg ha⁻¹) of four test clone and their control at immature stage.

Test Clones	UP (2008)			Pruned (2009)		Skiff (2010)	
Ph/9/68A	1044.27c			604c		1321ab	989.43c
Sh/D/11/18	1251.98bc			892b		2225c	1172.66bc
A/8/61	1210.26bc			573c		1996a	1259.89b
SC/12/28	1476.68b			619c		1618b	1274.12b
BT2	1740.15a			1072a		1693b	1544.75a
LSD at 0.05 level of significance	146.86			158.22		186.34	91.46

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

Year wise made tea production (kg ha⁻¹) are presented in Table 5 for mature stage. Highest average made tea was obtained from test clone SC/12/28 (3341.75 kg ha⁻¹) which is followed by A/8/61 (3320.37 kg ha⁻¹) and Sh/D/11/18 (3304.15 kg ha⁻¹); production of both test clones A/8/61 and Sh/D/11/18 (Figure 2 of Appendix I) were statistically similar and higher than control BT2 (3051.25 kg ha⁻¹). Lowest average production was found in test clone Ph/9/68A (2764.35 kg ha⁻¹).

Table 5. Made tea production (kg ha⁻¹) of four test clone and their control at mature stage.

Test Clones	DSK		MSK		LSK		LP	
Ph/9/68A	2633.0a		2310.4e		3480.32bc		2633.7b	2764.35c
Sh/D/11/18	2598.3b		3509.1b		3879.41ab		3229.8a	3304.15ab
A/8/61	3336.3ab		3226.1c		3572.80b		3146.3ab	3320.37ab
SC/12/28	3275.5ab		3007.9d		3955.40a		3128.2ab	3341.75a
BT2	3112.5c		3612.0a		3314.10c		2166.4c	3051.25b
LSD at 0.05 level of significance	156.34		178.95		207.43		145.64	108.57

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

Quality performance

As determined by traditional organoleptic test, Table 6 illustrates the overall quality of test clones in comparison to the BT2 as control. All the test clones and the control were found to be of "Above average" cup quality except Sh/D/11/18. Test clone Sh/D/11/18 possess bright infusion with very useful strength and briskness. It has excellent creaming down quality (Figure 3 of Appendix I). From the quality category of Table 01 and the total scoring of Sh/D/11/18 from Table 6, it can be categorized as 'excellent quality' (having 34 or more than 34 quality score out of 50 is considered as excellent quality).

Table 6. Cup quality of different test clones and their control.

Test Clones	Infusion (10)	Liquor Colour (10)	Briskness (10)	Strength (10)	Creaming down (10)	Total (50)	Remarks
Ph/9/68A	7.48 ab	7.38 bc	7.64 b	7.28 d	2.61 d	32.39 bc	AA
Sh/D/11/18	7.49 a	7.41 ab	7.55 c	7.52 b	4.51 a	34.48 a	E
A/8/61	7.44 b	7.32 c	7.42 d	7.39 c	2.84 b	32.41 bc	AA
SC/12/28	7.22 c	7.41 ab	7.66 ab	7.27 d	2.82 b	32.38 c	AA
BT2	7.28 bc	7.46 a	7.68 a	7.72 a	2.71 c	32.85 b	AA
LSD at 5% level of significance	0.003	0.06	0.005	0.06	0.008	0.11	

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

A: Average, AA: Above Average, E: Excellent cup quality

There are many factors affecting liquor quality of a particular test clone. Number of pubescence and length of a single unopen bud are most fundamental criteria for liquor quality. Variety with numerous pubescence provide better 'cup quality' liquor (Wilson & Clifford, 1992) while variety with larger leaf bud has special interest for white tea and orthodox tea manufacturing. Number of pubescence and length of a single unopen bud of this experiment are given in Table 7. It was observed that, highest pubescence number was found Sh/D/11/18 test clone (1725) which is followed by SC/12/28 (1631). Higher number of pubescence of the test clone Sh/D/11/18 is the main reason for being 'excellent quality'. On the other hand, test clone SC/12/28 could have special interest of the tea planters for white tea and orthodox tea manufacturing for having longest leaf bud (4.31 cm) amongst the test clones (Figure 4 of Appendix I).

Table 7. Number of pubescence and length of a single bud of test clones and their control.

Test clones	Number of pubescence	length of a single unopen bud (cm)
Ph/9/68A	1452bc	2.56bc
Sh/D/11/18	1725a	3.32b
A/8/61	1386c	2.35c
SC/12/28	1631b	4.31a
BT2	1682ab	3.38b
LSD at 0.05 level of significance	61.98	0.48

Within row values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

Drought tolerance

The drought performances of the test clones and their control are given at Table 8. Test clone SC/12/28 provides highest values in Average depth of root at 3rd year, Proline Content, Total Chlorophyll, Chlorophyll Stability Index, Photosynthesis, Water Use Efficiency and Relative Leaf Water Content which was followed by test clone Sh/D/11/18, but both were higher than control BT2. On the other hand, in case of Leaf Water Potential and Transpiration both test clones SC/12/28 and Sh/D/11/18 provide lowest values than the control BT2. Kaloyereas (1958); El-Nadi (1969); Bota et al. (2004); Damayanthi et al. (2010) also reported that higher values for the average root depth at the

third year, the chlorophyll stability index, the proline content, the efficiency of photosynthesis and water utilization, and lower values for transpiration and leaf water potential suggest an increase in the degree of drought tolerance. So, the test clone SC/12/28 has been observed to be highly tolerant to drought while Sh/D/11/18 was found to be drought hardy than other test clones and the control.

Table 8. Drought performances of the test clones and their control.

Characteristics	Ph/9/68A	Sh/D/11/18	A/8/61	SC/12/28	BT2	LSD at 5% level of significance
Avrg. depth of root at 3 rd year (cm)	25.64c	31.14ab	26.61bc	33.18a	28.72b	2.45
Root Shoot Ratio	0.33	0.32	0.29	0.34	0.28	NS
Proline Content ($\mu\text{mol/g fr. wt}$)	0.51bc	0.56ab	0.48c	0.67a	0.55ab	0.09
Leaf Water Potential (LWP-bar)	11.96a	9.91bc	11.89ab	9.12c	10.33b	1.02
Total Chlorophyll (mg g^{-1})	6.12c	6.68ab	6.13c	7.67a	6.66b	0.003
Chlorophyll Stability Index (CSI%)	68.00bc	79.00b	67.00c	89.00a	88.00ab	1.31
Photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	8.28c	8.55bc	8.29c	9.55a	8.92b	0.80
Transpiration ($\text{m.mol m}^{-2} \text{s}^{-1}$)	2.22a	2.05c	2.24a	2.01c	2.15b	0.02
Water Use Efficiency ($\mu\text{mol/m mol}$)	5.32b	5.89ab	5.31b	5.97a	5.85ab	0.005
Relative Leaf Water Content (RWC %)	61.24c	69.14ab	62.35bc	69.41a	68.22ab	1.82

Within row values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

Conclusion

Morphologically test clone Sh/D/11/18 is the light-leaved Assam agrotype which is key indicator for better quality while test clone SC/12/28 is China Monipuri Hybrid agrotype is for more drought tolerance. It was also found that, Sh/D/11/18 and SC/12/28 both test clones possess excellent rooting ability. On the other hand, test clone Sh/D/11/18, A/8/61 and SC/12/28 gave higher results in 100 fresh shoot weight, shoot length, leaf area, lamina length, serration/leaf, pruning sticks/bush at FFP-2 and plucking point /bush/year in most cases than the control BT2. But, in case of yield performance at mature stage, test clone SC/12/28 was found superior to other test clones which was followed by A/8/61 and Sh/D/11/18, both were statistically similar. However, for quality performance, Sh/D/11/18 test clone has been found to be 'Excellent' cup quality. On the basis of drought parameters, SC/12/28 has been observed to be highly tolerant to drought while Sh/D/11/18 was found to be drought hardy than other test clones. Considering the morphological, yield, quality and drought parameters, test clone Sh/D/11/18 were released as 'Improved Quality Clone' 'BT22' while SC/12/28 were released as 'Drought Tolerant Standard Clone' 'BT23' in the BT-series to the industry in 2021.

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Appendix I: Pictorial representation of BT22 (Sh/D/11/18) and BT23 (SC/12/28)



Fig. 1. Excellent rooting of BT22 (Sh/D/11/18)



Fig. 2. Pluckable section of BT23 (SC/12/28)



Fig. 3. Excellent cup quality of BT22 (Sh/D/11/18)



Fig. 4. Longest single unopen buds of BT23 (SC/12/28)



VALIDATION OF DIFFERENT IPM TECHNIQUES FOR SUSTAINABLE TEA PRODUCTION IN BANGLADESH

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Abstract

The tea plant is subjected to the infestation of pests and diseases. To combat these maladies different control measures have been practiced in the tea garden of Bangladesh. Bangladesh Tea Research Institute has been successfully completed a sub-project entitled "Integrated pest management (IPM) approaches to major pests of tea for sustainable tea production" to minimize the use of synthetic pesticides by adopting IPM practices. BTRI has been developed IPM approaches for the control of four major pests of tea i.e. tea mosquito bug, red spider mite, thrips and looper caterpillar of tea in Bangladesh. Furthermore, a validation trial on different developed IPM techniques for sustainable tea production was done by Project Development Unit (PDU), Bangladesh Tea Board, Sreemangal, Moulvibazar during January to December 2022 at Baraooora Tea Estate, Sreemangal, Moulvibazar; Amtali Tea Estate, Bahubal, Habiganj and Six small tea gardens, Panchagarh Sadar, Panchagarh. The validation trials were set up following RCBD with five replications. Traditional/Conventional practice i.e. use of chemical pesticides to control pests in the tea garden was considered as control. Solar light trap and yellow sticky trap as IPM components were used for the controlling of thrips, jassid, white flies, and moths of caterpillar. Data were collected regularly at respective method and analyzed by means using SAS programme. The results showed that the solar-powered light trap and yellow sticky trap successfully trapped more thrips (112.32/trap) and (434.84/trap) followed by moths of looper caterpillar (34.64/trap) and (17.56/trap), respectively. Bio-control agent, *Bracon hebetor* as a larval parasitoid was very effective against looper caterpillar and significantly reduced the pest population. Result revealed that the parasitism of *B. hebetor* was found to be very effective, and the maximum mortality (98.42%) of looper caterpillars by the larval parasitoid of *B. hebetor* was found 21 days after release in the field condition. Therefore, the developed IPM techniques in tea such as solar power light traps, yellow sticky traps and *Bracon hebetor* have been validated and found to be satisfactory against different insect pests of tea. These would eventually eliminate the need for pesticides, lower production costs, and improve environmental safety.

Keywords: Tea, IPM, Solar light trap, Yellow sticky trap, Bio-control, *Bracon hebetor*, Looper caterpillar, Thrips, Sustainable tea production

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Introduction

Tea is a major cash crop as well as an important export commodity in Bangladesh. Tea is a long established plantation crop of enormous importance to Bangladesh, meeting almost the entire domestic demand for this cheapest health beverage. Now, there are 168 tea estates with about 66,000 hectares of tea plantations producing about 93.83 million kg of finished tea per annum with an average yield of about 1,700 kg per hectare in Bangladesh (BTB, 2022).

Tea plants are attacked by a variety of insects, mites, nematodes, fungal diseases, and weeds. A variety of pests attack tea plants, causing significant economic losses. Tea, as a long-lived woody perennial and monoculture, provides a stable microclimate and a steady source of food for the rapid growth of phytophagous organisms such as insects, mites, and eelworms. So far, 25 insects, 4 mites, and 12 nematode species have been identified in tea gardens of Bangladesh (Ahmed, 2005; Mamun, 2009). Among them, tea mosquito bug, red spider mites and termites are the major pests in mature tea plantation; while thrips, aphids, jassids, flushworms and nematodes are the major pests in nursery and young tea plantation. About 10-15% of its crop could be lost by various pests particularly insects, mites and nematodes. It may be extended to as high as 50% when pest attack/disease incidence is severe (Mamun, 2011).

Tea pests may be classified into three categories on the basis of the site of attack/infestation viz. *root pests* like nematode, termites, cockchafer grub; *stem pests* like red coffee borer, stem borer and *leaf pests* like tea mosquito bug, thrips, jassid, aphid, flushworm, looper caterpillar, leaf roller and all mite species (Mamun and Iyengar, 2010). Management of pests, diseases and weeds are an important operation in sustainable tea cultivation. To combat these problems different groups of chemical pesticides like organochlorine, organophosphate, pyrethroids, carbamates and some unclassified group have been used in the tea fields since 1960 (Mamun & Ahmed, 2011b; Ali et al., 2021). In this perspective, chemical control of pests is a dominating feature in Bangladesh tea (Alam, 1999). Chemical pesticides have been used for a long time, but have serious drawbacks, such as direct toxicity to beneficial insects, fishes and human being; pesticide induced resistance, health hazard and increased environmental, social costs and undesirable pesticide residue in made tea (Shrestha & Thapa, 2015). Therefore, managing these pest populations within economic threshold level is important for which application of pesticides becomes imperative in Integrated Pest Management (IPM) system. According to Food and Agricultural Organization (FAO, 1967), IPM is defined as “a pest management system, that, in the context of associated environment and population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains pest populations at levels below those causing economic injury”.

A sub-project entitled “Integrated pest management (IPM) approaches to major pests of tea for sustainable tea production” under Competitive Research Grant (CRG) of NATP-2 of PIU, BARC was successfully done by Bangladesh Tea Research Institute to minimize the load of synthetic pesticides by adopting IPM practices during May 2017 to September 2018. BTRI has developed the IPM approaches for the control of four major pests of tea i.e. tea mosquito bug, red spider mite, thrips and looper caterpillar infesting tea in Bangladesh. Among the different components of IPM, different IPM techniques were validated during the validation period. The important components of IPM strategy i.e. Use of solar light traps, sticky traps and *Bracon hebetor* as larval parasitoid for major pests of tea which were validated in the experimental period that described below:

Solar light traps: Use of light trap is one of the important mechanical controls in IPM programme. Mechanical control means controlling pests and diseases with the help of mechanical measures. The behavior of certain species of insects being attracted to light could be advantageously used in their management. Light trap has often been used in the ecological studies of Lepidopteran insect pests in agroecosystems. Light trap is a cost effective and environment-friendly monitoring tool of Lepidopteran pests in tea plantations (Mamun & Ahmed, 2011a). Fluorescent light traps are useful in attracting the moths of caterpillars and other insects (Ahmed et al., 2010). They can be set up the seasons of moths’ emergence. These traps are useful for monitoring the activity of the pests and as a means of control. Solar power light trap captured greater number of thrips and moths of looper caterpillar at different intervals (Mamun & Alam, 2018).

Sticky traps: In the tea ecosystem two types of traps, viz. Sticky traps (Adhesive traps) and Light traps may be used for monitoring and managing three upcoming major pests such as tea thrips, green hoppers and looper caterpillars. Yellow and blue sticky traps are also cost effective and eco-friendly monitoring tool for thrips, jassid, and Lepidopteran moths in tea plantations (Mamun et al., 2018). More number of thrips, jassids and moths of looper caterpillar were caught in the yellow sticky trap. However, the traps also captured a large number of non-target insects.

Bracon hebetor as larval parasitoid is under the biological control in IPM programme. It is one of the important components of IPM in many crops. Biological control involves the conservation, preservation and introduction of natural enemies like predators, parasitoids and pathogens for suppression of pests within tolerable levels. Biological control is one of the oldest pest management strategies that play a vital role in the control of many tea pests below economic injury level. The minor status of several pests such as aphids, scale insects, flushworms, leaf rollers and tea tortrix is due to the action of these natural enemies (Muraleedharan, 1992; Hazarika et al., 2001; Hazarika et al., 2009; (Kawai, 1997).

Within the framework of pest management, research has been conducted on cultural control, bio-control, resistant agrotypes, biopesticides, and any other IPM approaches against important insect pests of tea under CRG sub-project. So, attempts were made to validate developed IPM techniques for sustainable tea production in Bangladesh by Project Development Unit (PDU), Bangladesh Tea Board. The validation trial was conducted to evaluate the following IPM techniques viz.

- a. Use of yellow sticky traps against thrips, aphids, jassids, white flies.
- b. Use of light traps against thrips and moths of looper caterpillar
- c. Use of *Bracon hebetor* as larval parasitoid against looper caterpillar in tea.

Materials and Methods

A validation trial on different IPM techniques for sustainable tea production was done by Project Development Unit (PDU), Bangladesh Tea Board, Sreemangal, Moulvibazar during January to December 2022 at Baraoora Tea Estate, Sreemangal, Moulvibazar; Amtali Tea Estate, Bahubal, Habiganj and Six small tea gardens, Panchagarh Sadar, Panchagarh. The validation trials were set up following RCBD with five replications. Two experimental plots have been set up at Amtoli Tea Estate, Bahubal, Habiganj in 2.47 acre area from where 1.23 acre was used for IPM plot and 1.23 acre was used for control plot. For the same reason, a different site at Baraoora Tea Estate in Sreemangal, Moulvibazar and six farmers' plots in Panchagarh District were chosen. The IPM techniques i.e. yellow sticky traps @ 100 pieces per ha, solar light traps and *Bracon hebetor* were used in IPM research plot. The conventional method of tea pest control like spraying of chemical insecticide was applied in the control plot area. Data were collected regularly at respective method. The data on the number of infested shoots from both IPM research and control plots were analysed by two way ANOVA as well as the data on the number of healthy shoots from both of the plots were analysed by paired T test using SAS programme (Version 9.4). Means were separated with LSD test at 0.05 level of significance. On the other hand, the number of insect-pest captured from different traps were analysed by MS Excel.

Evaluation of solar light trap and yellow sticky traps against thrips and looper caterpillar in tea

An experiment was carried out to evaluate the solar light traps and sticky traps as mechanical control measures against thrips and looper caterpillar infesting tea at Baraoora Tea Estate, Sreemangal, Moulvibazar; Amtali Tea Estate, Bahubal, Habiganj and Six small tea gardens, Panchagarh during January-December 2022. The experiment was set up following Randomized Complete Block Design (RCBD) with five replications. Three treatments such as light trap (T₁), yellow sticky trap (T₂) and control (T₃) were considered in the study. Solar power light trap were used at night to attract the flying pests of tea i.e. thrips and moths of caterpillar. UV lights (220 V, 18 W, 5.2 nm; Philips) in the light traps were used in the experiment. The two light traps in the pair were placed approximately 600 m apart. Yellow sticky traps both sides coated with a thin film of a sticky adhesive were placed in the infested sections. The Yellow sticky card traps should be placed in the tea field 6 inch above the bush canopy (plucking table) at an angle 60° facing against wind by securing them on top of bamboo sticks. 10 sticky traps per 1000 m² were used in the study. A total of 100 traps are required per hectare. Post treatment observations on the attraction of pests were recorded at weekly interval up to 12 weeks.

Potential effects of Bracon hebetor as a bio-control agent for sustainable management of looper caterpillar

An experiment was carried out to determine the efficacy of bio-control agents i.e. *Bracon hebetor* as a larval parasitoid against Looper caterpillar infesting tea in field conditions. The trial was set up at Baraoora Tea Estate, Sreemangal, Moulvibazar and Amtali Tea Estate, Bahubal, Habiganj. The adult female of *B. hebetor* @ 1 jar consisting around 800-1000 *Bracon* considered as treatment with three replications. The source of the bio-control agent is Ispahani Biotech Limited, Zareen Tea Estate, Srimangal, Moulvibazar. The potentiality of parasitoid on mortality of looper caterpillar was studied. Mortality data of looper caterpillar were collected at weekly interval.

Results and Discussion

Field evaluation of solar light trap and yellow sticky trap against thrips and looper caterpillar in tea

Result revealed that solar power light trap (T₁) captured greater number of thrips (112.32/trap) followed by white fly (64.42/trap) and moths (34.64/trap) of looper caterpillar, *B. suppressaria* at Sylhet region during January-December 2022 (Table 1). A few amounts of thrips, aphid, jassid, white flies, *Helopeltis* and termites are also captured in solar power light trap. In case of Panchagarh region, similar trend of captured of insect pests of tea was found (Table 2). [Sinu et al. \(2013\)](#) found the similar results. They found that male moths constitute more than 77% of the overall catches of looper caterpillar, *H. talaca*. Overall, our results highlight that light trap is an effective, bias-free monitoring tool of moth pests of tea, which also captures the variability due to habitat type. [Al Mamun et al. \(2020\)](#) reported that Installing one light trap in an acre attracts at least more than 1000 adult pest for a day.

On the other hand, Yellow sticky traps are excellent tools for precision monitoring of thrips, jassids, white flies and leaf miners in tea. Yellow sticky traps (T₂) captured greater number of thrips (434.84/trap) followed by Jassid (252.36/trap) at Sylhet region during January-December 2022 (Table 1). However, the traps also captured a few number of non-target insects. In case of Panchagarh region, similar trend of captured of insect pests of tea was found (Table 2). [Prema et al. \(2018\)](#) found the similar results. They found that thrips catches on yellow sticky trap after 45, 60, 75 and 90 days of sowing of cotton were very high with 89.65, 120.00, 124.00, 91.90 numbers per trap, respectively. In another experiment, [Roy et al. \(2011\)](#) found that the field trials reflected that yellow sticky trap attracted more number of thrips compared to other colours. [Bian et al. \(2016\)](#) reported that lawn green and lime coloured traps are considered suitable to trap thrips in the tea plantations of southern China.

Table 1. Number of pest captured by the different IPM techniques evaluated at Sylhet region during January-December 2022.

Treatments	Number of pest captured by the IPM techniques evaluated at Sylhet region						
	Moth of looper caterpillar	Thrips	Aphid	Jassid	White fly	<i>Helopeltis</i>	Termite
T1 Solar light trap	34.64±0.84a	112.32±3.42b	26.48±0.54b	36.26±0.84b	64.42±0.60a	10.52±0.32b	4.84±0.12b
T2 Yellow sticky trap	17.56±0.68b	434.84±5.36a	48.18±0.82a	252.36±0.66a	42.60±0.76b	12.16±0.56a	8.08±0.34a

Means with the different letter are significantly different at $P>0.05$ using LSD.

Table 2. Number of pest captured by the different IPM techniques evaluated at Panchagarh region during January-December 2022.

Treatments	Number of pest captured by the IPM techniques evaluated at Panchagarh region						
	Moth of looper caterpillar	Thrips	Aphid	Jassid	White fly	<i>Helopeltis</i>	Termite
T1 Solar light trap	28.82±0.44a	86.34±1.38b	24.32±0.88b	30.66±0.47b	48.26±0.56a	8.24±0.44a	6.46±0.22a
T2 Yellow sticky trap	13.62±0.38b	294.32±2.36a	32.06±0.74a	164.42±0.54a	36.48±0.30b	6.10±0.28b	5.38±0.48b

Means with the different letter are significantly different at $P>0.05$ using LSD.

The proportion (%) of different insects captured on yellow sticky traps in the tea garden is presented in Figure 1. The study revealed that the yellow sticky traps captured the highest number of thrips (59.03%) followed by jassid (35.42%) and others insects (Fig. 1).

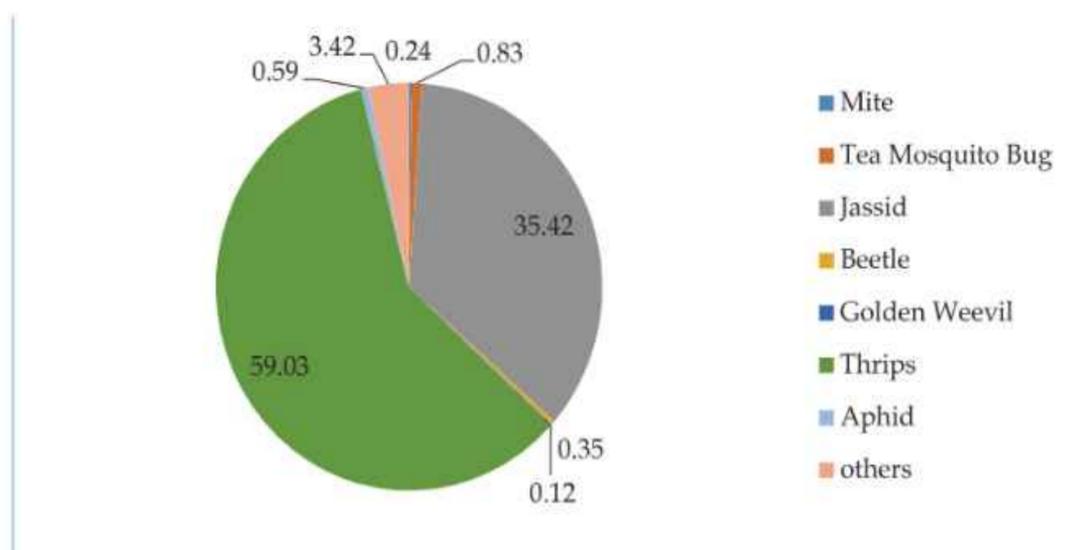


Fig. 1. Proportion (%) of different insect pests of tea trapped on yellow sticky traps in a tea garden at Sylhet region.

Field evaluation of *Bracon hebetor* as a bio-control agent for sustainable management of Looper caterpillar

Result revealed that parasitism of *B. hebetor* was found very effective and the maximum mortality (98.42%) of looper caterpillar by the larval parasitoid of *B. hebetor* was found at 21 days after release in the field condition (Fig. 2). However, the *B. hebetor* may be used as a larval parasitoid for the control of looper caterpillar infesting tea as one of the strong IPM component.

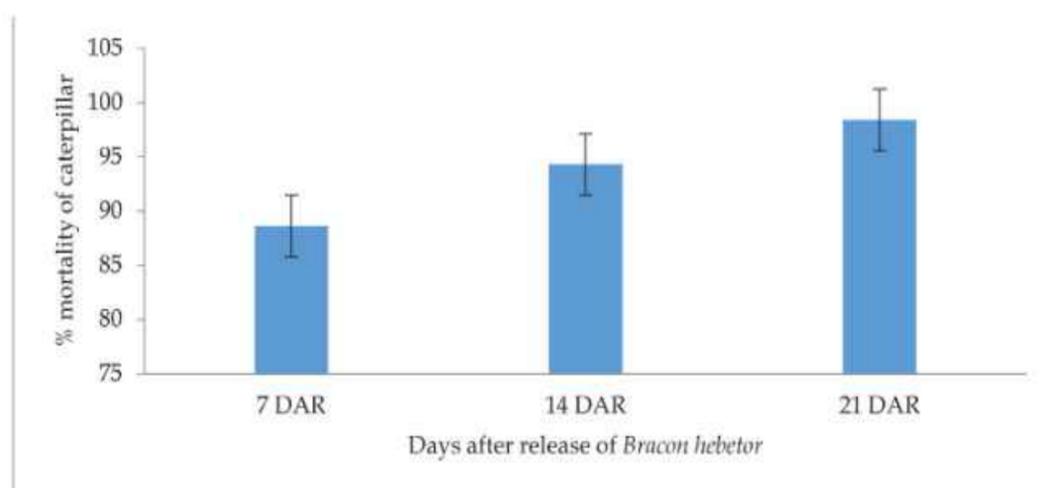


Fig. 2. Mortality (%) of looper caterpillar parasitized by larval parasitoid *Bracon hebetor* at field condition

Comparison between combine effect of IPM techniques and conventional method on shoot infestation

Result revealed that there was no significant difference in the number of infested shoot by different insects between IPM plot and control plot (Table 3). It revealed that the control of insect-pest was similar in both plots. Therefore, these 2-3 IPM techniques such as use of yellow sticky traps, light traps and *B. hebetor* showed good performance rather than conventional control method in controlling insect-pest specially thrips, jassid and looper caterpillar. The results also indicated that the number of infested shoot in whole season by thrips, jassid, looper caterpillar, aphid, flushworm and leaf roller was very low as 8.10, 4.30, 6.80, 0.30, 0.90 and 0.20, respectively (Table 3). But the number of infested shoot by *Helopeltis* was very high (1059.30) in the whole cropping season. It suggested that the mentioned 2-3 IPM techniques performed excellent over controlling thrips, jassid, looper caterpillar, aphid, flushworm and leaf roller except *Helopeltis*.

Table 3. Effects of treatment (A) and insect (B) on the number of infested shoot in tea.

Treatment	Infested shoot
Treatment (A)	
IPM research plot	152.94a
Control plot	155.60a
Insect (B)	
Aphid	0.30b
Flushworm	0.90b
<i>Helopeltis</i>	1059.30a
Jassid	4.30b
Looper caterpillar	6.80b
Leaf roller	0.20b
Thrips	8.10b
Significance level	
Treatment	NS
Insect	**
Treatment*Insect	NS
Mean	154.27
CV (%)	67.65

Means with the same letter are not significantly different at $P>0.05$ using LSD.

In Amtoli Tea Estate, chemical pesticides have been used for a long time to control looper caterpillar, thrips and jassid but these chemical pesticides have had serious drawbacks, such as direct toxicity to beneficial insects, fishes and human being; increased environmental and social costs, and undesirable pesticide residue in made tea. Besides these, the usage of chemical pesticides in the tea garden required more labour and cost.

Cost involvement of the new system and compare with earlier system

The total cost for 123 decimal of validation trial plot was 30,010 taka. On the other hand, the total cost for the same area of control plot was 27,135 taka. Here, the cost was higher in the trial plot than the control plot as become of the tea production was higher in the trial plot, which resulted more labor cost and ultimately higher cost in trial plot.

Table 4. Comparison between IPM validation trial plot and control (Conventional) plot per hectare area at three locations during January-December 2022.

Location	IPM plot				Control plot			
	Yield	Total cost	Net Income	BCR	Yield	Total cost	Net Income	BCR
Amtali T.E.	2184	419328*	535342	1.28	1944	373248*	476514	1.28
Baraora T.E.	980	147000*	223440	1.52	1214	182100*	276792	1.52
Panchagarh	4932	207046	399600	1.93	4826	234810	390960	1.67

* Based on cost of production of the garden

Financial benefit earned from the technology and comparison with earlier similar technology

In the validation trial plot at Amtali T.E., the total made tea production in IPM plot was 2184 kg and total income was 535342 taka. In contrast with validation trial plot, the total made tea production in control plot was 1944 kg and total income was 373248 taka (Table 4). A total of 162094 taka (30.27%) more income achieved in validation trial plot rather than control plot. The benefit cost ratio (BCR) was also found 1.52

i.e. economically viable. More numbers of plucking resulted higher production in IPM trial plot. Plucking gap increased in control plot due to minimizing the residual effect of pesticides which results low tea production. Similar trends were found in case of Panchagarh. But a little difference was observed in the trial plot of Baraooora T.E. In addition, in the eco-friendly environment healthy tea production was possible, which is the most demandable issue in the today's world.

Interested number of people demanded to practice the technology

Field day program was done where tea garden managers were present and after visiting Amtoli tea estate's validation trial plot, many tea garden managers eagerly showed their interests to practice these IPM techniques in their tea garden in the next year. In the meantime, about 8 tea gardens in Moulvibazar area have been using yellow sticky trap as an IPM technology for controlling specially thrips and jassid in mature tea and nursery area.

Future plan to implement or continue the practice

The management of Amtoli Tea Estate already has made a plan to implement these IPM techniques in their tea estate for the area of 5 hectare of tea plantation. Project Development Unit of Bangladesh Tea Board usually monitors the development activities of 168 tea estates of Bangladesh yearly and suggests the garden authority to use IMP techniques in their tea gardens.

Conclusion and Recommendation

IPM strategy especially solar light trap, yellow trap and bio-control agent i.e. *Bracon hebetor* parasitoid against pests of tea has been successfully evaluated, validated and implemented in tea gardens. Solar power light traps and yellow sticky traps as mechanical control measures have been validated and found to be very effective against moths of looper caterpillar and thrips. This sticky traps worked best for sucking pests and helps in trapping the population of thrips, jassids, white flies and adults of tea mosquito bugs. They are environment-friendly and cost-effective. Bio-control agent, *B. hebetor* as larval parasitoid against looper caterpillar has been validated and found to be satisfactory to control of the larvae of looper caterpillar. These IPM techniques were found very effective for the control of thrips and looper caterpillar which significantly reduced the risk of pesticide and chemical uses on tea, besides improving quality, health and welfare of the environment. IPM promotes sustainable bio-based pest management alternatives in pest management programme in the tea garden. IPM protects non-target species through reduced impact of pest management activities. It reduces or eliminates issues related to pesticide residue in made tea. It also decreases worker, tenant and public exposure to pesticides and leads to safe livelihoods. IPM maintains or increases the cost-effectiveness of a pest management programme. Moreover, if made tea produced from IPM treated field is to be sold in the auction separately, then the planters/managers/small growers would get better price and users would be eagerly interested to use IPM techniques in tea plantation in Bangladesh.

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Appendix I. Some pictorials of validation trial on Solar light trap as IPM technique at different locations.



Appendix II. Some pictorials of validation trial on Yellow sticky trap as IPM technique at different locations.





EFFECTS OF DIFFERENT TYPES OF COMPOST ON YIELD AND YIELD RELATED PARAMETERS OF YOUNG TEA [*Camellia sinensis* (L.) O. Kuntze]

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Abstract

A field experiment was carried out to observe the suitability of alternate sources of organic manure for application in the planting pit during tea plantation. Generally, it is advised to use 2 kg well decomposed cowdung with 30 g TSP and 15 g MOP in the pit. In this experiment, we used three different types of compost as treatment viz. Vermicompost (T₁), Farm yard compost (T₂), Water hyacinth compost (T₃) and Decomposed cowdung as the Control (T₄) along with recommended chemical fertilizers. Growth, development and yield related different parameters were observed and recorded during the first five years after planting. Base diameter, number of branches and fresh weight per plant were not significantly different for treatments at decentering stage but in the successive years these were varied significantly with treatments. At the end of 5th year after planting, highest number of branches found in T₄ (21) and found statistically similar with T₁ (18) and T₃ (18) whereas the lowest was found in T₂ (16). Similarly, base diameter was maximum in T₄ (33.0 mm) which was statistically similar with T₃ (32.9 mm) and T₁ (32.5 mm) but different with T₂ (28.9 mm). However, yield of tea was not significantly different for treatments.

Keywords: Compost, Branch number, Base diameter, Young/Immature tea, Yield

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Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is processed from its tender shoots which is prevalent and healthy beverage because of its excellent value and high palatability (Ma et al., 2019). Tea is consumed worldwide largely for its extensive variety of bioactive compounds, like amino acids, catechins, polyphenols, caffeine, and terpenes (Jiang et al., 2020). There is now a global shift to environmentally safe and economically viable alternatives for good production rather than using inorganic fertilizers. Therefore, to fulfill the upward consumption demand of tea, we need to give emphasize to increase the production within a small area. In developing countries the strategy for improving agricultural production through supplementing nitrogen and phosphorous by microbial processes should be considered. Through applying the biological fertilizers it can be accomplished (Ambachew & Fatih, 2018). Production of tea depends on several factors comprising of those that are inherent in the plant and those exerted on the tea crop by nature including pests and diseases; soil and climatic conditions and human through crop husbandry and cultural managements. In tea plantation fertilizer is one of the major agro-inputs that contribute to the productivity and cost of production. To achieve expected production of tea, a well-balanced fertilization throughout the year is the key contributing factor and it is important for proper maintenance of the health of tea bushes. The combined use of organic and inorganic fertilizers will ensure that the problems associated with the use of either organic or inorganic fertilizers are greatly reduced as the combination of organic and inorganic fertilizers complement each other (Anjaneyulu & Bhattacharjee, 2019).

As a source of nutrients, manure is using from the very beginning of cultivation. All the necessary macronutrients and many micronutrients can be added through the application of manure while increasing organic matter. In soil fertility enrichment and for supplying primary, secondary and micronutrients for crop production application of cowdung, poultry manure and vermicompost may play a vital role. Poultry manure contains high amount of secondary and micronutrients (1.6%N,

potassium 1.85-2.25% and phosphorus 1.55-2.25%), micronutrients, beneficial soil microbes and also contain 'plant growth hormones & enzymes (Sinha et al., 2009). There are some evidences that describes, Vermicompost could act as 'miracle growth promoter & also plant protector' from pests and diseases. While the traditional compost fails to supply the necessary amount of macro and micro nutrients including the vital NKP to plants in shorter time and in that case vermicompost retains nutrients for long time (Sinha et al., 2009). In tea cultivation, use of vermicompost has become popular and it is giving tremendous results in VP nursery and the producers getting their expected outcomes.

Even a decade earlier, tea was an important commodity of international trade for Bangladesh, but during the recent years it had a little share to export due to high domestic consumption. In 2021, total tea production of the country was 96.56 million kg (BTB, 2022) and consumption was also almost the similar. To meet up the internal consumption as well as for increasing the share for export of tea, Bangladesh Tea Board has taken a project in 2018 entitled "Road map for the tea industry of Bangladesh" and it's execution is going on. Main target of the project is to produce 140 million kg made tea by 2025. One of the main tools of the project is to extend and infill the tea area with high yielding varieties which is already initiated. It is also needed to mention here that having cultivable land for tea, extension of 2.5% tea area is mandatory for such types of gardens.

The critical value of organic matter for tea soil in Bangladesh has been fixed at 1% (Alam, 1999). However at present, its actual status is less than that. Leaching and surface run-off during monsoon season might be the prime reasons for poor nitrogen and organic matter content in tea soils of North Sylhet and Chittagong (Sana, 1989). Tea, being a perennial crop is recommended to apply certain amount of organic manures and fertilizers in pit during the time of plantation. Hence during the plantation of tea in Bangladesh, it is advised to mix 2 kg decomposed cowdung, 30 g Triple superphosphate (TSP) and 15 g Muriate of potash (MOP) with top soil for each planting pit (Alam, 2003).

However, sometimes decomposed cowdung is not available in all the tea growing area. As an alternative source of cowdung many tea growers are interested to use different organic manures produced from different origin depending on its availability adjacent to the tea growing area. Hence, the experiment was conducted to know the effects of different composts on young tea. On the basis of availability of organic manures in the growing area, vermicompost, farm-yard compost and compost prepared from water hyacinth were used as treatments in comparison with the control (decomposed cowdung).

Materials and Methods

The experiment was conducted at the Bangladesh Tea Research Institute (BTRI) Main Farm, Srimangal and the area was completely flat (24°29'N, 91°75'E and 29 m amsl). Required data were collected after planting to its formative pruning stage. One year old BT2 clonal tea plants were collected from BTRI Vegetative Propagation (VP) nursery and planted in the main field on September, 2017. In the planting pit, different types of compost were applied as treatments. According to BTRI, 2 kg well decomposed cowdung with 30 g TSP and 15 g MOP per pit during tea plantation showed the better result. However, in this experiment three different composts were used as treatments and cowdung used as control. Vermicompost was collected from vermicompost unit of Soil Science Division, Bangladesh Tea Research Institute and others were prepared at the main farm of BTRI. Randomized Complete Block Design was followed to conduct the experiment with four replications. It was double hedge plantation system and spacing was 60cm × 60cm × 120cm. In each plot there were 20 BT2 clonal tea plants.

The experiment continued from 2017 to 2022 that means from planting to frame formation pruning. During this period, all the intercultural operations including manuring, pruning, tipping, disease and pest management, etc. were continued according to BTRI recommendation (Alam, 2003) which were similar for each treatment. During the entire period of experiment, data collected regularly. During the decentering in the 1st week of May 2018, base diameters of main stems were recorded. Number of branches emerged as primaries after decentering were also recorded at the end of 2018. First frame

formation pruning (FFP-1) accomplished in the experimental field at the last week of December 2018. Required data related to agronomy like harvested green leaf yield, branch number etc. were collected in 2019. At the beginning of 2020 tea plants of the experiment were pruned as skiff condition and after attaining the tipping height (24 inches) green leaf as yield from each plot was collected. At the end 2020, Second frame formation pruning (FFP-2) was done for fourth year of plants age at 18 inches height from ground. After pruning, number of branches and base diameter of tea plants were recorded for every treatment. After attaining the tipping height (28 inches) and during that year (2021) green leaf from each plot was harvested. Age of tea plants was five and it was the final year as young tea. Hence at the beginning of 2022, tea plants of the experiment were pruned as skiff condition at 28 inches and after attaining the tipping height (30 inches) and throughout the year green leaf from each plot was collected. At the end of the season, first light pruning (LP) was done at 22 inches height of tea plants. Then number of branches and base diameter of tea plants were recorded for every treatment. Collected growth parameters and yield data were analyzed properly.

Results and Discussion

After plantation, decentering was done at the age of 8 months of tea plants. During the pruning and few weeks after the pruning, data were collected on different growth parameters viz. base diameter, fresh weight of the plant and number of branches emerged after pruning per plant, were observed non-significant for treatments at $p=0.05$ (Table 1).

Table 1. Effect on different growth parameters after decentering.

Treatments	Base diameter (mm)	Plant fresh weight (g)	No. of branches per plant
T ₁ (Vermicompost)	8.5	54.25	6
T ₂ (Farm-yard compost)	9.5	50.75	6
T ₃ (Water hyacinth compost)	8.5	53.25	7
T ₄ (Decomposed cowdung, Control)	9.0	51.75	7
LSD (P=0.05)	2.5	13.25	2.0
CV (%)	18	16	20

At the end of 2nd year after plantation FFP-1 was done and different morphological data especially on growth parameters were collected during the time of pruning and few weeks after the pruning. Base diameter of plant found significantly different for treatment at $p=0.05$ while the number of branches per plant was showed non-significant effect (Table 2). Both the base diameter and number of branches per plant were minimum in T₂ which was treated with Farm-yard compost.

Table 2. Effect on different growth parameters at the time of FFP1.

Treatments	Base diameter (mm)	No. of branches per plant
T ₁ (Vermicompost)	18.9ab	7.3b
T ₂ (Farm-yard compost)	16.6b	7.0b
T ₃ (Water hyacinth compost)	18.3ab	8.0ab
T ₄ (Decomposed cowdung, Control)	19.1a	9.0a
LSD (P=0.05)	2.37	1.6
CV (%)	11	17

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

During the successive years (3rd and 4th year of young tea plants), pruning was done following BTRI recommendation (Alam, 2003) and data were collected regularly. At this stage, collected data particularly the yield was collected and found non-significant for treatments. At the final year of the experiment i.e. 5th year of young mature tea plants, data were collected on harvested green leaf yield which was converted into made tea yield with 23% recovery (De Costa et al., 2007). Yield was not

significant for treatments at $p=0.05$ (Figure 1). However, number of branches per plant and base diameter of the main stem (collar zone) were found significantly different for treatments after the formative pruning. Maximum number of branches per plant and base diameter were observed in T_4 which was similar with T_3 and T_1 but different with T_2 (Table 3).

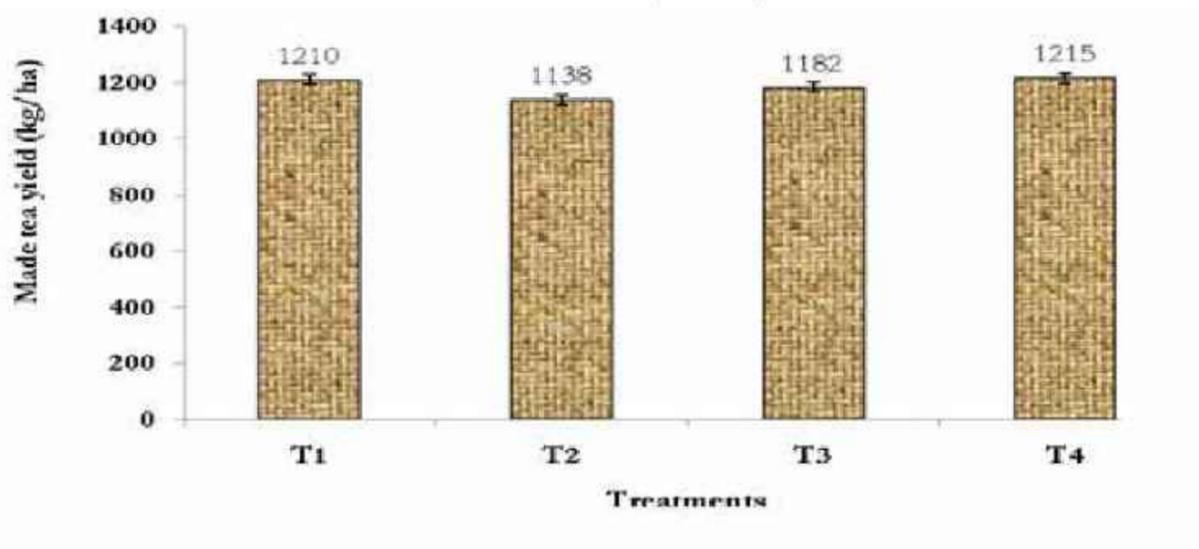


Figure 1. Made tea (kg ha^{-1}) under different treatments at fifth year.

Table 3. Effect on number of branches and base diameter at fifth year (after first light pruning)

Treatments	Base diameter (mm)	No. of branches per plant
T ₁ (Vermicompost)	32.5ab	18ab
T ₂ (Farm-yard compost)	28.9b	16b
T ₃ (Water hyacinth compost)	32.9a	18ab
T ₄ (Decomposed cowdung, Control)	33.0a	21a
LSD ($P=0.05$)	3.7	3
CV (%)	10	15

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

Conclusion

Findings of present study showed that apart from decomposed cowdung, compost produced from different organic sources can be applied in pit during the plantation. Particularly in the areas where prevailing scarcity of cowdung, vermicompost and compost produced from water hyacinth can be used extensively during the plantation of tea in addition with recommended doses of chemical fertilizers.

Further research direction

Similar study can be continued in collaboration with Soil Science Division to observe the soil characteristics, soil microbe status, etc. before and after the experiment.

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DETERMINATION OF RESIDUES OF TWO ORGANOPHOSPHATES AND THEIR DISSIPATION PATTERN BY QuEChERS METHOD

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Mainuddin Ahmed⁴ and Md. Ismail Hossain⁵

Abstract

An experiment was carried out at the main farm of Bangladesh Tea Research Institute (BTRI) to find the residue levels of commonly used two organophosphate insecticides, namely chlorpyrifos and quinalphos in green leaf and black tea, at various plucking intervals after spraying during 2017-2018. The safe harvest interval was also calculated based on the degradation of pesticide residue from green leaf to processed tea. Pesticides were applied in the experimental plots following BTRI recommended dose (Chlorpyrifos 48 EC @ 1.0 L ha⁻¹ and Quinalphos 25 EC @ 0.5 L ha⁻¹). Green leaf and black tea samples were analyzed for pesticide residue of the above insecticides at the pesticide analytical laboratory (ISO/IEC 17025 accredited lab) of Bangladesh Agricultural Research Institute (BARI). The samples were extracted and cleaned up using the QuEChERS extraction method. Flame Thermionic Detector (FTD) with ATTM-1 capillary column (30 m x 0.25 mm x 0.25 μm) was used to determine the pesticide residue. The initial (0 days) pesticide residue of chlorpyrifos was 7.822 mg kg⁻¹ in green leaf and 0.893 mg kg⁻¹ in black tea, respectively. These were reduced to an undetectable level in the green leaf on the 10th day and 0.013 mg kg⁻¹ in the black tea on the 7th day. On the other hand, quinalphos residue levels were initially (0 day) detected in green leaf and black tea at 4.973 and 0.193 mg kg⁻¹, respectively. It decreased to undetectable levels on the 5 and 10 days in both cases. The result showed that pesticide residue decreased considerably in the process stage. Chlorpyrifos and quinalphos had pesticide residue degradation rates ranges from 70.70-92.07% and 94.21-96.12%, respectively.

Keywords: Chlorpyrifos, Quinalphos, Residue, Tea, QuEChERS, MRL

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Introduction

Tea is the most consumed non-alcoholic beverage after water worldwide. It is made from the tender shoots of *Camellia sinensis* (L) O. Kuntze. About 6,455.19 million kg of tea is produced annually in over 50 countries worldwide (ITC, 2022). Tea provides Bangladesh's entire domestic demand, which is of immense economic significance. Bangladesh is ranked 9th in terms of production (ITC, 2022).

The tea plant is subjected to the attack of pests and diseases. If appropriate management measures are not done in time, numerous pests, especially insects, mites, and nematodes, could cause the loss of about 15% of its crop (Ahmed, 2005; Mamun, 2022). A variety of pests can damage any part of the tea plant. Pests, including termites, thrips, jassids, aphids, mites, and tea mosquito bugs, thrive in the prevailing warm climate (Sana, 1989; Ahmed, 2005). To keep these insect populations below the economic threshold level, it is crucial to implement an Integrated Pest Management strategy that includes pesticide application (Mamun & Ahmed, 2011; Mamun, 2022). It is well known that the chemical management of pests benefits tea-producing countries around the world. Hence, chemical pest management is a key aspect of Bangladesh tea production (Alam, 1999). Since 1960, many groups of pesticides, including Organophosphate, Pyrethroids, Carbamates, Avermectin, Neonicotinoid, and other unidentified groups, have been applied in tea fields to tackle these pest problems (Ahmed et al., 2009a; Ahmed et al., 2009b; Mamun, 2019; Ali et al., 2021). It is impossible to completely avoid using pesticides in tea cultivation because of the complex pest condition. Therefore, the fixation of Maximum Residue Level (MRL) of pesticides is essential. However, the use of pesticides on food crops could leave toxic residues in those finished products. Pesticides should be used correctly following Pre Harvest Interval (PHI) to avoid harmful residues.

Food safety, i.e., pesticide residue, is a burning issue worldwide. Pesticide residue in processed tea refers to what is left over or still present in the processed tea after a pesticide has been applied to the tea bushes in the field before harvesting. The pesticide residue in made tea harms human health as tea is a beverage (Dharmadi, 2007; Zhongmao, 2013). Bangladesh exports tea to several countries, primarily West and East European as well as Middle Eastern countries. The awareness of pesticide residue among consumers in these countries has increased. Hence, the EPA, the Codex Commission, the FAO, the WHO, the EU, German law, etc., have imposed restrictions on the production and purchase of tea that contains pesticide residue (Ahmed, 2007; Ahmed et al., 2010; Ahmed & Mamun, 2010; Ahmed & Mamun, 2012). Bangladesh is one of the signatories to the FAO code of conduct on the distribution and use of pesticides. Bangladesh is working to implement the role of pesticides in IPM, particularly as defined in the FAO code, for ensuring usage as low-volume crop protection agents to ensure a friendly environment. In addition, the government has already banned some highly dangerous chemicals.

Chlorpyrifos (O, O-diethyl O-[3,5,6-trichloro-2-pyridyl] phosphorothioate) is a chlorinated organophosphate insecticide with a broad spectrum of activity against different foliar and soil-borne insects. Plants may take up chlorpyrifos through leaf surfaces; however, most applied chlorpyrifos is often lost through volatilization, and very little is translocated within the plant. Chlorpyrifos has documented half-lives of between 7 and 120 days and is stable in soils. A higher pH of the soil makes chlorpyrifos less persistent. Chlorpyrifos is most likely to be lost through volatilization from water, with half-lives of between 3.5 and 20 days predicted for pond water. Temperature and alkalinity both speed up the process of hydrolysis.

On the other hand, quinalphos (O, O-diethyl-O-[2-quinoxaliny] phosphorothioate) is one of the most widely used organophosphorus insecticides in the world (Hu et al., 2010). It is categorized as a 'moderately hazardous' insecticide by the World Health Organization (WHO), and hence, most countries either ban or limit the use of quinalphos (Gangireddygaru et al., 2017). It is the most widely used pesticide for combating rice pests in India. Due to its widespread usage in agriculture, ease of availability, and farmers' lack of awareness of its negative consequences, the danger of quinalphos exposure to humans is particularly high in developing countries (Kumar et al., 2010; Singh & Khurana, 2009). Two-thirds of the applied insecticides evaporate after 14 days, while the leaf surface absorbs one-third and penetrates the plants. It breaks down quickly in the soil in aerobic conditions.

Chlorpyrifos and quinalphos work against insect pests by direct contact and ingestion. These pesticides are used to control leaf-sucking (tea mosquito bug, aphid, Jassid, and thrips), leaf-eating (looper caterpillar), and root feeder (termite) pests in Bangladesh tea. Judicious use of insecticides has the least harmful impact on biocontrol agents, ensuring the eco-friendly management of insect pests in tea plantations. Considering the above facts, a study was undertaken to determine the residues with PHI of widely used two organophosphate insecticides (Chlorpyrifos 48 EC and Quinalphos 25 EC) in Bangladesh tea.

Materials and Methods

The experiment on chlorpyrifos (Gola 48 EC) and quinalphos (Quicklac 25 EC) was conducted on the mature tea at BTRI main farm during 2017-18 to determine residue and fix the safe harvest interval (PHI). The experiment was laid out in RCBD with three replications. Chlorpyrifos and quinalphos were applied in all the experimental plots at the BTRI-approved dose of 1.0 L and 0.5 L per ha in 500 L of water.

Sampling

Green leaf samples weighing about 3 kg were taken from plots treated with chlorpyrifos and quinalphos at 0, 1, 3, 5, 7, and 10 days following pesticide application (FAO, 2009). After applying the insecticide, leaf samples for the first day (0 days) were taken 3 hours later, and samples for the remaining days were taken at the respective intervals. Each sample had at least 70% of two leaves and a bud. For leaf analysis, 250 g of green leaves were taken from each plucking and preserved at -20°C in the laboratory. In addition, black tea was made in the Miniature Tea Factory (MTF) of the Institute following CTC (Crush, Tear &

Curl) process using 2.0 kg green leaves. Withering, rolling, oxidation (fermentation), and drying were followed in the manufacturing process. Before spraying, leaves from the experimental plots were collected for the blank sample, then processed in the MTF using the same procedure.

Extraction and Clean up

The pesticide residue from green leaf and black tea samples was determined at the pesticide analytical laboratory (ISO/IEC 17025 accredited lab) of BARI, Gazipur. The QuEChERS extraction method was used to extract and clean up samples, modified by Prodhon et al. (2015).

The blender was used to grind the samples finely. Ten-gram sample was taken in a 50 mL polypropylene centrifuge tube. Acetonitrile (MeCN) of 10 ml was then added to the centrifuge tube. A vortex mixer was used to agitate the centrifuge tube for 30 seconds after it had been correctly closed. The centrifuge tube was filled with 1 g of NaCl and 4 g of anhydrous MgSO₄. The vortex mixer rapidly shook it for 1 minute to avoid the development of magnesium sulfate aggregates.

The extract was then centrifuged at 5000 rpm for 5 minutes. A 15 mL microcentrifuge tube containing 600 mg anhydrous MgSO₄, 150 mg charcoal, and 120 mg Primary Secondary Amine (PSA) was filled with an aliquot of 3 mL of the MeCN layer. The mixture was then vigorously mixed for 30 seconds in a vortex and centrifuged for 5 minutes at 4000 rpm (Laboratory Centrifuges, Sigma-3K30, Germany). A clean GC vial was used to transfer a 1 ml supernatant that had been centrifuged and filtered through a 0.2 m PTFE filter.

Detection and quantification residues of chlorpyrifos and quinalphos

GC-2010 (Shimadzu) machine was selected to analyze the concentrated extracts using an ATTM-1 column (30 m x 0.25 mm x 0.25 µm) with a Flame Thermionic Detector (FTD). Both the carrier gas and the make-up gas were helium. Sigma-Aldrich Laborchemikalien, Seelze, Germany, provided the standard for chlorpyrifos and quinalphos via S. F. Scientific Pvt. Ltd., Dhaka, Bangladesh. The purity of the standards of these insecticides was 99.6 percent. The purity of formulated insecticide was also examined and determined to be 100% pure. Standard solutions of various concentrations of chlorpyrifos and quinalphos were prepared before the injection of the sample extract, and each was injected with the aforementioned instrument parameters individually. The samples' retention duration, peak area, and other measurements were calibrated using a five-point calibration curve of the standard insecticide solution. The retention time of each peak served as an identification of that insecticide. The GC program automatically expressed sample results in mg kg⁻¹, which indicated the concentration of the final volume injected. This value was used to calculate the actual amount of chlorpyrifos and quinalphos residue in the sample using the formula below:

$$\text{Residue in the sample (mg kg}^{-1}\text{)} = \frac{\text{Conc. obtained in injected volume (mg kg}^{-1}\text{)} \times \text{Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

Results and Discussion

Pesticide residue of chlorpyrifos and quinalphos in green leaf and made tea

Tables 1 and 2 provide a summary of the analytical findings for the residues of chlorpyrifos and quinalphos detected in green tea leaf and made tea samples. Chlorpyrifos was found in green tea leaves up to 7 DAS, and the amounts were above MRL. At 0, 1, 3, and 5 DAS, the quantities of chlorpyrifos residue were 7.822 mg kg⁻¹, 1.975 mg kg⁻¹, 0.645 mg kg⁻¹, 0.446 mg kg⁻¹, and 0.164 mg kg⁻¹, respectively (Table 1). On the other hand, chlorpyrifos was found in made tea up to 7 DAS, and the amounts were above MRL up to 5 DAS. At 0, 1, 3, and 5 DAS, the quantities of chlorpyrifos residue were 0.893 mg kg⁻¹, 0.531 mg kg⁻¹, 0.189 mg kg⁻¹, and 0.107 mg kg⁻¹, respectively (Table 1). In addition, the sample of 7 DAS had a residue concentration of 0.013 mg kg⁻¹, which was below the MRL established by the EU.

Table 1. The residue level of chlorpyrifos (mg kg^{-1}) in green leaf and made tea.

Days after spraying (DAS)	Sample weight (g)	Injected volume (μL)	Amount of residue in green leaf (mg kg^{-1})	Amount of residue in made tea (mg kg^{-1})	EU MRL (mg kg^{-1})
0	10	1	7.822	0.893	0.1
1	10	1	1.975	0.531	
3	10	1	0.645	0.189	
5	10	1	0.446	0.107	
7	10	1	0.164	0.013	
10	10	1	ND	ND	

ND=Not Detectable

The result showed that quinalphos residue might be found in green tea leaves up to 7 DAS. At 0, 1, 3, and 5 DAS, the residue amounts were 4.973 mg kg^{-1} , 1.364 mg kg^{-1} , 0.64 mg kg^{-1} , and 0.058 mg kg^{-1} , respectively and all these amounts were over the MRL up to 5 DAS (Table 2). The amount of residue 0.022 mg kg^{-1} in the sample of 7 DAS was less than the MRL (0.05) established by the EU. At 10 DAS, no residue was detected. Therefore, 7 DAS, might be chosen as the PHI of quinalphos for green tea leaves. The findings showed that made tea samples contained quinalphos residue up to 3 DAS. The residue amounts were 0.193 mg kg^{-1} and 0.079 mg kg^{-1} at 0 and 1 DAS, respectively, and were above MRL up to 1 DAS (Table 2). The amount of residue 0.025 mg kg^{-1} in the sample from the 3 DAS was less than the MRL (0.05) established by the EU. At 5 and 7 DAS, no residue was found.

Table 2. The residue level of quinalphos (mg kg^{-1}) in green leaf and made tea.

Days after spraying (DAS)	Sample weight (g)	Injected volume (μL)	Amount of residue (mg kg^{-1}) in green leaf	Amount of residue (mg kg^{-1}) in made tea	EU MRL (mg kg^{-1})
0	10	1	4.973	0.193	0.05
1	10	1	1.364	0.079	
3	10	1	0.64	0.025	
5	10	1	0.058	ND	
7	10	1	0.022	ND	
10	10	1	ND	ND	

ND=Not Detectable

Degradation of pesticide residue from green leaf to black tea

In both green and black tea, the pesticide residue assessed degraded over repeated picking intervals (Fig. 1-2). Even seven days after spraying, it was observed that the pesticide residue on green leaves was higher than the MRL. However, compared to green leaf, the residue is substantially lower in black tea. Thus, manufacturing plays a significant role in lowering the pesticide content of black tea produced from green leaves. The levels of selected pesticide residues in black tea dropped below MRL within 7 days. The degradation rate of chlorpyrifos and quinalphos residues varies from 70.70-92.07% and 94.21-96.12%, respectively (Table 3).

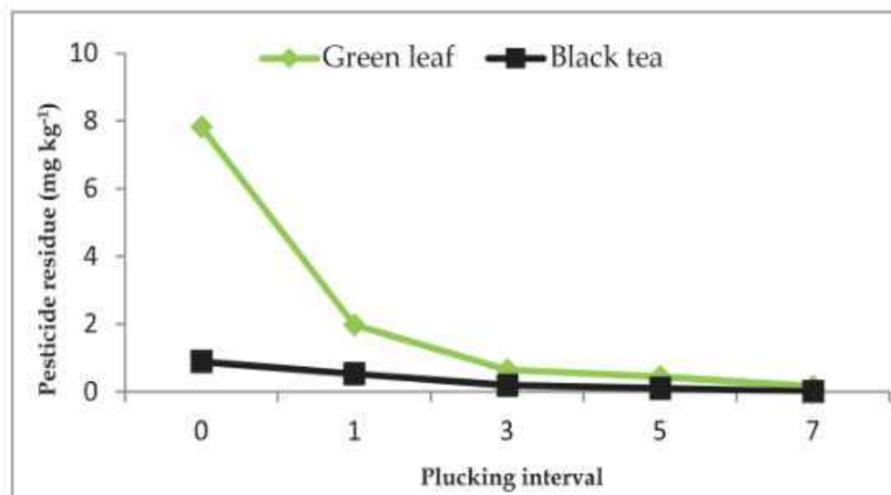


Fig. 1. Degradation of chlorpyrifos residue in green leaf and black tea at different plucking intervals.

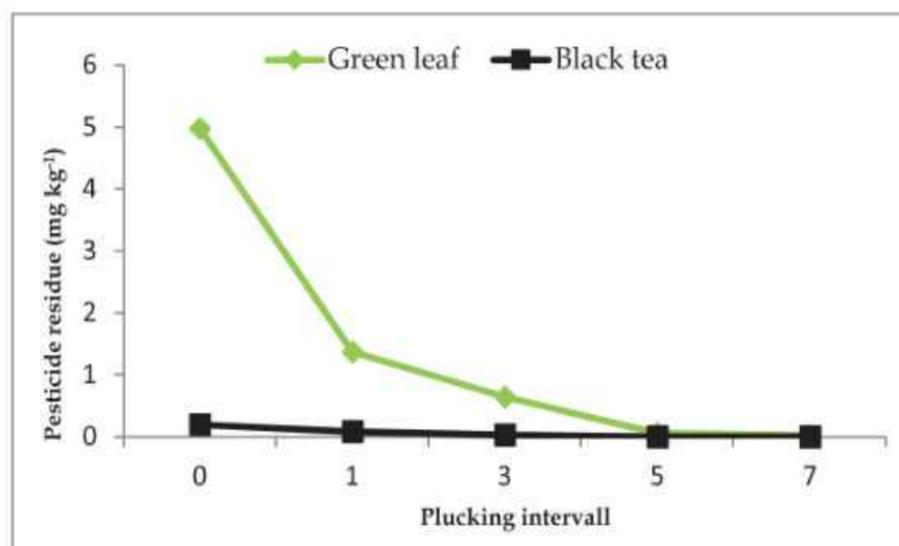


Fig. 2. Degradation of quinalphos residue in green leaf and black tea at different plucking intervals.

Table 3. Percent degradation of pesticide residue from green leaf to black tea at different plucking intervals.

Days after spraying (DAS)	Chlorpyrifos			Quinalphos		
	Green leaf	Black tea	% degradation	Green leaf	Black tea	% degradation
0	7.822	0.893	88.58	4.973	0.193	96.12
1	1.975	0.531	73.11	1.364	0.079	94.21
3	0.645	0.189	70.70	0.640	0.025	96.09
5	0.446	0.107	76.01	0.058	ND	-
7	0.164	0.013	92.07	0.022	ND	-
10	ND	ND	-	ND	ND	-

Safe harvest interval

It is normal to harvest tea leaves at various intervals following regrowth from earlier plucking. According to the aforementioned studies, the residue content increases with closer harvest intervals, while residue levels decrease with wider harvest intervals (Table 4).

Table 4. Safe harvest intervals for the tested pesticides in Bangladesh tea.

Chemical	Dose ha ¹	Residue level* (mg kg ⁻¹)	Safe harvest interval (days)
Chlorpyrifos	1.0 lit.	0.013	7
Quinalphos	0.5 lit.	ND	7

* Black tea at 7 PHI (Pre Harvest Interval)

Table 5 compares the residue levels of the selected pesticides in Bangladeshi tea to those of several international organizations. Seven days after picking, the residue levels of the aforementioned pesticides are below the MRL established by the EPA, Codex Commission/FAO, EU, Japan, and German Law.

Table 5. Residue level of two commonly used organophosphate pesticides in Bangladesh tea compared to the international organizations.

Pesticides	MRL (mg kg ⁻¹) fixed by different organizations					
	EU	FAO/WHO	EPA	German Law	Japan	Bangladesh
Chlorpyriphos	0.1	0.1	-	0.1	3.0	0.013
Quinalphos	0.05	-	-	0.1	0.1	ND

Discussion

According to the results of [Seenivasan and Muraleedharan \(2011\)](#), [Bishnu et al. \(2009\)](#), [Amaraweera & Wickramasinghe \(2019\)](#), and [Amirahmadi et al. \(2013\)](#), none of the pesticides tested in the current study do not exceed the maximum residue levels specified by the EU, FAO/WHO, EPA, German Law, and Japan. In a three-year, extensive survey conducted by [Seenivasan & Muraleedharan \(2011\)](#), 912 samples of tea produced in south Indian factories were examined for pesticide residues from various pesticide classes, including organochlorine, organophosphate, and synthetic pyrethroid. Only 0.5 percent of the samples had traces of the targeted insecticides, according to the laboratory results. The residues in the remaining samples were within the upper limits for tea established by the EU, FAO/WHO, and the Prevention of Food Adulteration Act of the Indian government. Chlorpyrifos, cypermethrin, deltamethrin, ethion, heptachlor, dicofol, and endosulfan residue levels in tea plantations of the Dooars regions of West Bengal, India, were quantified by [Bishnu et al. \(2009\)](#). They reported that the consumption of tea that contains the residues of banned pesticides, such as heptachlor, may be harmful to the consumer's health.

[Amaraweera & Wickramasinghe \(2019\)](#) analyzed 15 tea samples from the leading tea exporters in Sri Lanka using five black tea grades (OP, BOP, FBOP, BOPF, and PEKOE). Some of the samples that were examined contained detectable pesticide residues. However, no samples went beyond the upper limits for residue set by Codex, the EU, and Japan. [Amirahmadi et al. \(2013\)](#) observed pesticide residue of tea in a market in Tehran. They examined 53 samples from several pesticide classes including synthetic pyrethroid, organochlorine, and organophosphate. Out of 53 samples, 15 (28.3%) were found to be contaminated with pesticides. None of the samples showed contamination over the maximum residue limit established by the EU and India.

In the current study, it was observed that the pesticide residues of chlorpyrifos and quinalfos gradually declined in following plucking intervals after spraying. Black tea made from the corresponding green leaf had far less pesticide residue. Therefore, tea production has a higher impact on lowering pesticide residues. [Sood et al. \(2004\)](#) reported a similar result. They also mentioned that the natural processes of rain, dew, evaporation, photolysis, biodegradation, growth dilution, and the interval between the application of pesticides and the harvesting of the tea leaves caused the pesticides sprayed on tea bushes to deteriorate. During manufacture, insecticides in the green leaves deteriorate as a result of evaporation and heat degradation. When making tea, mostly drying, [Chen & Haibin \(1988\)](#) observed a 30–60% reduction in pesticide residue in tea during manufacturing process.

The amount of pesticide residue, quality, and tea yield are all affected by the time between the last application and harvest of the crop (PHI). According to GAP (Good Agricultural Practice), PHI is 7 days (Chaudhuri, 2008). Following the approved PHI, the maximum residue level (MRL) in made tea is taken into consideration.

Conclusion and Recommendations

To circumvent non-tariff trade obstacles under World Trade Organization (WTO) regulations, all necessary steps must be taken for this export-oriented item to maintain residue levels below the MRL. According to FAO recommendations, other criteria of residue-level such as the No-observed-Adverse Effect Level (NOAEL), Acceptable Daily Intake (ADI), Food Factor, and Maximum Permissible Intake (MPI) should be taken into account. These requirements make it possible to strictly adhere to the MRL and PHI in order to gain access to the global market. Therefore, the scientific method should be consistent in its practical and regular application for the sake of the tea sector. The GAP states that the PHI is 7 days. The approved PHI is used to fix the MRL in made tea. The study revealed that 7 day PHI may be suggested for the aforementioned pesticides in Bangladeshi tea. When necessary, BTRI-approved insecticides with prescribed dosage should be used.

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ASSESSING BIOCHEMICAL AND SENSORY CHANGES OF TEA DURING STANDARDIZATION OF A MODERN GREEN TEA PROCESSING UNIT FOR MANUFACTURING QUALITY GREEN TEA

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Abstract

A programmable logic controller (PLC) based modern green tea factory was recently launched at Bangladesh Tea Research Institute to meet the demand of some good quality green tea for the people of the country and also to strengthen research in this area to support the tea industry. This necessitated to carry out research with this processing unit to standardize the manufacturing technique for the production of quality green tea. Different parameters were changed during processing according to the treatments and the product green tea samples were analyzed biochemically for the determination of quality. From the results, it was observed that total polyphenols and caffeine contents decreased significantly with the increase of withering period from 1 hour to 3 hours. If the fixation temperature increased from 250°C to 270°C, the caffeine content almost remained unchanged but the total polyphenols content increased, though not significantly. The total polyphenols and caffeine contents increased as the rolling time increased from 45 minutes to 55 minutes. Immediate after rolling, application of high temperature to dry the leaves was found detrimental for the final green tea quality and thus lowering the roll-drying temperature from 220°C to 200°C significantly enhanced the quality. It was observed that after cooling the roll-dried leaves, increase of temperature in the dryer from 100°C to 120°C enhanced tea quality. From the findings of the present study, it can be suggested that 1 hour Withering → Fixation at 270°C → 55 min Rolling → Roll Drying at 200°C → Final Drying at 120°C can be set as a common procedure for manufacturing quality green tea using the newly established green tea factory of Bangladesh Tea Research Institute.

Keywords: Green tea, Processing, Polyphenols, Caffeine, Sensory

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Introduction

Green tea is a popular non-alcoholic beverage that originated in China and is now consumed all over the world. The young tender leaves of the plant *Camellia sinensis* are used to make green tea. Green tea contains higher quantities of bioactive compounds than other types of teas (Oliveira, 2012). It provides a high concentration of antioxidants called polyphenols (Amin et al., 2023). The growing health awareness level among the households has led to the increasing popularity of green tea in recent times (Adhikary et al., 2023). Green tea is widely recognized for its many health benefits, such as boosting metabolism, improved digestion, decrease type II diabetes, immune system enhancement, enhancing mental alertness, reducing the risk of cardiovascular diseases, and lowering the risk of certain types of cancer (Chacko et al., 2010). Most of these beneficial effects of green tea are mainly attributed to the presence of high contents of polyphenols (Hsu et al., 2011).

Green tea is a nonoxidized tea that is produced by steaming or pan-firing the fresh leaves to inactivate the enzyme polyphenol oxidase which is responsible for the oxidation of tea polyphenols and cause the formation of the brown or black color (Tadesse et al., 2015). Different methods of processing are used for producing green tea. After plucking, according to Chinese tradition, a small amount of indoor withering is permitted to remove the moisture from the leaves but in Japanese style no withering is done. Withering allows to develop some flavor compounds in tea (Singh et al., 2014). After withering, the leaves are promptly steamed or pan-fired to inactivate the enzymes responsible for breaking down the color pigments in the leaves and thus this process preserves the tea's green colour during the subsequent rolling and drying operations. Rolling is also an important process in

green tea manufacturing. The objective of rolling is to sufficiently break the cell walls of the tea leaves so that some interior components can interact with the air. This process increases flavor and aroma. Additionally, rolling controls the release of natural substances and flavor during steeping in the cup. Rolling also allows the leaves to be shaped (Anonymous, 2020). After rolling is completed, the leaves are dried by gently applying heat that reduces the moisture content of the tea leaves to less than 5%. This drying process finalizes the tea, by locking in fragrance and flavor, and enables the leaves to be stored at room temperature. In green tea processing, only minor transformation takes place during the fixing, rolling and drying steps and therefore the majority of biochemical constituents that are present in green leaf are retained in the finished product (Bortolini et al., 2021). Thus, the processing technology of green tea allows to preserve natural polyphenols with respect to the health-promoting properties (Chacko et al., 2010). In addition to polyphenols, green tea also contains the stimulating agent caffeine which is attributed to a reduction in mental fatigue (Joseph et al., 2011).

Bangladesh is an important tea producing country. However, majority of the tea planters of Bangladesh are still unfamiliar about the standard green tea processing technique. Due to lack of proper knowledge about green tea processing, planters are not being able to produce green tea; and those who are trying to produce some, are not able to produce good quality green tea. As the demand for domestically produced green tea has shown an upward trend, the Bangladesh Tea Board has recently launched a programmable logic controller (PLC) based modern green tea factory at Bangladesh Tea Research Institute (BTRI) not only to meet the demand of some good quality green tea for the people of the country but also to strengthen research in this area to support the industry. Therefore, the present study was carried out to standardize the green tea processing technique through the newly launched green tea production unit by assessing the biochemical and sensory properties of the final product. The objectives of the study were - (i) to observe the effect on green tea quality by changing different parameters at the critical stages of processing, and (ii) to establish a standard procedure of production of quality green tea through this processing unit.

Materials and Methods

Experimental site

The site of the study was the newly launched green tea factory of BTRI, Srimangal, Moulvibazar.

Description of the newly established green tea processing unit

The whole processing unit was purchased from the Yaojiangyuan Mechanics Co., Ltd., Ningbo, Zhejiang, China. The machineries of the unit are controlled by PLC (programmable logic controller). The main processing stages in this unit are Withering → Fixation → 1st Re-humidification → Rolling → Roll Drying → 2nd Re-humidification → Final Drying. The machineries used for these stages are shown in the Figure 1.

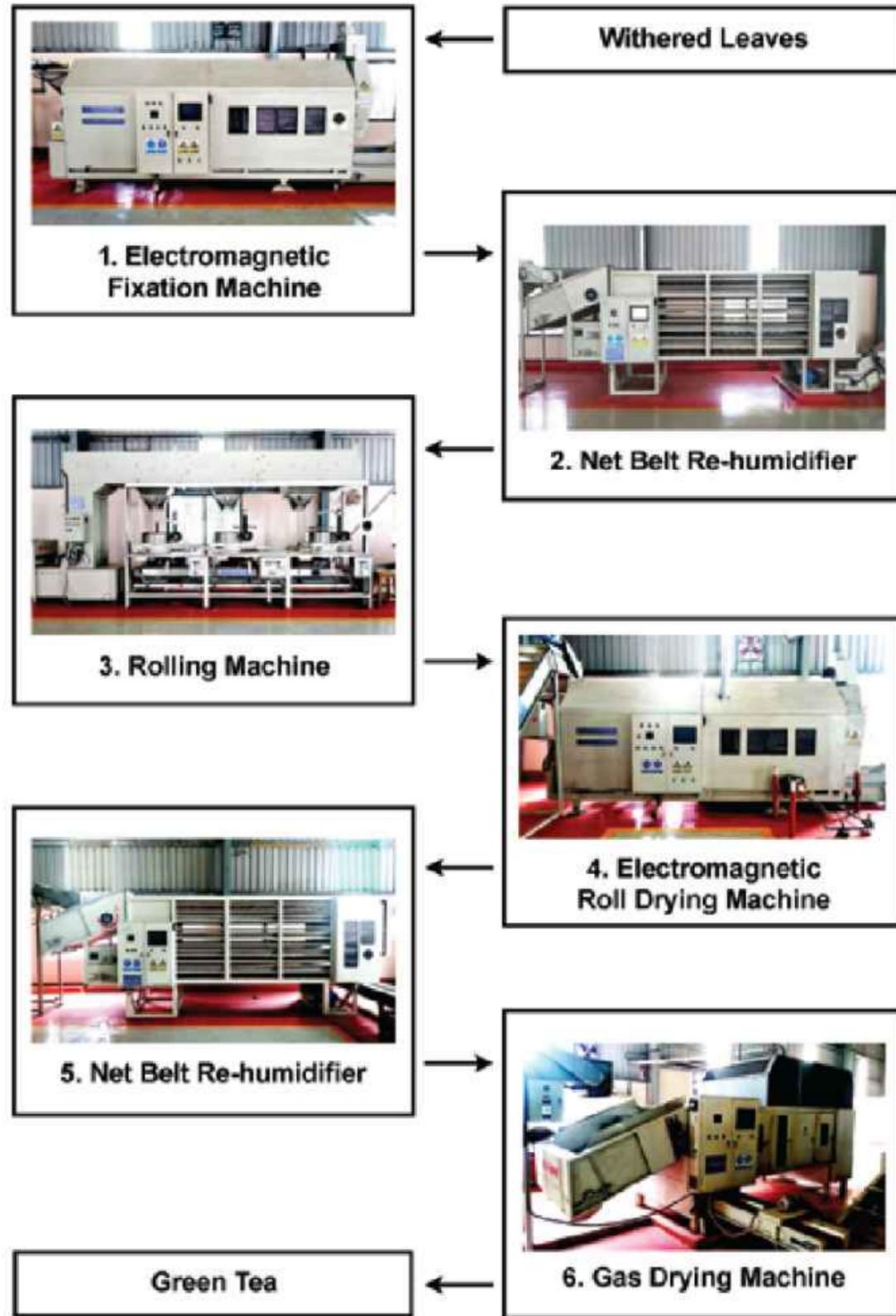


Fig. 1. Flow diagram of green tea manufacturing (sequentially the main machineries used) at the BTRI green tea factory.

For the processing of green tea using this unit, the fresh tea leaves are first withered for a certain period over a tarpaulin laying on the floor and blowing air over the leaves by fans. When withering is completed, the leaves are then ready to go for fixation to kill the enzymes of the leaves. Fixation is done by the Electromagnetic Fixation Machine. After fixation, the 1st Re-humidification is done by the 1st Net Belt Re-humidifier for cooling and softening the leaves. The softened leaves are then transferred to the Rolling Machine to roll the leaves for rupturing the cells of the leaves. After rolling, the leaves are roll dried in the Electromagnetic Roll Drying Machine. The roll dried leaves are then transferred to the 2nd Net Belt Re-humidifier for 2nd Re-humidification. Finally, the leaves are sent to the Gas Drying Machine for final drying.

Green leaf quality and tea manufacturing

Young tea shoots, mostly comprising of single leaf and a bud and two leaves and a bud, were plucked from the BTRI Main Farm and Bilashcherra Experimental Farm of BTRI for all of the processing trials carried out during July to September 2021. Freshly plucked green leaves were then immediately transferred to the factory for manufacturing. Among the processing stages of the newly launched processing unit, five stages i.e. withering, fixation, rolling, roll-drying and final drying were identified as the critical stages of manufacturing that can affect the final green tea quality. Therefore, the tentative relevant time, temperature and duration for withering, fixation, rolling, roll-drying and final drying were pre-fixed based on the manufacturer's instruction and by few processing trials with green leaves and assessing the quality characteristics of processed green teas. Then different treatments were set for all of the mentioned critical stages; and thus, there were five sets of treatments. When all are set, starting from the withering stage, different treatments were employed one by one. When the treatments of a specific stage were employed, the parameters of other stages were remained unchanged to get the treatment effect of that particular stage only. All of the mentioned processing trials were conducted in a similar environment.

In brief, according to the treatments, the fresh tea leaves were first withered for 1-3 hours and then passed through the fixation machine at 250-270°C (for five minutes). After cooling the fixed leaves through the 1st Net Belt Re-humidifier, they were transferred to the rolling machine. The leaves were then rolled in the roller for 45-55 minutes. After rolling, the leaves were discharged from the roller and the balls or lumps developed during the rolling process were broken with hands to separate the shoots. Then the leaves were passed through the electromagnetic roll drying machine at 200-220°C (for five minutes) to partially dry the leaves. After cooling the roll-dried leaves through the 2nd Net Belt Re-humidifier, they were transferred to the final drying machine. In the drying machine, the leaves were dried at 100-120°C, for 17 minutes. After cooling the dried green tea samples, they were packed in airtight bags. The five sets of treatments used in this study are summarized below-

- a) Withering periods-
 - W1 : 1 hour
 - W2 : 2 hours
 - W3 : 3 hours
- b) Fixation temperatures-
 - F1 : 250 °C
 - F2 : 260 °C
 - F3 : 270 °C
- c) Rolling periods-
 - R1 : 45 minutes
 - R2 : 50 minutes
 - R3 : 55 minutes
- d) Roll drying temperatures-
 - RD1 : 200 °C
 - RD2 : 210 °C
 - RD3 : 220 °C
- e) Final drying temperatures-
 - FD1 : 100 °C
 - FD2 : 110 °C
 - FD3 : 120 °C

Data collection

Only one set of treatments were employed during manufacturing in a single processing day due to the limited amount of raw materials. Different treatments were employed one by one and the product green tea samples were collected and preserved in the refrigerator within airtight bags for further biochemical and sensory analysis. The samples were biochemically analyzed in the biochemistry lab of BTRI. Sensory (organoleptic taste) assessment was done in the miniature tea factory and tea tasting room of BTRI.

Chemicals and reagent

Folin-ciocalteu's reagent (FCR), Sodium carbonate anhydrous pure (99.5%), Gallic acid (98%) and Methanol HPLC grade (99.8%) used in this study were purchased from the Sisco Research Laboratories Ltd., India.

Biochemical analyses

The polyphenols and caffeine are the most important compounds present in tea which are of considerable pharmacological significance (Tadesse et al., 2015). The polyphenols in tea include mainly flavanols, hydroxyl-4-flavanols, flavones, flavonols, anthocyanins and phenolic acids (Mukhtar et al., 2000). The important tea flavanols are catechins (flavan-3-ols), of which the major ones are (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (+)-catechin (C), and (+)-gallocatechin (GC) (Chen et al., 2003). The levels of polyphenols in tea has been regarded as a quality indicator of tea (Obanda et al., 1997). Caffeine also has pharmacological effects on central nervous system, heart, and respiratory system. Caffeine is known to decrease fatigue and improve performance (Altimari et al., 2000). Therefore, in this study, we tried to optimize the process with respect to maximal polyphenols and caffeine contents as an indicator of tea quality.

Analysis of total polyphenols content

Total polyphenols content (TPC) was assessed by following the method described by International Organization for Standardization (ISO, 2005) and Anesini et al. (2008). In a mortar and pestle, green tea samples were finely ground, and 0.2 gm was then transferred to graded extraction tubes. Then 5 mL of a hot, 70% v/v methanol/water solution was added, capped, and vortexed. The tubes were heated to 70°C in a hot water bath for 10 min, vortexed after 5 and 10 min, and then cooled to room temperature for 10 min before being centrifuged at 3500 rpm. A second extraction of the residue was performed using 5 mL of a hot, 70% methanol/water solution. The extracts were then mixed together and made up to a volume of 10 mL using cold methanol/water solution. This extracted solution was further diluted by placing 1 mL of extract into a 100 mL volumetric flask and topping it up with distilled water. Gallic acid stock solution @ 1 mg/ml was made by dissolving gallic acid monohydrate into distilled water. Gallic acid standard solutions were made by transferring 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of stock solutions to 100 mL volumetric flasks and making the volume up to the mark with distilled water which corresponds to 10, 20, 30, 40, and 50 µg/ml gallic acid. One mL of the diluted tea samples and gallic acid standards were placed into separate tubes and 5 mL (10% v/v) Folin-Ciocalteu phenol reagent was added to each tube. They were mixed by vortexing and 4.0 mL (5.7% w/v) sodium carbonate solution was added to each tube. After that, the tubes were left to stand in the dark for 60 minutes at room temperature. A UV-VIS spectrophotometer (Hitachi, U-2900) set to 765 nm against water was used to measure the absorbance values of the standards and samples. Using the gallic acid standard curve, the total amount of polyphenols in a sample was quantified as mg/g dry matter basis.

Determination of caffeine

Caffeine content was estimated by following the method as described by Shrestha et al. (2016). Caffeine was analysed using an HPLC system (Hitachi-chromaster) equipped with a C18 Column (Column Size: 4.6×250 mm, Pore size: 5µm). The temperature of the column and the UV detector were set to 40°C and 275 nm, respectively. For each sample, a 10 µL volume was injected. A flow rate of 1 mL/min was used using 40% methanol (methanol: water = 40: 60) as a mobile phase. In a mortar and pestle, green tea samples were finely ground. A 300 mg grounded tea sample was taken into a conical flask and 200 ml of distilled water was added. The flask was then placed on a hot water bath at 100°C for half an hour. After cooling to room temperature, the solution was filtered using Whatman filter paper. Using distilled water, the solution was diluted to a volume of 250 ml. The solution was further diluted by taking 1 ml of that solution into a 10 ml volumetric flask and made the volume up to the mark by adding distilled water. The solution then filtered through 0.2 µm micro-filter and filled the vial for HPLC analysis. Working standard caffeine solutions were prepared by serial dilutions with HPLC grade water to make different concentrations (2, 4, 6, 8 and 10 ppm); which were used for standard calibration curve preparation (peak area vs concentration). The caffeine content was calculated from the regression equation. Figure 2 illustrates a representative chromatogram of Caffeine for a green tea sample.

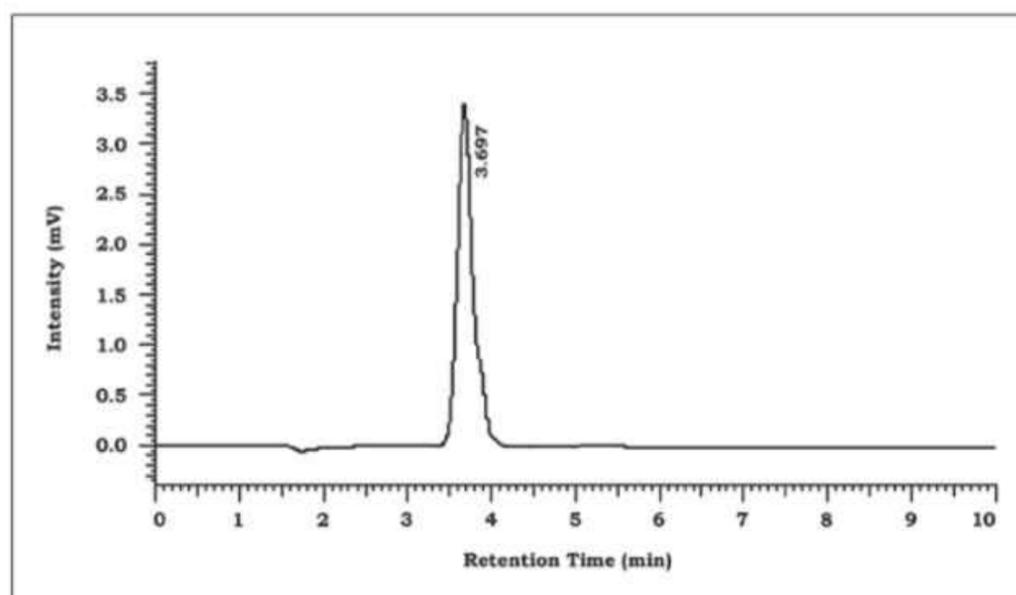


Fig. 2. A representative chromatogram of Caffeine of a green tea sample.

Sensory (organoleptic taste) assessment

Sensory assessment of the product green tea samples was done by conventional organoleptic tasting method and scored numerically on the basis of liquoring characteristics (Hossain et al., 2017). The liquor was prepared by pouring boiling water in a mug of a capacity of 142 ml in which 2.5 g tea was contained. After 3 minutes of brewing, the liquor was poured into a bowl and the taster evaluated the liquor. Liquoring characteristics were assessed by scoring numerically out of 50 points against Liquor color (10 points), Strength (10 points), Brightness (10 points), Bitterness (10 points) and Astringency (10 points). All the quality scores were recorded and they were analyzed statistically.

Statistical analysis

Data were analyzed statistically using the software package SPSS (SPSS, Inc., Chicago, IL, USA). For the comparison of means, one-way ANOVA was used. DMRT was used to test for significant differences at 5% level of probability.

Results and Discussion

Effects of different withering periods

It was noted that total polyphenols and caffeine contents were significantly highest when withering was done for one hour (Figure 1). The total polyphenols and caffeine levels gradually declined significantly as withering periods were extended. According to Kosińska & Andlauer (2004), development of aroma and partial oxidation occurs due to the breakdown of cell walls caused by moisture loss during withering. This suggested that some polyphenols undergo oxidation process during withering that reduces the total polyphenols content. So, longer periods of withering might increase the partial oxidation of polyphenols and thus reduces the total polyphenols contents in the product green tea. In this study, caffeine contents also decreased with extending the withering periods; which is not in agreement with Thombs et al. (1997) who reported that caffeine content increased during withering. The aim of withering is to remove moisture to such an extent that the leaves become softened and ready for further rolling. From sensory analysis, significant changes were noticed in liquor color and brightness due to different withering periods (Appendix 1). When the withering periods were increased, the liquor color and brightness points were decreased. Similarly, total points have been found to be significantly decreased with the increase of withering time. These results imply that, assuming all other variables remain constant, 1 hour of withering is sufficient and preferable to 2 to 3 hours of withering for the production of high-quality green tea.

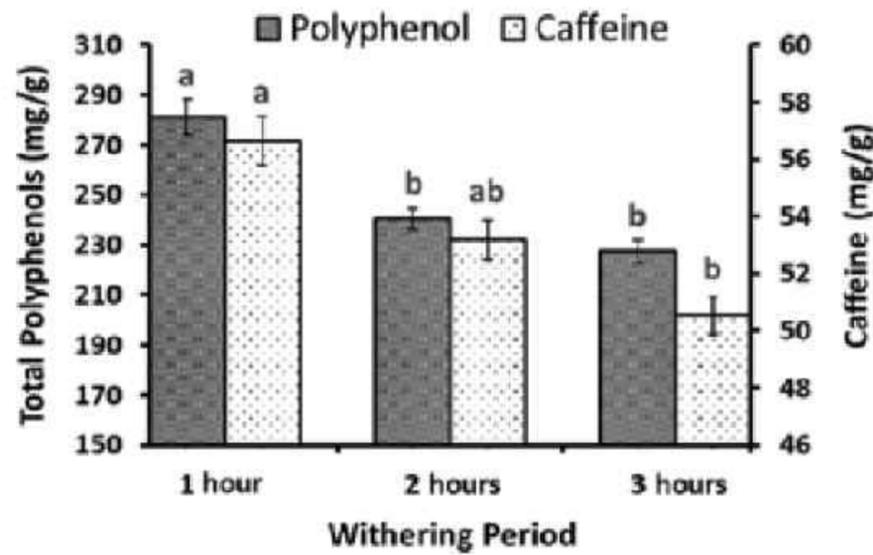


Fig. 3. Effects of different withering periods on the amount of total polyphenols and caffeine in the product green tea.

Effects of different fixation temperatures

To determine the best fixation temperature, three different temperatures were examined. It was observed that the total polyphenols contents increased progressively, but not significantly, when the fixation temperatures were raised from 250 to 270 °C (Figure 4). Caffeine contents did not vary due to different fixation temperatures. Fixation is done to deactivate the enzymes, which initiates oxidation of polyphenols (Kosińska & Andlauer, 2004). Thus it can be assumed that higher temperature deactivated the enzymes efficiently that resulted reduced or no oxidation of polyphenols. However, it is observed that if the fixation temperature is increased further, the leaves become so dry that they break during rolling which adversely affects its physical appearance. These findings suggested that within tolerable limit (so that the leaves do not become friable), higher fixation temperature favored the manufacture of high quality green tea. From sensory analysis, no significant differences on liquor characteristics were observed due to different fixation temperatures (Appendix II).

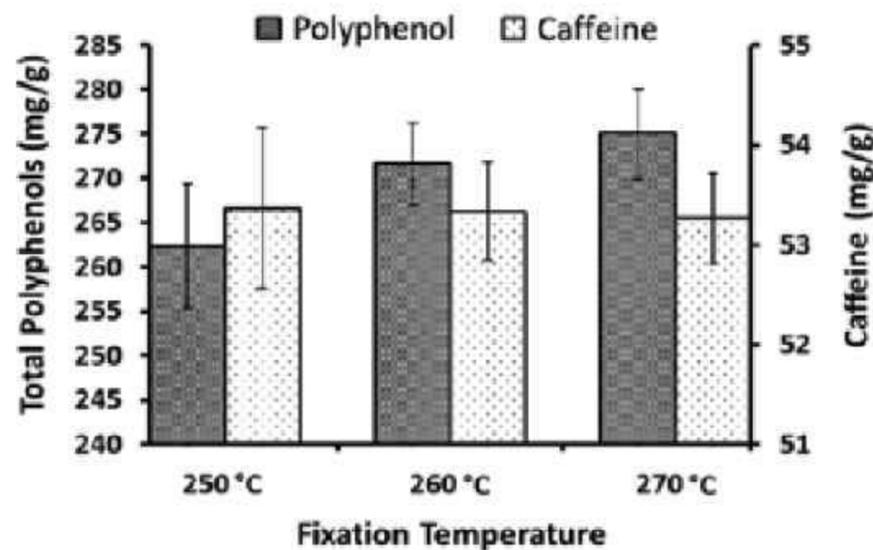


Fig. 4. Effects of different fixation temperatures on the amount of total polyphenols and caffeine in the product green tea.

Effects of different rolling durations

Following fixation, which stops fermentation (oxidation), and allowing the leaves to cool, rolling is the next step in the manufacturing of green tea. In rolling, the leaves are rolled by a rolling machine. Duration of rolling was identified as a crucial factor significantly affecting the green tea quality. When rolling periods were extended from 45 to 55 minutes, there was a significant increase in the amount of

total polyphenols and caffeine (Figure 5). Thus, if all other conditions are constant, 55 minutes rolling performed best in terms of total polyphenols and caffeine concentrations. Sensory analysis also showed significant effect of rolling durations on green tea liquor characteristics (Appendix III). The strength and bitterness as well as the total points were found to be increased with the increase of rolling durations. The purpose of rolling is to sufficiently break down the cell walls of the tea leaves so that some internal components can interact with the air to enhance the flavor and aroma. Rolling also allows the leaves to be shaped (Anonymous, 2023). It is observed that duration of rolling is somewhat dependent on the previous withering and fixation stages. If the optimum temperatures and durations were maintained in the previous stages, 55 minutes rolling gave best result. The newly established processing unit is such a setup that produces whole leaf green tea. If the rolling period is further extended, the rate of splitting of a single shoot into multiple segments increases which adversely affects the appearance of the shoot within the infusion.

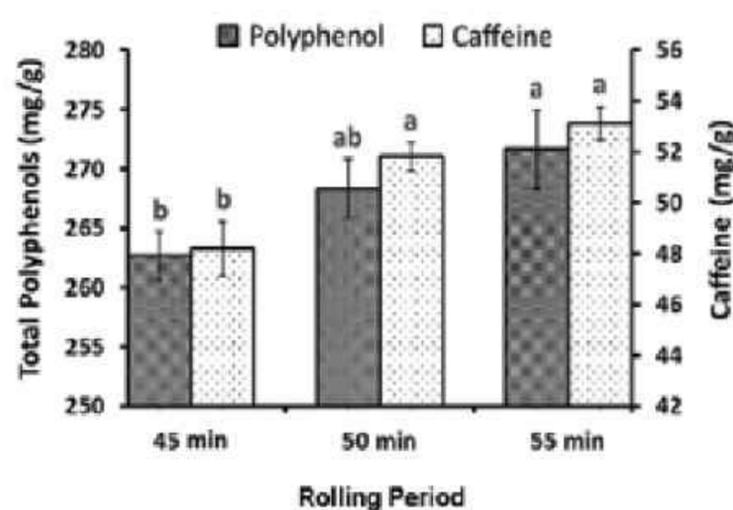


Fig. 5. Effects of different rolling durations on the amount of total polyphenols and caffeine in the product green tea.

Effects of different roll drying temperatures

When rolling is completed, the next task is to dry the leaves gradually. For betterment of final product, removal of moisture and enhancement in flavour, drying is usually repeated several times (Singh et al., 2014). Through this newly established processing unit, drying is completed in two stages i.e. (i) partial drying through the roll drying stage and (ii) complete drying through the final drying stage. Green tea quality was significantly influenced by the roll drying temperature. The roll drying temperature was found inversely correlated with the green tea quality. The total polyphenols and caffeine concentrations dropped when the roll drying temperature increased from 200 to 220 °C (Figure 6). Compared to 200 °C, the decrease was not significant at 210 °C but was significant at 220 °C. Therefore, it can be suggested that the temperature for roll drying shouldn't be more than 210 °C. This result is in accordance with the statements of some researchers in this area who mentioned that if the drying method is improper, heat-sensitive polyphenols may be oxidized and destroyed (Kaur & Singh, 2014; Nistor et al., 2017), resulting in the loss of the majority of the product's antioxidant properties (Hwang & Thi, 2014; Li et al., 2017). Furthermore, Maillard & Berset (1995) and Méndez-Lagunas et al., (2017) described three potential mechanisms that reduce the phenolic content during drying at high temperatures, including the release of bound phenolic compounds, partial degradation of lignin leading to the release of phenolic acid derivatives, and the start of thermal degradation of the phenolic compounds. Hence, the decrease of the phenolic content at high temperature in this study could be due to the above mechanisms. From, sensory analysis, no significant differences on liquor characteristics were noted due to different roll drying temperatures (Appendix IV).

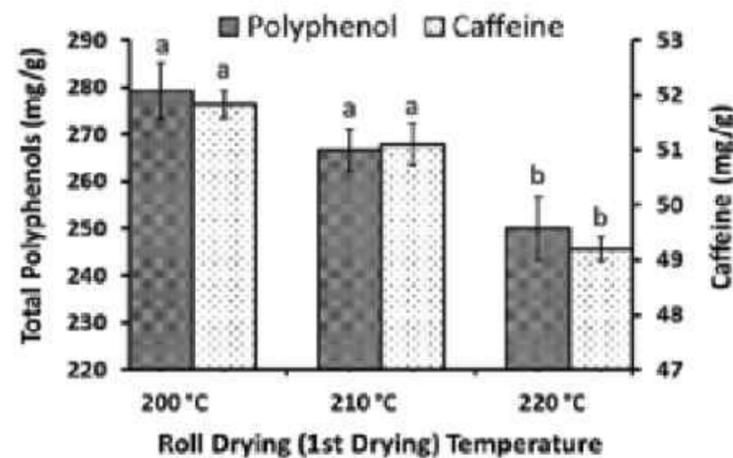


Fig. 6. Effects of different roll drying temperatures on the amount of total polyphenols and caffeine in the product green tea.

Effects of different final drying temperatures

The purpose of drying is to set the color, fragrance, taste and shape in the final product; and to preserve it easily. According to Li et al. (2006), drying procedures, particularly high temperatures and lengthy drying times, may cause some of the phenol components to be destroyed. The duration of final drying is fixed (17 minutes) in the newly established processing unit. In our initial trial, we noticed that drying temperature around 130 °C resulted some burnt smell in the final product. Therefore, comparatively lower drying temperatures (<130 °C) were chosen for this study. It was observed that higher drying temperatures had a favorable impact on the quality of the green tea within the tested range. The total polyphenols and caffeine concentrations were increased as the final drying temperature increased from 100 to 120 °C (Figure 7). The increase was significant for total polyphenols, but insignificant for caffeine. Sensory analysis also showed significant effect of final drying temperatures on green tea liquor characteristics (Appendix V). Liquor color and astringency points as well as the total points were found to be increased with the increase of final drying temperatures. From the finding of the current study, it can be suggested that regardless of other conditions, the final drying temperature can be kept at 120 °C for the manufacture of better quality green tea through the newly established processing unit of BTRI.

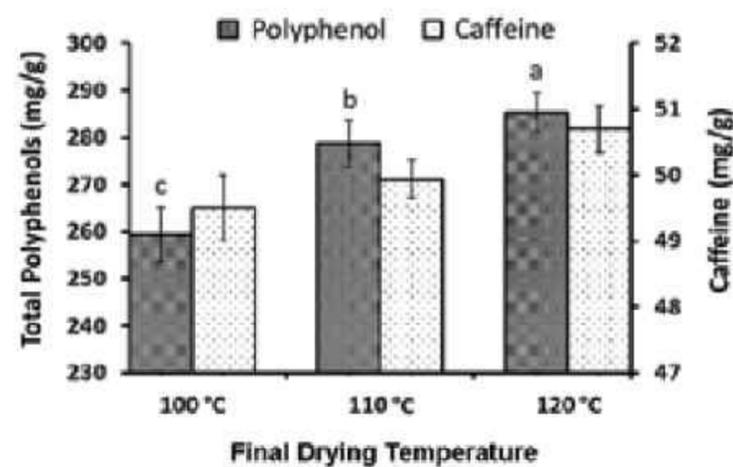


Fig. 7. Effects of different final drying temperatures on the amount of total polyphenols and caffeine in the product green tea.

Conclusion

In this study, a standard procedure for the production of quality green tea through the newly established green tea processing unit of BTRI was established for the main cropping season. Based on the findings, it can be suggested that 1 hour Withering → Fixation at 270°C → 55 min Rolling → Roll Drying at 200°C → Final Drying at 120°C can be set as a common procedure for manufacturing quality green tea. However, slight adjustment is necessary due to the variations in the raw materials and the environmental conditions.

Limitations of this study

The findings obtained and recommendations made in the present study were based on the raw materials collected and the environmental conditions prevailed during the main cropping season (July to September). There will be a variation in shoot quality and environmental conditions during the early and late seasons. Therefore, further studies in early and late seasons may be helpful in standardizing the specific production strategy for those seasons.

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Appendix I. Effects of different withering periods on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
W1	7.54a	6.46	6.55	6.43	5.46a	32.44a
W2	7.23b	6.65	6.22	6.56	5.38ab	32.04b
W3	6.84c	6.84	6.13	6.11	5.17b	31.09c
LSD at 0.05 level of significance	0.18	NS	NS	NS	0.06	0.24

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix II. Effects of different fixation temperatures on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
F1	7.49	6.61	6.49	6.43	5.42	32.44
F2	7.48	6.63	6.48	6.47	5.41	32.47
F3	7.49	6.71	6.53	6.29	5.43	32.45
LSD at 5% level of significance	NS	NS	NS	NS	NS	NS

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix III. Effects of different rolling durations on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
R1	7.43	6.51b	6.43	6.38b	5.39	32.14b
R2	7.45	6.58ab	6.48	6.42ab	5.41	32.34ab
R3	7.48	6.68a	6.53	6.58a	5.55	32.82a
LSD at 5% level of significance	NS	0.06	NS	0.11	NS	0.23

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix IV. Effects of different roll drying temperatures on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
RD1	7.33	6.52	6.46	6.51	5.49	32.31
RD2	7.38	6.48	6.51	6.48	5.48	32.33
RD3	7.28	6.51	6.52	6.47	5.49	32.27
LSD at 5% level of significance	NS	NS	NS	NS	NS	NS

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix IV. Effects of different roll drying temperatures on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
FD1	7.24b	6.53	6.28b	6.54	5.54	32.13c
FD2	7.36ab	6.48	6.49ab	6.47	5.46	32.26b
FD3	7.45a	6.47	6.54a	6.54	5.52	32.52a



POPULATION DYNAMICS OF PLANT PARASITIC NEMATODES AND THEIR RELATION TO SOIL PROPERTIES IN TEA

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Abdul Qayyum Khan⁴, Md Mozammel Hoque⁵ and Md Ahasanur Rahman⁵

Abstract

A study was conducted to assess the occurrence of plant parasitic nematodes (PPNs) and their relationship with soil properties from July to December 2015 at the Lackatoorah, Malnicherra, and Ali Bahar tea estates in the North Sylhet region of Bangladesh. Rhizosphere soil samples were taken at a depth of 23 cm from the primary nursery bed, secondary nursery bed, first-year plantation, and second-year plantation of the selected tea estates. Root samples were also collected from four locations of the above tea estates. Nematodes were extracted from the soil samples using the Baermann funnel method. Physico-chemical properties of the soil like textural class, sand (%), silt (%), clay (%), pH, and organic matter (%) from different locations of the aforementioned tea gardens were also determined to find out their relationship with nematodes population. The result revealed that out of 60 collected soil samples from different locations of selected tea estates, 47 were infested with PPNs with frequencies of 78.33%. The average frequency of occurrence was maximum (90.00%) and minimum (65.00%) in the soil samples of the Malnicherra and Lackatoorah tea estates, respectively. The highest frequency of PPNs was observed in the soil samples of the secondary nursery bed of selected tea gardens, with a frequency of 93.00%. The maximum frequency of occurrence (80.00%) was observed in the root samples of the secondary nursery bed, and that was the minimum (26.67%) in the second-year plantation. The highest numbers of PPNs were observed in the soil of the Malnicherra tea estate, followed by Lackatoorah and Ali Bahar tea estates. The mean numbers of PPNs were lowest in soil and root samples of second-year plantations from all the tea estates. According to pH, the selected tea estates were under moderately acidic soil with a range of 4.3-5.0. Three types of soil texture were found in different tea estates: sandy, sandy loam, and sandy clay loam. The highest nematode population was observed in sandy loam soil, and the lowest was in sandy soil. Maximum PPN was found in the sand, silt, and clay within the range of 70-80%, 8-13%, and 9-18%, respectively. The population of PPNs was linearly and positively correlated with soil pH and organic matter (%) in all three tea estates.

Keywords: Tea, Plant parasitic nematodes, Rhizosphere soil, Soil properties

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Introduction

Nematodes are a type of soil pest also known as eelworms. They may be parasitic, feeding on the roots of plants as ectoparasites or endoparasites, or saprophagous, feeding on decomposing organic materials (Mamun, 2019; Paul et al., 2017; Roy & Rahman, 2013). It can be found worldwide in a wide range of habitats. The PPNs are an important pest of many cultivated plants, including rice, fruits, vegetables, legumes, tea, coffee, cocoa, etc. Nematode infestation is a slow change; plants don't show symptoms until a substantial portion of the root system has been lost or stopped functioning. The first indicators of nematode attack are a slowdown in growth, then yellowing and wilting of leaves. In severe conditions, die-back and death could occur (Mamun et al., 2014; Paul et al., 2014). In various tea-growing nations around the world, there are over 40 species of PPNs from 20 genera (Gnanapragasam, 2014; Mamun et al., 2011; Mohotti et al., 2023). Twelve species of PPNs, namely *Xiphinema* sp., *Tylenchus* sp., *Rotylenchulus* sp., *Pratylenchus* sp., *Meloidogyne* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., *Eutylenchus* sp., *Dolichodoros* sp., *Criconemoides* sp., *Aphelenchoides* sp., and *Aphelenchus* sp., in tea soil, are recorded from Bangladesh (Paul et al., 2019). The PPNs cause crop losses of more than 100 billion US\$ each year all over the world (Dias-Arieira et al., 2021). The PPNs cause a loss of 5-10%, or even up to 50%, in producing healthy tea seedlings, depending on the severity of the infestation (Roy & Rahman, 2013). One of the important ways that infestation spreads among tea estates is through infected nursery plants. Understandably, nematodes cannot be eliminated after they have been introduced to an area. Additionally, harm to young seedlings at their crucial growth stage has a long-term effect on the plant's health throughout its lifetime (Gnanapragasam, 2014).

Tea can be cultivated on soils with varying geological origins. The majority of the soils in Bangladesh are alluvial sedimentary soils. Bangladesh tea soil is highly acidic and extensively degraded, with lower fertility. Most soils have a medium texture and low nitrogen (1.0-1.2 percent) and organic matter (0.70-0.90 percent) contents. Most of Bangladesh's tea soils are loamy in texture. Additionally, the exchangeable sites in Bangladesh tea soil have more than 85% aluminium (Alam, 1999; Sana, 1989). Numerous abiotic and biotic factors may impact the spatial pattern of nematodes in the soil (Kemboi et al., 2022; Olabiyi et al., 2009). Most plant-parasitic nematodes tend to have their maximum population numbers in the top 45 cm of the soil profile (Fajardo et al., 2011), with their vertical distribution somewhat influenced by the host's rooting pattern (Bhattacharya et al., 2012). According to Ferris et al. (1996), the host plant, with its food source, is the most significant factor influencing nematode dispersion in the soil. Moreover, abiotic factors, viz. temperature, soil physico-chemical properties, and moisture, may substantially impact nematodes' quantity, distribution, and structure in the host's roots and soil (Krif et al., 2020; Upadhaya et al., 2019).

The information on the status of population dynamics of PPNs and their relationship with soil physico-chemical properties in the North Sylhet region is scanty. Therefore, the present study was undertaken to know the population dynamics of PPNs in tea soil of the North Sylhet region and to determine the relationship between nematode populations and soil properties in tea.

Materials and Methods

The study was conducted in the Ali Bahar, Lackatoorah, and Malnicherra tea estates of the North Sylhet region from July to December 2015. Soil samples were collected from four different locations of nursery and young tea plantations from each tea estate, such as primary and secondary nursery beds as well as first and second-year plantations.

Collection of soil samples

Using a sampling auger, soil samples were taken from the root zone of the tea plants up to a depth of 23 cm and up to 5 cm from the base of the plants. Five beds from primary and secondary nursery areas and five spots from first and second-year plantations from each tea estate were randomly selected for soil sample collection. Every sample comprised a composite of 5 soil cores from each location of the selected tea estates. Twenty composite samples were prepared from each tea estate. In the case of root samples, 5 plants were selected arbitrarily from each location of the selected tea estates. Twenty root samples were also collected from each tea estate. Thus a total of 120 soil and root samples were collected from different locations of the above tea estates. The samples were placed in plastic bags, sealed to avoid desiccation, and kept out of sunlight. They were transported in a shielded bag and stored at 4°C to minimize changes in nematode populations. Every time the samples were mixed thoroughly, 10 g of soil and 10 g of root sample were made for nematode extraction. For analysis of soil properties, one composite sample was prepared by mixing 5 subsamples for each location with the rest of the soils after nematode extraction. Thus, 12 soil samples were made, and a 250 g sample was analyzed for soil properties.

Nematode extraction from soil

Nematode extraction and identification from soil and root were carried out in the Nematology Laboratory of the Entomology Division, BTRI, Sreemangal, Moulvibazar. Nematodes were extracted from the samples following the Baermann funnel technique with some modifications (Mian, 1998). Nematodes were taken in a glass slide with a drop of water by loosening the pinch clamp. Nematodes were examined using a Primostar Trinocular compound microscope (Carl Zeiss) with 100X objectives to distinguish between plant-parasitic (PPNs) and non-parasitic (NPNs) nematodes. Plant parasitic nematodes bear stylet at the anterior end of their mouth part, which can be protruded and used to penetrate plant cells like a hypodermic needle. A mean of three counts was taken in each case.

Analysis of soil properties

Soil properties viz. pH, sand%, silt%, clay%, texture, and organic matter were analyzed in the Soil Science Division of BTRI to know their relationship with the nematode population. A pH meter was used to calculate soil pH (soil: distilled water = 1: 2.5). Sand, silt, and clay fractions were determined by the hydrometer method. Textural classes were selected from the Marshall (1947) triangular diagram. The Walkley and Black wet oxidation method was used to calculate the soil organic carbon (Huq & Alam, 2005), and organic matter was calculated by multiplying the organic carbon by 1.72.

Data analysis

The average number of nematodes in 3 different drops of water was used to calculate the number of nematodes. The frequency of PPN was determined from the relationship between the number of samples in which PPNs were observed divided by the total number of samples taken from that tea estate multiplied by 100 to express as a percentage (Norton et al., 1978). Regression analysis determined the relationship between nematode population and soil properties. All collected data were subjected to analysis using the MS Excel program.

Results and Discussion

Nematodes associated with soil and root samples

Among 60 soil samples, 13 samples have found the nonexistence of PPNs. Regarding root samples, 22 samples have been found free of PPNs. The frequencies of the nematodes in all surveyed tea gardens varied from one garden to another. The overall frequency of PPN occurrence was 78.33% and 63.33% in soil and root, respectively. The frequency of occurrence was maximum (90.00%) in the soil samples of the Malnicherra tea estate and minimum (65.00%) in the Lackatoorah tea estate. On the other hand, the frequency of occurrence was maximum (75.00%) in the root samples of the Malnicherra tea estate and minimum (50.00%) in the Lackatoorah tea estate (Table 1).

Table 1. Frequency of occurrence of PPNs in soils and root samples of three tea estates in the North Sylhet region.

Name of tea estate	Sample analyzed		Sample with PPN		Frequency of PPN (%)±SE	
	Soil	Root	Soil	Root	Soil	Root
Ali Bahar Tea Estate	20	20	16	13	80.00±2.13	65.00±1.95
Lackatoorah Tea Estate	20	20	13	10	65.00±1.52	50.00±1.13
Malnicherra Tea Estate	20	20	18	15	90.00±2.51	75.00±2.21

PPN= Plant Parasitic Nematode; SE= Standard Error

Table 2 shows the frequency of occurrence of PPNs in different locations of selected tea estates in the North Sylhet region. Results revealed that the soil samples from secondary beds of chosen tea gardens were infested with PPNs with a frequency of 93.00%, followed by the soil samples from first-year plantations (86.66%). The lowest frequency of PPNs (60.00) was found in the soil samples of the second-year plantation. In the root samples, the maximum frequency of occurrence (80.00%) was observed in the secondary nursery bed, which was the lowest (26.67%) in the second-year plantation.

Table 2. Frequency of occurrence of PPNs in soils and root samples in different locations of three tea estates in the North Sylhet region.

Location	Sample analyzed		Sample with PPN		Frequency of PPN (%)±SE	
	Soil	Root	Soil	Root	Soil	Root
Primary nursery bed	15	15	12	10	80.00±1.11	66.67±1.25
Secondary nursery bed	15	15	14	12	93.00±2.01	80.00±2.12
First-year plantation	15	15	13	09	86.66±1.53	60.00±1.44
Second-year plantation	15	15	09	04	60.00±1.65	26.67±1.02

PPN= Plant Parasitic Nematode; SE= Standard Error

Table 3 shows the mean number of nematodes found in soil and root samples, including the frequency of occurrence of PPNs in different locations of selected tea estates. In the case of the Ali Bahar tea estate, the highest frequency of PPNs was found in the soil of the secondary bed (21.83%),

followed by the first-year plantation (18.21%), and the lowest was found in the second-year plantation (10.54%) (Table 3a). In the Lackatoorah tea estate, the highest frequency of PPNs was found in first-year plantations (22.09%), and those were 20.47% and 15.78% in secondary beds and primary beds, respectively (Table 3b). It was also observed that the soil samples from the secondary bed of the Malnicherra tea estate contained the highest frequency of PPNs (27.49%), followed by first-year plantation (26.36%) and primary bed (19.92%) (Table 3c). Results also showed that the frequency of occurrence of PPNs in the entire root samples of selected tea gardens was 100%.

Table 3. Mean number and percentage of PPN in different locations.

Location	PPN (no.)±SE		NPN (no.)±SE		Total (no.)±SE		Frequency of PPN (%)±SE	
	Soil	Root	Soil	Root	Soil	Root	Soil	Root
a. Ali Bahar Tea Estate								
Primary nursery bed	10.11±1.12	6.20±0.14	50.11±2.33	0.00	60.22±3.01	6.20±0.14	16.79±2.01	100.00
Secondary nursery bed	11.21±1.35	8.14±0.36	40.15±1.52	0.00	51.36±1.84	8.14±0.36	21.83±2.53	100.00
First-year plantation	13.41±1.03	4.03±0.27	60.23±2.74	0.00	73.64±2.14	4.03±0.27	18.21±1.43	100.00
Second-year plantation	5.32±1.54	2.01±0.22	45.16±1.47	0.00	50.48±1.71	2.01±0.22	10.54±1.12	100.00
b. Lackatoorah Tea Estate								
Primary nursery bed	12.22±1.02	7.13±0.27	65.24±2.17	0.00	77.46±2.89	7.13±0.27	15.78±1.13	100.00
Secondary nursery bed	15.01±1.55	11.18±0.34	58.33±1.52	0.00	73.34±2.24	11.18±0.34	20.47±1.47	100.00
First-year plantation	13.46±1.32	9.27±0.14	47.47±1.14	0.00	60.93±1.42	9.27±0.14	22.09±2.01	100.00
Second-year plantation	6.15±1.17	3.16±0.11	70.00±2.46	0.00	76.15±1.76	3.16±0.11	8.08±1.05	100.00
c. Malnicherra Tea Estate								
Primary nursery bed	15.23±0.74	12.13±0.11	61.23±1.14	0.00	76.46±2.33	12.13±0.11	19.92±1.22	100.00
Secondary nursery bed	14.03±0.51	9.22±0.24	37.00±1.21	0.00	51.03±1.72	9.22±0.24	27.49±1.59	100.00
First-year plantation	17.34±0.83	10.11±0.31	48.45±1.43	0.00	65.79±1.86	10.11±0.31	26.36±1.33	100.00
Second-year plantation	8.12±0.39	3.07±0.14	73.31±1.33	0.00	81.43±2.55	3.07±0.14	9.97±1.04	100.00

PPN= Plant Parasitic Nematode; NPN= Non-Parasitic Nematode; SE= Standard Error

Figure 1a shows the mean population number of PPNs in different locations of three tea estates. The mean numbers of PPNs were highest in the secondary nursery bed and the lowest was in the second-year plantation. Figure 1b demonstrates the average number of PPNs in three tea estates of the North Sylhet region. The Malnicherra tea estate had the most PPNs, followed by Lackatoorah and Ali Bahar tea estates.

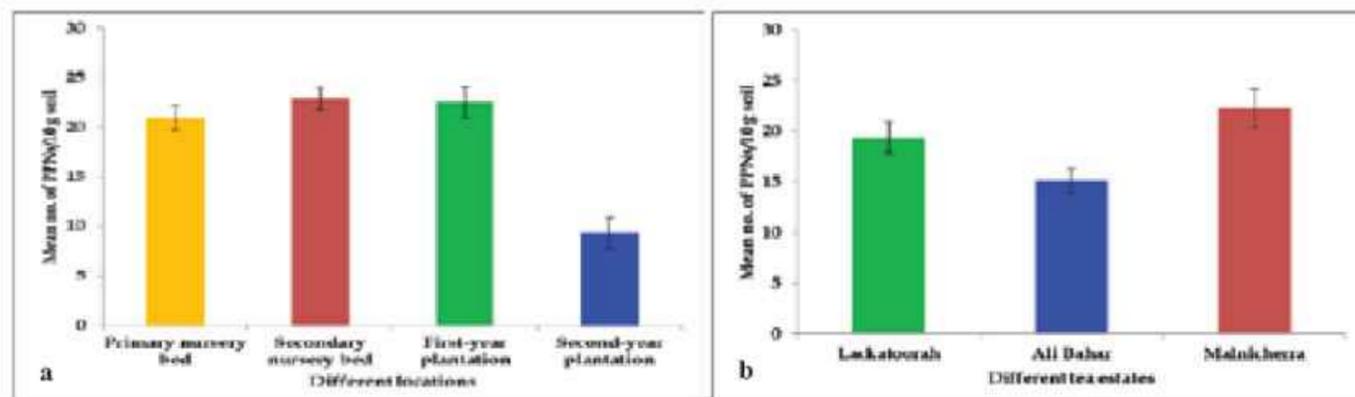


Fig. 1. The mean number of PPN in different locations (a) of selected tea estates (b). Vertical lines at each bar indicate the SE value

It was observed that the nematode population varied with different locations, tea estates, and ages of tea plants in all the surveyed tea estates. The survey provided information on the frequency and average population of PPNs associated with tea soil in the North Sylhet region. In general, 78.33% of the samples had a PPNs population, which was high enough to initiate a potential for damage and contribute the most significant portion of the risk. The tea plant is a good host of PPNs, and different species of nematodes infect the tea plant (Ahmed, 2005; Bhattacharya et al., 2012; Khan et al., 2006; Nalini et al., 2005; Paul et al., 2019; Roy & Rahman, 2013). This study found that the root samples of second-year plantations from all the tea estates had the lowest number of PPNs. This may be because the PPNs cannot easily suck the roots of second-year tea plants due to their hard epidermis. This corroborates earlier works that reported soil depth and crop growth stage affect the population density of nematodes in corn fields (Simon et al., 2023).

Relationship between nematode population and soil properties

Data presented in Table 4 elucidate the textural class, the percentage of sand, silt, clay, organic matter (%), and pH of the Lackatoorah, Malnicherra, and Ali Bahar tea estates in the North Sylhet region. The tested soils from the Lackatoorah tea estate, with a pH range of 4.4-5.0, were moderately acidic (Table 4a). Similarly, the pH range of the Malnicherra tea estate was 4.5-5.1 (Table 4b), and that of the Ali Bahar tea estate was 4.5-5.0 (Table 4c), which is also moderately acidic.

The soil textural class in different locations of the Lackatoorah tea estate was found in sandy loam, sandy clay loam, and sandy (Table 4a). On the other hand, sandy loam and sandy clay loam textured soil were found in Malnicherra and Ali Bahar tea estates (Table 4b, c). Results revealed that soil textural class affected the average number of nematode populations in different locations of selected tea estates. The highest PPNs were observed in sandy loam soil next to sandy clay loam, and the lowest PPNs were observed in sandy soil (Figure 2a).

Different textural types were found due to the varying amounts of sand, silt, and clay. In sandy loam soil, the percent of sand, silt, and clay ranged from 70.00-81.00%, 8.00-12.80%, and 7.00-17.75%, respectively (Table 4). Whereas the percent of sand, silt, and clay were 90.00%, 0.00%, and 10.00%, respectively, in sandy soil, and those were within the range of 53.40-54.30%, 16.30-17.40%, and 28.30-30.30% respectively (Table 4). Maximum PPNs were found in the percent of sand, silt, and clay within the range of 70-80%, 8-13%, and 9-18%, respectively (Figure 2b, c, d). On the other hand, the populations of PPNs were positively correlated with soil pH with co-efficient of determination (R^2) values were 0.79, 0.84, and 0.79 for Lackatoorah, Malnicherra, and Ali Bahar tea estates, respectively (Figure 3a, c, e). Organic matter (%) was also positively correlated with PPN populations, with R^2 values were 0.84, 0.79, and 0.81 for Lackatoorah, Malnicherra, and Ali Bahar tea estates, respectively (Figure 3b, d, f).

Table 4. Soil physico-chemical properties of selected tea estates in the North Sylhet region.

a. Lackatoorah Tea Estate						
Location of soil sample	Textural class	Sand(%)±SE	Silt(%)±SE	Clay(%)±SE	pH±SE	OM(%)±SE
Primary nursery bed	Sandy clay loam	55.16±0.51	17.65±0.23	27.19±0.21	4.6±0.11	1.46±0.01
Secondary nursery bed	Sandy loam	80.00±0.73	10.00±0.32	10.00±0.11	5.0±0.08	1.72±0.08
First-year plantation	Sandy loam	70.40±0.42	11.85±0.53	17.75±0.33	4.7±0.04	1.55±0.02
Second-year plantation	Sandy	90.00±0.65	1.03±0.17	8.17±0.28	4.4±0.05	1.34±0.09
b. Malnicherra Tea Estate						
Primary nursery bed	Sandy loam	81.00±0.65	12.00±0.31	7.00±0.23	4.8±0.07	1.48±0.11
Secondary nursery bed	Sandy loam	78.20±0.48	12.80±0.22	9.00±0.17	5.1±0.09	1.69±0.05
First-year plantation	Sandy clay loam	53.40±0.34	16.30±0.14	30.30±0.21	4.5±0.06	1.20±0.08
Second-year plantation	Sandy clay loam	54.30±0.25	17.40±0.42	28.30±0.33	4.7±0.04	1.38±0.06
c. Ali Bahar Tea Estate						
Primary nursery bed	Sandy clay loam	55.00±0.41	16.10±0.23	28.90±0.14	4.5±0.10	1.29±0.03
Secondary nursery bed	Sandy loam	70.00±0.33	9.40±0.11	15.20±0.08	5.0±0.08	1.72±0.05
First-year plantation	Sandy loam	76.80±1.01	8.00±0.09	17.20±0.25	4.7±0.04	1.53±0.07
Second-year plantation	Sandy loam	76.23±0.74	10.47±0.12	13.30±0.09	4.8±0.06	1.48±0.04

SE= Standard Error

Soil pH, texture, organic matter content, moisture, rainfall, temperature, and plant type influence the population density of nematodes in tea (Bhattacharya et al., 2012; Khan et al., 2006). Diverse soil pH, texture, and organic matter (%) were found in different locations of the tea estates during this study. Asif et al. (2015) and Choshali et al. (2015) reported that some species of PPNs were higher in sandy loam or lighter soils, which enhanced nematode movement. This report supports the findings of this study. Another study by Paiko et al. (2019) reported that PPNs were negatively correlated with sandy soil, similar to the present study's findings. The report further showed that soil pH was positively correlated with certain species of PPNs. Khan et al. (2006) and Bhattacharya et al. (2012) found a positive correlation between the PPNs population and the organic matter content of tea soil.

followed by the first-year plantation (18.21%), and the lowest was found in the second-year plantation (10.54%) (Table 3a). In the Lackatoorah tea estate, the highest frequency of PPNs was found in first-year plantations (22.09%), and those were 20.47% and 15.78% in secondary beds and primary beds, respectively (Table 3b). It was also observed that the soil samples from the secondary bed of the Malnicherra tea estate contained the highest frequency of PPNs (27.49%), followed by first-year plantation (26.36%) and primary bed (19.92%) (Table 3c). Results also showed that the frequency of occurrence of PPNs in the entire root samples of selected tea gardens was 100%.

Table 3. Mean number and percentage of PPN in different locations.

Location	PPN (no.)±SE		NPN (no.)±SE		Total (no.)±SE		Frequency of PPN (%)±SE	
	Soil	Root	Soil	Root	Soil	Root	Soil	Root
a. Ali Bahar Tea Estate								
Primary nursery bed	10.11±1.12	6.20±0.14	50.11±2.33	0.00	60.22±3.01	6.20±0.14	16.79±2.01	100.00
Secondary nursery bed	11.21±1.35	8.14±0.36	40.15±1.52	0.00	51.36±1.84	8.14±0.36	21.83±2.53	100.00
First-year plantation	13.41±1.03	4.03±0.27	60.23±2.74	0.00	73.64±2.14	4.03±0.27	18.21±1.43	100.00
Second-year plantation	5.32±1.54	2.01±0.22	45.16±1.47	0.00	50.48±1.71	2.01±0.22	10.54±1.12	100.00
b. Lackatoorah Tea Estate								
Primary nursery bed	12.22±1.02	7.13±0.27	65.24±2.17	0.00	77.46±2.89	7.13±0.27	15.78±1.13	100.00
Secondary nursery bed	15.01±1.55	11.18±0.34	58.33±1.52	0.00	73.34±2.24	11.18±0.34	20.47±1.47	100.00
First-year plantation	13.46±1.32	9.27±0.14	47.47±1.14	0.00	60.93±1.42	9.27±0.14	22.09±2.01	100.00
Second-year plantation	6.15±1.17	3.16±0.11	70.00±2.46	0.00	76.15±1.76	3.16±0.11	8.08±1.05	100.00
c. Malnicherra Tea Estate								
Primary nursery bed	15.23±0.74	12.13±0.11	61.23±1.14	0.00	76.46±2.33	12.13±0.11	19.92±1.22	100.00
Secondary nursery bed	14.03±0.51	9.22±0.24	37.00±1.21	0.00	51.03±1.72	9.22±0.24	27.49±1.59	100.00
First-year plantation	17.34±0.83	10.11±0.31	48.45±1.43	0.00	65.79±1.86	10.11±0.31	26.36±1.33	100.00
Second-year plantation	8.12±0.39	3.07±0.14	73.31±1.33	0.00	81.43±2.55	3.07±0.14	9.97±1.04	100.00

PPN= Plant Parasitic Nematode; NPN= Non-Parasitic Nematode; SE= Standard Error

Figure 1a shows the mean population number of PPNs in different locations of three tea estates. The mean numbers of PPNs were highest in the secondary nursery bed and the lowest was in the second-year plantation. Figure 1b demonstrates the average number of PPNs in three tea estates of the North Sylhet region. The Malnicherra tea estate had the most PPNs, followed by Lackatoorah and Ali Bahar tea estates.

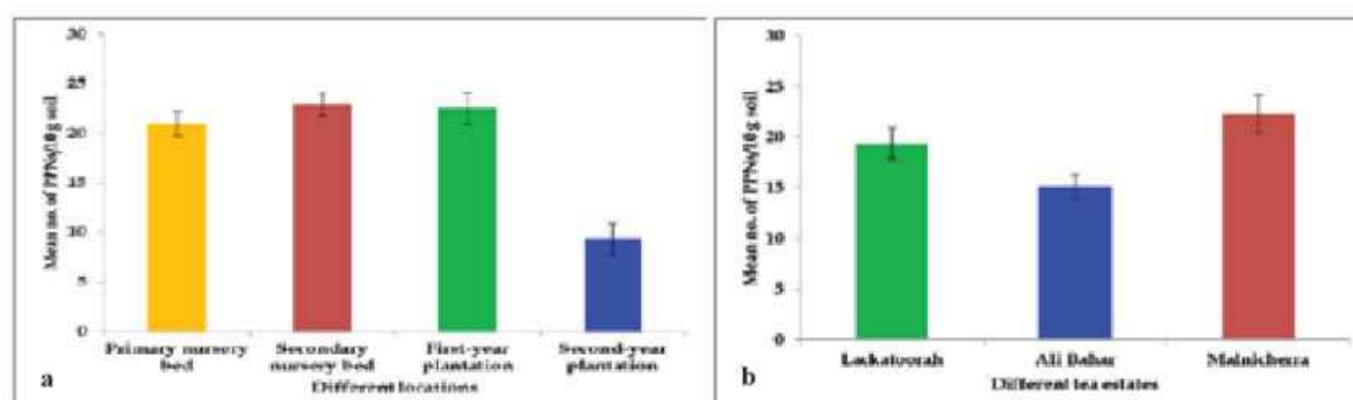


Fig. 1. The mean number of PPN in different locations (a) of selected tea estates (b). Vertical lines at each bar indicate the SE value

It was observed that the nematode population varied with different locations, tea estates, and ages of tea plants in all the surveyed tea estates. The survey provided information on the frequency and average population of PPNs associated with tea soil in the North Sylhet region. In general, 78.33% of the samples had a PPNs population, which was high enough to initiate a potential for damage and contribute the most significant portion of the risk. The tea plant is a good host of PPNs, and different species of nematodes infect the tea plant (Ahmed, 2005; Bhattacharya et al., 2012; Khan et al., 2006; Nalini et al., 2005; Paul et al., 2019; Roy & Rahman, 2013). This study found that the root samples of second-year plantations from all the tea estates had the lowest number of PPNs. This may be because the PPNs cannot easily suck the roots of second-year tea plants due to their hard epidermis. This corroborates earlier works that reported soil depth and crop growth stage affect the population density of nematodes in corn fields (Simon et al., 2023).

Conclusion

This study provides the population dynamics of PPNs and shows their relationship to soil properties in tea plantations. Overall, 78.33% of soil samples were infected with PPNs. The average frequency of occurrence was maximum (90.00%) and minimum (65.00%) in the soil samples of the Malnicherra and Lackatoorah tea estates, respectively. The highest frequencies of occurrence were observed in soil (93.00%) and root (80.00%) samples of secondary nursery beds of selected tea gardens. The highest nematode numbers were found in sandy loam soil. Maximum PPN was found in the percent of sand, silt, and clay within the range of 70-80%, 8-13%, and 9-18%, respectively. A linear and positive correlation existed between the PPN population and organic matter (%) and soil pH in all three tea plantations. The study on PPNs in tea would be helpful to know the density of nematodes in soil and their degree of damage. The influence of soil properties on the nematode population would be beneficial to ensure a better selection of soil medium and optimization of soil properties in tea plantations. Further research on the relationship of physico-chemical parameters with major plant PPNs in Bangladesh tea can be done extensively in other tea plantations of different locations.

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EFFECT OF GREEN LEAF ON VOLUME AND QUALITY OF BLACK TEA

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and Muhammad Abid Hasan Chowdhury⁴

Abstract

Tea is one of the most popular beverages globally and majority of our produced tea is black tea. This study is conducted in Black Tea Factory of Bangladesh Tea Research Institute with three different leaf quality (GL_{80%}, GL_{50%}, GL_{30%}) to assess the difference of made tea volume and made tea quality due to different green leaf quality. The volume of made tea varies with the green leaf quality. Tea volume of individual each grade (FBOP, BOP, GBOP, OF and Dust) was lowest in GL_{80%} while highest in GL_{30%} leaf quality. Total polyphenols (16.23%), theaflavin (0.89%), brightness (27.77%) and caffeine (3.17%) found highest in green leaf with 80% good leaf. A fundamental quality criterion for determining the body, color, strength, brightness, and briskness of black tea beverage is the TF/TR ratio. Best ratio of TF:TR (1:10.39) was found in green leaf with 80% while the low quality of made tea with 1:12.47 ratio was found in low quality of green leaf (30% good leaf). Strongest positive relation (0.93^{***}) was found between total polyphenols and good leaf percentage. Correlation Coefficient (r) were +0.88^{***} and +0.80^{***}, respectively for caffeine and organoleptic score against good leaf (%) also explaining the presence of strong positive relationship between them. It can be concluded that, the volume and the quality, both biochemical and organoleptic of made tea mostly depends on the green leaf quality.

Keywords: Tea, Good leaf (%), Volume, Biochemical properties, Organoleptic quality

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Introduction

Tea is one of the most popular beverages globally consumed (Cheng, 2004; Vinson, 2000) with a per capita intake of around 120 ml per day (Mckay & Blumberg, 2002). It is mostly grown in China, Japan, India, Bangladesh and Sri Lanka as well as other many countries (Fatima & Rizvi, 2011). About 20% of tea is produced as green tea, while 80% of the nearly 2.5 million metric tons of produced teas are black teas and majority of consumed tea is black tea and green tea (Boehm et al., 2009). ITC (2021) estimates that global production, retention, imports and consumption of tea were 6,269 million kg, 4,444 million kg, 1,735 million kg, and 5,879 million kg, respectively in 2020. Since 1854, Bangladesh has been cultivating tea commercially with the establishment of country's first tea estate, the 'Malnicherra Tea Estate' in Sylhet. In 1947, there were only 28,734 hectares of tea cultivated area with a yield of 656 kg per hectare. By contrast, in 2020, there were 62,168.46 hectares of tea cultivation with a yield of 1561 kg per hectare (BTB, 2021). In 2020, there are 86,394 million kg of tea production in total, of which 79 million kg are CTC tea, 7 million kg are orthodox tea, and almost 1% are green tea (ITC, 2021).

There are four major separate steps in the production of black tea: withering, rolling or maceration, fermentation, and drying (Tomlins & Mashingaidze, 1997). Green leaf quality is an important criterion which is responsible for made tea quality. When first two leaves and the bud are plucked is generally termed as 'fine plucking', while 'coarse plucking' standard is called when three or four leaves and the bud are plucked (Wright, 2005). The optimal leaf quality compromise between yield and quality is thought to be two leaves and a bud (Banerjee, 1992). However, planters sometimes compromise with leaf quality by plucking comparatively less soft and younger shoots for extra production in a cropping year (Okinda & Bowa, 2012). But coarse plucking standard reduces made tea quality. For the development of flavored precipitates during the infusion process, the caffeine concentration of tea has a high correlation with its quality (Smith et al., 1993). The amounts of substances like amino acids, and catechins, as well as other substances like thearubigins and theaflavins, also significantly influence the sensory properties of tea (Kato et al., 2008). The sensory

qualities of tea, particularly the brightness of the tea color, have been reported to be influenced by thearubigins and theaflavins (Owuor & Obanda, 2001). It has been observed that made tea density changes through the year although the manufacturing processes remain more or less the same (Jose, 1999). This study is conducted to assess the difference of made tea volume and made tea quality due to different green leaf quality.

Materials and Methods

This experiment was carried out at Black Tea Factory of Bangladesh Tea Research Institute (BTRI) Sreemangal, Moulvibazar from March 2022 to December 2022. Three categories of green tea leaves, (i) containing 80% good (fine) leaf (GL_{80%}), (ii) containing 50% good (fine) leaf (GL_{50%}) and (iii) containing 30% good (fine) leaf (GL_{30%}) of BTRI released clonal tea variety BT2 were the different treatments of the experiment. After the arrival of the green tea leaves into the factory, leaf analysis was immediately carried out to determine the good (fine) leaf percentage. The 'Ballometric method' was followed for assessing the good leaf percentage of the green tea leaves. In brief, fresh tea shoots were picked up at random and weighed to equivalent weight of 100 numbers of metal balls of equal size. Then the soft tender leaves (two leaves and a bud, soft single banji), which can be easily separated by slight hand pressure from the remaining portion of the shoots are separated manually and kept as good or fine leaves. When all the fine leaves were separated, the good leaves were weighed to equivalent weight against the same sized balls. At this time, the number of balls equivalent of the good leaf weight represent the good leaf percentage. For the manufacturing of black tea, BTRI standard manufacturing technique was followed which is Green Leaves→ Withering→ Rolling→ Oxidation→ Drying→ Sorting. In this experiment, made tea samples were sorted by FBOP, BOP, GBOP, OF and Dust grades according to standard mesh sizes. Made tea of each grade (FBOP, BOP, GBOP, OF and Dust) from three different green leaf quality (GL_{80%}, GL_{50%}, GL_{30%}) were used for volume measurement. After volume measurement, each grade from different green leaf quality were used for biochemical assessment. Total Polyphenols (%), Theaflavin (%), Thearubigin (%), TF:TR ratio, Total Color (%), Brightness (%) and Caffeine (%) of each sample were determined by following methods:

Method for Total Polyphenols (PL) determination (ISO 1402-1, 2005; Anesini et al., 2008)

In short, a test tube was filled with 1 ml of diluted sample. Then 5 ml of 10% Folin-Ciocalteu's reagent (FCR) and 4 ml of sodium carbonate solution (7.5% w/v) was added. Then this mixture in test tube was allowed to stand for 60 minutes at room temperature. After this period, absorbance was measured at 765 nm by using UV-vis spectrophotometer. Galic acid was used as standard. Using a standard curve for gallic acid, the total amount of polyphenols was represented as a percentage of mass on a dry matter basis for the sample.

Method for Theaflavin, Thearubigin, Total Color and Brightness (Borse, 2012)

Theaflavin (TF), Thearubigin (TR), Total Color and Brightness were estimated from tea brew by following the UV-vis spectrophotometric method with slide modification. A 9 g sample of tea was boiled in 375 ml of water for ten minutes. After that, a cotton cloth was used to filter the tea infusion. Infusion was kept at room temperature to cool it down. Disodium hydrogen phosphate 1% (w/v) aqueous solution was added to 6 ml of the infusion. 10 ml of ethyl acetate was added to that mixture by rapidly repeating inversion for one minute. The ethyl acetate layer containing the TF fraction was obtained after the bottom layer was drained away. Then, 5 ml of ethyl acetate was used to dilute the TF fraction. Absorbance measurements at 380 nm and 460 nm were used to determine the optical densities (E1, E2, and E3). To generate 25 ml of methanol (E1), 10 ml of TF extract was diluted. A 1 ml of infusion was diluted to ten ml with water and made up to 25 ml with methanol (E2). A 1 ml of tea infusion was mixed with 1 ml of 10% (w/v) of oxalic acid and diluted to 10 ml with water, the made up to 25 ml with methanol (E3). Optical densities of E1, E2 and E3 were measured at 380 nm and 460 nm. At 380 nm, the percent of TF and TR was computed, and at 460 nm, the percent of total color and brightness were calculated.

$$\text{TF (\%)} = 2.25 \times E1;$$

$$\text{TR (\%)} = 7.06 \times (4 E3 - E1);$$

$$\text{Total colour (\%)} = 6.25 \times 4E2;$$

$$\text{Brightness (\%)} = E1 / 4E2 \times 100;$$

Method for caffeine determination

The caffeine content in black tea was by following Belay et al. (2008) method with slide modification. 200 ml of water and 2.5 g of black tea sample were boiled for ten minutes while being stirred. It was then cooled at room temperature after filtering through cotton wool. With distilled water, this solution's volume was increased to 250 ml. Dichloromethane was added to this diluted mixture in a ratio of 1:1 in order to extract the caffeine using a separating funnel. With 25 ml of dichloromethane added to each round, the operation was carried out four times, and the solution were collected in a volumetric flask. The absorbance of the extracted solution was measured at 270 nm using a UV-vis spectrophotometer. By using a standard calibration curve, the caffeine (%) was calculated.

Organoleptic Quality assessment (BTRI, 2009; BTRI, 2021)

The traditional organoleptic tasting procedure was used to evaluate the organoleptic quality of manufactured teas and scoring was done on the basis of liquor characteristics. In order to make the liquor, boiling water was poured into a mug with a 142 ml (0.25 pint) capacity which contained 2.5 g of tea. The liquor was then poured into a bowl after 5 minutes of brewing as well as the infused leaf had to be taken from the mug into the inverted lid, which was then placed on top of the mug. Both the infused leaf and the liquor were then tasted by taster panel. Infused leaf appearance and liquor characteristics were assessed by scoring numerically out of 50 points as below:

- Infusion (10)
- Liquor characteristics (40 points): Liquor colour (10 points), Briskness (10 points), Strength (10 points), Creaming down (10 points)

The Duncan's Multiple Range Test (DMRT) was used to compare means after the analysis of variance was calculated. The degree of relationship was measured using the correlation coefficient (r) by RStudio version 2022.07.2 and linear regression graph was prepared by using Microsoft Office Excel version 2019.

Results and Discussion

Effect of green leaf quality on made tea volume and quality (biochemical properties and organoleptic quality) were described below:

Effect of green leaf quality on made tea volume

It was observed that, in case of any particular grade, the volume differs with the green leaf quality. From Table 1 it was observed, tea volume of individual each grade was lowest in GL_{80%} while highest in GL_{30%} leaf quality. The density of tea is inversely correlated with volume, meaning that the density is higher when the volume is smaller and vice versa. In this experiment, made tea became more flaky or lighter with the decrease of green leaf quality. Flaky means lower density or high-volume tea which is subjected to problems in packaging and transportation because it covers more volume (area). On the other hand, grainy or low volume tea was made from higher quality green leaf which had more density and quality. The correlation coefficient (r) was (-) 0.99 explaining as a strong negative correlation between green leaf quality and made tea volume and the linear regression graph was made (Figure 1) where regression equation was $y = -0.2115x + 277.09$.

Table 1. Volume (cc) of 100 gm different graded tea from different leaf quality.

Green leaf quality (Good leaf %)	Volume (cc) per 100 gm				
	FBOP	BOP	GBOP	OF	Dust
GL _{80%}	297.56c	282.97c	265.29c	255.47c	200.74c
GL _{50%}	303.24b	288.18b	271.47b	259.37b	207.39b
GL _{30%}	308.39a	293.64a	275.39a	264.89a	213.19a
P value	0.046	0.022	0.031	0.026	0.011
CV (%)	7.45	8.28	6.36	8.61	7.84
LSD at 5% level of significance	4.17	5.04	2.94	3.97	5.81

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

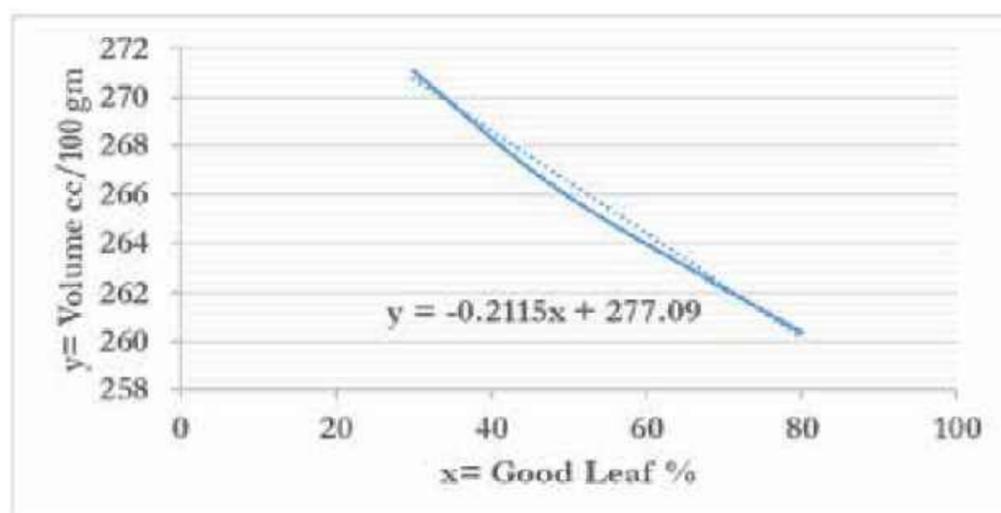


Fig. 1. Linear regression graph of $y = \text{Volume cc/100 gm}$ against $x = \text{Good Leaf } (\%)$.

Effect of green leaf quality on quality of made tea

Quality of made tea is defined by both biochemical properties and organoleptic quality of made tea. Effect of green leaf quality on biochemical properties and organoleptic quality are described below:

Biochemical properties of made tea

With an increase of coarse plucking standards; the caffeine, theaflavin, total ash, and total water soluble solids contents of black tea decreased, although thearubigins (Amiri & Asil, 2007), crude fiber (Śmiechowska & Dmowski, 2006) and floride (Lu et al., 2004) content was found to be increased. Green leaf quality i.e. the 'Good leaf (%)' affects the biochemical properties of made tea (Table 2 and Table 3). From Table 3 it was observed that total polyphenols (PL), theaflavin (TF), thearubigin (TR), brightness (BR) and caffeine (CF) percentage were found to be decreased significantly with the decrease of good leaf percentage. The PL (16.23 %), TF (0.89 %), BR (27.77 %) and CF (3.17 %) found highest in green leaf with 80% good leaf. There was no significant change in TC. Since the concentrations of TF and TR are important quality indicators, the appropriate ratio between them is crucial to achieving the right balance of the black tea liquor's briskness, strength, brightness, body and color (Rahman et al., 2020). Gill et al. (2011) reported that the standard ratio of TF:TR is 1:10. In this study, the ratio of TF:TR was found to be increased with the decrease of good leaf percentage. Best ratio of TF:TR (1:10.39) was found in green leaf with 80% while the low quality of made tea with 1:12.47 ratio was found in low quality of green leaf (30% good leaf). Due to the fact that coarse plucking is attributed for the higher polyphenol oxidase level in mature shoots, a coarse plucking standard produced very high TR concentrations and low TF value (Teshome, 2019).

Table 2. Biochemical properties of different graded tea from different leaf quality.

Name of the tea grades	Green leaf quality (Good leaf %)	Total Polyphenol (%)	Theaflavin (%)	Thearubigin (%)	TF:TR	Total Color (%)	Brightness (%)	Caffeine (%)
FBOP	GL _{80%}	15.41	0.91	10.07	1:11.06	4.03	24.69	3.46
	GL _{50%}	12.94	0.73	8.64	1:11.83	3.53	23.31	2.69
	GL _{30%}	9.40	0.9	8.06	1:8.95	3.15	21.36	2.43
BOP	GL _{80%}	17.91	0.81	8.2	1:10.12	4.35	26.66	3.32
	GL _{50%}	14.76	0.83	9.14	1:11.01	4.1	23.13	2.83
	GL _{30%}	10.27	0.7	9.01	1:12.87	3.78	22.87	2.67
GBOP	GL _{80%}	16.73	0.92	9.5	1:10.32	5.23	33.73	3.27
	GL _{50%}	14.13	0.93	10.46	1:11.24	5	27.44	2.75
	GL _{30%}	10.15	0.7	9.53	1:13.61	4.85	24.13	2.48
OF	GL _{80%}	16.59	1.23	12.84	1:10.43	4.93	26.65	2.95
	GL _{50%}	13.99	0.94	11.98	1:12.74	4.38	24.14	2.72
	GL _{30%}	10.11	0.91	12.55	1:13.79	3.98	18.75	2.39
Dust	GL _{80%}	14.54	0.56	5.66	1:10.10	4.08	27.14	2.87
	GL _{50%}	13.42	0.93	9.66	1:10.38	3.85	22.89	2.56
	GL _{30%}	9.5	0.69	9.49	1:13.75	3.75	19.84	2.29

Table 3. Biochemical properties of tea from different leaf quality.

Green leaf quality (Good leaf %)	Total Polyphenol (%)	Theaflavin (%)	Thearubigin (%)	TF:TR	Total Color (%)	Brightness (%)	Caffeine (%)
GL _{80%}	16.23 a	0.89 a	9.25 c	1:10.39 c	4.52	27.77 a	3.17 a
GL _{50%}	13.84 b	0.87 a	9.98 a	1:11.47 b	4.17	24.18 b	2.71 b
GL _{30%}	9.88 c	0.78 b	9.73 b	1:12.47 a	3.9	21.39 c	2.45 c
P value	0.0001	0.045	0.0405	0.006	0.523	0.0075	0.0001
CV (%)	6.62	9.23	8.24	7.51	6.56	10.63	6.34
LSD at 5% level of significance	1.21	0.02	0.09	0.82	Ns	1.64	0.11

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

Organoleptic quality of made tea

Organoleptic quality of tea also differs with the quality of green leaf (Table 4). Highest quality (32.892) made tea was found from GL_{80%} leaf quality while lowest quality (32.182) was observed in GL_{30%} leaf quality. Intermediate quality (32.570) made tea was found in GL_{50%} leaf quality.

Table 4. Organoleptic quality of tea from different leaf quality.

Green leaf quality (Good leaf %)	Infusion	Liquor colour	Briskness	Strength	Creaming down	Total
GL _{80%}	7.340a	7.604a	7.480a	7.432a	3.036a	32.892a
GL _{50%}	7.310b	7.504b	7.444b	7.380b	2.932b	32.570b
GL _{30%}	7.298c	7.330c	7.328c	7.338c	2.888c	32.182c
P value	0.0097	0.048	0.0036	0.0041	0.007	0.0068
CV (%)	3.94	4.87	2.39	3.18	3.72	2.63
LSD at 5% level of significance	0.004	0.09	0.003	0.04	0.009	0.18

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

Organoleptic quality of made tea varies due to more good leaf percentage which possesses more biochemical compounds. As the good leaf percentage decreases, biochemical compounds also decrease with the overall liquor quality. The amount of reaction substrates (catechins) and enzymes (polyphenol oxidase and peroxidase) in fresh tea leaves is a key factor influencing the production of theaflavins. These elements also impact the made tea beverage's sensory quality (Mizani et al., 2011). With two leaves and a bud, the theaflavins (TFs) concentration was at its highest. But, lower amounts of polyphenols and polyphenol oxidase activity likely contributed to the decrease in TFs levels produced by additional leaves (Tüfekci & Güner, 1997). According to the age of the leaves, there are differences in the catechin distribution within a shoot (Robertson, 1983). Lin et al. (1996) reported that, higher amount of total catechins was found in the soft leaves (5.86%) while concentration was found to be declined in old leaves (2.15%) and stem (0.85%).

Relationship between biochemical properties with organoleptic quality of made tea and green leaf quality

Black tea's quality decreased regarding both of the plain and aroma quality characteristics with the decrease of plucking standards (Mahanta, 1988). Relationship between biochemical properties with organoleptic quality of made tea and green leaf quality was determined by Pearson's Correlation Coefficient (r) value. From Figure 2, it was observed that strongest positive relation (0.93^{***}) was found between total polyphenols (%) and good leaf (%). Correlation Coefficient (r) were +0.88^{***} and +0.80^{***}, respectively for caffeine and organoleptic score against good leaf (%) also explaining the presence of strong positive relationship. The relation between total color and good leaf percentage

was insignificant where brightness had moderate relation (+0.75**). Negative but significant relationship was observed between TF:TR ratio and good leaf percentage. Significant strong relation also found between total polyphenols and caffeine content (+0.87***) as well as between total polyphenols and organoleptic score (+0.86***).

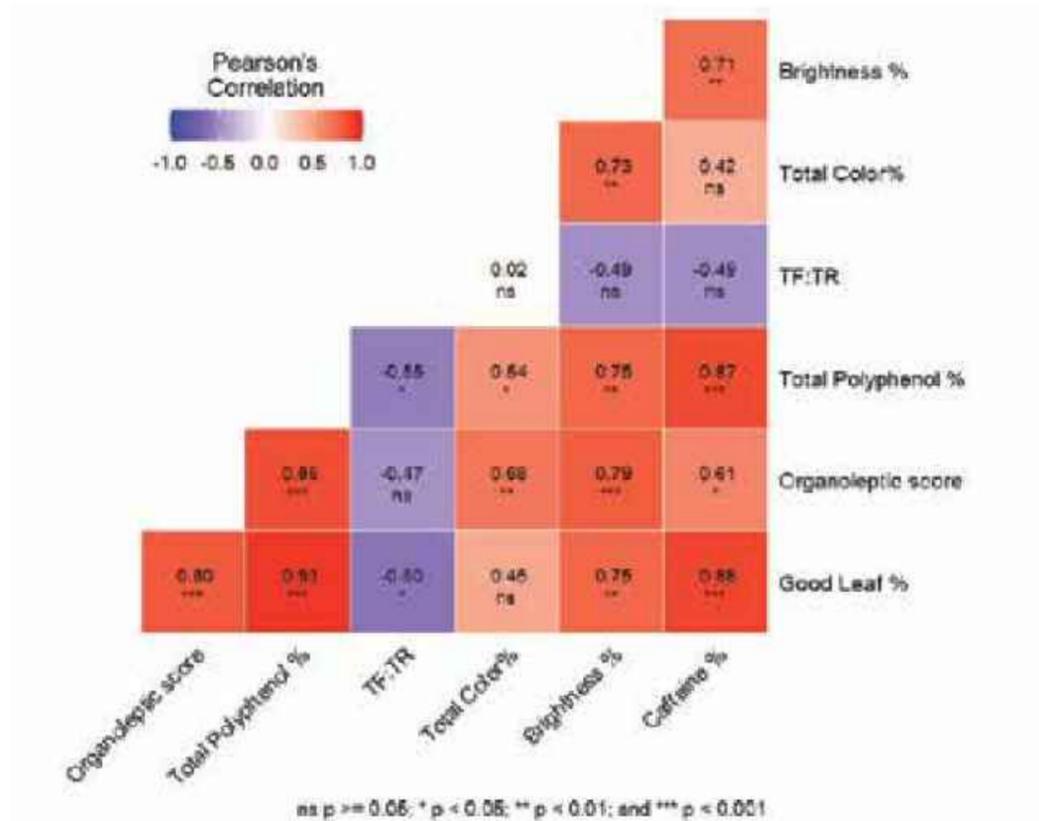


Fig. 2. Relationship between quality of made tea and green leaf quality.

Linear regression graph of Total Polyphenols (%), TF:TR ratio, Total Color (%), Brightness (%), Caffeine (%) and Organoleptic score against good leaf (%) was presented in Figure 3-8. Linear regression graph of Total Polyphenols (%), TF:TR ratio, Total Color (%), Brightness (%), Caffeine (%) and Organoleptic score were $y = 0.1172x + 6.3321$, $y = -0.0412x + 13.638$, $y = 0.0123x + 3.5384$, $y = 0.127x + 17.675$, $y = 0.0144x + 2.0074$, $y = 0.0139x + 31.804$ respectively.

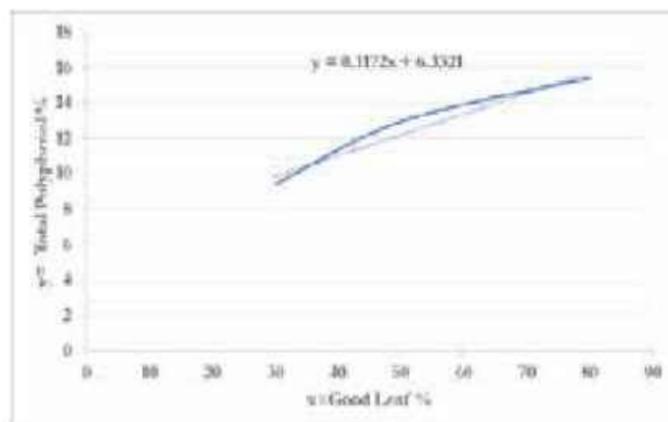


Fig. 3. Linear regression graph of Total Polyphenols (%) against x= good leaf (%)

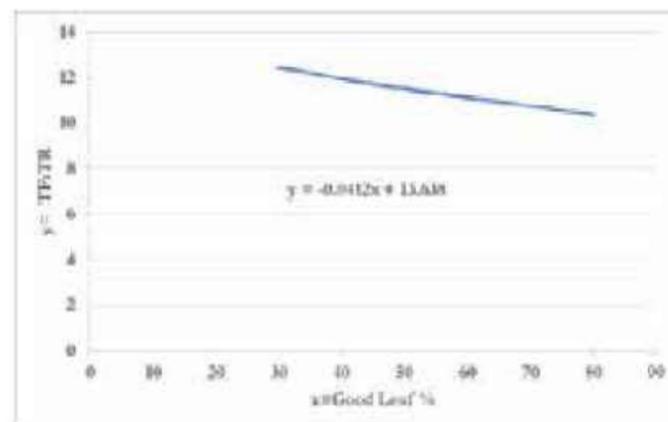


Fig. 4. Linear regression graph of TF:TR against x= good leaf (%)

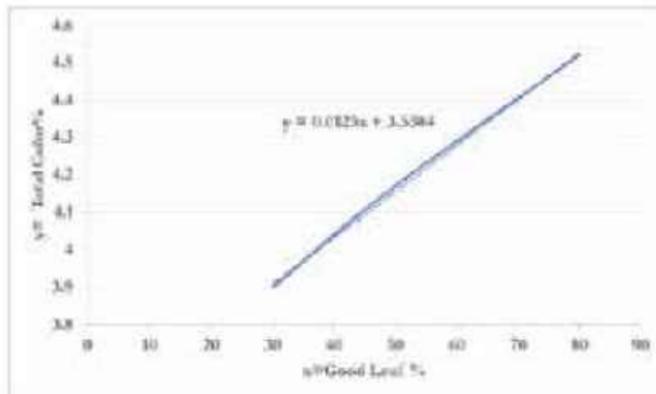


Fig. 5. Linear regression graph of Total Color (%) against x= good leaf (%)

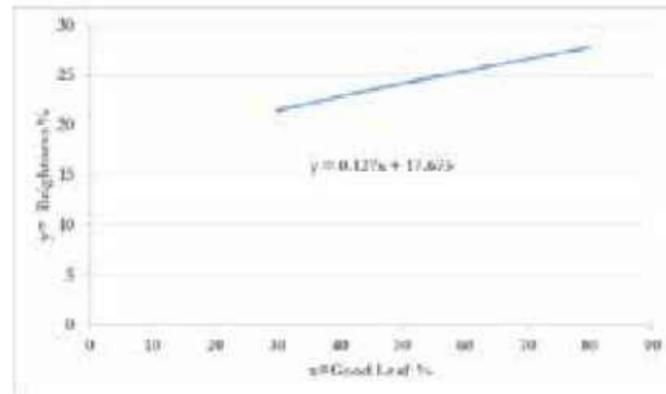


Fig. 6. Linear regression graph of Brightness (%) against x= good leaf (%)

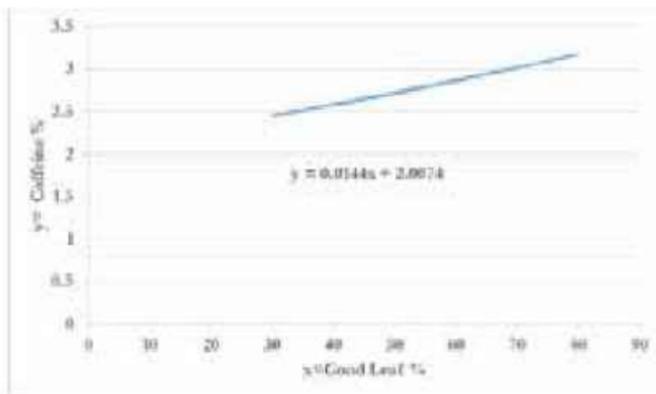


Fig. 7. Linear regression graph of Caffeine (%) against x= good leaf (%)

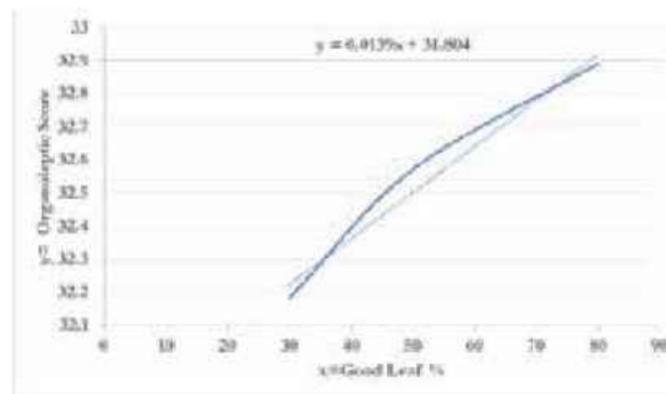


Fig. 8. Linear regression graph of Organoleptic score against x= good leaf (%)

The findings were explained by the fact that tender and soft tea shoots have significant quantities of polyphenols, which causes the simple quality criteria of the made tea (Owuor & Obanda, 1995). When the more mature leaves of the tea shoots are utilized as the raw material for manufacturing, the quality of black tea is significantly reduced (Teshome, 2019).

Conclusion

The volume of made tea varies with the green leaf quality. Tea volume of individual each grade (FBOP, BOP, GBOP, OF and Dust) was lowest in GL_{80%} while highest in GL_{30%} leaf quality. Volume is inversely proportional to the density of tea i.e. larger volumes have lower densities, whereas smaller volumes have higher densities. Total polyphenols (%), theaflavin (%), thearubigin (%), brightness (%) and caffeine (%) were found to be decreased significantly with the decrease of good leaf percentage. Total polyphenols (16.23 %), theaflavin (0.89 %), brightness (27.77 %) and caffeine (3.17 %) found highest in green leaf with 80% good leaf. A fundamental quality criterion for determining the body, color, strength, brightness, and briskness of black tea beverage is the TF/TR ratio. Best TF/TR ratio (1:10.39) was found in green leaf with 80% while the low quality of made tea with 1:12.47 ratio was found in low quality of green leaf (30% good leaf). Strongest positive relation (0.93^{***}) was found between total polyphenols (%) and good leaf (%). Finally, it can be concluded that, the made tea quality i.e. both biochemical properties and organoleptic quality mostly depends on the green leaf quality. The quality of the tea is increased in accordance to the quality of the green leaf. So, our planters must put special consider on good quality green leaf to make best quality and high density made tea as well as to get more auction price.

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IDENTIFICATION OF IDEAL LIGHT PRUNING TIME FOR SMALL HOLDING TEA PLANTATIONS IN THE CHATTOGRAM HILL TRACTS

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Abstract

One of the most crucial operations in tea cultivation is pruning, in which the branches of the tea bushes are slash down at a predetermined height to reinvigorate the bush and bring it within the pluckers range. Pruning also helps to maximize the yield and quality. Among the pruning operations practiced, Light Pruning (LP) is one of the drastic and vital operations that has significant impact on tea production and quality. Recovery potential of a tea plant after pruning depends on many factors among which time of pruning plays an important role because of the surrounding environmental conditions. In this context, the current study was conducted to identify the best time of LP in the Chattogram Hill Tracts of Bangladesh. Starting from 1st November, seven different dates were tested maintaining about 20 days interval. Results showed that significantly highest number of plucking points/bush/round (42.33) and yield (2400 kg ha⁻¹) was obtained when LP was done on 10th December, followed by 30th December and 20th November. Significantly the lowest number of plucking points/bush/round (27.33) and yield (1267 kg ha⁻¹) was obtained when LP was done on 1st March and it was followed by 10th February. Considering the mortality percentage after pruning, it was observed that no mortality was observed when pruning was done on 1st November, 20th November, 10th December and 30th December. On the contrary, highest mortality (4.44%) was observed when pruning was done on 10th February followed by 1st March and 20th January. From the findings, 10th December was identified as the ideal time of LP for the tea plantations in the Chattogram hill tracts of Bangladesh for higher productivity.

Keywords: Tea, Pruning time, Light Pruning, Hill tracts

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Introduction

One of the most popular beverages consumed worldwide is tea. *Camellia sinensis*, the tea plant, is grown in roughly 30 different nations (Adnan et al., 2013). Although it originated in South Eastern China, it is now grown in several tropical and subtropical areas across the world and contains more than 82 genetically distinct species (Krafczyk & Glomb, 2008; Sultana et al., 2008; Akhlas et al., 2003). A tea plant naturally develops into a tiny tree, but successive pruning and other silvicultural techniques, such as tipping, plucking, and harvesting the best vegetative product, transform it into a bush (Kumar et al., 2015). Next to plucking, pruning is one of the most crucial operations that affect productivity and quality of tea (TTRA, 2008). To rejuvenate and bring tea bushes within reach of the pluckers, pruning is the clipping of tea bush branches at a certain height and at a predetermined interval. Pruning is necessary to control apical dominance, maintain bushes in the vegetative stage, and direct energy toward the formation of leaves. Additionally, it promotes branching, which results in more tender leaves (Ravichandran, 2004).

Generally, the tea planters of North East India and Bangladesh consider December to January as optimal time to prune tea bushes. During this period tea plant has optimum level of starch reserve in tea roots (Sarma, 2023). The regeneration of the tea bush as well as the carbohydrate balance of the root and shoot system is greatly impacted by deep pruning (Bore et al., 2003). Qamar et al. (2011) reported the highest shoot growth (71.33 cm) and fresh leaf output from tea plantations that were pruned in December. Maximum plucking points in tea plants pruned in early November and receiving 240 kg N ha⁻¹ were also recorded by Hamid et al. (2000). According to Hajra (2001), the optimal period for tea pruning in North East India is between December and January when the starch reserve in the roots is completely grown.

In the 1840s, tea farming in Bangladesh began in the area of the Chittagong Club. The first tea garden in Bangladesh was established in Sylhet in 1854 and it began producing tea commercially in 1857 (BTB, 2023). Since 2002, the tea cultivation was also introduced in the northern parts of the country namely Panchagarh, Lalmonirhat, Thakurgaon, Nilphamari, Dinajpur and the southern district Bandarban in the Chattogram Hill Tracts. To extend small holding tea cultivation and to provide suitable technologies to the growers of Chattogram Hill Tracts (Bandarban sadar, Rowangchari and Ruma upazila), Ministry of Commerce of Bangladesh has launched a project titled as 'Extension of Small Holding Tea Cultivation in Chattogram Hill Tracts in the year 2016 (BTB, 2023). Pruning operations mostly depends on time, topography and climate. As the topography and climate of Chattogram Hill Tracts is completely different than the other tea growing regions of the country, it is necessary to identify the ideal pruning time for this region. Among the pruning operations practiced, Light Pruning (LP) is the most vital one and has a substantial impact on yield and quality. Therefore, the current research was carried out to observe the effect of different light pruning time on crop yield and identify the best time for higher productivity in the Chattogram Hill Tracts of Bangladesh.

Materials and Methods

The experiment was carried out in a 12-years old mature tea (BT2) section of small holding tea planter Mr. Osman Goni in Bandarban sadar, Bandarban Hill Tracts during the period of November 2020 to December 2021. Three replications of the experiment were done using a randomized complete block design (RCBD). There were seven treatments of the experiment. Starting from 1st November 2020, light pruning completed at different dates maintaining about 20 days interval were the treatments of the experiment. The treatments were denoted as T1 (LP on 1st November), T2 (LP on 20th November), T3 (LP on 10th December), T4 (LP on 30th December), T5 (LP on 20th January), T6 (LP on 10th February) and T7 (LP on 1st March), respectively. Pruning of tea bushes and fertilizer applications were done according to the BTRI recommendation (BTRI, 1994; BTRI, 1986). There was no irrigation facility in the experimental site and hence the experiment was fully depended on rain water. Five (5) layers of tipping allowance were given to each bush. Plucking was carried out mostly at weekly interval and the related data were collected. Data were collected on (a) Number of shoots plucked per bush (plucking points) per round, (b) Weight of the shoots per bush per round to calculate the green leaf yield (kg ha⁻¹) and (c) Mortality (%) of the tea bushes after pruning. Data were analyzed statistically with the help of MSTATC software.

Results and Discussion

Effect of light pruning time on number of plucking points

It was observed that the number of plucking points is significantly impacted by pruning time (Table 1). The treatment T3 (LP on 10th December) produced significantly highest number of plucking points/bush/round (42.33), which was followed by T4 (LP on 30th December), T2 (LP on 20th November), T1 (LP on 1st November), T5 (LP at 20th January) and T6 (LP on 10th February). Significantly the lowest number of plucking points/bush/round (27.33) was obtained in T7 (LP on 1st March). The plucking points obtained in T3 and T4 are statistically comparable to each other. Similarly, the plucking points obtained in T4, T2, T1 and T5 are comparable to each other. The lowest plucking points obtained in T7 are also comparable with T6.

Table 1. Effect of light pruning at different times on the number of plucking points.

Treatments	Plucking points (per bush per round)
T1 (LP on 1 st November)	34.66bc
T2 (LP on 20 th November)	37.66b
T3 (LP on 10 th December)	42.33a
T4 (LP on 30 th December)	41.33ab
T5 (LP on 20 th January)	34.33bc
T6 (LP on 10 th February)	30.66cd
T7 (LP on 1 st March)	27.33d
LSD at 5% level of significance	8.33
CV (%)	13.16

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

From the results, a gradual decline in the number of plucking points/bush/round was observed in the treatments after 10th December (T3). This result is in accordance with the result of [Ahmad et al. \(2014\)](#) who reported that most plucking points were produced by tea bushes when pruned on December 10th.

Effect of light pruning time on green leaf yield

Satisfactory recovery of the tea bushes after pruning and the productivity of the bushes depends on many factors including the type and level of intensity of pruning, condition, activity, and reserves of the root system, timing of pruning, tea bush health which reflects pruning techniques used in the past, nutrition of the bush, soil quality, rainfall patterns, and temperature ([TRIT, 2004](#)). In this study, different time of LP was tested to identify the best time specific to the Chattogram Hill Tracts of Bangladesh as the topography and climate of this region is completely different than the other tea growing regions of the country. From the result (Table 2), it was observed that significantly highest green leaf yield of 2400 kg ha⁻¹ was obtained in the treatment T3 (LP on 10th December), which was followed by T4 (LP on 30th December), T2 (LP on 20th November), T5 (LP on 20th January), T1 (LP on 1st November) and T6 (LP on 10th February). Significantly the lowest yield of 1267 kg/ha was obtained in T7 (LP on 1st March). Statistically, the highest yield from T3 is comparable with the yields from T4, T2 and T5. Again, the yields from T4, T2, T5 and T1 are comparable with each other. The lowest yield from T7 is also comparable with the yield from T1.

Table 2. Effect of light pruning at different times on the yield of tea.

Treatments	Yield (kg ha ⁻¹)
T1 (LP on 1 st November)	1767bcd
T2 (LP on 20 th November)	2033abc
T3 (LP on 10 th December)	2400a
T4 (LP on 30 th December)	2350ab
T5 (LP on 20 th January)	1950abc
T6 (LP on 10 th February)	1500cd
T7 (LP on 1 st March)	1267d
LSD at 5% level of significance	621.39
CV (%)	14.29

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

According to [Satyanarayana et al. \(1994\)](#), effective pruning promotes improved branching, which results in more tender leaves. If timing of pruning is appropriate, maximum number of shoots can be generated that is helpful to maximize production. In this study, plucking point was highest in treatment T3 (Table 1) and the yield was also highest in the same treatment (Table 2). It is known that for proper recovery, pruning should be done in such a time when the bushes are in dormant stage and have enough starch reserve to withstand the shock and further regrowth. It was observed that the bushes of the experimental site were at complete dormant stage in early December. Most probably the starch reserve was highest at this time and hence the highest yield was obtained in the treatment T3 (LP on 10th December). At the NTRI tea garden, [Hamid et al. \(2002\)](#) found that pruning during the dormant season resulted in the largest production of fresh tea leaves per bush.

Effect of light pruning time on mortality (%) of the tea bushes

It was shown that the timing of pruning had a significant impact on the mortality (%) of tea bushes (Table 3). Significantly the highest mortality (%) of 4.44 was recorded in the treatment T6 (LP on 10th February), which is statistically similar with the mortality from the treatments T5 (LP on 20th January) and T7 (LP on 1st March). No mortality was found in the treatments T1 (LP on 1st November), T2 (LP on 20th November), T3 (LP on 10th December) and T4 (LP on 30th December).

The tea plant's starch reserves, according to Chakrabarty & Barpujari (1992), are a key factor in determining when to prune since new growth depends on the reserves, and pruning in any form without an appropriate store of carbohydrates might be devastating. With pruning operation, a large volume of the foliage and young shoots is removed off resulting in cessation of carbohydrate synthesizing activities of the plants. Thus, the initial growth of new developing shoots for formation of bush frame and recover from pruning is supported by the carbohydrate reserve in the roots (Selvendran & Selvendran, 1972). After pruning, it was seen that the amount of root reserves significantly decreased (Wijeratne et al., 2002). Availability of adequate soil moisture is also important for translocation of starch from root-serve to the growing points. Severe moisture stress may reduce root carbohydrate concentrations but may faster its consumption in the droughty areas (Marimuthu et al., 1996; Barman et al., 1998).

Table 3. Effect of light pruning at different times on mortality (%) of tea bushes.

Treatments	Mortality (%)
T1 (LP on 1 st November)	0
T2 (LP on 20 th November)	0
T3 (LP on 10 th December)	0
T4 (LP on 30 th December)	0
T5 (LP on 20 th January)	2.22a
T6 (LP on 10 th February)	4.44a
T7 (LP on 1 st March)	2.22a
LSD at 5% level of significance	6.34
CV (%)	11.22

Within column values followed by different letter (s) are significantly different by DMRT ($p \leq 0.05$)

In the Chattogram hill tracts, rain flush is generally available till late November and starts again from April-May. Therefore, acute water scarcity is observed from the beginning of the year to till April-May. In the present study, the plants light pruned after December most probably suffered due to shortage of food reserve due to drought condition, and hence the mortality is high in the treatments T5 (LP on 20th January), T6 (LP on 10th February) and T7 (LP on 1st March). On the other hand, no mortality was observed in the treatments where light pruning was completed before or within December, probably due to the better starch reserve at the time of pruning and the bushes could alleviate the drought condition easily as demand of water was less due to defoliation by pruning.

Conclusion

Light pruning at different times had significant effect on the yield of tea. Considering the number of plucking points/bush and yield, higher performances were observed when light pruning was completed within December; and more specifically, the highest performances was on 10th December. Higher bush mortality (%) was observed when light pruning was carried out after December. Therefore, it can be concluded that mid-December (within 10th December) is the ideal time for Light Pruning for maximum crop production and lower bush mortality (%) in the Chattogram hill tracts of Bangladesh.

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