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C O N T E N T S

Editorial

The 47th volume of Tea Journal of Bangladesh contains five research articles.

The first article is about yield and quality performances of six vegetative propagated test clones of tea namely MZ/39, E/4, D/13, B2T1, BR2/97 and SDL/1 with control BTRI released renowned variety named BT2. It was found that among these test clones, MZ/39 gave significantly highest yield of 1612.4 kg/ha and 3447 kg/ha made tea at immature and mature stages respectively. The cup quality of test clone MZ/39 was found to be above average while cup quality of control and other test clones were also above average. Considering yield and quality potentials, the test clone MZ/39 appeared quite promising and finally it has been released as 'BT21' in 2018.

The second article is on foliar application of a Liquid fertilizer as a supplementary dose on the yield of mature tea. While we believe foliar feeding is useful and effective in correcting deficiencies, it is also used primarily to increase yield and quality by overcoming the limitations of the soil. Foliar fertilizers with different origins have been successfully used as foliar spray in different crops but as far as we know no beneficial or harmful effects of these liquid fertilizers is documented yet in Bangladesh tea. Therefore, one of the commercial liquid fertilizers "Liquid Gold/Excel Super" was evaluated in this study. The findings of the present study revealed that along with the BTRI recommended doses of Urea, TSP and MOP as ground application, the foliar application of the liquid fertilizer "Liquid Gold/Excel Super" at the dose of 2 ml/L water (2 L/ha, mixed with 1000 L water) with five rounds application (at 5 weeks interval) during the cropping season might be considered as an effective and economic viable dose for enhancing tea yield.

The third article was addressed as "Field factors on *Corticium theae* Bernard a causal agent of Black rot disease in tea". Dense shade, bad drainage and sanitation, high humidity etc. are usually considered the predisposing factors for the prevalence and severity of the disease. Microclimate of an area under tea plantation is greatly influenced by the architectures of plantation. In the same area there are tillah, flat and hillock directly effect on penetration of solar radiation, humidity, temperature and air circulation, thus influence plant growth as well as diseases development. The research work was achieved on the pattern of distribution and severity of the Black rot disease in the commercial tea fields to minimize gaps in knowledge of the progress of the disease and our understanding of the complex relationships between various dimensions. The result reflects the impact as- seedlings, mature tea bushes, hillock areas are the more prone to Black rot disease for incidence while the heavy shade condition is for disease severity. The findings of this study will help understand the pattern of distribution of the disease in the commercial tea fields so that the planters can escape the disease easily.

The forth research article is concerned to the impact of red rust infection on the physiological and biochemical changes in tea leaves as well as quality parameters of made tea. The crop loss in respect of yield due to the disease was assessed in different regions of the world but there is very little information on the physiological and biochemical changes as well as deterioration of quality of made tea caused by the said disease. The researcher examined the physiological parameters like Chlorophyll-a, Chlorophyll-b & Carotenoids and quality evaluating parameters like Theaflavins (TF), Thearubigins (TR), High polymerized Substances (HPS), Color index (CI), Total liquor color (TLC) and Caffeine was assessed in case of red rust infected shoot and healthy shoot. The result reflects a significant reduction in quality of tea in terms of Chlorophyll-a, Chlorophyll-b and Carotenoids in infected shoot. Contents of TR, HPS, TLC and Caffeine decreased considerably but CI increased in red rust infected shoots. The findings may grow a keen attention to the planters to ensure the quality of made tea by controlling the red rust disease in their tea gardens.

The final article reports to identify the plant parasitic nematodes associated with tea in Bangladesh soil. A total of 12 plant parasitic nematode genera under 10 families were identified at different tea estates of six valley circles in greater Sylhet, Chattogram and Panchagarh regions. Author studied the nematodes up to genus level based on morphological characters and measurement. Nematode is one of the major soil pests of Bangladesh tea in the nursery and new clearing and invades tea seedlings, upto 1 year old, in young plantation. The information generated in this article will help to make a clear concept about the species and structure of plant parasitic nematode associated with tea soil in Bangladesh.

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. Mohammad Ali**)
Chief Editor

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YIELD AND QUALITY ASSESSMENT OF SIX TEST CLONES OF TEA

M.A. Aziz[1], M.I. Hossain[2*] and M.R. Arefin[3]

Abstract

The present study was conducted to investigate the long term yield and quality performances of six test clones of tea namely MZ/39, E/4, D/13, B2T1, BR2/97 and SDL/1 with control BT2. The results of the experiment revealed that, at immature stage i.e. 1st year to 5th year after plantation, their yield differences were significant. The test clone MZ/39 gave highest yield of 504.8 g/plant and 1612.4 kg/ha made tea compared to control. Again, at mature stage amongst the six test clones MZ/39 gave the highest significant yield of 1079.2 g/plant and 3447 kg/ha made tea compared to control. The overall cup quality of the test clones and the control was assessed by conventional organoleptic test. The cup quality of test clone MZ/39 was found to be above average while cup quality of control was also above average. Considering yield and quality potentials, the test clone MZ/39 appeared quite promising and finally it has been released as 'BT21' in 2018.

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

Introduction

Tea is one of the most consumed non-alcoholic as well as medicinal beverage in the world. Now, there are 167 tea estates having about 63,500 hectare of tea plantation producing about 96.07 million kg of made tea per annum with an average yield of about 1769 kg per hectare in Bangladesh (BTB, 2020).

Our present yield per hectare is quite low compared to other tea growing countries of the world. One of the major reasons is around 35% of our tea growing area is covered with seedling plants with over 60 years old plant which are of lower productivity (PDU, 2015). The internal consumers of the country are presently consuming about 98% of its produce. Consequently, this increase in internal consumption causes decrease of exportable surplus with a slow rate of increase in production, which in turn causes to decline in export of tea. So, developing high yielding varieties and seed stock seems to be our only way to mitigate both demands as Bangladesh is a land hungry country. Emphasis should be given on selection and planting of vegetatively propagated material i.e. high yielding quality clones for better yield and quality of tea (Dutta and Alam, 2001).

The increasing cost of production as well as adverse climatic conditions have led to marginal economic return to the tea industry. In these circumstances the industry needs to replant and extend new tea areas with improved planting materials of higher yield and good quality. Due to the heterogenous nature of tea seedlings, the seed available except biclonal stocks, could not guarantee the production of improved planting material (Njuguna, 1990).

With an objective of evolving planting materials with high yield and quality potential BTRI has put its priorities on clonal selection and hybridization programme since its inception. The clonal selection programme was initiated in 1959 and hybridization programme in 1965 (Rashid and Alam,

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1990). As an outcome of these studies, the institute so far released twenty one vegetative clones in the BT-series to the industry. The present experiment was carried out to study the long term yield and quality performances of six vegetative propagated test clones of tea with a view to find out a new vegetative clone of higher yield and better quality.

Materials and Methods

The experiment was carried out with six test clones of tea in the experimental field of BTRI experimental farm, during the period from 2001 to 2016. After rooting trial in the nursery the selected test clones, namely MZ/39, E/4, D/13, B2T1, BR2/97 and SDL/1were put to long term yield and quality trial during 2001 at BTRI Farm. The experiment was laid out by following Randomized Complete Block Design (RCBD) with 105cm x 60cm spacing of plot size. BT2 was used as a standard (control) for yield and quality comparison. The experiment was conducted in rain-fed condition. Yield data was collected during the cropping seasons throughout the experimental period. Fertilizer mixture was applied as per BTRI recommendations (BTRI Pamphlet nos. 21 & 22). Young and mature tea pruning were followed as per BTRI recommendations (BTRI Pamphlet nos. 79); for young tea, as follow: Decentre-Prune-Skiff-Prune-Skiff and for mature tea, as follow: Light prune-Deep skiff-Medium skiff-Light skiff (BTRI Pamphlet nos. 111). The green leaf was harvested at weekly interval during the plucking season starting from mid-March to mid-December throughout the experimental period. Yield data were recorded and analyzed statistically using MSTAT programme in a microcomputer. The mean values were adjusted by DMRT. The yield was expressed as mean yield of green leaf g plant^{-1} and is presented separately for immature (1st - 5th year) and mature (6th-14th year) stage. The made tea (kg ha^{-1}) was also calculated on the basis of 23% recovery from green leaf. Tea of each test clones from standard sized green leaves were manufactured by CTC method in the BTRI miniature tea factory. Quality of all the test clones and control were assessed weekly by conventional organoleptic test and scored numerically. General characteristics of six test clones (MZ/39, E/4, D/13, B2T1, BR2/97 and SDL/1) and control BT2 is given in Table 1.

Table 1. General characteristics (Aziz *et al*., 2011) of six test clones and BT2 as control

Test Clone	Bush characters	Leaf type	Pruning recovery	Nursery rooting	Cup quality	Manu. Pref.
MZ/39	Ortho-plagotropic, compact bush, densely branched with heavy girth and floriferous.	Patina is quite glossy, leaves are semi-dark green, medium in size, texture is fairly thick and hard, prominent long apex, serration uniform, leaf blade is slightly wavy and leaf pose is semi-erect and moderately embossed.	High	Excellent	Above average	CTC
E/4	Plagiotropic, loose frame, not very compact but having effective plucking points and poorly floriferous.	Leaves are medium in size, light green, quite glossy, leaf texture thin and soft, semi-erect leaf pose, apex is less prominent, long lamina, slightly boat shape, serration uniform and venation is less prominent.	High	Good.	Above average	CTC

D/13	Plagiotropic, compact plucking table, fairly dense plucking points, heavy girth with good branching behaviour and poorly floriferous.	Patina is glossy, leaves are light green, broad in size, texture is thick, soft and leathery, and apex is less prominent, leaf pose semi horizontal.	High	Good.	Above average	CTC
B2T1	Biclonal progenyof BT2 and TV1. Ortho-plagiotropic, compact bush with dense plucking points, very uniform flushing behaviour.	Patina is quite glossy, dark green foliage, texture is thick and hardy, erect leaf pose, apex is prominent, uniformly dentate serration and venation is fairly prominent.	High	Good.	Above average	CTC
BR2/97	Orthotropic, fairly compact bush, good branching, fair growth and quite hardy.	Patina quite glossy, texture is fairly thick, dark green foliage, blades are wavy with prominent apex, serration is uniform but dent bluntish, leaves are long with erect pose.	Good	Good	Above average	CTC
SDL/1	Typically plagiotropic, very compact bush and profuse branch, highly plucking density.	Patina is glossy, light green foliage, semi erect leaf pose, leaves are small in size and texture is thin and soft. Apex is less prominent; serration is less uniform and venation is fairly prominent.	Good	Good	Excellent	CTC
BT2	Orthotropic, not 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 and Assam-China hybrid type.	High	Excellent	Above average	CTC

Note: Pruning recovery & Nursery rooting: Excellent, very good, good, fair/moderate
Cup quality: Excellent, Above average, Average, Below average.

Table 2. The categories of tea clones as yield, standard and quality clones

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

* Quality score: E = Excellent (34 to >34 out of 50)
AA = above average (32 to <34 out of 50)
A = average (30-32 out of 50)
BA = Below Average (<30 out of 50).

Results and discussion

The performance of test clones are discussed under three categories (ie. Yield, Quality and Drought tolerance) which are given below:

Yield performance:

The mean yield of green leaf (g per plant) over sixteen experimental years are presented in Table 3 for immature stage (1^{st} - 5^{th} year) and in Table 4 for mature stage (6^{th}- 16^{th} year). From Table 3 it reveals that at initial stage of growth, only two test clones (MZ/39 and B1T2) showed significant higher yield trend than control BT2 while rest of the test clones gave lower yield. When the data were analyzed individual year wise (both mature and immature stages), their yield differences were significant.

Table 3. Mean yield of green leaf (g/plant) of six test clone and their control at immature stage (1^{st} – 5^{th} year)

Clone	1st Year Up (2001)	2nd Year Prune (2002)	3rd Year UP (2003)	4th Year Pruned (2004)	5th Year Skiff (2005)	Average
MZ/39	192.34 a	448.50 a	581.43 a	482.97 b	818.8 a	504.8 a
E/4	63.93 d	146.23 d	278.73 c	283.60 d	463.5 d	247.2 c
D/13	112.14 c	219.52 c	439.37 b	364.83 c	622.7 bc	351.7 bc
B2T1	112.33 c	286.67 bc	541.22 ab	536.63 a	800.8 ab	455.5 b
BR2/97	106.80 c	202.40 c	263.53 c	350.53 c	534.6 c	291.6 c
SDL/1	130.44 b	293.44 bc	461.11 ab	354.80 c	657.6 b	379.5 bc
BT2	122.25 bc	320.01 b	455.95 b	394.27 bc	606.1 bc	379.7 bc
LSD at 0.05	48.00	117.07	188.97	128.20	203.7	46.1

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

Table 4. Mean yield of green leaf (g/plant) of six test clone and their control at mature stage (6^{th} – 16^{th} year)

Clone	6^{th} Year LP 2006	7^{th} Year DSK 2007	8^{th} Year MSK 2008	9^{th} Year LSK 2009	10^{th} Year LP 2010	11^{th} Year DSK 2011	12^{th} Year MSK 2012	13^{th} Year LSK 2013	14^{th} Year LP 2014	15^{th} Year DSK 2015	16^{th} Year MSK 2016	Avg.
MZ/39	860.8a	986.9a	999.8a	1397.2a	1016ab	1024.1a	1017ab	1259.4a	801.5a	1267.5a	1240.6a	1079.2a
E/4	436.1d	549.1cd	554.3d	855.6c	686	761.2bc	806c	874.5c	623.7bc	969.6bc	925.6bc	731.1c
D/13	585.6c	725.4c	641.6c	888.9c	791	797.4bc	845.1bc	892.3bc	399.4d	805.5c	848.3d	747.3c
B2T1	876.1a	863.3b	790.3bc	1137.8b	860b	866.6b	958b	1068.9b	700.6b	1003.2b	1048.9bc	924.9b
BR2/97	512.7cd	603.6d	558.9d	877.5c	744	749.7c	805c	975.6bc	601.5c	889.5bc	875.9cd	744.9c
SDL/1	731.8b	845.6b	801.8b	1240.7ab	945ab	952.7ab	1039a	1100ab	618.8bc	1173.4ab	903c	941.1ab
BT2	682.2bc	800.2bc	765.9bc	1215ab	1042a	912ab	966.1ab	1149ab	612.3bc	1079.8ab	1105.6b	939.1ab
LSD at 0.05	132.9	124.1	185.7	222.5	153.6	131.2	67.2	188.2	96.7	221.9	113.95	149.8

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

Estimated made tea production (kg^{-ha}) both for immature and mature stage was done and presented in Table 5 and Table 6. Average value of made tea production for immature stage (1^{st} year to 5^{th} year) and mature stage (6^{th} year to 16^{th} year) are presented in Fig 1 & Fig 2. Fig 1 clearly indicates that MZ/39 is the highest yielder (1612.4 kg^{-ha}) during immature stage followed by B2T1 (1454.9 kg^{-ha}).

But during mature stage (Fig 2) it indicates that, yield of MZ/39 is 3447 kg^{-ha} which is followed by the SDL/1 (3005.9 kg^{-ha}), BT2 (2999.6 kg^{-ha}) and B2T1 (2954.2 kg^{-ha}).

Table 5. Estimated made tea (kg^{-ha}) at immature stage ($1^{st} – 5^{th}$ year)

Clone	1st Year Up 2001	2nd Year Prune 2002	3rd Year UP 2003	4th Year Pruned 2004	5th Year Skiff 2005
MZ/39	614.4a	1432.6a	1857.2a	1542.7ab	2615.4a
E/4	204.2c	467.1e	890.3c	905.9c	1480.5c
D/13	358.2ab	701.2c	1403.4b	1165.3bc	1989bc
B2T1	358.8ab	915.7bc	1728.8ab	1714.1a	2557.9ab
BR2/97	341.1ab	646.5d	841.8c	1119.7bc	1707.6d
SDL/1	416.7b	937.3bc	1472.9b	1133.3bc	2100.5b
BT2	390.5bc	1022.2b	1456.4b	1259.3b	1936bc
LSD at 0.05	153.3	273.9	365.6	409.5	519.7

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

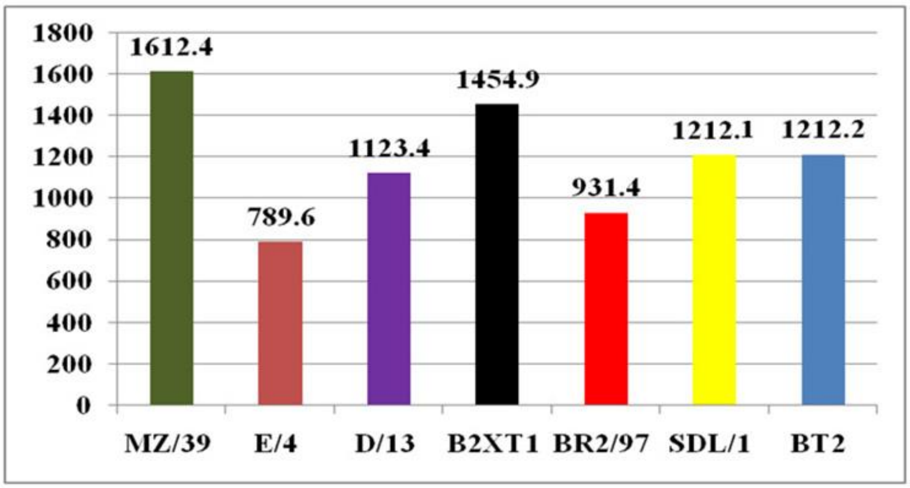

Fig 1. Average estimated made tea (kg^{-ha}) at immature stage ($1^{st} – 5^{th}$ year)

Table 6. Estimated made tea (kg^{-ha}) at mature stage ($6^{th} – 16^{th}$ year)

Clone	6^{th} Year LP 2006	7^{th} Year DSK 2007	8^{th} Year MSK 2008	9^{th} Year LSK 2009	10^{th} Year LP 2010	11^{th} Year DSK 2011	12^{th} Year MSK 2012	13^{th} Year LSK 2013	14^{th} Year LP 2014	15^{th} Year DSK 2015	16^{th} Year MSK 2016
MZ/39	2749.6a	3152.3a	3193.6a	4462.9a	3245.3ab	3271a	3248.5ab	4022.8a	2560.1a	4048.6a	3962.7a
E/4	1393f	1753.9d	1770.5d	2732.9c	2191.2c	2431bc	2574.5c	2793.3c	1992.2bc	3097.1bc	2956.6bc
D/13	1870.5e	2317.1c	2049.4c	2839.3c	2526.6bc	2547bc	2699.4b	2850.2bc	1275.8d	2572.9bc	2709.6bc
B2T1	2798.4a	2757.6b	2524.4b	3634.4b	2747b	2768b	3060ab	3414.3b	2237.9b	3204.4bc	3350.4b
BR2/97	1637.7d	1928cd	1785.2d	2802.9c	2376.5bc	2394.7c	2571.3c	3116.3bc	1921.3c	2841.2c	2797.8c
SDL/1	2337.5b	2701b	2561.1b	3963bab	3018.5ab	3043.1ab	3318.8a	3513.6ab	1976.6bc	3748.1ab	2884.4bc
BT2	2179.1c	2556bc	2446.4bc	3880.9ab	3328.3a	2913.1ab	3085.9ab	3670.1ab	1955.8bc	3449.1b	3531.5ab
LSD at 0.05	508.37	521.89	736.97	610.71	622.52	564.16	646.52	601.13	398.35	489.84	619.59

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

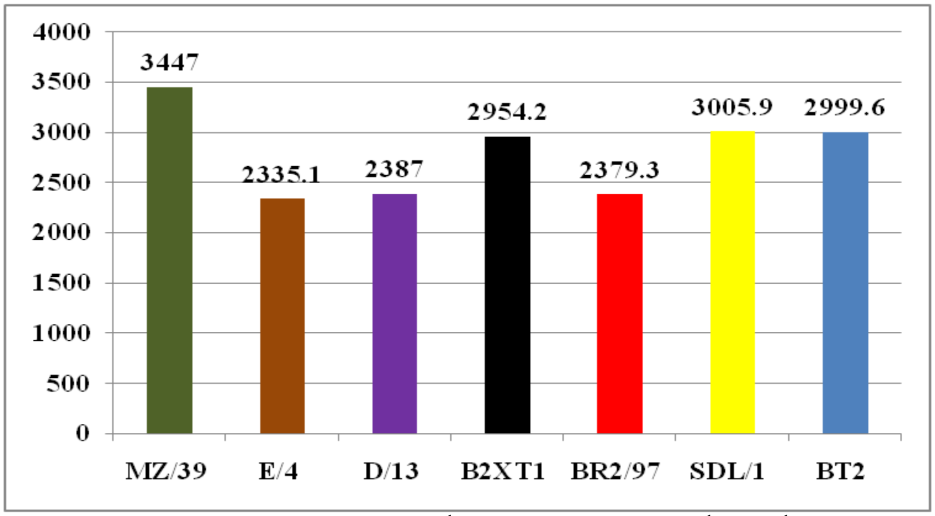

Fig 2. Estimated made tea (kg^{-ha}) at mature stage (6th – 16th year)

Quality performance:

The overall quality performances of the test clones and BT2 control were assessed by conventional organoleptic test are shown in Table 7. It was observed that the cup characters of all the test clones and control were categorized as "Above average". The test clones consistently produced tea of above average quality. They have bright infusion, colour liquour with useful strength and briskness.

Table 7. Cup quality of different test clones and their control (Average score of 15 years, From 2002 to 2016)

Test Clone	Infusion (10)	Liquour Colour (10)	Briskness (10)	Strength (10)	Creaming down(10)	Total	Overall Quality
MZ/39	7.5b	7.4b	7.5a	7.4a	2.8b	32.6ab	AA
E/4	7.4b	7.6a	7.4ab	7.3b	2.7c	32.4bc	AA
D/13	7.7a	7.6a	7.5a	7.4a	3.0a	33.5a	AA
B2T1	7.1d	7.5ab	7.3b	7.3b	2.7c	32.1c	AA
BR2/97	7.4bc	7.4b	7.4ab	7.3b	2.9ab	32.5b	AA
SDL/1	7.3c	7.5ab	7.3b	7.3b	2.9ab	32.5b	AA
BT2	7.3c	7.5ab	7.4ab	7.3b	2.7c	32.3bc	AA
LSD at 0.05	0.21	0.16	0.14	0.09	0.25	0.6	

The test clone D/13 appeared superior to other test clones which was followed by MZ/39 in case of quality performance. But overall quality of these test clones were similar type of 'Above Average (AA)' quality. On the other hand, considering yield parameter at both mature and immature stage, MZ/39 was more superior to other test clones. In 2018, the test clone MZ/39 has been released as 'BT21' in the BT-series to the industry.

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EFFECT OF FOLIAR APPLICATION OF A LIQUID FERTILIZER AS A SUPPLEMENTARY DOSE ON THE YIELD OF TEA

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Abstract

Tea was sprayed with four different doses (2, 3, 4 & 5 ml/L water) of a liquid fertilizer "Liquid Gold/Excel Super" along with one control (no application) as a supplementary dose for tea. Comparatively higher yield of 2012 kg/ha was obtained from the dose 3 ml/L water (3 L/ha, mixed with 1000 L water) with five applications per cropping season. The highest yield was closely followed by the yield of 2002 kg/ha from the dose 2 ml/L water (2 L/ha, mixed with 1000 L water) and lowest yield was obtained from the control where no spray with the liquid fertilizer was done. Considering economic viewpoint, use of liquid fertilizer as a supplementary dose along with the BTRI recommended dose was found profitable in most of the applied doses (except 5 ml/L water) and the dose 2 ml/L water (2 L/ha) was found most effective.

Keywords: Liquid fertilizer, foliar spray, supplementary dose, tea yield.

Introduction

Tea **(***Camellia sinensis* L.**)** is a perennial evergreen shrub belonging to the *Camellia* genus of the Theaceae family (Ravichandran, 2002). Tea is normally grown as a long-term monoculture and fertilizer plays a vital role for its economic production. Like other crops, performance of the tea primarily depends on the nutrients available in soil as well as their utilization by the plant. Tea shoots are plucked at regular intervals (6-25 days) and removed a certain amount of various elements from the plant-soil system (Verma, 1997). Hence, certain major elements need to be supplemented through fertilizer application. In the flush shoot the nitrogen content is the highest followed by potassium (K), calcium (Ca), phosphorus (P), sulfur (S), magnesium (Mg) and zinc (Zn) (Sedaghathoor *et al.*, 2009). Tea yield increases sharply with the application of increased levels of N and K to a certain point (Barbora, 1996). In fact, tea plants need large amounts of N, P, K and Mg for growth. The deficiency of these nutrients could adversely affect the yield and quality (Li and Pan, 2004; Yu *et al.*, 1997; Zheng, 1999). In Bangladesh, the deficiencies of these nutrients are generally met by broadcasting Urea, TSP, MOP and Dolomitic lime into the soil based on the soil analysis report.

Foliar application of fertilizers is becoming increasingly the most effective way to increase yield and plant health. Foliar fertilization is a technique of feeding plants by applying liquid fertilizer directly to their leaves. Foliar feeding has been used in different crops as a means of giving supplemental doses of major and minor nutrients, plant hormones, stimulants and other beneficial substances (Singh, 2016). Foliar fertilization is generally used for better management of nutritional status, growth, to correct deficiencies quickly, and improve disease resistance for better crop quality. While we believe foliar feeding is useful and effective in correcting deficiencies, it is also used primarily to increase yield and quality by overcoming the limitations of the soil. Experiments have shown that foliar feeding can increase yield from 12 to 25 percent

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when compared to conventional soil fertilizer application (Woldegebriel, 2007). Foliar applications are best suited for new flush as the young leaves readily absorb nutrients (Bonheure and Willson, 1992). The leaves are green factories where the complex chemical process of photosynthesis occurs to produce food using the raw materials. Foliar fertilizers are absorbed right at the site where these are used as quite fast acting, whereas much of soil fertilizers may never go to plants (Woldegebriel, 2007). In the foliar method very small amounts of fertilizers are required to apply per hectare and there is no or few risk of ground water contamination. Furthermore, interest in foliar sprays increased because of the development of high concentration soluble fertilizers and the increasing use of machinery for spraying fungicides, herbicides, and insecticides and overhead irrigation further facilitate the application of nutrients to crops in the form of sprays. To develop a sound foliar fertilization policy for a specific crop, it requires different kinds of experimentation like supplementation or substitute of ground application, how much to supplement, at what concentration, application frequency etc. According to Savoy (1999), foliar nutrition can supplement soil nutrition at certain circumstances, but it cannot be a substitute for soil application. Again, despite many advantages of foliar feeding, it is also reported that leaf damage due to foliar fertilization sometimes was severe enough to cause yield reductions (Fageria *et al.*, 2009). This is probably due to the use of high nutrient concentrations as spray that damaged the leaf tissue.

Liquid fertilizers with different origins are available in the market that have been successfully used as foliar spray in different crops but as far as we know no beneficial or harmful effects of these liquid fertilizers is documented yet in Bangladesh tea. Therefore, one of the commercial liquid fertilizers “Liquid Gold/Excel Super” was selected for this study to observe its effect as a supplementary dose on tea and to find out the most effective and economical dose for balanced nutrition and higher yield of tea.

Materials and Methods

The study was conducted on a Deep Skiffed mature tea clone (BT2) area at the main farm of Bangladesh Tea Research Institute, Srimangal, Moulvibazar. Five different doses of the liquid fertilizer “Liquid Gold/Excel Super” were used as the treatment of the experiment. The liquid fertilizer was a balanced mixed fertilizer that contains almost all primary and secondary nutrients (composition of the fertilizer as indicated in the container label- Primary nutrients: N-14.2%, P_2O_5-8.0%, K_2O- 8.0%; Secondary nutrients: Ca- 400 mg/L, Mg- 400 mg/L, S-3000 mg/L, Fe- 4 mg/L, Mn- 1 mg/L, Zn-145 mg/L, Cu- 1 mg/L, B- 2 mg/L, Mo- 0.1 mg/L and Na- 75 mg/L). This trial was laid out in a Randomized Complete Block Design with three replications. The size of the unit plot was 143 m^2 (13.2 m × 10.8 m) and the total number of plots was 15.

Spraying dates were set at the beginning of the study and all the doses of the liquid fertilizer were applied five times during the cropping season as foliar spray onto the tea plants. Each time, 15L of solution was sprayed on each plot (@ 1000L solution per ha). Broadcasting of general fertilizers (Urea, TSP and MOP) were made in two splits according to the BTRI recommended dose considering tea yield as 2600 kg/ha (BTRI Pamphlet No. 21). Intercultural operations like plucking, weeding, insect pest and disease management were done as and when necessary and were the same for all plots. Yield data of tea were collected from each plot as green leaf kg/plot during the study period maintaining an interval of seven days in general. The collected data were then converted to made tea in kg/ha. Finally, the compiled data were analyzed statistically.

Treatments:

T_1 = Control (No application)
T_2 = 2 ml / L water (2 L/ha, mixed with 1000 L water)
T_3 = 3 ml / L water (3 L/ha, mixed with 1000 L water)
T_4 = 4 ml / L water (4 L/ha, mixed with 1000 L water)
T_5 = 5 ml / L water (5 L/ha, mixed with 1000 L water)

Application frequency:

First application : 20th April
Second application : 25th May
Third application : 29th June
Fourth application : 3rd August
Fifth application : 7th September

Results and discussion

Yields obtained from different treatments suggests that comparatively higher mean yield of 2012 kg/ha was obtained from the treatment T_3 (3 ml/L water) that was closely followed by the yield of 2002 kg/ha from the treatment T_2 (2 ml/L water)(Table 1). Yield obtained from the treatment T_2 was followed by the yield from the treatment T_4 (yield 1920 kg/ha) and T_5 (yield 1858 kg/ha). The lowest yield of 1774 kg/ha was obtained from the treatment T_1 where no spray with liquid fertilizer was done. The yield differences among the treatments were not statistically significant. However, from the result it was observed that the highest yield increase was in the treatment T_3 (3 ml/L water) which is 13.42% over control. Similarly in an experiment during 1996-97 by Phu Tho farmers in Vietnam showed that applying a combination of the foliar fertilizer Komic + a growth stimulant (Atonic) improved bud density of tea by 43% compared to control (Anonymous, 2020). From the yield data of this study (Table 1), it can be said that 2-3 Lit/ha of Liquid Gold could be the effective dose for each spray to get higher yield of tea. The dose larger than these might had some toxic effects that have resulted progressively reduced yields.

Table 1. Tea yield obtained from different treatments

Treatment	Dose	Mean yield of green leaf (kg/plot)	Mean yield of green leaf (kg/ha)	Mean yield of made tea (kg/ha)
T_1	Control	110.267	7711	1774
T_2	2 Lit/ha	124.483	8705	2002
T_3	3 Lit/ha	125.083	8747	2012
T_4	4 Lit/ha	119.350	8346	1920
T_5	5 Lit/ha	115.517	8078	1858
Level of significance		NS		
LSD at 5% level		19.521		
CV (%)		11.25		

NS = Not significant.

From the partial budget analysis (Table 2) it can be seen that highest gross margin was in the treatment T_2 (390400 Tk/ha), which was followed by the treatment T_3 (389950 Tk/ha), T_4 (369100 Tk/ha), T_1 (354800 Tk/ha) and the lowest was in the treatment T_5 (354250 Tk/ha). Therefore, compared to the control T_1, almost all of the applied doses were economically viable except the dose applied in T_5. Again, the highest yield of tea was obtained from the treatment T_3, but the highest gross margin was not found in the same treatment and the highest gross margin was in the treatment T_2. Hence, the dose 2 L/ha (mixed with 1000 L water) is more effective considering economic viewpoint. Similarly, in an experiment with Kenyan highland tea, Njogu *et* *al.* (2015) reported that among the two foliar fertilizers (FF1 and FF2) tested, full dose of FF1 significantly increased tea yield compared to the control but the FF2 did not. However, in economic viewpoint the half rate of FF2 was found viable.

Table 2. Partial budget analysis of Liquid Gold/Excel Super fertilizer on the yield of tea

Treatment	Yield of made tea (kg/ha)	Variable cost (Tk/ha)			Gross return (Tk/ha)	Gross margin (Tk/ha)
		Fertilizer	Labour	Total		
T_1	1774	0	0	0	354800	354800
T_2	2002	4900	5100	10000	400400	390400
T_3	2012	7350	5100	12450	402400	389950
T_4	1920	9800	5100	14900	384000	369100
T_5	1858	12250	5100	17350	371600	354250

[Assuming, price of liquid fertilizer = 490 Tk/lit; price of made tea = 200 Tk/kg; labour wage = 102 Tk/day]

Conclusion

The findings of the present study revealed that along with the BTRI recommended doses of Urea, TSP and MOP as ground application, the foliar application of the liquid fertilizer "Liquid Gold/Excel Super" at the dose of 2 ml/L water (2 L/ha, mixed with 1000 L water) with five rounds application (at 5 weeks interval) during the cropping season might be considered as an effective and economic viable dose for enhancing tea yield.

Limitations of the study

The optimum dose may vary from place to place due to soil heterogeneity, climate, plant type and age of the plant. The result was made on the basis of yield data of one cropping season; it was necessary to observe the plants response in the following years. The composition of liquid fertilizer mentioned in the label of the container was not verified through chemical analysis. Any changes in the quality of made tea due to the application of liquid fertilizer was not monitored.

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FIELD FACTORS ON *CORTICIUM THEAE* BERNARD A CAUSAL AGENT OF BLACK ROT DISEASE IN TEA

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Abstract

Impact of varieties, topography, age of tea plants and shade condition were studied on the incidence and severity of Black rot diseases of tea by using 0-5 scoring scale. In all cases of attributes, the maximum level of incidence with Black rot disease was found in September and severity with the same was found in July. The highest 26.05% incidence of the disease showed in seedlings and 22.96% in clone. The highest & identical severity (PDI 20.86) of the disease was observed in seedlings compared to clone. Disparate impact of ages of tea bush was found on the incidence of Black rot disease by 25.00% in immature and 26.93% in mature tea plants with similar variation in severity of the disease. The highest and different incidence of the disease was found in hillock areas by 27.88% followed by flat and tillah areas as 26.30 and 23.67% respectively. Severity of the disease was also found in same areas by 20.56, 18.72 and 17.58 respectively. Similar impact of shade condition was found on the incidence of Black rot disease by 20.85% in shaded and 20.71% in un- shaded condition of tea section with significant variation in severity of the disease. In concluding the impact, seedlings, mature tea bushes, hillock areas are the more prone to Black rot disease for incidence while the heavy shade condition is for disease severity. The findings of this study will help understand the pattern of distribution of the disease in the commercial tea fields so that the planters can escape the disease easily.

Key wards: Incidence, Severity, Black rot, Tea

Introduction

Tea is an important cash crop of Bangladesh. It is one of the largest agro-based industries in the country. There are 167 tea estates having about 61.597 thousand hectares of land under tea plantation producing about 96.07 million kg of made tea during 2019 (BTB, 2020). The geographical location of tea growing area is restricted only to between $21^0\,30'$ and $26^0\,15'$ north latitude and between $89^0\,0'$ and $92^0\,41'$ east longitude (Ahmed, 2005). Tea ecosystem is a complex agro-ecosystem. It comprises tea, shade trees, green crops, forest etc. The intensive mono culture of a perennial crop like tea over an extensive and contiguous area in apparently isolated ecological zones in Bangladesh has formed virtually a stable ecosystem which provided unlimited opportunity for perpetuation and spread of endemic and introduced diseases (Alam, 1999). The architecture of tea plantation, variability of plant types and the systemic interaction of various agro-techniques, intercultural operations etc. impose a significant impact on development of diseases. The loss of tea in Bangladesh tea due to various pests, diseases and weeds has been estimated to be about 10-15% (Sana 1989). More than 400 pathogens cause various diseases in tea (Chen & Chen, 1990) viz. foliage, stem and root. Among the diseases, Black rot is a most destructive leaf disease of tea caused by *Corticium theae* Bernard. (Ali, 1992). Dense shade, bad drainage and sanitation, high humidity etc. are usually considered predisposing factors for the prevalence of the disease. Hillock areas and North tillah slops are also conducive to the disease (Ali, 1992). Tea zone of Bangladesh is divided into three

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units, viz. Tillah- a low hills are normally up to 300 feet height steeply rounded, Flat are categorised as high flat and low flat and Hillock- a place in between of two adjacent tillah (Sana, 1989).

Microclimate of an area under tea plantation is greatly influenced by the architectures of plantation. In the same area there are tillah, flat and hillock directly affect on penetration of solar radiation, humidity, temperature and air circulation in an area, thus influence plant growth as well as diseases development (Islam and Ali, 2010). There still remain many gaps in our knowledge of the process of different diseases in tea and our understanding of the complex relationships between various dimensions. So that, this research work was taken to define the pattern of distribution and severity of the Black rot disease in the commercial tea fields towards the appropriate control strategies.

Materials and Methods

A field survey was carried out during 2014-2016 at Bangladesh Tea Research Institute (BTRI) main station, Bilashcherra Experimental Farm (BEF) under Balishera tea valley to ascertain the impact of Varieties, topography, age of plants and shade condition on the prevalence and severity of the diseases. Data were collected monthly from 300 tea bushes selected randomly for each attribute. This survey was replicated thrice for each. Data were recorded on the prevalence and severity of the diseases by observing the typical symptom. These were done by using the following 0-5 scoring scale like no infection= 0, 1- 20% infection= 1, 21- 40% infection= 2, 41- 60% infection= 3, 61- 80% infection= 4 and 81- 100% infection= 5 (Islam and Ali 2011). The severity of the disease was expressed in Percent Disease Index (PDI), which was computed following a standard formula as described below (Singh 2000).

$$\text{Percent Disease Incidence} = \frac{\text{No.of infected plants}}{\text{Total number of plants counted}} \times 100$$

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

Data subjected to analysis of variance by MSTAT computer programe. Mean separation was done by Duncan's Multiple Rang Test (DMRT) and Least Significant Difference (LSD).

Results and Discussion

Though Black rot disease was seen from the month of January- February in the field but this was very negligible. Significant (P= 0.05) variations in disease incidence and severity for the disease was found due to variations of variety, age of plants, topography and shade condition. For all cases of attributes, the maximum level of incidence of Black rot was found in the month of September but severity with the same was found in July (Tables 1, 2, 3 and 4).

Effect of variety:

The highest incidence of Black rot disease was found in September both for clone (31.08%) and seedlings (35.25%) while the severity was in July with PDI 40.54 in clone and 42.12 in seedlings (Table 1). Statistically (P= 0.05) dissimilar impact of variety was found on the incidence of Black rot disease by showing 26.05% in seedlings and 22.96% in clone. The highest severity (PDI 20.86) of the disease was observed in seedlings which is identical with that of clone (Table 5). In concluding the varietal impact, seedlings are the more prone to Black rot disease for incidence.

Effect of age of tea bush:

The highest incidence of Black rot disease was found in September both for mature (36.44%) and immature (33.81%) while the severity was in July by PDI 42.98 in mature and 40.86 in immature tea plants (Table 2). Statistically (P= 0.05) disparate impact of ages of tea bush was found on the incidence of Black rot disease by showing 26.93% in mature and 25.00% in immature tea plants. No significant variation with the variety was found on severity of the disease (Table 5). In concluding the impact of age, Black rot disease was high in mature tea bush though the severity of the disease was identical statistically both in mature and immature tea.

Topographical variation:

In case of topographical variation, the highest incidence (37.63%) of Black rot disease was found in hillock areas followed by flat (35.24%) and tillah (32.02%) areas in September. The severity of the disease was found in same topographies as PDI 43.08, 38.84 and 37.50 respectively in the month of July (Table 3). The highest and different (P= 0.05) incidence of the disease was found in hillock areas by 27.88% followed by flat and tillah areas as 26.30 and 23.67% respectively (Table 5). Severity of the disease was also found 20.56, 18.72 and 17.58 in hillock, flat and tillah respectively with identical statistic (Table 5). In concluding the impact of land topography, the incidence and severity of Black rot of tea was higher in hillock than flat areas.

Effect of shade condition:

In case of shade condition, the highest incidence of the disease was found in September both for shaded (28.18%) and un- shaded (28.04%) conditions while the severity was in July by 34.79 and 31.28 for the same (Table 4). Statistically (P= 0.05) similar impact of shade condition was found on the incidence of Black rot disease by showing 20.85% in shaded and 20.71% in un- shaded condition of tea section. Table 5 showed significant variation in severity of the disease was found in shaded condition (16.60) and un-shaded condition (14.87). In concluding the impact of shade condition, no statistically impact of shade condition on the incidence but significant variation was found for severity of the disease.

Table 1. Monthly fluctuation of disease incidence and severity of Black rot on different varieties of tea

Month	Clone		Seedlings	
	% Disease incidence	Disease severity (PDI)	% Disease incidence	Disease severity (PDI)
March	9.60 h	3.31h	10.89 h	3.69 g
April	12.29 g	4.99 g	13.93 g	6.58 f
May	16.05 f	31.18 c	18.21 f	34.70 c
June	23.53 e	36.39 b	26.68 e	36.52 b
July	27.51 c	40.54 a	31.20 c	42.12 a
August	30.08 b	28.22 d	34.13 b	30.69 c
September	31.08 a	14.69 e	35.25 a	18.07 d
October	30.00 b	8.09 f	34.04 b	10.42 e
November	26.57 d	4.01 g	30.13 d	5.03 g

Table 2. Variation of disease incidence and severity of Black rot due to different ages of tea

Month	Mature		Immature	
	% Disease incidence	Disease severity (PDI)	% Disease incidence	Disease severity (PDI)
March	11.26 h	3.57 g	10.30 g	3.53 g
April	14.40 g	6.50 f	13.55 f	6.05 g
May	18.83 f	34.25 c	17.61 e	31.69 c
June	27.58 e	37.68 b	25.31 d	36.41 b
July	32.25 c	42.98 a	30.07 c	40.86 a
August	35.28 b	31.29 c	32.54 b	29.08 d
September	36.44 a	17.17 d	33.81 a	15.13 e
October	35.19 b	10.32 e	32.71 b	8.90 f
November	31.16 d	3.77 g	29.11 c	4.63 g

Table 3. Topographical variation on disease incidence and severity of Black rot of tea

Month	Tillah		Flat		Hillock	
	% Disease incidence	Disease severity (PDI)	% Disease incidence	Disease severity (PDI)	% Disease incidence	Disease severity (PDI)
March	9.89 h	2.36 g	10.64 f	2.63 f	11.38 g	3.75 f
April	12.65 g	4.70 f	13.58 e	4.92 ef	14.54 f	6.56 ef
May	16.54 f	26.85 c	18.26 d	31.83 c	19.42 e	32.75 c
June	24.24 e	32.48 b	27.50 c	34.46 b	28.66 d	36.93 b
July	28.35 c	37.50 a	31.31 b	38.84 a	33.40 c	43.08 a
August	31.08 b	26.66 c	35.03 a	26.54 c	36.42 b	30.45 c
September	32.02 a	15.60 d	35.24 a	16.56 d	37.63 a	17.07 d
October	30.92 b	7.64 e	34.64 a	8.18 e	36.78 ab	9.79 e
November	27.38 d	4.50 f	30.51 b	4.55 f	32.72 c	4.74 g

Table 4. Impact of Shade condition on the fluctuation of disease incidence and severity of Black rot disease

Month	Shaded		Un shaded	
	% Disease incidence	Disease severity (PDI)	% Disease incidence	Disease severity (PDI)
March	8.71 g	3.01 f	8.54 g	2.27 f
April	11.23 f	5.30 ef	11.14 f	3.74 ef
May	14.61 e	25.70 c	14.56 e	23.69 c
June	21.34 d	29.82 b	20.99 d	27.90 b
July	24.95 c	34.79 a	24.94 c	31.28 a
August	27.29 b	24.59 c	26.99 b	21.61 c
September	28.18 a	14.10 d	28.04 a	13.37 d
October	27.22 b	7.91 e	27.12 b	6.35 e
November	24.14 c	4.14 f	24.11 c	3.63 f

Table 5. Variation of Black rot disease of tea with the varies of variety, age, topography and shade condition

Parameter		% Disease incidence	Disease severity (PDI)
Variety	Clone	22.96	19.05
	Seedlings	26.05	20.86
	LSD (P= 0.05)	0.014	1.90
	CV (%)	0.02	2.72
Age of plants	Mature	26.93	20.84
	Immature	25.00	19.58
	LSD (P=0.05)	0.255	3.81
	CV (%)	0.34	6.45
Topography	Tillah	23.67	17.58
	Flat	26.30	18.72
	Hillock	27.88	20.56
	LSD (P=0.05)	0.479	3.535
	CV (%)	0.97	9.76
Shade condition	Shaded	20.85	16.60
	Un-shaded	20.71	14.87
	LSD (P=0.05)	0.302	0.69
	CV (%)	0.41	1.52

The Fungus *Corticium* survives in pruning litres, plant debris and soil during dry season. In the following year depending on the first rain, fruiting bodies of the pathogen infect the plants. Generally there is very little or no rainfall from November to February in Bangladesh. This might be the reason behind less recording of the disease during the month of February. Black rot is a serious disease in tea plantation. The disease became evident after about four weeks following the onset of rain. Towards the end of the monsoon the fungus of the Black rot disease *Corticium theae* Bernard produce resting bodies in the creaks and crevices of the infected stems. The active mycelium dies during the dormant season which produces new mycelium again during the next monsoon (Ali, 1992). Islam and Ali (2010) quantified the highest incidence of Black rot was only on seedling and highest in flat areas. The shade reduces sunlight and particularly UV-B, which plays an important role on some plant diseases such as blister blight of tea (Gunasekera *et al.,* 1997); shade modifies the micro-environmental conditions and creates a "phylloclimate" able to perturb interactions between pathogens and target organs (Chelle, 2005); and shade can also work as a barrier and can limit the splash dispersal of the pathogen (Ntahimpera *et al.,* 1998). Mooi (1965) and Stewart (1990) described as very young plants are susceptible to late blight, plants of intermediate age are the most resistant, and old plants become more susceptible again. A study conducted on *Chrysanthemum morifolium* found that leaf age affected the number and size of lesions caused by *Pseudomonas cichorii*, with older leaves being less susceptible to infection than younger, immature leaves (Jones *et al.,* 1985). Young fruit and leaf tissues of Citrus are more susceptible to *Xanthomonas axonopodis* pv. *citri* (citrus canker) than mature tissues (Verniere *et al*., 2003). The intimate mixing of varieties is a proven method in certain cropping systems of introducing diversity to reduce plant disease (Wolfe, 1985, Akem *et al*., 2000 and Zhu *et al*., 2000). Survival of the pathogen, nature of infection, mode of penetration of the pathogen etc. are greatly influenced by the various environmental factors. Temperature, humidity, light penetration, ventilation etc. are the important factors for disease development. These factors varies from section to section and also varies of tea plant architecture, shade condition, topography, age and varieties.

Conclusion

In concluding the impact, seedlings, mature tea bushes, hillock areas are the more prone to Black rot disease for incidence while the heavy shade condition is for disease severity. The findings of this study are in close conformity with findings of different researchers. The findings of this study will help understand the pattern of distribution of the disease in the commercial tea fields so that the planters can escape the disease easily.

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CHANGES IN QUALITY OF TEA LEAVES AND MADE TEA DUE TO RED RUST (*CEPHALEUROS PARASITICUS KARST*) INFECTION

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Abstract

An analytical experiment was conducted at Bio-chemistry laboratory of Bangladesh Tea Research Institute (BTRI) during 2016-17 to find out the impact of red rust infection on the physiological and biochemical changes in tea leaves as well as quality parameters of made tea. Physiological and biochemical parameters of green leaves and made tea were analyzed using UV-visible spectrophotometer in the laboratory. Result revealed that the physiological and biochemical contents were significantly reduced with the increase of infection of red rust. Photosynthetic pigments such as chlorophyll a, b and carotenoids in infected green leaves were found lower than the fresh leaves. There was a significant reduction in quality of tea in terms of Chlorophyll-a, Chlorophyll-b and Carotenoids by 76.98%, 82.78% and 50% respectively in red rust infected tea. A reduced percent of thearubigin (TR), highly polymerized substances (HPS) and total liquor color (TLC) were observed by 34.35%, 18.52% and 3.417% respectively in red rust infected leaves. No change was found in case of theaflavin (TF) in both fresh and infected leaves but color index (CI) was found to be increased in infected leaves by 35.32%. Caffeine content was decreased by 15.97% and pH value was found almost same for fresh and infected leaves. It can be concluded that the infection of red rust deteriorate the physiological and biochemical contents of tea leaves as well as quality of made tea.

Key words: Tea leaves, Red rust, Infection, Physiological and biochemical change

Introduction

Tea is the most popular and inexpensive beverage produced from the young shoots of the commercially cultivated tea plant (*Camellia sinensis* (L.) O. Kuntze). Tea being a perennial monocultural crop consists of a stable microclimate for a number of pests and diseases. The perennial habit of the tea plant, unique cultural conditions and warm humid climate of the tea growing areas are highly favorable for diseases. A number of diseases have been recorded in tea of which the majority is of fungal origin. A few are caused by bacteria, algae and viruses (Agnihothrudu, 1964). In recent study on tea diseases, Chen and Chen (1990) described nearly 400 pathogens of tea. Irrespective of the pathogens and the parts affected the disease symptoms manifest as debilitation, defoliation and sometimes death of the bushes (Baby, 2001). Leaf diseases are important due to the noticeable reasons that tea plant is cultivated for its young succulent leaves. Leaf diseases affect the crop by its indirect effect on bush health, but if both young and mature leaves are attacked, the quantity of harvest is reduced. Leaf blight, leaf spot, leaf rot and leaf rust are the common leaf diseases of the tea bush. Among the leaf diseases, red rust is more serious problem than other leaf diseases.

Red rust disease of tea is caused by an alga *Cephaleuros parasiticus* Karst. When tea plants are affected by red rust disease, the leaves become variegated (yellow or white). In extreme cases, red rust causes severe damage to young tea plants by killing stem tissues in patches.

Red rust can attack both young and old tea plants under adverse conditions of soil and climate. The predisposing factors of the disease are mainly poor fertility, alkalinity and lack of aeration of the soil, hard pan, inadequate or complete absence of shade, drought and water logging (Sarmah,

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1960). The disease is widely distributed in the zones of North-East India, Srilanka, and Bangladesh (Sana, 1989). It is also one of the major leaf diseases found in southern India as well as northeast India (Muraleedharan and Chen, 1999). The disease is most found in young tea, old tea bushes in seed baries and unhealthy tea sections. In Bangladesh, about 8-25% tea estates are affected by this disease (Sana, 1989).

The algae can infect the branches at any stage of growth. Once the branch is infected, the algae remain latent for a year. In the following year, depending on the first rain fruiting bodies of the pathogen are produced (Huq *et al.,* 2007).

Red rust infection may adversely affect the quality of processed tea. The quantity of crop loss in respect of yield due to the infection of red rust was assessed in different regions of the world (Sana, 1989), but there is very scanty information on the physiological and biochemical as well as quality deterioration of made tea caused by red rust. Therefore, present study was undertaken to determine the effect of red rust infection on the physiological and biochemical changes in green leaves as well as quality of made tea.

Materials and Methods

Pluckable shoots both red rust infected and fresh were collected separately from BTRI main farm. In another way, tea shoots were plucked bulkly from the field. These leaves were separated as fresh leaves and infected leaves (Fig 1) in the plant pathology laboratory of BTRI, Moulvibazar, Bangladesh. These leaves were collected for the analysis of both green leaf and made tea. Made tea were prepared manually in the Biochemistry laboratory of BTRI. Physiological and biochemical parameters of green leaves and made tea were analyzed using UV-visible spectrophotometer.

(a)

(b)

Fig 1 (a). Showing red rust infected shoot- variegated with green-yellow patches. **(b)** Fresh healthy shoot

Estimation of chlorophyll and carotenoids contents:

Chlorophyll content of tea shoots was measured following Wellburn (1994) by using UV-visible spectrophotometer. Chlorophyll a, b and carotenoids were calculated using the formulae like chlorophyll a = (15.65 A666 – 7.34 A653), chlorophyll b = (27.05 A653 – 11.21 A666) and carotenoids = (1000 A470 – 2.86 chlorophyll a – 129.2 chlorophyll b)/221, while the chlorophyll and carotenoids values were expressed as milligram per gram (mg/g) fresh weight basis.

Contents of TF, TR, HPS and TLC were calculated by the following formulae :

TF (%) = (4.313 × C × 2 × 100)/(Sample weight × dry matter content), TR (%) = (13.643 × (B+D-C) × 2 × 100)/(Sample weight × dry matter content), HPS (%) = (13.643 × E × 2 × 100) / (Sample weight × dry matter content) and TLC (%) = (10× A × 2 × 100)/(Sample weight × dry matter content). Multiplication factors of TF and TR were derived from molar extinction coefficients of pure compounds and dilution factor (Roberts and Smith, 1963). In the case of TLC, value 10 is the dilution factor (Thanaraj and Seshadri, 1990). Color index was derived using the formula as CI = (TF * 100) / (TR + HPS) as reported by Ramaswamy (1986).

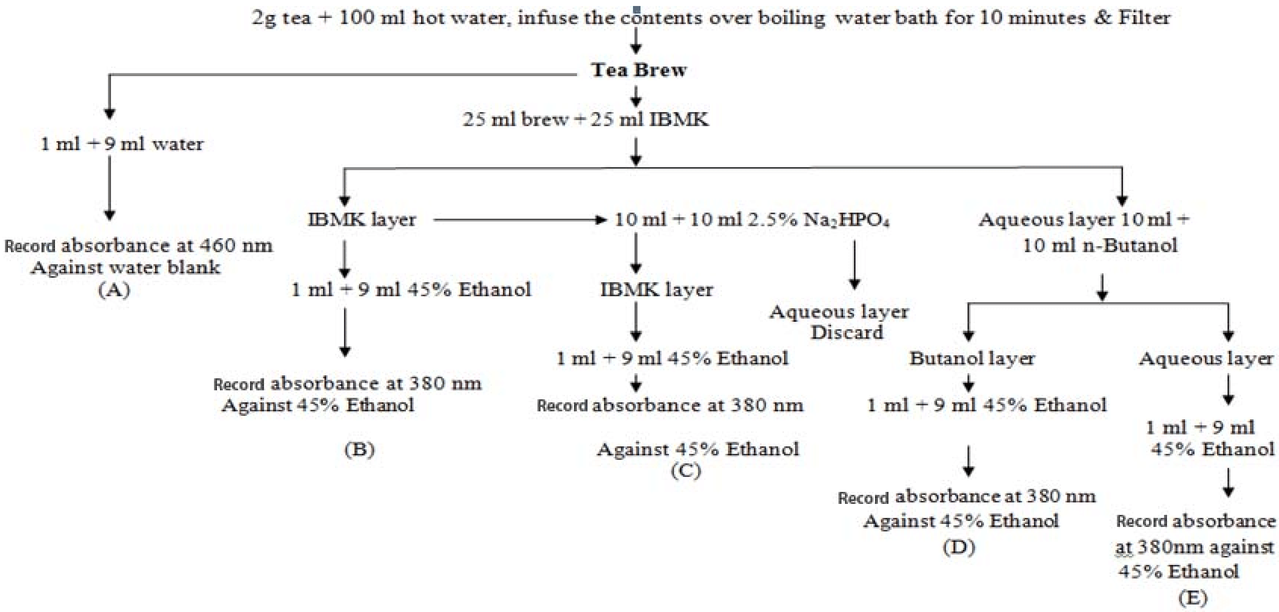

Fig 2. Estimation of TF, TR, HPS and TLC in black tea by spectrophotometer. (Ahmed *et al.*, 2016)

Estimation of caffeine:

The presence of caffeine in the tea samples was measured through UV-visible spectrophotometer and absorbance was compared in 260 nm. Quantity of caffeine present in tea leaves was computed using the standard calibration curve derived from known concentrations (0 to 20 ppm) of caffeine and the results were expressed as per cent caffeine equivalents (Maidon *et al.*, 2012, Mamun *et al.,* 2016).

Statistical analysis:

All measurements and analyses were analyzed statistically using the MS Excel program to determine the average value and standard error of the mean (SEM).

Results and Discussion

Chlorophyll and carotenoids contents: Chlorophyll a, b and carotenoids of red rust infected and fresh tea leaves were analyzed (Fig 3). Results revealed that fresh tea leaves contain 4.43 mg/g chlorophyll a, 1.80 mg/g chlorophyll b and 1.02 mg/g carotenoids. Infected tea leaves contain 1.02 mg/g chlorophyll a, 0.31 mg/g chlorophyll b and 0.51 mg/g carotenoids. In this case it was found that there was a significant reduction in quality components of tea in terms of Chlorophyll-a, Chlorophyll-b and carotenoids by 76.98%, 82.78% and 50% respectively in red rust infected tea by *Cephaleuos parasiticus*.

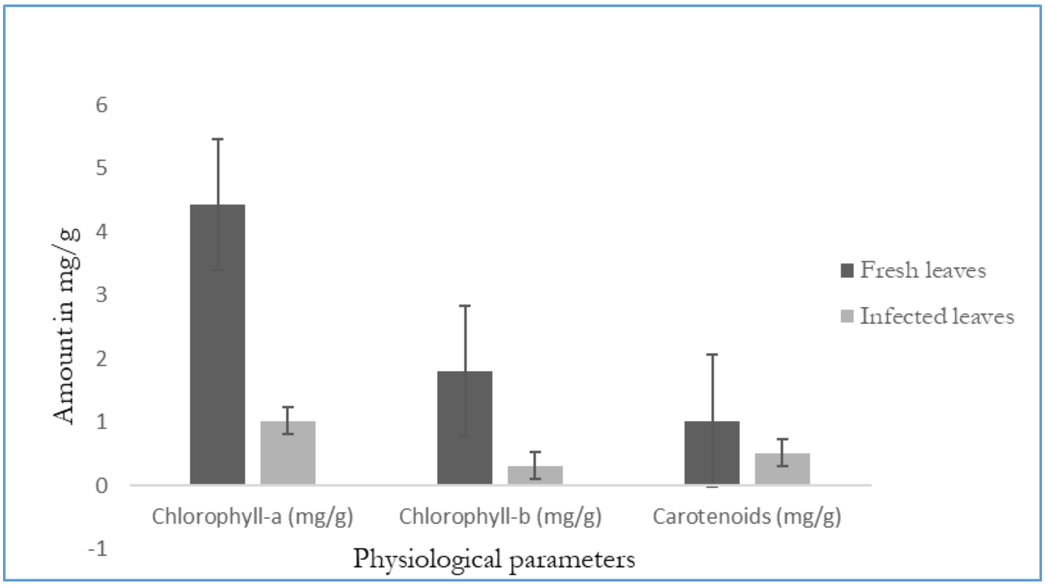

Fig 3. Chlorophylls and carotenoids contents of fresh and red rust infected leaves

Quality of made tea: In infected tea leaves, a reduced percent of thearubigin (TR), highly polymerized substances (HPS) and total liquor color (TLC) were observed by 34.35%, 18.52% and 3.417% respectively. No change was found in case of theaflavin (TF) in both fresh and infected leaves but color index (CI) was found to be increased in infected leaves by 35.32% (Fig 4). Caffeine content was decreased by 15.97% and pH value was found almost same for fresh and infected leaves (Table 1). Whereas the better quality of tea is considered between the ratio of TR and TF must be 10 and in this research the appropriate ratio was also found in fresh leaves but gradually decreased in red rust infested leaves.

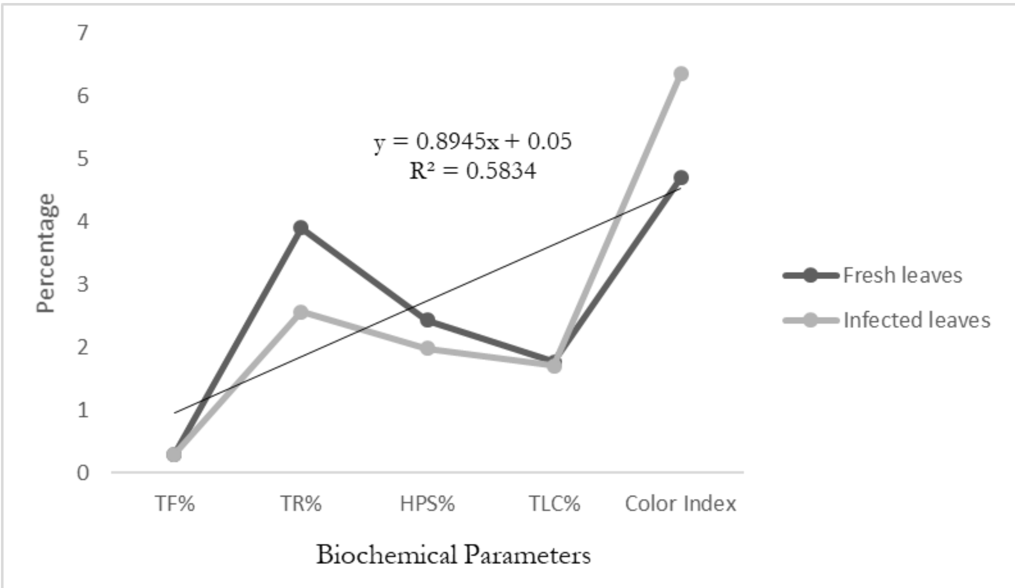

Fig 4. TF, TR, HPS levels and color changes in fresh and red rust infected tea leaves

Table 1. Caffeine, pH and liquor color level in fresh and red rust infected tea leaves

	Amount of caffeine (mg/L) at specific absorbance		pH of brewed tea		Color of brewed tea	
	Absorbance at 260 mm	Amount of caffeine	Before boiling	After boiling	Before boiling	After boiling
Fresh leaves	1.81	47.45	4.84	4.81	Reddish	Light red brownish
Infected leaves	1.52	39.87	4.93	4.80	Yellowish	Deep red brownish
$t_{(0.05)}$ =	0.001	0.001	0.004	0.004		

Dalvi and Sardeshpande (1993) reported, chemical analysis of heavily red rust infected mango leaves revealed significant decrease in chlorophyll a, b and total chlorophyll and carotenoid pigments, indicating its adverse effect on photosynthesis. However, the infected leaves contain more reducing sugars and total sugar than healthy leaves (Ponmurugan and Baby, 2008). In blister blight infection of tea, similar results were found by Rajalakshmi and Ramarethinam (2000), where chlorophyll contents and carotenoids were severely reduced due to infection. Ponmurugan *et al* (2007) found 34.47%, 38.31% and 23.53% reduction in case of chlorophyll, reducing sugar and polyphenol contents respectively during brown blight infection. They also found 33.9%, 45.66% and 26.39% reduction in terms of chlorophyll, reducing sugar and polyphenol contents respectively due to red rust infection which was quite similar to the findings of this study.

Conclusion

It can be concluded that physiological and biochemical parameters such as chlorophyll, carotenoids, theaflavin, thearubigin, caffeine, highly polymerized substances, color index, etc. were found much lower in the infested leaves compared to fresh leaves. By deteriorating the quality and production, red rust may negatively affect the national economy of Bangladesh. Further research is needed to sustainable control of the red rust in our tea gardens to ensure the quality and production of tea.

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IDENTIFICATION OF PLANT PARASITIC NEMATODES ASSOCIATED WITH TEA SOIL IN BANGLADESH

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Abstract

An investigation was carried out at different tea estates of six valley circles in greater Sylhet, Chattogram and Panchagarh regions to identify the plant parasitic nematodes associated with tea soil during 2015-16. Soil samples were collected from rhizosphere of tea seedlings of secondary nursery bed using a soil sampling auger. Baermann Funnel Technique with some modifications was followed to extract nematodes from the soil samples. Plant parasitic nematodes were identified up to genus level based on morphological characters and measurement. A total of 12 plant parasitic nematode genera under 10 families like *Aphelenchus* sp., *Aphelenchoides* sp., *Criconemoides* sp., *Dolichodorus* sp., *Eutylenchus* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Meloidogyne* sp., *Pratylenchus* sp., *Rotylenchulus* sp., *Tylenchus* sp. and *Xiphinema* sp. were identified. Among the identified nematode genera *Criconemoides* sp., *Dolichodorus* sp., *Eutylenchus* sp., *Rotylenchulus* sp. and *Xiphinema* sp. are the first time reported as pests of tea seedlings from the country. *Meloidogyne* sp., *Pratylenchus* sp., *Hoplolaimus* sp. and *Helicotylenchus* sp. were found predominantly. The highest nematode genera (10) were found in the tea estates of Balisera valley circle whereas the lowest nematode genera (03) were found in Panchagarh region.

Keywords: Plant parasitic nematode, tea soil, morphological characters, measurement

Introduction

Tea plants are subjected to the attack of several insects, mites, nematodes, fungal pathogens and weeds causing important economic losses. Amongst the various constraints to healthy seedlings production in nursery and survival of young tea, plant parasitic nematodes have a significant monetary importance (Muraleedhran, 1993 and Ahmed, 2005). Nematode is soil pest commonly referred to as eelworms. It is widely distributed throughout the world in different habitats (Nalini *et al.,* 2005 and Gnanapragasam, 2014). Most of them are microscopic. It usually ranges from 0.3 to 1.0 mm in length (sometimes 5.0 mm long). Nematodes are vermiform in shape, filiform or thread like body, tapering at both ends (but in certain groups females are saccular with a distinct neck). Males are always slender. Nematode attacking tea seedlings was first reported from South India in 1901 and then from Sri Lanka in1928 (Sivapalan, 1972).

Plant parasitic nematodes attack almost every part of the plant including roots, stems, leaves, fruits and seeds. They could feed either externally (ectoparasitic) or internally (endoparasitic) and they all possesses a hypodermic needle like stylet which they pierce the plant cells, secrete saliva containing digestive juices and suck back the digested plant tissue contents with the aid of a modified esophagus. Nematode infestation is a gradual process; the plants react with visible symptoms only when an appreciable part of the root system has been destroyed or ceases to function. Stagnation of growth followed by yellowing and wilting of leaves are the earliest signs of nematode attack. In severe cases, die-back and death may occur. The nature of nematode damage may be mechanical, chemical or physiological or combination of these (Mian, 1998).

Plant parasitic nematodes cause significant economic losses to a wide variety of crops. Crop losses due to nematodes range from 8 to 20% on major crops around the world (Sasser and Fackman, 1987).

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In Sri Lanka, due to nematode infestation the crop loss is estimated to be about 15-20% plant injury and 350-500 kg of made tea per hectare per year (Sivapalan, 1972). Infested nursery plants are one of the main sources of spread of infestation amongst tea estates. It is well known fact that once nematodes are introduced into a field, it cannot be eradicated thereafter. Besides, the damage done to the young seedlings during the critical growth period has a long lasting effect throughout the plant's life (Gnanapragasam, 2014).

More than 40 species of plant parasitic nematodes, belonging to 20 genera have been recorded in different tea growing countries of the world (Sivapalan, 1972; Nalini *et al.,* 2005; Gnanapragasam, 2014) while Chen and Chen (1989) reports 82 species of nematodes are associated with tea plants. In Bangladesh tea, so far 10 species of nematodes have been recorded. Among them the species of *Meloidogyne*, *Pratylenchus*, *Hoplolaimus* and *Tylenchus* are predominant in Bangladesh tea (Ahmed, 2005 and Khan *et al*., 2006).

Nematode is one of the major soil pests of Bangladesh tea in the nursery and new clearing and invades tea seedlings, upto 1 year old, in young plantation (Sana, 1989 and Ahmed, 2005). In Bangladesh tea, nematode free soils are not available to establish tea nursery or to fillup polybag for vegetative propagation. An appreciable amount of crop is lost due to plant parasitic nematodes in Bangladesh tea. There is a great influence of plant variety, age of plant, climatic condition, phyico-chemical properties of soil and topography on the diversity of plant parasitic nematodes in tea (Nalini *et al.,* 2005; Bhattacharya *et al*., 2012). Diverse soil properties, rainfall pattern, temperature etc. are found in different tea growing area of Bangladesh (Alam, 1999). But the information on the diversity of plant parasitic nematodes in different tea valley circles of Bangladesh is very limited. That's why, the present work was undertaken to survey plant parasitic nematodes associated with tea soil in different valley circles and to study their morphological characters and measurement.

Materials and Methods

The investigation was carried out in the nursery areas of different valley circles of greater Sylhet, Chattogram and Panchagarh region during 2015-2016. Soil samples were collected from Baraoora T.E, BTRI, Horincherra T.E., Jagcherra T.E and Mazdehee T.E. of Balisera; Phooltullah T.E., Rasheedabad T.E. and Silloah T.E of Juri; Ameenabad T.E., Chandbagh T.E. and Luayuni-Holicherra T.E. of Lungla; Amo T.E., Brindabon T.E. and Chandpore T.E. of Lusherpore; Allynugger T.E., Chatlapore T.E. and Phulbari T.E. of Monu-Doloi; Alibahar T.E., Lackatoorah T.E. and Malnicherra T.E. of North Sylhet; BTRI Sub-Station, Oodalea T.E. and Waggachara T.E. of Chattogram; BTRI Sub-Station and Koratoa T.E. of Panchagarh valley circles. Soil samples were collected from rhizosphere of tea seedlings grown in polytube from secondary nursery bed. Five secondary beds were selected arbitrarily measuring 5m x 30m each from the above tea estates. Samples were collected using a soil-sampling auger at a depth of 23 cm and about 3 cm from the base of the plants. Each sample was a composite of 10 soil cores from each location. The soil samples sent to the Nematology Laboratory, BTRI by the different tea estates also analysed for nematode identification. Every time the soil sample was mixed thoroughly and 10g sub sample was made for nematode counting.

Extraction of nematodes

Baermann Funnel technique with some modifications (Mian, 1998) was followed to extract nematodes from the soil samples in Nematology Laboratory under Entomology Division, BTRI. Glass funnel (20 cm diameter) was mounted on a funnel stand. A rubber tube of 15 cm long was fitted with the stem of each funnel. The rubber tube was closed with a pinch clamp. An amount of 10g sub sample of soil was drawn from each composite sample and was taken into 50 ml beaker. Then the beaker was covered with muslin cloth and kept in the glass funnel in reverse for overnight. Tap water was added in the funnel until the water level is 2.5 cm below the funnel rim. The active nematodes moved through the muslin cloth leaving the soil. They were concentrated at the bottom of the rubber tube. Nematodes were collected in a petridish by releasing the pinch clamp along with small quantity of water.

Morphological characters and measurement of plant nematodes

From the petridish small quantity of nematode suspension was transferred into a syracuse dish and observed under a sterio-disecting microscope. Individual nematodes were picked up from the syracuse dish and temporary mount was prepared in water on glass slide. The slide was then passed over the flame of an alcohol lamp to kill the nematodes by heating at 60-70^{0}c. After placing a cover slip, the nematodes were observed under a compound microscope (Primostar Trinocular microscope of Carl Zeiss Microscopy) with 40X-100X objectives. TUCSEN-ISH-500, camera was used to take digital images of nematodes. Morphological characters of head, tail, digestive system, reproductive system, stylet and cuticular markings were recorded. Body shape, body length, maximum body width, stylet length, esophageal length, tail length etc. were measured by an ocular micrometer. The important morphometrics viz. L, a, b, c, MB and V were measured by using the following formula (Mian, 1998):

L = Overall body length

$$a = \frac{\text{Body length}}{\text{Maximum body width}} \quad b = \frac{\text{Body length}}{\text{Esophageal length}} \quad c = \frac{\text{Body length}}{\text{Tail length}}$$

$$MB = \frac{\text{Distance from anterior body to median bulb}}{\text{Esophageal length}} \times 100 \qquad V = \frac{\text{Distance from head end to vulva}}{\text{Body length}} \times 100$$

Identification of plant parasitic nematodes

Plant parasitic nematodes were identified up to genus level based on morphological characters and measurement. Characteristics of the specific nematode were compared with standard key books (Mai and Lyon, 1975; Mian, 1998). CIH (Commonwealth Institute of Helminthology) descriptions of plant parasitic nematodes were also being studied for confirmation of identification.

Data analysis

Nematode numbers present in the suspension were determined by taking the average number of nematodes present in three different drops of nematode suspension. Ecological indices of different nematode species in the samples were calculated by using the formulae of Norton (1978).

$$\text{Absolute frequency} = \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Sum of frequencies of all species}} \times 100$$

$$\text{Relative density} = \frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$$

$$\text{Absolute density} = \frac{\text{Number of individuals of a species in a sample}}{\text{Volume or mass units of the sample}} \times 100$$

$$\text{Prominence value} = \frac{\text{Absolute density x } \sqrt{\text{Absolute frequency}}}{100}$$

Results and Discussion

A total of 12 plant parasitic nematode genera viz. *Xiphinema* sp., *Dolichodorus* sp., *Aphelenchus* sp., *Aphelenchoides* sp., *Criconemoides* sp., *Eutylenchus* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Meloidogyne* sp., *Pratylenchus* sp., *Rotylenchulus* sp. and *Tylenchus* sp. were identified (Table 1). The identified plant nematode genera were under ten families of two orders, Tylenchida and Dorylaimida. All the families except Longidoridae were under the same order Tylenchida and the later one under that of Dorylaimida. A good number of non-parasitic nematodes were also found to be associated with the soil samples.

Table 1. Common name, Orders and Families of the identified Nematode species

Nematode species	Common name	Orders	Families
1. *Aphelenchus* sp.	-	Tylenchida	Aphenchida
2. *Aphelenchoides* sp.	Dwarf nematode		Aphelenchoididae
3. *Criconemoides* sp.	Ring nematode		Criconematidae
4. *Dolichodorus* sp.	Awl nematode		Dolichodoridae
5. *Eutylenchus* sp.	-		Atylenchidae
6. *Helicotylenchus* sp.	Spiral nematode		Hoplolaimidae
7. *Hoplolaimus* sp.	Lance nematode		Hoplolaimidae
8. *Meloidogyne* sp.	Root knot nematode		Heteroderidae
9. *Pratylenchus* sp.	Root lesion nematode		Pratylenchidae
10. *Rotylenchulus* sp.	Reniform nematode		Hoplolaimidae
11. *Tylenchus* sp.	Citrus nematode		Tylenchidae
12. *Xiphinema* sp.	Dagger nematode	Dorylaimida	Longidoridae

Morphological characters and measurement of individual plant parasitic nematodes

(1) *Aphelenchus* sp.

This nematode was identified through the characters described by Chanu *et al.* (2017). Female is medium sized (Fig. 1d) and lips slightly offset. Stylet small without basal knobs (Fig. 1a). Median bulb well developed and squarish (Fig. 1a). Tail long, cylindrical and slightly ventrally concave (Fig. 1b). Vulva located posteriorly of the body length (Fig. 1c). The following morphometrics were recorded, ♀♀**:** L= 510.05 µm, a= 26.5, b= 9.8, c= 16.0, V= 69.8%, MB= 57.8%, Stylet = 10.6 µm, Tail = 31.9 µm.

(2) *Aphelenchoides* sp.

The genus was identified comparing the characters described by Mai and Lyon (1975). Medium sized slender nematodes (Fig. 2a) and head region weakly sclerotized. Stylet weak with basal swellings (Fig. 2b). Median bulb well developed, rounded-rectangular in shape filling the body width (Fig 2b). Dorsal esophageal gland duct opening within bulb, just anterior to the valve plates. Vulva posterior, genital tract single, anteriorly directed (Fig 2c). Tail medium conoid without terminal mucron (Fig 2a). The following morphometrics were recorded, ♀♀**:** L= 340.39 µm, a= 23.59, b= 11.74, c= 19.11, V= 58.09%, MB= 61.82%, Stylet = 12.0 µm, Tail = 51.66 µm.

(3) *Criconemoides* sp.

Identification of the genus was confirmed on the basis of report by Brzeski *et al.* (2002). Female nematodes ventrally arcuate (Fig. 3c). Esophagus overlap intestine ventrally. Stylet long, robust, with anchor shaped knobs (Fig. 3a). Tail uniformly conical with small rounded end annulus (Fig. 3b). Vulva open with anterior vulva lip. The following morphometrics were recorded, ♀♀**:** L= 272.80 µm, a= 13.78, b= 8.27, c= 15.50, V= 40.0%, MB= 76.17%, Stylet = 35.20 µm, Tail = 17.80 µm.

(4) *Dolichodorus* sp.

Description of the plant nematodes by Williams (1986) was studied for identification. Due to lack of sufficient specimen detailed morphology was not studied. Some morphological characters were studied partially with limited specimen as follows: Lip region rounded. Basal esophageal bulb not overlapping intestine. Stylet weak (Fig. 4a). Tail of male with wing shaped bursa (Fig. 4b).

(5) *Eutylenchus* sp.

It was compared with the characteristic features described by Williams (1979). Body curved, elongate, cylindrical (Fig. 5c). Cephalic region set off by deep constriction bearing setae. Lip region projecting slightly from head contour. Stylet medium with flattened knobs (Fig. 5a). Tail long, tapering regularly to a pointed terminus (Fig. 5b). The following morphometrics were recorded, ♀♀**:** L= 781.0 µm, a= 44.38, b= 9.43, c= 15.43, V= 64.25%, MB= 69.23%, Stylet = 21.36 µm, Tail = 50.60 µm.

(6) *Helicotylenchus* sp.

The characteristics of the genus were compared with that of described by Siddiqi (1972). Body vermiform with tapering towards both terminus. Body remained spiral shape on thermal death (Fig. 6a). Stylet well developed (Fig. 6b). Median esophageal bulb oval, basal bulb overlaped intestine ventrally and appeared strong. Ovaries paired, outstretched. Tail dorsally convex-conoid to a narrow terminus, which formed a slight ventral projection (Fig. 6d). The following morphometrics were recorded, ♀♀**:** L= 617.4 µm, a= 31.5, b= 6.3, c= 42.0, V= 66.7%, MB= 70.0%, Stylet = 24.5 µm, Tail = 14.7 µm.

(7) *Hoplolaimus* sp.

The diagnostic features were as described by Khan and Chawala (1975) and Mian and Tsuno (1988). Body vermiform, cylindrical, and 'C' shaped on relaxed due to thermal death (Fig. 7a). Head distinctly set-off from the body and strongly hexaradiate. Stylet strong, with backwardly projected knobs (Fig. 7b). Esophagus overlaped intestine ventrally. Vulva conspicuous and transverse silt (Fig. 7d). Tail short, with bluntly rounded terminus (Fig. 7c). The following morphometrics were recorded, ♀♀**:** L= 1146.6 µm, a= 39.0, b= 9.75, c= 58.5, V= 47.9%, MB= 80%, Stylet = 39.2 µm, Tail = 19.6 µm.

(8) *Meloidogyne* sp.

In the study adult male and female were not found in soil sample. Identification was done based on characteristic of larva described by Hirschmann (1985). Body translucent white, vermiform, slender, tapering posteriorly (Fig. 8c). Head region elevated, distinctly set off from body. Stylet weakly developed, knobs elongated (Fig. 8a). Tail slender, ending in slightly rounded tip (Fig. 8b). The following morphometrics were recorded, larva**:** L= 360.51 µm, a= 25.68, b= 4.61, c= 15.79, MB= 45.91%, Stylet = 8.21 µm, Tail = 22.79 µm.

(9) *Pratylenchus* sp.

It was identified studying the characteristic features described by Seinhorst (1977). Female body slender, cylindrical to anus and ventrally curved after thermal death (Fig. 9a). Stylet medium and sclerotized. Stylet knobs rounded broadly fused to shaft (Fig. 9b). The vulval region flat and position high (Fig. 9c). Tail tapering gradually to smooth conical tip (Fig. 9a). The following morphometrics were recorded, ♀♀**:** L= 441.0 µm, a= 22.5, b= 5.0, c= 18.0, V= 80.0%, MB= 55.6%, Stylet = 14.7 µm, Tail = 24.5 µm.

(10) *Rotylenchulus* sp.

The nematode was identified comparing the explanation of Linford and Oliveira (1940). Body vermiform, slender and ventrally curved after fixation (Fig. 10a). Lip region not set off from the body. Stylet well developed with rounded knob (Fig. 10b). Esophagus and median bulb was well developed and slightly overlapping intestine laterally and ventrally. Lumen clearly visible (Fig. 10b). The vulva not prominent (Fig. 10c). Tail tapered to a narrow rounded terminus. The following morphometrics were recorded, ♀♀**:** L= 345.6 µm, a= 23.72, b= 2.93, c= 13.6, V= 68.09%, MB= 35.07%, Stylet = 12.56 µm, Tail = 22.93 µm.

(11) *Tylenchus* sp.

The genus was identified studying the characteristic features described by Andrassy (1977). Body relatively smaller, vermiform and slightly curved ventrally due to thermal death (Fig. 11a). Cephalic framework moderately set off and round. Stylet medium sized with round knobs (Fig. 11b). Basal bulb of esophagus not overlapped the intestine (Fig 11c). Vulva slightly transversed (Fig. 11d). Tail filiform, long with pointed terminus (Fig. 11a). The following morphometrics were recorded, ♀♀**:** L= 539.9 µm, a= 30.5, b= 8.32, c= 4.82, V= 66.7%, MB= 50.0%, Stylet = 11.8 µm, Tail = 112.1 µm.

(12) *Xiphinema* sp.

It was identified studying the characteristic features illustrated by Schindler (1957). Body long, cylindrical, ventrally curved after fixation, more at posterior region (Fig. 12a). Head region not offset. Stylet very long with sclerotized basal flanges. Guiding ring located near the base of stylet (Fig. 12b). Esophagus consisting of a long, narrow, procorpus and a short glandular bulb. Female vulva located near about the middle of the body length (Fig. 12c). Tail filiform (Fig. 12d). The following morphometrics were recorded, ♀♀**:** L= 1506.6 µm, a= 40.0, b= 7.2, c= 45.8, V= 51.2%, MB= 79.4%, Stylet = 115.3 µm, Tail = 32.9 µm.

Community analysis of plant parasitic nematodes

Among the identified nematode *Meloidogyne* sp. was the most abundant species in the soil with 81.82, 150.0, 16.85 and 75.0% absolute frequency, absolute density, relative frequency and relative density respectively. While that of the lowest was observed for *Dolichodorus* sp. and its absolute frequency, absolute density, relative frequency and relative density were 5.45, 30.0, 2.25 and 66.67% respectively. Four plant parasitic nematodes such as *Meloidogyne* sp*., Pratylenchus* sp., *Hoplolaimus* sp. and *Helicotylenchus* sp. were predominant with prominence values of 13.58, 11.09, 9.58 and 7.39 respectively (Table 2).

Table 2. Community analysis of plant parasitic nematodes associated with tea soil

Nematode species	Absolute frequency (%)	Absolute density (%)	Relative frequency (%)	Relative density (%)	Prominence Value (PV)
Aphelenchus sp.	27.27	70.0	7.87	70.00	3.65
Aphelenchoides sp.	45.45	90.0	10.11	64.29	6.07
Criconemoides sp.	18.18	50.0	5.62	62.50	2.13
Dolichodorus sp.	5.45	30.0	2.25	66.67	0.70

Eutylenchus sp.	9.09	40.0	4.49	57.14	1.20
Helicotylenchus sp.	54.55	100.0	11.24	66.67	7.39
Hoplolaimus sp.	63.64	120.0	13.48	66.67	9.58
Meloidogyne sp.	81.82	150.0	16.85	75.00	13.58
Pratylenchus sp.	72.73	130.0	14.61	108.33	11.09
Rotylenchulus sp.	36.36	60.0	6.74	75.00	3.62
Tylenchus sp.	45.45	80.0	8.99	88.89	5.39
Xiphinema sp.	8.18	50.0	5.62	75.0	1.43

The highest Ten (10) nematode genera were recovered from the tea estates of Balisera valley circle. The lowest three (03) nematode genera were obtained from Panchagarh region (Table 3). Different nematode genera were commonly found in tea estates of different valley circles. Among eight valley circles, *Meloidogyne* sp. was commonly found in four valley circles viz. Balisera, Lungla, Mono-Doloi and North Sylhet. Where as the plant nematode, *Pratylenchus* sp. was commonly observed in three valley circles such as Balisera, Lushkerpore and North Sylhet. On the other hand, *Helicotylenchus* sp. and *Hoplolaimus* sp. were recovered from two valley circles each.

Table 3. Distribution of plant parasitic nematodes associated with tea seedlings in different tea estates

Nematode genera	Name of valley circles							
	Balisera	Juri	Lungla	Lushkerpore	Monu -Doloi	North Sylhet	Chattogram	Panchagarh
Aphelenchus sp.	+	-	+	+	+	++	+	+
Aphelenchoides sp.	+	-	+	+	+	+	+	+
Criconemoides sp.	-	+	-	+	+	-	++	-
Dolichodorus sp	+	-	-	-	-	-	-	-
Eutylenchus sp.	-	-	-	-	+	+	-	-
Helicotylenchus sp.	++	+	++	+	-	+	+	-
Hoplolaimus sp.	++	+	+	+	++	+	+	-
Meloidogyne sp.	++	+	++	+	++	++	+	+
Pratylenchus sp.	++	-	+	++	+	++	-	-
Rotylenchulus sp.	+	-	-	-	-	-	-	-
Tylenchus sp.	+	-	-	+	+	+	-	-
Xiphinema sp.	+	-	-	-	+	-	-	-

- = not recorded; + = present; ++ = common

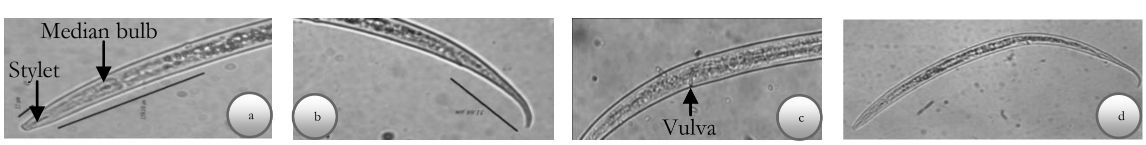

Fig 1. *Aphelenchus* sp. (a) stylet & median bulb, (b) tail, (c) vulva and (d) adult female

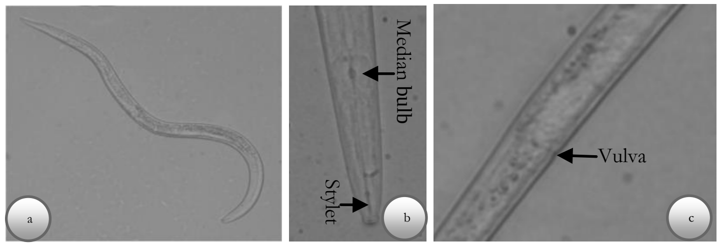

Fig 2. *Aphelenchoides* sp. (a) adult female, (b) stylet & median bulb, (c) vulva

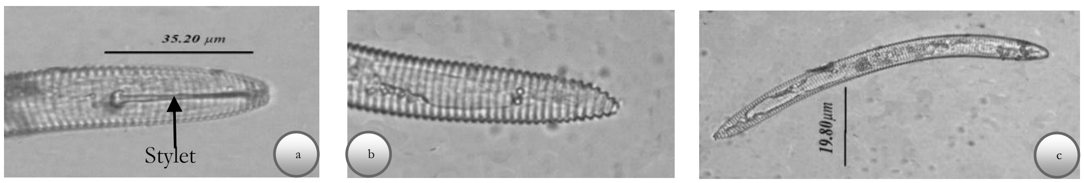

Fig 3. *Criconemoides* sp. (a) stylet, (b) tail and (c) adult female

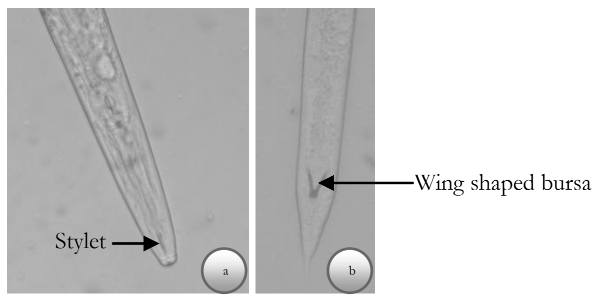

Fig 4. *Dolichodorus* sp. (a) stylet with head; (b) mail tail

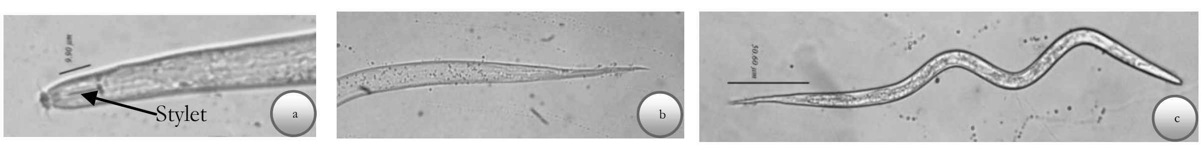

Fig 5. *Eutylenchus* sp. (a) stylet, (b) tail and (c) adult

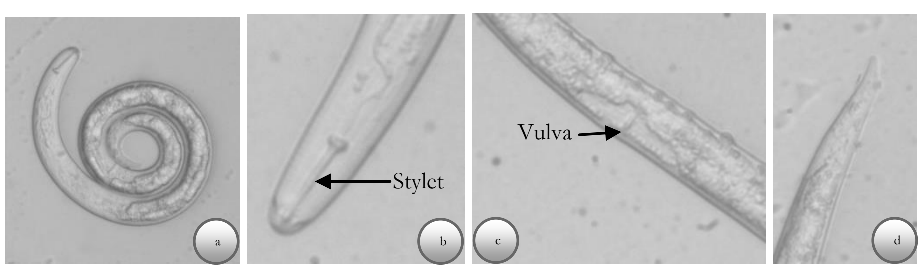

Fig 6. *Helicotylenchus* sp. (a) adult female, (b) stylet with head, (c) vulva and (d) tail

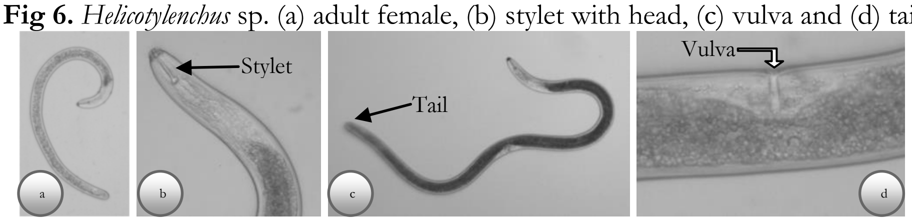

Fig 7. *Hoplolaimus* sp. (a) larvae, (b) stylet with head, (c) adult female and (d) vulva

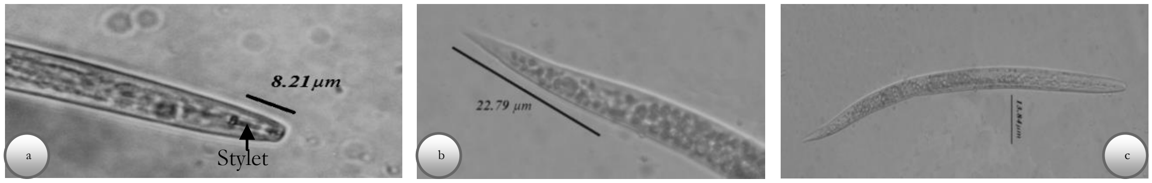

Fig 8. *Meloidogyne* sp. (a) stylet, (b) tail and (c) larvae

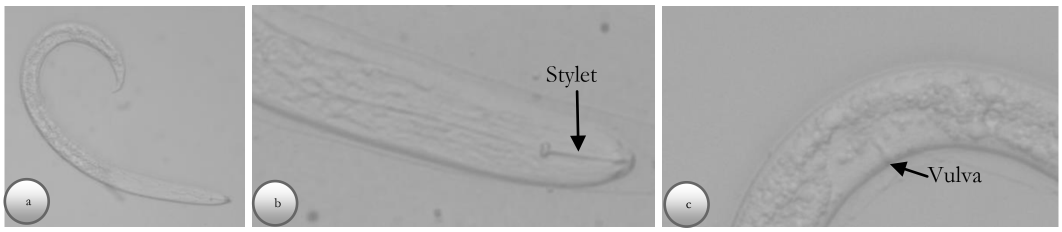

Fig 9. *Pratylenchus* sp. (a) adult female, (b) stylet with head and (c) Vulva

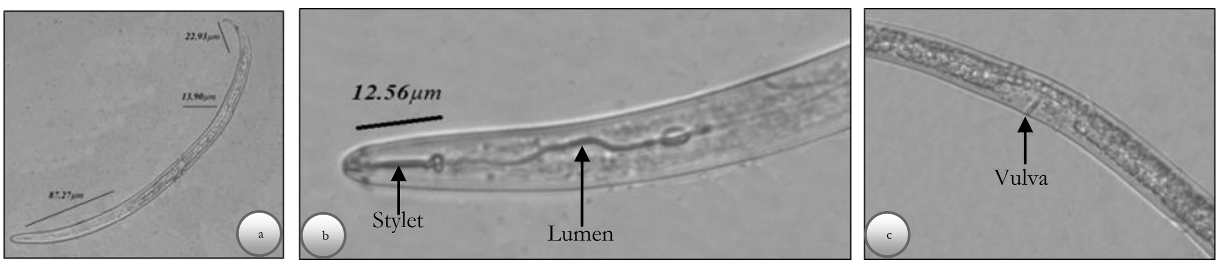

Fig 10. *Rotylenchulus* sp. (a) adult female, (b) stylet and (c) vulva

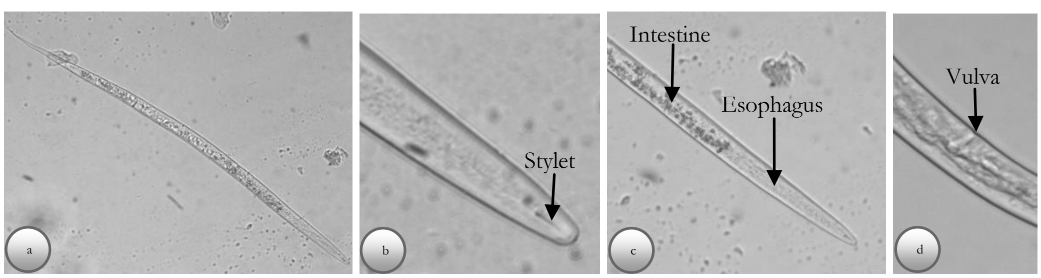

Fig 11. *Tylenchus* sp. (a) adult female, (b) stylet with head, (c) esophago-intestine and (d) vulva

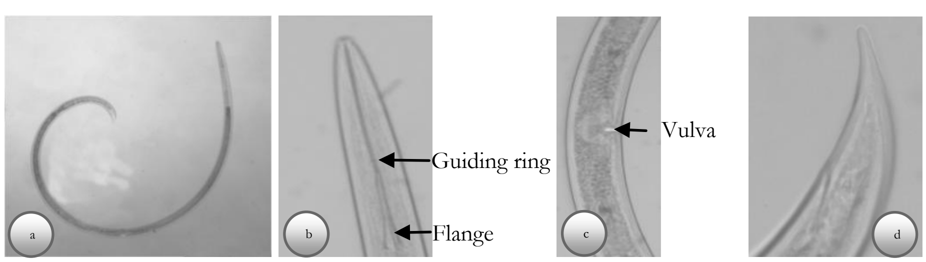

Fig 12. *Xiphinema* sp. (a) adult female (b) stylet with flange & guiding ring, (c) vulva and (d) tail

In this study twelve species of pant parasitic nematodes were found to be associated with tea soils in the tea estates of different valley circles. All of them have been reported as pests of tea in many countries (Sivapalan, 1972; Nalini *et al.,* 2005; Gnanapragasam, 2014). So far, five plant parasitic nematodes species viz. *Helicotylenchus sp.*, *Meloidogyne sp.*, *Pratylenchus sp., Tylenchus sp.* and *Aphelenchus sp.* have been reported as pests of tea from Bangladesh (Sana, 1989). Later, Ahmed (2005) and Khan *et al.* (2006) identified ten species of plant parasitic nematodes such as *Aphelenchoides* sp., *Helicotylenchus* sp., *Hemicriconemoides* sp., *Hoplolaimus* sp., *Meloidogyne* sp., *Paratylenchus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Tylenchus* sp. *and Zygotylenchus* sp. in tea soil. *Aphelenchus* sp., *Aphelenchoides* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Meloidogyne* sp., *Pratylenchus* sp. and *Tylenchus* sp. are also recorded in the present study. So other nematodes such as *Criconemoides* sp., *Dolichodorus* sp., *Eutylenchus* sp., *Rotylenchulus* sp. and *Xiphinema* sp. are the new records from the country. Among the plant parasitic nematodes, the occurrence of *Meloidogyne* sp*., Pratylenchus* sp., *Hoplolaimus* sp. and *Helicotylenchus* sp. was higher as compared to other plant parasitic nematodes. The findings of Khera & Chaturvadi (1977), Ahmed (2005), Khan *et al.* (2006), Orisajo (2012) and Roy & Rahman (2013) support the present results. The information on plant parasitic nematodes diversity and their distributional pattern in the tea estates of different valley circles gathered in this study would be useful to take appropriate management strategies for specific location.

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