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EDITORIAL

The present issue (volume 42) of Tea Journal of Bangladesh contains five research papers on different aspects of tea science.

The first paper deals with the effects of different plucking systems on shoot growth dynamics and yield thereby in two cultivars of tea having some differing (opposing) characteristics. The paper reveals that shoot growth dynamics of yield related parameters for ultimate yield of tea are different for different plucking treatments and vary from cultivar to cultivar. Though the original research work was carried out In Sri Lanka, it also holds true for Bangladesh condition too. The work would have some implications in upgrading the concept of plucking system in our tea.

The second article contains the evaluation of some indigenous plant extracts for controlling *Helopeltis* commonly known as Tea Mosquito Bug. The work indicated that most of the studied plants extracts were effective in controlling the target pest *Helopeltis*. The order of their effectiveness was:

Bur weed>Lantana>Datura>Neem>Mahogani>Katamehedi.

All these plants are available in and around tea growing areas of Bangladesh. The extracts are environment friendly and very effective. Those could be used safely both in organic and inorganic tea fields.

The third paper is on the development of a suitable protocol for *in vitro* culture of tea from shoot tip and nodal explants. The authors tried to establish a protocol for micro-propagation of tea. It has been a long awaited technique to grab for the commercialization of an easier, economic and suitable propagation method for our tea clones.

The next paper is on the integrated management of Tea Galls - a minor disease in tea. The work hints that Deep Skiff (DSK) operation followed by the application of a systemic fungicide Carbendazim @ 750 gm per hectare is the most effective method to minimize gall disease in tea.

The last paper is on the effect of poultry manure on the yield of mature tea as well as on soil properties. It reveals that comparable yield can be achieved even after 75% reduction of conventional chemical fertilizer (BTRI recommended dose) if poultry manure is added in appropriate dose. Thus this widely available manure from poultry droppings can minimize the use of chemical fertilizer and has a potential in reducing the cost of inorganic fertilizer as well as ill effects on tea soil properties.

(Dr. Mainuddin Ahmed)
Chief Editor

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EFFECTS OF DIFFERENT PLUCKING SYSTEMS ON SHOOT GROWTH DYNAMICS AND YIELD IN CONTRASTING TWO CULTIVARS OF TEA (*CAMELLIA SINENSIS* L.)

T. Ahmed[1*], W.A.J.M. De Costa[2] and M.A. Wijeratne[3]

Abstract

A field experiment was conducted to study the effects of different plucking systems on shoot growth dynamics and yield of tea in two contrasting cultivars (drought tolerant TRI2025 and moderately drought tolerant TRI2026). Depending on the severity of plucking, four plucking systems were used as treatments namely, mother-leaf plucking, fish-leaf plucking, janam-leaf plucking and estate practice. Between the two cultivars, TRI2026 showed significantly higher yield because of its greater number of harvested shoots m^{-2}, higher average shoot fresh weight (g) and less banji shoots (%) than the drought tolerant cultivar TRI2025. In TRI2025, estate practice produced greater yield among the treatments, while TRI2026 gave the highest yield in fish-leaf plucking system. It is concluded that shoot growth dynamics, yield related parameters and yield of tea are affected by different plucking treatments which vary due to cultivars.

Keywords: Tea cultivar, Severity of plucking, Shoot growth dynamics, Shoot density, Yield

Introduction

The operation of harvesting crop in tea, termed plucking, is the removal of the tender apical portions of secondary and higher order shoots which are subsequently processed to give the commercial product. Shoot growth is the major physiological process which determines the tea yield. The number of plucked shoots per unit land area (shoot density) and the mean weight per shoot are the components of yield of tea (Rahman, 1977). Of the two components mentioned, shoot density is the major yield contributing factor which determines more than 80% of the variation in yield of tea (Wijeratne, 2001). Although the number of harvested shoot has been identified as the main factor responsible for the observed variation in yields between different genotypes, variation in the rate of shoot growth is the main parameter that causes season-to-season yield variation in a given genotype (Squire and Callander, 1981). The number of harvested shoots per unit land area is determined by the rate of shoot initiation whereas the mean weight per shoot is determined by the rate of shoot expansion.

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These parameters largely depend on the quality of cultivar, plant density (spacing), pruning and plucking policies, fertilizer management, pest and disease control as well as climatic and soil conditions. Having the uniformity of tea bushes and high yield potential, clonal tea produced higher yield in comparison to seedling tea (Gunasekare, 2008). Tanton (1979) stated that semi-erect leaf possessing China-type tea (var. *sinensis*) produced up to six times the number of shoots per unit area than the horizontal Assam-type (var. *assamica*) and could be responsible mainly for yield variation. Hadfield (1975) showed that China-type tea with a photosynthetically favourable semi-erect leaf pose, produced four times the yield of Assam tea which has a horizontal leaf pose, concluding that this difference was related to photosynthetic efficiency.

The plucking of tea acts as a stimulus for further shoot production by temporarily destroying apical dominance (Tubbs, 1937; Portsmouth and Rajiah, 1957). The axillary bud immediately below the point of plucking is activated and develops into a new shoot. At times more than one shoot is regenerated which may depend on cultivar, environmental conditions and cultural practices. Severity of plucking determines the post-harvesting point of a shoot. Different plucking systems are followed in different agro-climatic regions due to difference in the rate of shoot growth. Some follow mother-leaf plucking, while others adopt fish-leaf and janam-leaf plucking. Mother-leaf plucking was the standard estate practice in Sri Lanka (Portsmouth, 1957) but now the estate practice plucking system (a combination of mother-leaf plucking and fish-leaf plucking) is popular while in North-East India janam-leaf plucking is practiced due to high growth rate (Wettasinghe *et al*., 1981). The appropriate plucking system may vary from cultivar to cultivar. In order to maximize the crop production and for the sustainability of a bush, it is necessary to find out the appropriate plucking system for a particular tea field. Therefore, this study was undertaken to know the effects of different standard of plucking on shoot growth dynamics and yield in two contrasting tea cultivars.

Materials and Methods

The experiment was conducted at the Tea Research Institute, Low Country Station, Ratnapura ($6^0$40'N, $80^0$25'E and 30 m amsl) and data were collected about a period of one year (from March 2011 to February 2012). The experiment was consisted of a two-factor factorial treatment structure. The treatment factors were a) two contrasting tea cultivars and b) four plucking standards. The contrasting two tea cultivars, drought tolerant TRI2025 (Assam-type) and moderately drought tolerant TRI2026 (China-type) were used. The four different standard of plucking were mother-leaf plucking (shoot is plucked leaving the oldest normal leaf), fish-leaf plucking (plucking to the fish-leaf), janam-leaf plucking (plucking below the fish-leaf) and estate practice. In the estate practice, about 60-75% of tea crop shoots were plucked to the fish-leaf and the balance to the mother leaf (Wettasinghe *et al*., 1981).

There were four replicate plots for each cultivar x plucking standard treatment combination. Altogether there were 32 experimental plots and each plot having 10 bushes were demarcated. So, the experimental area was laid in a randomized complete block design. Tea bushes were 11 years old with double hedge spacing (0.6m x 0.9m x 1.5m) and were in the third year of its pruning cycle. Application of fertilizers and cultural practices were carried out according to the recommendations of TRI, Sri Lanka.

Plucking was done in the morning on weekly rounds for estimation of the plot yield (g $plot^{-1}$) and it was used to extrapolate as tea yield in g m^{-2} and during the plucking following data were collected e.g. harvested shoot population density (No. m^{-2}), basal shoot population density (No. m^{-2}) and total shoot population density (No. m^{-2}).

To know the shoot growth dynamics, five shoots each having three leaves and active bud were randomly selected from the centre of the bush from each plot at two months intervals. Selected shoots were plucked according to the treatment and tagged for future identification. Tagged shoots were plucked when they reached at the standard of two leaves with active bud and the following measurements were made on these harvested shoots e.g. length of shoot replacement cycle (L_{SRC}), fresh weight per shoot at two leaves with active bud stage and number of shoot regenerated from the plucking point. Collected data were statistically analyzed using SAS computer package.

Results and Discussion

Shoot growth dynamics

The time taken by an axillary bud to grow into a harvestable shoot is termed as the shoot replacement cycle. The L_{SRC} was significantly longer for cultivar TRI2026 than the TRI2025 (Table 1). In TRI2025, mother-leaf plucking showed the highest duration for shoot replacement cycle (36.1 days) which was significantly different with others. However in TRI2026, janam-leaf plucking had the highest L_{SRC} (36.7 days) which was similar with mother-leaf plucking and fish-leaf plucking but different with estate practice. Between two cultivars, greater fresh weight per shoot was observed in TRI2026 (0.49 g) than TRI2025 (0.46 g). For both cultivars, mother-leaf plucking showed greater fresh weight per shoot and the lowest was in janam-leaf plucking (Table 1). In both cultivars, janam-leaf plucking regenerated greater number of shoots which was significantly different and followed by fish-leaf plucking, estate practice and mother-leaf plucking, respectively (Table 1).

Table 1. Effect of different plucking systems on length of shoot replacement cycle (L_{SRC}) and fresh weight per shoot at two leaves with active bud stage and number of shoots regenerated

Plucking system	L_{SRC} (Days)		Shoot fresh weight (g)		Number of shoot generations	
	TRI2025	TRI2026	TRI2025	TRI2026	TRI2025	TRI2026
Mother-leaf	36.1(±2.8)^{a}	36.3(±2.4)$^{a\,b}$	0.50(±0.04)^{a}	0.50(±0.04)^{a}	1.0(±0.04)^{b}	1.0(±0.01)^{b}
Fish-leaf	33.9(±2.2)^{b}	35.8(±2.8)$^{a\,b}$	0.45(±0.05)$^{b\,c}$	0.49(±0.06)$^{a\,b}$	1.1(±0.05)^{b}	1.1(±0.02)^{b}
Janam-leaf	34.2(±2.4)^{b}	36.7(±3.2)^{a}	0.42(±0.06)^{c}	0.47(±0.04)^{b}	1.4(±0.11)^{a}	1.3(±0.09)^{a}
Estate practice	34.8(±2.5)^{b}	35.6(±3.4)^{b}	0.48(±0.05)$^{a\,b}$	0.49(±0.04)$^{a\,b}$	1.1(±0.03)^{b}	1.1(±0.02)^{b}
Mean	34.7 ^{B}	36.1 ^{A}	0.46 ^{B}	0.49 ^{A}	1.2	1.1
Level of significance 0.05						
LSD	1.01	1.10	0.03	0.03	0.09	0.08
CV (%)	10	11	25	22	27	27

The difference of shoot growth dynamics may be due to the variation of genetic characteristics between two cultivars. Similar findings observed by Wijeratne (1994) that shoot replacement cycle was shorter in drought tolerant TRI2025 for its early bud break and faster rate of leaf appearance than the drought susceptible cultivar TRI2023. Between two cultivars, greater fresh weight per shoot was observed in TRI2026 than TRI2025. This may be due to the variation of L_{SRC} or for genetic characteristics resulting the variation of shoot weight between two cultivars most probably caused by the inherited different characteristics of shoot internodes and leaves as suggested by Wickramaratne (1981). The cultivar that takes longer period to complete the shoot replacement cycle, naturally assimilates more photosynthates and absorb more nutrients for its growth and the vice-versa. In agreement with present findings, Wettasinghe *et al*. (1981) showed janam-leaf plucking resulted in a 26% drop in average shoot weight. In both cultivars, mother-leaf plucking spent a longer time period for L_{SRC} in contrast to other systems. Number of shoot generations were higher in janam-leaf plucking which is supported by Visser (1960), that harder plucking systems influenced multiple shoot growth than the lighter system of plucking. This would have resulted from the weak apical dominance over the second axillary buds below the fish leaf in a region where internodes are very short.

Shoot density (Number m^{-2})

The difference in number of harvested shoot population density, basal shoot population density and total shoot population density were highly significant for cultivars, treatments and between their interaction effects. Greater number of shoots m^{-2} were observed for all the above parameters in TRI2026 than TRI2025, presented in the Table 2. In cultivar TRI2025, greater number of harvested shoots m^{-2} were observed in estate practice (217) which was statistically similar with fish-leaf plucking and mother-leaf plucking but significantly different with janam-leaf plucking. However cultivar TRI2026 gave greater number of harvested shoots m^{-2} in fish-leaf plucking (243) which was similar with janam-leaf plucking and estate practice but significantly different with mother-leaf plucking.

Table 2. Effect of severity of plucking on harvested shoot density, basal shoot density and total shoot density (No. m^{-2})

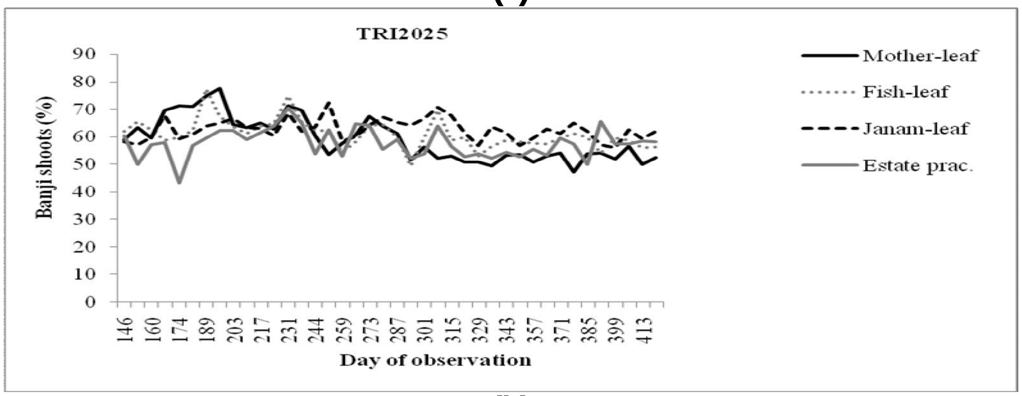
Plucking system	Harvested shoot density		Basal shoot density		Total shoot density	
	TRI2025	TRI2026	TRI2025	TRI2026	TRI2025	TRI2026
Mother-leaf	208(±4) ab	221(±6) b	190(±4) c	189(±4) c	398(±7) c	410(±9) b
Fish-leaf	215(±5) ab	243(±5) a	204(±4) b	224(±4) b	419(±7) b	467(±8) a
Janam-leaf	206(±5) b	242(±5) a	202(±5) b	231(±4) a	408(±9) bc	473(±9) a
Estate practice	217(±5) a	240(±6) a	222(±5) a	237(±5) a	438(±9) a	477(±11) a
Mean	212 B	237 A	205 B	220 A	417 B	457 A
Level of significance	0.05					
LSD	9.3	10.1	7.1	7.4	13.1	13.7
CV (%)	20	19	16	15	14	14

From the above results, it is clearly indicated that density of harvested shoot significantly varied due to cultivars. Similar findings observed by Stephens and Carr (1994) that shoot population density of tea almost depended on its genetic makeup. Although the numbers of shoots generated after plucking were greater in TRI2025, at the time of plucking a greater number of shoots were being harvested in TRI2026. This was due to the production of more than one secondary shoot generations which became smaller in size than the generation of one shoot and often they do not reach the plucking surface. This phenomenon happened especially in the drought tolerant cultivar of TRI2025 and subsequently reduced the harvested shoot density, which is supported by the findings of Wijeratne (1994). Plucking system also affected the harvested shoot population density which varied from cultivar to cultivar. In TRI2025, greater number of harvested shoot density observed in estate practice, while in TRI2026 that was under fish-leaf plucking.

Banji shoots (%) in the harvested shoots

Formation of banji shoots (%) varied for cultivars. Between the two cultivars, higher percentage of banji shoots observed in the drought tolerant TRI2025 than the TRI2026 at most of the plucking dates. Severity of plucking also had significant effect on the banji formation of shoots. In TRI2025, greater percentage of banji shoots observed in janam-leaf plucking (about 63% in an average) which was followed by fish-leaf plucking (61%), estate practice (59%) and mother-leaf plucking (58%), respectively [Figure 1 (a)]. Similarly for the cultivar TRI2026, lower percentage of banji shoots observed in mother-leaf plucking and estate practice (about 55% in an average) which was followed by janam-leaf plucking (56%) and fish-leaf plucking (57%) [Figure 1 (b)]. In the harvested leaf, percentage of banji shoots were varied at different plucking dates for all of the four treatments in both cultivars.

(a)



(b)

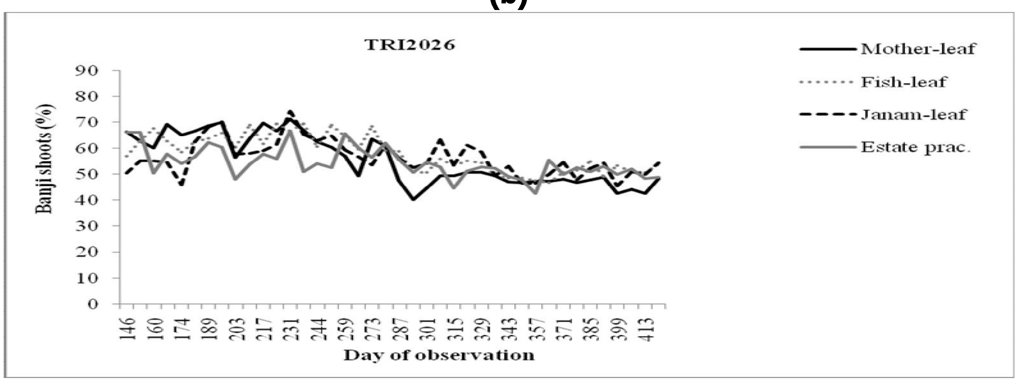


Fig. 1. Banji shoots (%) in the harvested leaf for the severity of plucking in (a) TRI2025 and (b) TRI2026 at different plucking days of the year

Formation of banji reduces production of transpiring leaves and fast growing apices, thus reducing the water requirement for growth. Hence, greater production of dormant shoots can be considered as a useful mechanism of drought tolerance by maintaining internal water balance of the bush (Odhiambo *et al*., 1993). Clonal differences in banji shoot production also have been reported by Stephens and Carr (1990) for Mufindi tea clones. Among the four treatments, mother-leaf plucking produced less number of banji shoots than others in both cultivars. It happened because results of this study showed that being the lightest system of plucking, mother-leaf plucking took longer period for its shoot replacement cycle. And consequently, banji percentage was greater in janam-leaf plucking.

Harvested yield records

Greater fresh weight and dry weight (g m^{-2}) were recorded in TRI2026 than TRI2025 (Table 3). In TRI2025, estate practice produced higher harvested fresh leaf weight and dry weight which was significantly different and followed by fish-leaf plucking, mother-leaf plucking and janam-leaf plucking respectively. However for the cultivar

TRI2026, fish-leaf plucking produced greater harvested leaf fresh weight and dry weight which was significantly different and followed by janam-leaf plucking, estate practice and mother-leaf plucking, respectively.

Table 3. Effect of severity of plucking on harvested fresh leaf weight and dry weight

Severity of plucking	Fresh leaf weight (g m^{-2})		Dry leaf weight (g m^{-2})	
	TRI2025	TRI2026	TRI2025	TRI2026
Mother-leaf	26.1(±0.8) ^{b}	25.9(±0.8) ^{c}	5.7(±0.2) ^{c}	5.4(±0.2) ^{c}
Fish-leaf	27.9(±0.9) ^{ab}	35.5(±1.3) ^{a}	6.4(±0.2) ^{b}	7.7(±0.3) ^{a}
Janam-leaf	22.6(±0.8) ^{c}	33.2(±1.0) ^{b}	5.4(±0.2) ^{c}	7.4(±0.2) ^{ab}
Estate practice	28.8(±1.0) ^{a}	31.1(±1.0) ^{b}	6.8(±0.2) ^{a}	7.0(±0.2) ^{b}
Mean	26.4 ^{B}	31.4 ^{A}	6.1 ^{B}	6.9 ^{A}
Level of significance	0.05			
LSD	1.87	2.29	0.43	0.50
CV (%)	32	33	33	33

As already pointed out, the yield of tea depends on the average shoot weight and the number of shoots harvested per unit area (harvested shoot density). The relative importance of these components in the determination of tea yield has been discussed by many researchers (Tanton, 1992; Odhiambo *et al*., 1993). Both the parameters are significantly affected by the characteristics of the cultivar which is largely under genetic control and also by the system of plucking. Having greater harvested shoot density, less banji shoots and greater shoot weight, the cultivar TRI2026 produced greater harvested leaf yield than TRI2025. For almost the similar reasons, greater yield of tea was observed under estate practice in TRI2025 and that was in fish-leaf plucking for TRI2026. In TRI2025, the lowest yield observed in janam-leaf plucking which supported with the findings of Wettasinghe *et al*. (1981) who stated that increasing severity of plucking increased the harvest index up to a certain period after which it will be decreased due to aging of maintenance foliage. Moreover, present study shows that in both cultivars, mother-leaf plucking producing lower yield and it was more pronounce in TRI2026 than TRI2025. The main reason of low yield in mother-leaf plucking was due to its lower number of harvested shoots per unit land area which is considered as the major yield component of tea.

Conclusion

An improved vegetatively propagated tea cultivar has significant effect to increase the yield due to its higher shoot density, greater average shoot weight and less number of banji shoots. To ensure the highest productivity from a tea area, it is essential to use the appropriate plucking policy which vary from cultivar to cultivar.

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EVALUATION OF SOME INDIGENOUS PLANT EXTRACTS AGAINST TEA MOSQUITO BUG, *HELOPELTIS THEIVORA* WATERHOUSE (HEMIPTERA: MIRIDAE) INFESTING TEA

M.S.A. Mamun[1*], M. Ahmed[2], S.K. Paul[3] and R.S. Chowdhury[4]

Abstract

An experiment was conducted to evaluate the toxicity of some indigenous plants viz., Bur weed (*Xanthium strumarium*), Datura (*Datura metel*), Katamehedi (*Duranta erecta*), Lantana (*Lantana camara*), Mahogani (*Swietenia mahagoni*) and Neem (*Azadirachta indica*) at 5.0, 7.5 and 10.0% (w/v) concentrations against tea mosquito bug, *Helopeltis theivora* Waterhouse under both laboratory and field conditions at Bangladesh Tea Research Institute, Srimangal, Moulvibazar during 2010-2011. The plant extracts were prepared with acetone and water solvents separately. Direct toxicity test was done by topical application method in completely randomized design under laboratory and spraying method in randomized complete block design under field condition. Results indicated that all the plant extracts had showed the toxic effect on *Helopeltis* under both laboratory and field conditions. Under laboratory condition, the toxicity of Bur weed (52.86%) was found to be highest whereas least action found in Katamehedi (27.24%). Among the solvents, acetone extract (40.30%) was slightly more effective than water extract (37.01%). The maximum average mortality (44.09%) was observed at the highest concentration (10.0%) of plant extracts and the mortality percentage was directly proportional to the level of concentration of plant extracts. In case of field evaluation, the aqueous extract of Bur weed showed the highest percent reduction in infestation (46.73%) over control and that of Katamehedi had the lowest action (36.71%) at 10% concentration. The effectiveness of most of the plant extracts were found to increase proportionally with the increase of doses and decreases proportionally with increase of time. The order of toxicity of the plant extracts on *Helopeltis* was: Bur weed>Lantana>Datura>Neem>Mahogani>Katamehedi. The above mentioned plants are available throughout the tea growing areas and the planters may use these plants in tea pest management especially for the control of *Helopeltis* as safer phytopesticidal products in both organic and inorganic tea estates.

Keywords: Tea, Plant extracts, Biopesticides, Toxicant, Tea mosquito bug, *Helopeltis theivora*

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Introduction

Tea is the most widely used non-alcoholic beverage all over the world, prepared from the tender shoot of tea plant, *Camellia sinensis* (L) O. Kuntze. Tea plants are subjected to the attack of several pests such as insects, mites and nematodes (Sana, 1989). In world tea, 1034 species of arthropods and 82 species of nematodes are associated with tea plants (Chen and Chen, 1989). Among them 25 species of insects, 4 species of mites and 10 species of nematodes are recorded from Bangladesh (Ahmed, 2005). Among the various insect pests that attack or cause severe damage to tea plant, one is the *Helopeltis theivora*, commonly known as Tea mosquito bug (Waterhouse, 1886). It is one the most serious pests of tea in almost all tea growing countries like Sri Lanka, India, Indonesia and Bangladesh. The crop loss due to *Helopeltis* to the tune of 10-15% is reported from Bangladesh (Ahmed, 2012). Both nymphs and adults of *Helopeltis* suck the cell sap from the buds, young leaves and tender stems. Generally infested shoots dry up which directly affect to crop loss. In a severe attack, the plantation turns black and growth of flush i.e. two leaves and a bud, is completely checked. In addition to direct crop loss, damage by *Helopletis* leads to debilitation of bushes resulting in die-back with crows-feet and delayed or meager flushing thereafter consequently almost the entire crop is lost.

To combat these problems different groups of pesticides have been used in the tea fields with approval of BTRI since 1960 (Mamun *et al.,* 2012). The large-scale use of synthetic insecticides in tea leads to adverse effects such as development of pesticide resistance, frequent pest outbreaks, emergence of new pests, environmental pollution and health hazards. As tea is a consumable commodity, the effect of residue of pesticides in made tea is harmful to human health. In this context, botanicals are being considered as environmentally safe, selective, biodegradable, economical and renewable alternatives for use in IPM programmes in tea (Mamun, 2011). Botanicals are natural plant products that belong to the secondary metabolites, which include alkaloids, terpenoids, phenolics, and minor secondary chemicals. It is estimated that the plants may contain as many as 4000,000 secondary metabolites (Jacobson, 1989). As many as 2121 plant species have been reported to posses' pest control properties; 25 of these plants species posses the characteristics required for an ideal botanical insecticide and are therefore more promising for use in organic pest control programmes (Radhakrishnan, 2005). Botanicals like neem, ghora-neem, mahogani, karanja, adathoda, sweet flag, tobacco, derris, annona, smart weed, bur weed, datura, calotropis, bidens, lantana, chrysanthemum, artemisia, marigold, clerodendrum, wild sunflower etc. are available surroundings of the tea garden or may be grown by the planters with minimum expense and extracted by indigenous methods (Mamun and Ahmed, 2011). These botanical materials can be used as an alternative to chemical pesticides. This will be very helpful in minimizing the undesirable side effects of synthetic pesticides.

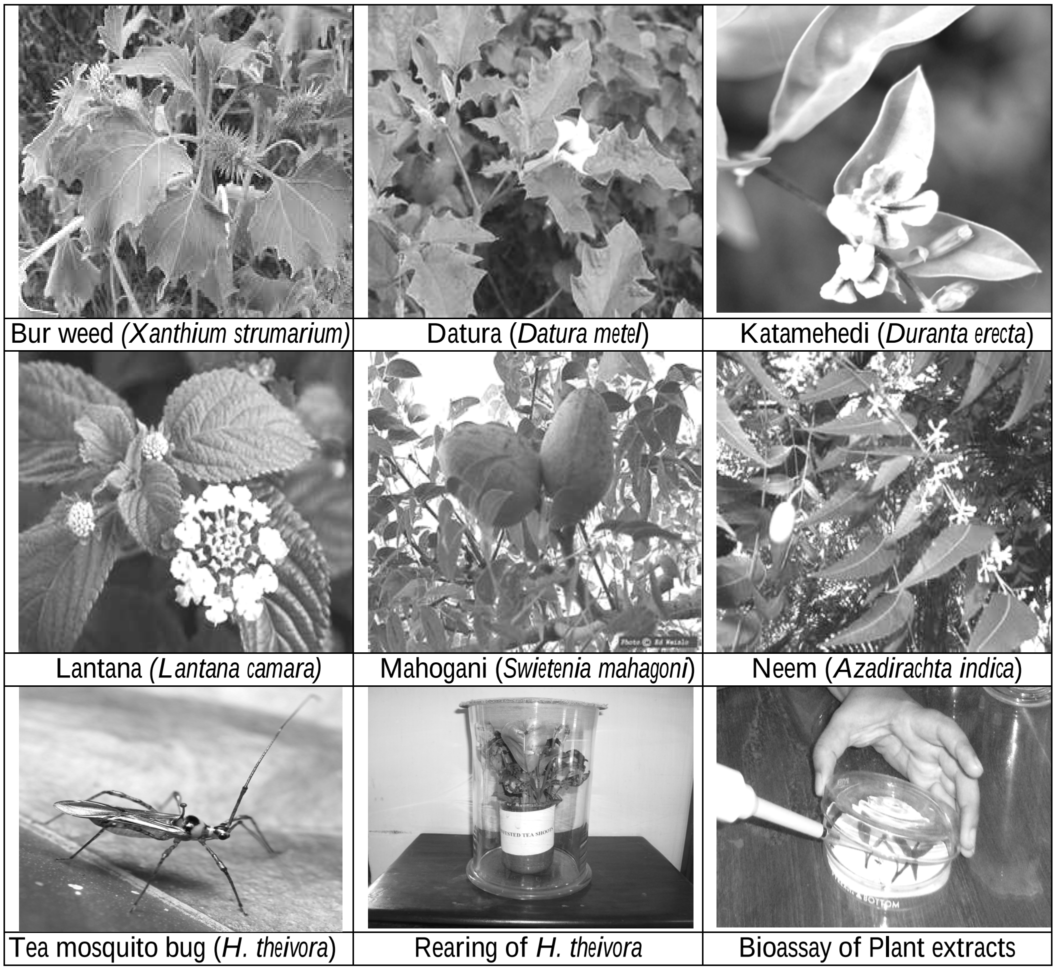
Based on the foregoing, the present study was undertaken to evaluate the toxic effect of six indigenous plant extracts namely Burweed (*Xanthium strumarium),* Datura *(Datura metel)*, Katamehedi (*Duranta erecta*), Lantana *(Lantana camara),* Mahogani *(Swietenia mahagoni)* and Neem *(Azadirachta indica)* against tea mosquito bug, *H. theivora,* a major insect pest of tea in Bangladesh.

Materials and Methods

An experiment was conducted both in the Entomology Laboratory and in the main farm of Bangladesh Tea Research Institute (BTRI), Srimangal, Moulvibazar during the period from January 2010 to December 2011. The *Helopeltis* was collected from different sections of BTRI main farm and was reared in the Entomology Laboratory, BTRI at 27 to 30°C temperature and 70-80% relative humidity on a susceptible tea clone, BT3 by following the method of Muraleedharan (2006). The insects were reared on tea shoots with three leaves and a bud in transparent glass jars (20 cm X 15 cm) and mouths of transparent jars were covered with nylon mesh. Each jar was set up with 20 pairs of adult insects and the newly emerged adults were utilized in the subsequent experiments of the present study.

Preparation of plant extracts

Fresh leaves, succulent stems, flowers, seeds and whole plants of Bur weed (*Xanthium strumarium*), Datura (*Datura metel*), Katamehedi (*Duranta erecta*), Lantana (*Lantana camara*), Mahogani (*Swietenia mahagoni*) and Neem (*Azadirachta indica*) were collected locally from nearby areas of BTRI main farm, Srimangal, Moulvibazar (Table 1 & Plate 1). Each plant material was dried under shade and powdered by using electric grinder and pass through a 20 mesh sieve and kept in a 1 kg capacity polypropylene bag. 300 g of each powdered plant material were taken into a 2 litre capacity conical flask and 1000 ml of distilled water and/or acetone was added to it separately and shaken for 8 h in a mechanical shaker and then kept it for 24 h. Acetone and distilled water were used as solvents. The extract was separated using fine Muslin cloth and then filtered. The filtrate was collected in a 2 litre capacity conical flask and volume was made up to 1000 ml. This was considered as stock solution. Required concentrations (5.0, 7.5 and 10.0%) were prepared from the stock solution.



Bur weed (*Xanthium strumarium)* | Datura (*Datura metel*) | Katamehedi (*Duranta erecta*)

Lantana *(Lantana camara)* | Mahogani (*Swietenia mahagoni*) | Neem (*Azadirachta indica*)

Tea mosquito bug (*H. theivora*) | Rearing of *H. theivora* | Bioassay of Plant extracts

Plate 1. Some indigenous plant extracts against tea mosquito bug

Table 1. Plants evaluated for insecticidal activities against *Helopeltis*

Common name	Scientific name	Family	Plant parts used
Bur weed	*Xanthium strumarium*	Compositae	Whole plant
Datura	*Datura metel*	Solanaceae	Leaves and fruits
Katamehedi	*Duranta erecta*	Verbenaceae	Leaves and flowers
Lantana	*Lantana camara*	Verbenaceae	Leaves and flowers
Mahogani	*Swietenia mahagoni*	Meliaceae	Seeds
Neem	*Azadirachta indica*	Meliaceae	Seeds

Laboratory evaluation of toxicity of plant extracts against *Helopletis*

A laboratory test for direct toxicity by topical application method was conducted according to the method of Talukder and Howse (1993) with slight modification. Three different concentrations of each plant extracts (5.0, 7.50 and 10.0%) were prepared with respective solvents (Acetone and Water). One microliter (µl) of prepared solution was applied to the dorsal surface of the thorax of each *Helopelits* using a micropipette. Ten insects per replication were treated and each treatment was replicated thrice. In addition, the same numbers of insects were treated with solvent only for control. After treatment, the insects were transferred into 9 cm diameter petri dishes (10 insects/petri dish) containing tea shoots (two leaves and a bud). Insect mortalities were recorded at 24, 48 and 72 hours after treatment (HAT). Original data were corrected by Abbott's (1987) formula:

$$P = \frac{P' - C}{100 - C} \times 100$$

Where,

P = Corrected mortality (%)
P´ = Observed mortality (%)
C = Control mortality (%)

Field evaluation of toxicity of plant extracts against *Helopeltis*

Field evaluation of aqueous extracts of the above six plants was also conducted against tea mosquito bug at the BTRI main farm in 2010-2011. No pesticide was applied to the bushes prior to the start of the experiment. The experiment was laid out in low skiffed sections following Randomized Block Design with three replications each having plot size of 5m x 5m. The treatments were T1= Bur weed, T2= Datura, T3= Katamehedi, T4= Lantana, T5= Mahogani, T6= Neem and T7= control.

Plots with heavy infestation of *H. theivora* were chosen for this study. After selection of the plots, pre treatment count was taken in the respective plots. The total quantity of each aqueous extract for spraying in each treatment was calculated on the basis of plot size. The plots were sprayed with 5.0, 7.5 and 10.0% concentrations of each of the extracts separately. Spraying was done early in the morning with knapsack sprayer where uniform coverage of each plot and bush was taken care of. Two rounds of foliar spray were given at 15 days interval and post treatment observations were taken in four consecutive weeks. One hundred shoots were randomly collected from the harvested shoots of each plot and infested shoots were counted. A shoot was considered as infested, if it contained even a single feeding spot. Effectiveness of the plant extracts was calculated by using Henderson & Tilton's (1955) formula:

$$\text{Percent effectiveness} = \left(1-\frac{cbxta}{caxtb}\right) \times 100$$

Where,

cb = No. of insect population in control before treatment

ca = No. of insect population in control after treatment

tb = No. of insect population in treated before treatment

ta = No. of insect population in treated after treatment

Statistical Analysis

The experimental data were statistically analyzed by Completely Randomized Design (factorial CRD) and Randomized Block Design in case of laboratory and field experiment respectively using MSTAT statistical software in a microcomputer. The results are expressed as Mean ± SE and data were statistically analyzed by one-way ANOVA, with the level of significance set at $p<0.05$. The mean values adjusted by Duncan's Multiple Range Test (DMRT) (Duncan, 1951).

Results and Discussion

Laboratory evaluation of toxicity of plant extracts against *Helopeltis*

The effects of different of plant extracts of Bur weed, Datura, Katamehedi, Lantana, Mahogani, Neem against tea mosquito bug, *H. theivora* are presented in Tables 2-5. Average mortality percentage of tea mosquito bug at 24, 48 and 72 hours after treatment indicated that Bur weed extract possessed the highest (52.86%) toxic effect, whereas Katamehedi leaf extract possessed the lowest (27.24%) toxic effect (Table 2). Among the solvents, acetone extract was found more toxic (40.30%) and it was significantly different from water (37.01%) extract (Table 3). The maximum average mortality (44.09%) was observed at higher concentration (10.0%) of plant extract and the mortality percentage was directly proportional to the level of concentration of plant extract (Table 4).

Table 2. Mean mortality percentage of tea mosquito bug, *H. theivora* treated with different plant extracts (Interaction of plant extracts and time)

Name of the plant	Mortality rate (%)			Mean (%)
	24HAT	48HAT	72HAT	
Bur weed	47.59	54.63	56.36	52.86a
Datura	35.18	44.02	47.59	42.26c
Katamehedi	22.59	27.96	31.48	27.24f
Lantana	38.70	45.68	49.26	44.55b
Mahogani	25.78	29.26	32.16	29.07e
Neem	32.13	35.69	39.70	35.84d
$S\bar{x}$	0.135			0.1051
Probability level	NS			0.01

HAT= Hours after treatment
NS = Not Significant
Within column and row values followed by different letter(s) are significantly different by DMRT

Table 3. Effect of different solvents used in preparing various plant extracts on the mortality of tea mosquito bug, *H. theivora* (Interaction of solvent and time)

Name of the solvent	Mortality rate (%)			Mean (%)
	24HAT	48HAT	72HAT	
Acetone	34.98	41.52	44.41	40.30a
Water	32.34	37.57	41.11	37.01b
Sx	0.0967			0.0821
Probability level	NS			0.01

HAT= Hours after treatment
NS = Not Significant
Within column and row values followed by different letter(s) are significantly different by DMRT

Table 4. Effect of different doses of plant extracts on the mortality of tea mosquito bug, *H. theivora* (Interaction of dose and time)

Dose (%)	Mortality rate (%)			Mean (%)
	24HAT	48HAT	72HAT	
5.0	28.67	34.38	37.44	33.50c
7.5	34.26	37.75	43.12	38.38b
10.0	38.06	46.50	47.71	44.09a
Sx	0.1732			0.0958
Probability level	NS			0.01

HAT= Hours after treatment
NS = Not Significant
Within column and row values followed by different letter(s) are significantly different by DMRT

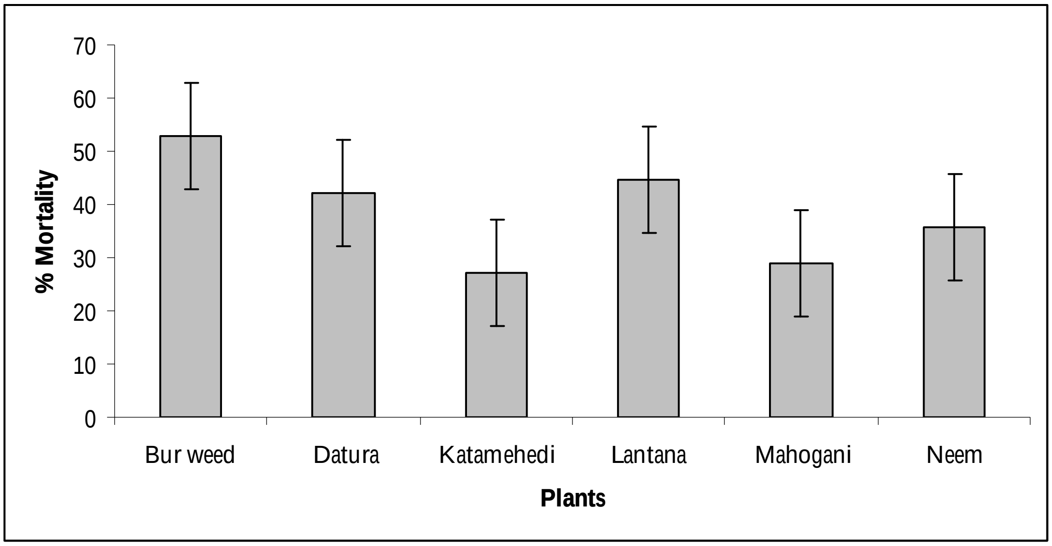


Fig. 1. Performance of plant extracts on the mortality of *H. theivora*

The order of toxicity of the plant extracts on tea mosquito bug, *H. theivora* was: Bur weed>Lantana>Datura>Neem>Mahogani>Katamehedi (Fig. 1). Among the test plants, Bur weed extracts showed the highest toxic effect, whereas Katamehedi showed the lowest toxic effect. Control of *Helopeltis* with some indigenous plant extracts were reported by Gogoi *et al.* (2003), Deka *et al.* (1998), Sarmah and Bhola (2008). The present results are in agreement with the findings of those workers.

Field evaluation of toxicity of plant extracts

The data for two years experiment at BTRI main farm reveal that at 5% aqueous extracts of the plants gave 28.30% reduction in infestation compared to control and those of 33.26% and 41.34% at 7.5 and 10% respectively (Fig. 2). Higher concentration of aqueous extract of all the tested plants showed higher percent reduction in infestation. The highest percent reduction in infestation was found 46.73% at 10% of concentration of aqueous extract of Bur weed (Table 5). Another plant, Lantana occupied the second position in effectiveness by reducing infestation which was 43.90% over untreated control at the same concentration and followed by Datura (41.82%), Neem (40.39%), Mahogani (38.50%) and Katamehedi (36.71%).

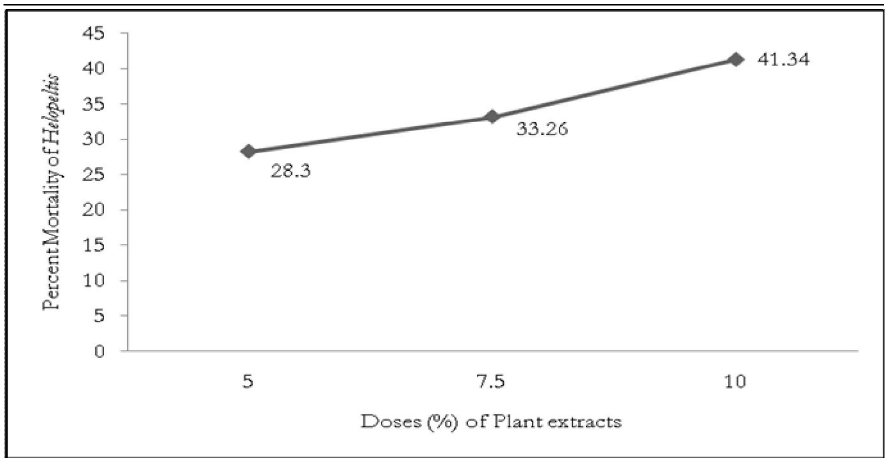


Fig. 2. Effect of doses of plant extracts on *Helopeltis*

Table 5. Field evaluation of different plant extracts against *Helopeltis theivora*

Treatments	Dose (%)	Pre treatment (% shoot infestation)	Post treatment observations (% reduction of infestation over control)				
			1st spray		2nd spray		Average
			7 days	14 days	21 days	28 days	
Bur weed	5.0	30	39.56	31.17	33.11	23.08	31.73i
	7.5	35	47.62	29.82	39.13	28.57	36.29f
	10.0	40	48.41	41.52	56.52	40.48	46.73a
Datura	5.0	28	35.06	23.44	32.81	25.32	29.16j
	7.5	32	38.78	32.33	37.89	28.57	34.39g
	10.0	36	46.43	39.47	45.65	35.71	41.82c
Katamehedi	5.0	38	28.57	21.05	29.35	19.64	24.65l
	7.5	43	36.51	23.98	37.20	20.63	29.58j
	10.0	34	46.43	29.82	42.03	28.57	36.71f
Lantana	5.0	42	38.10	26.32	33.33	23.81	30.39i
	7.5	37	40.48	32.75	39.61	28.57	35.35g
	10.0	40	54.55	35.41	50.59	35.06	43.90b
Mahogani	5.0	44	34.52	21.05	27.54	19.64	25.69k
	7.5	39	41.96	24.34	37.50	21.88	31.42i
	10.0	36	48.98	32.33	44.10	28.57	38.50e
Neem	5.0	34	38.10	22.81	30.43	21.43	28.19j
	7.5	30	39.29	28.95	36.96	25.00	32.55h
	10.0	37	48.05	35.41	44.66	33.44	40.39d
Control	-	29	36	42	49	57	-
CV%	-	-	8.77	6.45	7.08	5.36	-

Mean of three observations
Within column and row values followed by different letter(s) are significantly different by DMRT

The results of the studies showed that aqueous extracts of the test plants were effective in reducing the mosquito bug infestation. However, variations existed in their efficacy. Similar results were reported by Sing *et al*. (2012), Anonymous (2010), Roy *et al*. (2009), Sarmah *et al*. (2006) and Gogoi *et al.* (2003).

Conclusion

It has been observed from the result that the botanicals used in this experiment had direct toxic effect on tea mosquito bug, *H. theivora*. The tested indigenous plants are available throughout the tea growing areas and the planters may use these plants in tea pest management especially for the control of tea mosquito bug, *H. theivora*. However, before releasing it as a new technology further investigation is needed to confirm the result. Furthermore, natural products are notoriously variable, and therefore consistency of the final product will be much harder to achieve. Also, it may be necessary to determine both the shelf stability of the active ingredient and its fate in the environment or in animals. Such studies can be complicated enough when only a single compound is being tracked, but the effort required to track several putative active principles in a single product should not be underestimated. These insecticidal activities of tested plants can therefore be incorporated into other insect control techniques in the strategy of integrated pest management (IPM) in tea.

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DEVELOPMENT OF *IN VITRO* MICRO PROPAGATION SYSTEM IN TEA PLANT [*CAMELLIA SINENSIS* (L.) O. KUNTZE] USING SHOOT TIP AND NODAL SEGMENT EXPLANTS

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Abstract

A successful method of *in vitro* propagation system for tea plant [*Camellia sinensis* (L.) O. Kuntze] was developed. Shoot tip and nodal segment explants of elite tea clone BT2 were used for direct shoot regeneration. The experiments were carried out following the method of shoot initiation, multiple shoot proliferation, root induction and transfer of the plantlets to the soil after proper hardening. Among the different hormone supplements examined, combined application of BAP and IBA was found to be effective for shoot initiation and multiple shoot proliferation. The best shoot initiations (48% for shoot tip and 52% for nodal segment explants) were observed and the highest mean number and mean length of shoots/explant for shoot tip (13.2 and 4.5cm.) and nodal segment (15.1 and 5.0 cm.) explants were achieved on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l IBA. Addition of 0.5 mg/l GA3 on the shoot multiplication medium showed optimum results on shoot elongation. Moreover 85% rooting was achieved when the isolated microshoots were pretreated with high concentration of IBA (500 mg/l; 30 min) and transferred to the soil directly. After proper hardening rooted plantlets were transplanted to the pot and 80% plantlets were found to survive.

Keywords: BAP-6-benzyleaminopurine, IBA- indole-3-butyric acid, MS- Murashige & Skoog (1962) medium, micropropagation, explants, plantlet.

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the most popular mild stimulating, caffeine containing drink used all over the world. Medicinal values have increased its popularity as a health drink also. In Bangladesh it has been habituated as a daily consumption. Because of increasing demand world wide and in domestic level tea is now an important agricultural product in our country. Bangladesh is now facing the challenging competition in the international market as the demand of world tea consumers are on good quality. Considering tea as an important commercial crop, Bangladesh has to give emphasis on its improved production and quality as well. Intensive plantation with elite clones and seed stocks is one of the options to achieve the goal for the tea industries of Bangladesh.

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But conventional propagation system of tea plant is very slow and has some unavoidable limitations. Sufficient planting materials are not available throughout the year due to winter dormancy and season depending rooting ability of the cuttings results the poor survival rate at the nursery level. Besides, production of large number of cuttings is impossible immediately after releasing of an elite clone from the experimental plot as the number of mother plants are limited (80-100). At present 15000-18000 plants/ha is recommended to get optimum production and 10% more saplings are required for infilling. In this situation *in vitro* micropropagation can be considered as useful attempt to solve unavailability of sufficient planting materials for tea plantation. This technique can be an ideal choice for the circumvention of conventional multiplication system (Mondal *et. al.*, 2004) and commercially, it can be effectively utilized for introducing improved clones in a relatively short period of time (Sharma *et. al.*, 1999).

Several reviews on micropropagation of tea and related species have been reported in past (Creze 1980, Kato 1989, Phukan and Mitra 1984, Sharma *et. al.*, 1999, Das 2001, Mondal 2003). Haque *et. al.* (2002) tried to initiate shoot regeneration from shoot tip culture of some BT (Bangladesh Tea) clones as the initial work of BTRI. The present study is aimed to develop the complete regeneration protocol for the rapid propagation of desired clones of tea plant using shoot tip and nodal segment explants.

Materials and Methods

Shoot tips and nodal segments were collected from new shoots of the first flush of mature tea field of BT2 clone (growing in the Nucleus Clone Plot of Bangladesh Tea Research Institute). The explants were thoroughly cleaned and surface sterilized with 70% alcohol for 2 minutes followed by washing with 0.2% HgCl2 for 17 minutes. All traces of mercuric chloride were then washed off with sterile distilled water prior to cutting into shoot tip (0.5-1.0 cm.) and nodal segment (1.0-2.0 cm.) explants. The responsive explants were incubated for shoot initiation and multiplication on the basal medium of MS (Murashige and Skoog 1962) inorganic salts supplemented with 3% sucrose and 0.8% agar. After 30-35 days of culture in the multiplication media, individual microshoots (2.5-4 cm.) were isolated and used for rooting experiments. The final pH of MS basal medium was adjusted to 5.8 before autoclaving for 20 minutes at 121^{o} C. The incubation was made in a growth room under 16 hour photoperiod regime. The temperature and humidity of the culture room were maintained at 25±2º C and 65% respectively. Weekly visual observation was made and the data on shoot initiation, proliferation and root induction were used to calculate the percentage of cultures responding per treatment.

Results and Discussion

Different experiments were conducted to establish the direct regeneration system for tea plant using shoot tip and nodal segment explants. The findings of the experiments show that both the explants were responded towards regeneration on MS medium and different hormone compositions have significant impact on their regeneration system. In hormone free MS medium the response towards growth of incubated explants were found very slow even when kept maintained on subculture. 2.0 mg/l BAP showed best response towards regeneration for both the explants when various concentrations of cytokinins (Kn, BAP, 2ip and TDZ) alone were examined. Nakamura (1988) reported that the addition of cytokinins is essential for the growth of shoot tips of tea plant and he found more accelerated shoots with large leaves in MS medium with 3.0 mg/l BAP. Multiple shoot formation was not occurred when BAP was tried alone during this study. It indicates that cytokinin alone is not suitable for multiple shoot proliferation from both the explants. Similar observation was reported by George and Sherrington (1984). They stated that the application of just a cytokinin has been commonly effective but optimum rates of shoot initiation generally occur with the combinations of auxin and cytokinin. The results of combined application of different concentrations of cytokinins and auxins on multiple shoot proliferation are presented in Table 1. It reveals from the table that highest proliferation of shoots was recorded on the MS medium supplemented with different concentrations of BAP and IBA. Shoot initiation was observed in all the treatments of BAP and IBA although the percentages of shoot initiation and multiplication varied in different combinations. The best shoot initiation for shoot tip (48%) and nodal segment (52%) explants were observed within 30-40 days on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l IBA. Shoot growth and newly developed shoot buds were observed to initiate on the same medium combination after each subculture (Fig. 1, 2, 3, 4, 5 & 6). The highest mean number and mean length of shoots/explant for shoot tip (13.2 and 4.5cm.) and nodal segment (15.1 and 5.0 cm.) explants were achieved on this medium. Jha and Sen (1992) achieved multiple shoots using 5.0 mg/l BAP and 0.1 mg/l IBA. Combinations of BAP and IBA were also suggested in several works for multiple shoot proliferation in tea micropropagation (Kato 1985, Kuranuki and Shibata 1992, Nakamura 1990). Table 1 reveals that multiple shoot proliferation was also observed from both the explants in the combination of TDZ and NAA but lower than that of BAP and IBA. Rest combinations did not showed significant impact on shoot multiplication.

Table 1. Response of shoot tip and nodal segment explants to shoot initiation and multiple shoot formation using different concentrations of cytokinins and auxins

Concentration (mg/l)		No. of explants inoculated	% of responsive explants		Days required for shoot initiation		Average length of shoot (cm.)		Mean no. of shoots/explant after 4 month	
TDZ	NAA		ST	NS	ST	NS	ST	NS	ST	NS
2.0	0.1	25	22	20	40-43	40-43	1.5	2.2	4.3	4.6
2.0	0.5	25	28	26	33-37	38-40	2.6	2.6	4.3	4.8
2.0	1.0	25	29	28	32-36	36-41	3.3	3.4	6.0	5.8
2.0	**1.5**	**25**	**32**	**33**	**32-33**	**34-37**	**4.0**	**4.3**	**8.0**	**8.6**
2.0	2.0	25	28	27	33-38	36-40	3.4	3.6	6.5	7.0
2.0	2.5	25	24	23	35-37	35-38	2.5	3.0	4.7	4.8
BAP	Kn									
2.0	0.1	25	08	12	33-40	36-40	1.5	2.4	1.00	1.35
2.0	0.5	25	16	20	35-38	34-38	3.8	4.3	2.02	1.88
2.0	**1.0**	**25**	**24**	**27**	**32-37**	**31-36**	**3.64**	**3.87**	**4.3**	**4.8**
2.0	1.5	25	12	18	33-37	34-37	2.4	3.8	2.52	3.07
2.0	2.0	25	16	16	36-38	34-40	3.0	2.7	1.96	2.60
2.0	2.5	25	10	14	34-39	35-37	2.6	2.5	1.72	2.35
BAP	IBA									
2.0	0.01	25	32	36	30-40	32-40	3.4	3.6	4.70	5.70
2.0	0.05	25	36	32	28-38	33-38	3.8	3.8	9.80	8.80
2.0	**0.10**	**25**	**48**	**52**	**28-33**	**28-32**	**4.5**	**5.0**	**13.2**	**15.1**
2.0	0.50	25	36	40	30-35	30-33	4.3	4.2	10.3	10.5
2.0	1.00	25	28	32	30-33	32-35	3.2	4.3	8.50	7.50
2.0	1.5	25	28	36	30-35	32-35	2.2	3.2	5.30	6.30
BAP	NAA									
2.0	0.1	25	18	18	42-45	40-45	2.5	3.4	0.8	1.2
2.0	0.5	25	24	24	40-43	40-42	3.6	3.6	1.8	2.0
2.0	**1.0**	**25**	**27**	**30**	**37-40**	**35-38**	**3.0**	**3.4**	**4.6**	**5.0**
2.0	1.5	25	24	26	38-41	38-40	4.0	4.3	2.4	2.8
2.0	2.0	25	22	23	38-43	39-42	3.8	4.0	2.2	2.5
2.0	2.5	25	19	20	38-44	38-42	3.5	3.5	2.0	1.9

Effects of GA3 on shoot development and elongation of multiple shoots derived from shoot tip and nodal segment explants were also examined (Table 2). When GA3 added in the multiplication media the shoot growth was found stimulated. It was observed that the mean length of the longest shoots of shoot tip and nodal segment explants were 5.4 cm. and 5.8 cm. respectively after using 0.5 mg/l GA3 on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l IBA (Fig. 7 & 8). Similar results were found by Jacqueline and Torres (1986). The suitability of GA3 for the proliferation and shoot growth were also reported by Arulpragasum and Latiff (1986) and Jain *et. al.* (1993). Findings of these workers are in agreement with the present findings on multiple shoot proliferation and growth.

Table 2. Effect of GA3 on elongation of multiple shoots derived from shoot tip and nodal segment explants

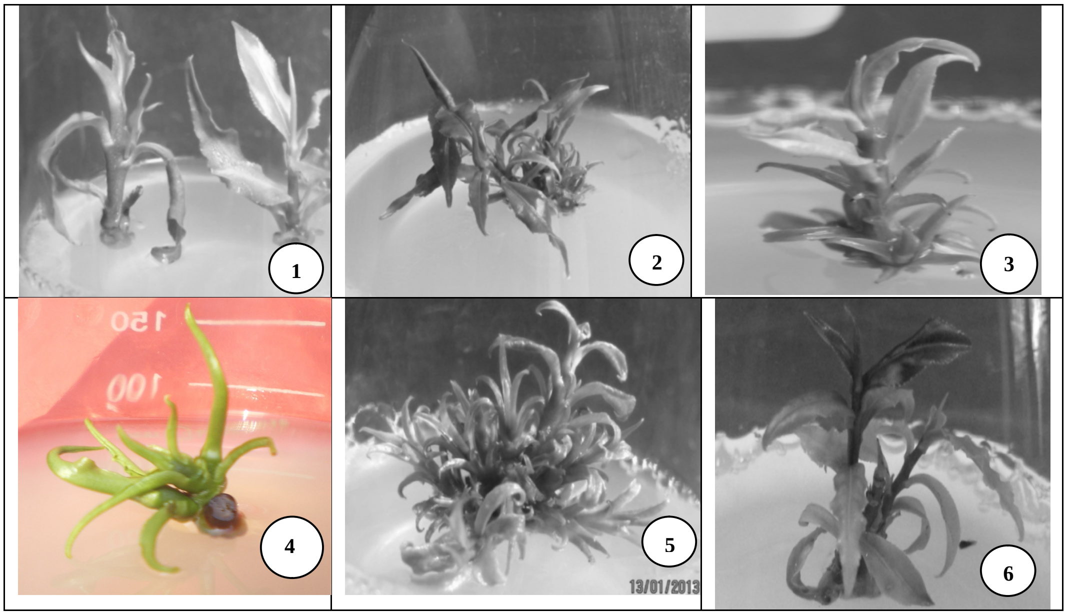
Name of the explants	Hormone concentrations (mg/l)			Average length of shoots before using GA_3 (cm)	Average length of shoots after using GA_3 (cm)
	BAP	IBA	GA_3		
Shoot tip	2.0	0.1	0.5	4.5	5.4
Nodal segment	2.0	0.1	0.5	5.0	5.8

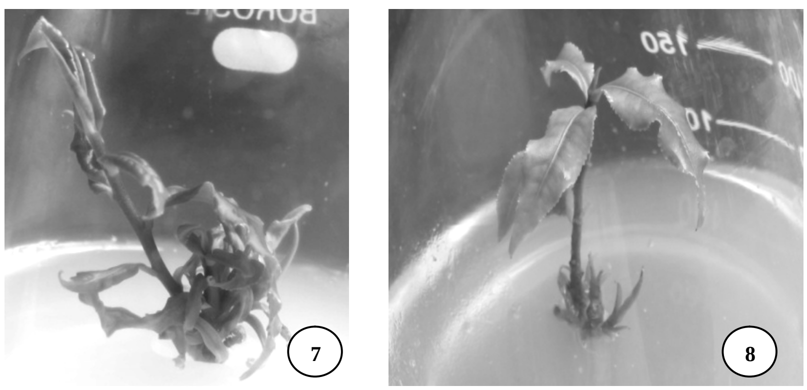
After successful development of microshoots, induction of healthy roots is an essential part for the establishment of plantlets in hardening chamber. Several researchers reported a significant increase in rooting percentage of micropropagated shoots of tea plant from the effect of high concentration of IBA (0.5-1.0 g/l) treatment (Bidarigh and Azarpour 2011, Sandal *et. al.*, 2001, Mondal *et. al.*, 2004). Jain *et. al.* (1993) reported that *ex vitro* rooting was better than all types of *in vitro* rooting methods. In the present study root induction was found when microshoots (2.5-4.0 cm) were treated with 500 mg/l IBA for 30 minutes followed by direct transplanting into the soil mix (Table 3). Root development was started to initiate within 45 days and after three months 85% rooting was achieved (Fig. 9, 10 & 11). Similar findings were reported by Sharma *et. al.* (1999). They got maximum 71.6% rooting after treating the microshoots in 500 mg/l IBA for 30 minutes prior to transfer into the soil. Ranaweera *et. al*. (2013) developed low cost method of micropropagation and achieved 100% rooting and survivability of plantlets using *ex vitro* rooting. They stated that *ex vitro* rooting of tea microshoots reduce the cost of production of micropropagated plants by 71% compared to that of micropropagation with an *in vitro* rooting step.

Table 3. Effect of microshoots treated with high concentrations of IBA (500 mg/l) on root formation

Dipping time in 500 mg/l IBA(min)	No. of shoots transferred to the soil	% of responsive shoots in rooting	Days taken to root initiation	Mean no. of roots/shoot (cm.) after 3 months	Mean length of root/shoot (cm.) after 3
5	10	-	-	-	-
10	10	20	65	3.5	2.6
20	10	48	60	4.2	3.2
30	10	85	45	8.5	5.4
40	10	30	68	2.0	1.0
50	10	nacrosis	-	-	-

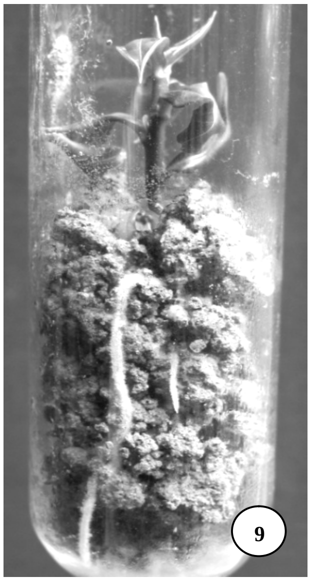
Success of survivality of tissue culture derived plants depends upon proper hardening. In the present study *ex vitro* rooted plantlets were transferred into small pots containing soil mix and kept deeply covered with transparent polythene. To reduce sudden shock, the pots were kept in growth room for three weeks. These plantlets were exposed to environment for one hour daily and then again placed in growth room for another week. When the regenerated plants were fully established in the small pots, they were kept inside a polyshaded chamber with intermittent watering. 80% plantlets were observed to survive. Sharma *et. al.* (1999) reported that survival percentage depends on maintaining a special designed hardening chamber with controlled soil pH, CO2 enrichment, light conditions and relative humidity. In this investigation rooted plants were kept inside a polyhouse with continuous maintaining of high humidity for further growth.





Figs. 1-6. Different stages of direct organogenesis from shoot tip and nodal segment explants on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l IBA. 1, 2 & 3. Shoot initiation multiple shoot formation and shoot elongation from shoot tip explants. 4, 5 & 6. Shoot initiation multiple shoot formation and shoot elongation from nodal segment explants.

Figs. 7 & 8. Shoot elongation from nodal segment and shoot tip explants on MS medium supplemented with 2.0 mg/l BAP, 0.1 mg/l IBA and 0.5 mg/l GA3.



Figs. 9-10. Different stages of *ex vitro* rooting. 9. Transfer the microshoot onto the soil in covered jar after 30 min 500 mg/l IBA treatment, 10 rooted plants after 4 months and 11. Transfer to the pot.

Conclusion

In conclusion, the present study clearly defines the establishment of a functional protocol for *in vitro* micropropagation of desired tea clones. *Ex vitro* rooting of microshoots is cost effective, profitable and time saving method for the multiplication of newly developed tea clones. Tissue culture technique provides the significant application for rapid clonal propagation as well as the tool of modern technology for crop improvement. Through this work for the first time a suitable protocol has been developed for *in vitro* regeneration of tea clones of Bangladesh.

Acknowledgement

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INTEGRATED MANAGEMENT OF GALL DISEASE IN TEA

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Abstract

An experiment was conducted at Bilashcherra Experimental Farm of Bangladesh Tea Research Institute during 2006- 2013 to find out the potential control methods of Gall disease in tea. The experiment was laid out in a RCBD design with ten treatments and three replications. The treatments consist of different pruning operations and followed by foliar application of Carbendazim based fungicide @ 750g ha^{-1}. All the treatments were applied during pruning operation. Repeated application of the said fungicide was applied after first rainfall of the year. Yield data and disease severity were recorded at seven days and thirty days intervals respectively. The disease severity was assessed using a 0-5 scale. All treatments caused significant reduction ($P=0.05$) in severity of the disease in terms of PDI and increase of yield of made tea as compared to control. The highest yield (2392.45 kg/ha) and highest reduction (65.17%) in PDI was obtained from T_5 followed by T_7, T_4 and T_3. The highest marginal rate of return (1,792%) was also found in same treatment. Deep Skiff (DSK) operation and following application of systemic fungicide Carbendazim @ 750 g /ha gave the better result to minimize the Gall disease as well as to achieve higher yield over other treatments.

Keywords: Gall disease, Integrated, Carbendazim, Deep Skiff

Introduction

Tea, the most popular beverages in the world, obtained from the flush shoots of the plant *Camellia sinensis* (L). O. Kuntze. It has been cultivated in more than 30 countries over the world. Most of the tea world production is produced in Asia (81.4%), second is in Africa (15.5%), which is being followed by Latin America (2.0%) (FAO, 1999). It is one of the largest agro-based industries in Bangladesh. There are about 55.25 thousand hectares (Anon., 2013) of land under tea plantation producing about 61.93 million kg of made tea (Anon, 2013). The average yield per hectare of tea in Bangladesh is 1,144 kg/ ha (Anon., 2013) which is quite low as compared to some other tea growing countries of the world. Many factors are associated with the low yield of our tea. Crop loss due to diseases is one of the key factors. Tea is a crop that

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is grown extensively under monoculture conditions, remote from its natural environment. The tea bush like any other living plant is susceptible to attacks by diseases, more so as it has been forced to grow, under varying climatic and soil conditions. Disease attack may be in a sporadic form or in an epidemic form. When the disease occurs in an epidemic form, it can cause colossal crop loss and can damage the national economy of a country. The majority of the diseases in tea are of fungal origin. More than 400 pathogens cause various diseases in tea (Chen and Chen, 1990) viz., foliage, stem and root all over the world. The disease spectrum of Bangladesh tea consists of one algal disease, 18 fungal diseases and a few epiphytes (Sana, 1989). The total crop loss in Bangladesh tea, though not yet precisely ascertained, a conservative estimate to the tune of 10-15% may be reckoned annually (Sana, 1989). Among the diseases, Gall is the major one. Several isolations were made in the laboratory to identify the causal agent of the disease but no other organisms were observed except *Fusarium oxysporum* Schlecht. The disease was first noticed in the year 1998 at Bilashcherra Experimental Farm of Bangladesh Tea Research Institute on a tea clone BT1. The disease was found to be confined on the upper canopy of bushes mostly within 5 cm of the plants. Galls were found to form at the base of leaf petioles, leaf scars, on pruning cuts, at the base of twigs, in between nodes particularly at the axial of branches. In some cases galls were found to develop on the edges of thick cut surfaces of branches. The disease causes massive gall formation on the upper canopy of the tea bushes resulting scanty and weak flushes (shoots). With the advancement of time infected upper twigs die and falloff. Severe gall formation restricts emergence of new shoots, consequently substantial crop loss may occur if not controlled at the initial stage.

Integrated Disease Management (IDM) is an approach that attempts to use all available methods of control of a disease or of all the diseases and pests of a crop plant for best control results with the least cost and the least damage to the environment. Cultural, Physical, Biological and Chemical control are the key components of Integrated Disease Management. Available literatures in relation to control of the disease in tea cultivation are very limited. Different chemical groups like carbendazim, copper oxychloride, copper hydroxide, hexaconazole, propiconazole are recommended for controlling the disease in Bangladesh (Huq *et al*. 2007). Effective, economically viable, environment friendly and acceptable management practices are yet to be developed. So, a research work has been under taken to establish potential control measures of the leading disease.

Materials and Methods

A field experiment was conducted at the Bilashcherra Experimental Farm (BEF) of Bangladesh Tea Research Institute (BTRI) during 2006 to 2013 to establish potential control measures of the disease. The experiment was laid out in a RCBD design with ten treatments each of replicated thrice. The treatments were T_0= Untouched (Control), T_1= T_0+ Carbendazim @ 750 gm ha^{-1}, T_2= LP, T_3= LP+ Carbendazim @

750 gm ha^{-1}, T_4= DSK, T_5= DSK+ Carbendazim @ 750 gm ha^{-1}, T_6= MSK, T_7= MSK+ Carbendazim @ 750 gm ha^{-1}, T_8= LSK and T_9= LSK+ Carbendazim @ 750 gm ha^{-1}. All the treatments were applied during pruning operation. After pruning and cleaning of litters Carbendazim based fungicide was applied @ 750 gm ha^{-1}. Repeated application of the said fungicide was applied after first rainfall of the year. Data were collected on the disease severity at monthly interval and on yield of green leaves at weekly interval against each treatment. The disease severity was assessed using a 0-5 scale; 0= no infection, 1= 1-20% infection, 2= 21-40% infection, 3= 41-60% infection, 4= 61-80% infection and 5= 81-100% infection of the disease (Rashid *et al.* 1987). Data were converted as production of made tea (kg ha^{-1}) and Percent Disease Index (PDI). Percent Disease Index (PDI) was computed following standard formula as described by Singh (2000).

$$PDI = \frac{\text{Sum of (disease score} \times \text{number of plants within that score)}}{\text{Highest score} \times \text{total number of plants checked}} \times 100$$

Data were analyzed using MSTAT C computer programme. Mean separation was done using Duncan's Multiple Range Test (DMRT).

Results and Discussion

All treatments with pruning and following foliar application of Carbendazim @ 750 gm ha^{-1} caused significant reduction in severity of Gall disease in terms of PDI and increase of yield of made tea as compared to control, where no pruning and fungicide was applied. Among the treatments the highest reduction in PDI was obtained with T_5, which is followed by T_7 and T_3. They caused 65.17, 58.95 and 43.53% reduction in PDI over control, respectively. The least effective treatment was T_1, which gave only 9.76% reduction in PDI (Figure 2). The highest yield of tea was achieved with the T_5, which was followed by T_7, T_4 and T_3. The yield was 2392.45, 2204.02, 2094.32 and 2080.62 kg ha^{-1} respectively. Treatments T_4 and T_3 are statistically similar. They gave 63.33, 50.47, 42.98 and 42.04% increase yield of made tea, respectively (Table 1). The yield of made tea was linearly and inversely correlated with severity of Gall disease in terms of PDI. There relation could be expressed by the regression equation Y = 40.792x + 1680.2, where Y represented yield of tea and x represented slop of the equation. The R^2 value indicated that their relationship may be expressed by 17.45% (Figure 1). The highest marginal rate of return (1,792%) was obtained in the treatment T_5 receiving DSK operation and following application of Carbendazim @ 750 gm ha^{-1} (Table 3). Results of the experiment revealed that foliar spray with Carbendazim @ 750 gm ha^{-1} after pruning reduced severity of the disease and increased yield of made tea considerably.

Carbendazim is a systemic fungicide that is absorbed through the foliage and is translocated within the plant. After removal the infected plant debris or pruning litters, the latent inocula within the plant may not be aggravated further due to fungi toxiccity (Pandey, 2001)

Table 1. Production of made tea (Kg ha^{-1}) and Percent Disease Index (PDI) against different treatments during (2006-2013)

Treatment	Made tea (Kg/ha) (Mean of 3 replications)	% Disease Index
T_0= Untouched (Control)	1,464.72 f	22.83 a
T_1= T_0+ Carbendazim @ 750 gm ha^{-1}	1,538.02 f	20.60 ab
T_2= LP	1,809.74 de	16.60 bcd
T_3= LP+ Carbendazim @ 750 gm ha^{-1}	2,080.62 bc	12.89 de
T_4= DSK	2,094.32 bc	14.38 cd
T_5= DSK+ Carbendazim @ 750 gm ha^{-1}	2,392.45 a	7.95 f
T_6= MSK	1,859.16 cde	14.96 cd
T_7= MSK+ Carbendazim @ 750 gm ha^{-1}	2,204.02 ab	9.37 ef
T_8= LSK	1,675.14 ef	18.38 bc
T_9= LSK+ Carbendazim @ 750 gm ha^{-1}	1,927.57 cd	15.95 cd

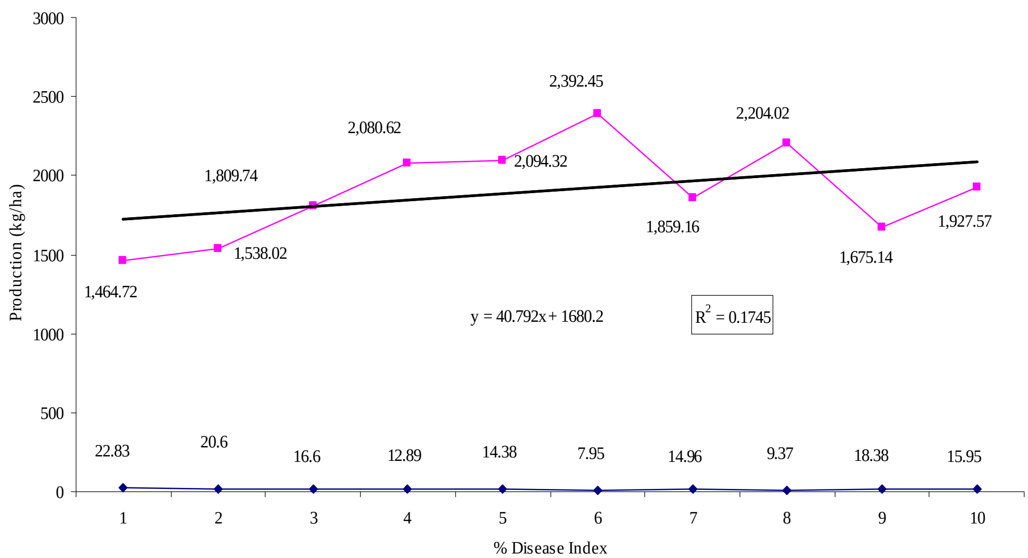

Fig.1. Effect of disease incidence on the production of made tea (kg/ha)

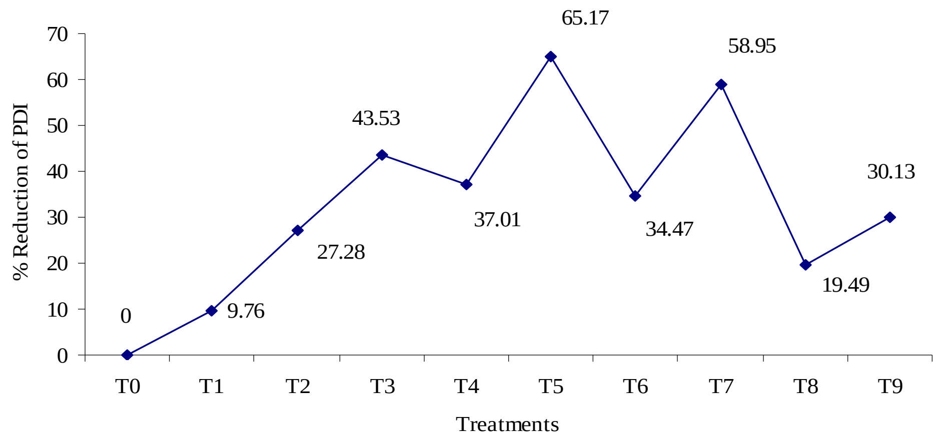

Fig. 2. Showing Reduction of PDI over the control

Table 2. Partial budget of different treatments used for controlling Gall disease in tea

Treatments	Yield (Kg/ha)	Variable cost (Tk/ha)	Gross Return (Tk/ha)	Gross Margin (Tk/ha)
T_0	1,464.72	0	2,93,000	2,96,000
T_1	1,538.02	1,565	3,07,600	3,06,035
T_2	1,809.74	7,752	3,62,000	3,54,248
T_3	2,080.62	9,317	4,16,200	4,06,883
T_4	2,094.32	5,574	4,18,800	4,13,226
T_5	2,392.45	7,036	4,78,400	4,71,364
T_6	1,859.16	3,484	3,71,800	3,68,316
T_7	2,204.02	5,049	4,40,800	4,35,751
T_8	1,675.14	3,049	3,35,000	3,31,951
T_9	1,927.57	4,613	3,85,600	3,80,987

Table 3. Marginal analysis of different treatments used for controlling Gall disease in tea

Treatment	Gross Margin (Tk/ha)	Variable cost (Tk/ha)	Margin Gross Margin(Tk/ha)	Margin Variable cost (Tk/ha)	Margin Rate of Return (%)
T_5	4,71,364	7,036	35,613	1,987	1,792
T_7	4,35,751	5,049	22,525	(-) 525	-
T_4	4,13,226	5,574	-	-	-

Conclusion

It can be concluded from the experiment that Deep Skiff (DSK) operation and following application of systemic fungicide Carbendazim @ 750 gm ha^{-1} gave better result to minimize the Gall disease as well as to achieve higher yield over other treatments.

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EFFECT OF POULTRY MANURE ON THE YIELD OF MATURE TEA *VIS-À-VIS* SOIL PROPERTIES

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Abstract

A trial was conducted at BTRI farm during January 2012 to December 2013 to evaluate the performance of poultry manure on the yield of mature tea and on soil properties. Poultry manure was applied in different rates (0, 0.25, 0.50, 0.75 ton/ha) with 100%, 75%, 50% and 25% of the BTRI recommended fertilizer doses. Poultry manure was applied once while BTRI recommended fertilizer was applied in two splits in a year. Yield of tea was recorded at seven days interval during the harvesting season. Soil samples were collected before starting the experiment as well as after completion of the experiment and analyzed accordingly. Highest yield (1442 kg made tea/ha) was recorded in T_4 where 0.75 ton/ha poultry manure was applied along with 25% of BTRI recommended chemical fertilizer. The yield increase was 21.69% over the control. A slight increase of the macro and micro nutrients were found. But the changes of the nutrient status due to the application of poultry manure were insignificant.

Keywords: Poultry manure, mature tea, soil properties

Introduction

Tea (*Camellia sinensis*) is an important cash crop as well as long established plantation crop of enormous economic importance to Bangladesh that can meet the domestic demand of this cheapest health beverage. It is an intensively managed perennial monoculture crop cultivated on large and small scale plantation.

Fertilizer is one of the major agro-inputs contributing to the productivity in tea plantation. For proper maintenance of the health of tea bushes and to obtain high yield, a well-balanced fertilization is necessary at certain intervals throughout the year (Sarwar *et al*, 2007). But the use of inorganic fertilizers has been observed to cause the destruction of soil texture and structure which often leads to soil erosion, acidity as a result of leaching effects of mineral nutrients (Ojeniyi, 2000). All these gave rise to reduced crop yields as a result of soil degradation and nutrient imbalance (Ojeniyi, 2000).

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The sustainable crop production needs proper management of nutrient resources and conservation of soil fertility. A combined application of balance inorganic fertilizer and supplementary dose of organic matter sustains the crop productivity and maintain the soil fertility. Integrated supply of nutrients to plants through organic and inorganic sources is becoming an increasingly important aspect of environmentally sound agriculture. Nutrients contained in organic manure are released slowly and are stored for a longer time in the soil, thereby ensuring a longer residual effect and persistence of nutrient availability (Singh *et al*, 2011). Improvement in environmental conditions with respect to public health has been observed as some of the major reasons for the need to adopt organic farming by farmers in the world (Eifediyi and Remison, 2010). Organic manures sustain cropping systems through better nutrient recycling and improvement in soil physical, chemical and biological properties (Ojeniyi, 2000). It increases water holding capacity of soil and nutrients will be made more readily available to crops where organic manures will be added to the soil (Dada and Fayinminnu, 2010).

Poultry manure is one of the main sources of organic plant nutrient has long been used by ancient farmers as a source of nutrition. It has a great potential for soil fertility maintenance. It has been used to amendment soil in order to provide nutrients for plant growth, increase organic matter in the soil, improve microbial populations in the soil, and to control plant pests such as nematodes and weeds (Shepherd and Withers, 2009). It supplies chelating agents that aid in maintaining the solubility of micronutrients (Sinnadurai, 1992).

If poultry manure is added in combination with chemical fertilizer, it will supplement all nutrients to crop and increase the productivity of the crop (Edward and Daniel, 1992). This study was therefore designed to investigate the effect of poultry manure on the yield of mature tea and on soil properties.

Materials & Methods

A trial was conducted at BTRI farm during January 2012 to December 2013 to evaluate the performance of poultry manure on the yield & soil properties of mature tea in a randomized block design with four treatments and three replications. The unit plot size was 36.36 m^2. The treatment combinations were T_1 = Control (No poultry manure) + BTRI recommended fertilizer dose (N_{130} P_{35} K_{75} Zn_{10} kg/ha); T_2 = Poultry manure (0.25 ton/ha) + 75% of BTRI recommended fertilizer dose; T_3 = Poultry manure (0.50 ton/ha) + 50% of BTRI recommended fertilizer dose; T_4 = Poultry manure (0.75 ton/ha) + 25% of BTRI recommended fertilizer dose. Urea (N) and Muriate of Potash (K) fertilizer were applied in two splits. First dose of fertilizer was applied in the month of April when soil contained sufficient moisture & the second dose was applied in the first week of August in the year 2012 and 2013 respectively. Poultry manure was applied once in a year.

Soil samples were collected from a depth of 0-23 cm before starting the experiment as well as after completion of the experiment. Yield of tea i.e. the weight of green leaves was recorded at seven days interval. Irrigation, pruning, pest control and other intercultural operations were done as and when necessary. Data on yield were recorded and analyzed statistically. Texture, pH, organic carbon (%), total nitrogen (%), available phosphorus (ppm), potassium (ppm), calcium (ppm), magnesium (ppm), sulphur (ppm), boron (ppm), copper (ppm), iron (ppm), manganese (ppm) and zinc (ppm) of the soil samples were determined before as well as after completion of the experiment. Soil texture was determined by hydrometer method. However, pH was determined by using pH meter (soil : distilled water = 1 : 2.5). Walkley and black wet oxidation method was applied for soil organic carbon determination (Imamul Huq and Alam, 2005). Total nitrogen was determined by Micro kjeldahl steam distillation method (Imamul Huq and Alam, 2005). Colorimetric estimation of available phosphorus was done by Bray-II ascorbic acid method (Imamul Huq and Alam, 2005). Available potassium, calcium and magnesium were extracted with 77% ammonium acetate solution. Available potassium was determined by flame photometer while calcium and magnesium was determined by AAS (atomic absorption spectrophotometer). Available zinc, manganese, copper and iron were extracted with 1M ammonium acetate (pH 4.8) and determined by AAS. Colorimetric determination of available boron was done by UV-spectrophotometer.

Chemical analysis of poultry manure was done. It was digested with nitric-perchloric acid mixture. After digestion of the poultry manure, phosphorus & sulfur was analyzed by UV-spectrophotometer while potassium was analyzed through flame photometer. Determination of zinc, copper, chromium, cadmium, lead and nickel was done by AAS. For nitrogen analysis poultry manure was digested with sulphuric-perchloric acid mixture. Then nitrogen was determined through micro-kjeldahl method (Imamul Huq and Alam, 2005). Organic carbon content of poultry manure was determined by dry combustion method.

Results and Discussion

Tea may be grown on soils of diverse geological origins. In Bangladesh most soils are quaternary and recent alluvia. Tea soils are highly weathered, extremely acidic and low of fertility status. Furthermore these soils do not receive deposits of fertile silt by flooding; rather they suffer from erosion (Alam, 1999). Table 1 shows analytical results of the soil samples which were collected from the experimental plots before setting up the experiment as well as after completion of the experiment. The soil was light textured with highly acidic in nature. After completion of the experiment a slight increase of all the macro and micro nutrient was found except copper. But from the statistical analysis (t-test) it is found that the changes of nutrient content in soil due to the application of poultry manure is insignificant.

Table 1. Soil analytical results

Soil parameters	Before experiment (Initial)	After experiment (Final)
Texture	Sandy loam	Sandy loam
pH	4.2	4.5
Organic carbon (%)	1.13	1.29
Total Nitrogen (%)	0.110	0.120
Available P (ppm)	3.36	4.22
Av. K (ppm)	28	32
Av. Ca (ppm)	76	93
Av. Mg (ppm)	12	19
Av. S (ppm)	11	15.6
Av. B (ppm)	0.46	0.47
Av. Cu (ppm)	7.60	6.66
Av. Fe (ppm)	198	201.3
Av. Mn (ppm)	35.6	37.8
Av. Zn (ppm)	1.16	1.19

Sana (1989) reported that the most suitable tea soil is thought to be light, friable and well drained having soil pH from 4.5 to 5.8. So, before setting up the experiment pH of the soil was below the critical limit while after the completion of the experiment pH increased slightly which was within the critical limit. The critical values in Bangladesh condition have been fixed at 0.1% for nitrogen and 1% for organic matter (Alam, 1999). Content of organic carbon and total nitrogen of the soil samples collected before as well as after the experiment was above the critical limit. Available phosphorus, potassium, calcium and magnesium content were below the critical limit in the soil samples which were collected before setting up the experiment. But after completion of the experiment only calcium content increased and it was above the critical limit. Alam(1999) reported that the critical limit of nutrient status of tea soil is 10 µg/g for P, 80 µg/g K, 25 µg/g for Mg and 90 µg/g for Ca.

The maximum permissible limits of micronutrients in soils are 2 to 250 mg/kg for copper and 10 to 300 mg/kg for zinc respectively (katabata-Pendias and Pendias, 1984). Before and after the experiment copper content was within the permissible limit but zinc content was below the permissible limit. Ma. *et al.*, (2003) reported that critical limit of available boron was 0.50 mg/kg from the analysis of tea growing soils of China. The boron content was found 0.46 ppm & 0.47 ppm in the soil samples collected before and after the experiment respectively. The critical limit of available sulphur is 40 ppm and the soil test values indicate lower sulphur content than the critical limit. Iron is the predominant metal with the concentration of 198 and 201.3 ppm in the soil whereas Mn content is 35.6 and 37.8 ppm.

Table 2. Chemical analytical results of poultry manure

Parameters	Poultry manure	Govt. Standard (critical limits)
Color	Grey	Dark grey to black
Physical condition	Non-granular	Non-granular
Odor	Absence of foul odor	Absence of foul odor
Moisture	22.1%	15-20%
pH	8.5	6.0-8.5%
Organic carbon	19.7%	10-25%
Total nitrogen	2.52%	0.5-4.0%
C : N	7.8 : 1	20 : 1
Phosphorus (P)	1.9%	0.5-3.0%
Potassium (K)	1.14%	0.5-3.0%
Sulphur (S)	0.33%	0.1-0.5%
Zinc (Zn)	0.02%	0.1%
Copper (Cu)	0.003%	0.05%
Chromium (Cr)	0.99 ppm	50 ppm (max)
Cadmium (Cd)	0.59 ppm	5 ppm (max)
Lead (Pb)	0.99 ppm	30 ppm (max)
Nickel (Ni)	1.02 ppm	30 ppm (max)
Inert material (max)	1%	1%

Analytical results of the poultry manure is presented in Table 2 which was applied during the experiment. From the phsico-chemical analysis of poultry manure it is found that all the physical properties as well as chemical constituents are within the critical limits. The content of toxic heavy metals such as chromium, cadmium, lead and nickel in the poultry manure which was applied in the experimental plots were 0.99, 0.59, 0.99, 1.02 ppm respectively. The permissible limits of chromium, cadmium, lead and nickel are 50, 5, 30, 30 ppm respectively (Fertilizer Recommendation Guide, 2012). So the presences of toxic heavy metals in the poultry manure were below the critical limit. Supplied poultry manure was a very good source of organic carbon (19.7%), total nitrogen (2.52%), phosphorus (1.9%), potassium (1.14%) and sulphur (0.33%) etc.

Table 3. Effect of poultry manure on the yield of tea

Treatment	Average yield (kg/plot)	Average yield (kg/ha)	Made tea (kg/ha)	Increase over control (%)
T_1	18.75	5156	1185	-
T_2	20.75	5704	1312	10.72
T_3	21.26	5846	1344	13.42
T_4	22.81	6272	1442	21.69

The effect of poultry manure on the yield of tea is presented in Table 3. Yield differences between the treatments were statistically insignificant. That means similar yield can be achieved even after 75% reduction of chemical fertilizer (BTRI recommended dose) if poultry manure is added in appropriate dose. Though not statistically significant, increased yield was recorded in all the treatments when poultry manure was applied. Highest yield was recorded in T_4 (1442 kg made tea/ha) which was 21.69% over the control. In case of T_2 and T_3, yield increase was 10.72% and 13.42% respectively.

Conclusion

Both organic and inorganic fertilization are essential to increase tea yield. Tea planters could minimize the use of NPK fertilizers with poultry manure to reduce the cost of inorganic fertilizers as well as to reduce their damaging effects on the soil. This trial clearly indicated that production of tea can be enhanced by the application of poultry manure. It may be recommended as organic fertilizer for improving the tea soil health as well as the production of mature tea. The highest rate of poultry manure (0.75 ton/ha) with 25% of the BTRI recommended fertilizer doses gave the highest yield (1442 kg/ha) of made tea which was 21.69% higher than the control.

A long term experiment on integrated nutrient management in combinations of poultry manure and NPK could also be undertaken to support general fertilizer application.

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