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Bangladesh Tea Research Institute
Srimangal-3210, Moulvibazar
An organ of
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C O N T E N T S

Editorial

The 45th volume of Tea Journal of Bangladesh contains eight research articles.

The first article is concern to judicious application of fungicides for controlling Black rot disease of tea. The causal organism of Black rot disease attacks maintenance leaves, causing gradually detoriation of bush health. The pathogen of the disease survives in pruning liters, plant debris and soil as well in dormant period. After first rain of the season, it regenerates and cause new infection. During June-July when the pathogen is forceful to regerminate and infection, time is optimum for controlling the pathogen by systemic fungicides which translocated within the plants and killed the pathogens and stopped further spreading. It is recommended in this article that two time spray with systemic fungicide after first monsoon rain at fortnight interval is better for controlling Black rot disease.

The research work of second article is on purification and characterization of polyphenol oxidase (PPO) from tea leaves and identification of some potent inhibitors to inhibit or stop the acitivity of PPO. PPOs are endogenous enzymes of tea shoots and are responsible for various characteristic quality of black tea during the manufacturing. This purified enzyme can be used in different industry such as textile, pharmaceuticals, cosmetic industry etc.

The third article is on the effect of foliar application of zinc on the yield of tea. Zinc is a micro nutrient which is essential for normal, healthy growth and reproduction of plants. Its deficiency is one of the most widespread micronutrient deficiencies in plants and causes severe reductions in crop production. Zinc is a heavy metal and several tea consuming countries have imposed restriction on its content in the processed tea. Keeping this in mind, authors reported that the annual dose of zinc sulphate should be restricted to 6 kg/ha.

The fourth paper on a case study on the impact of climate change on tea production in Bangladesh with special reference to Bilashcherra Experimental Farm, Bangladesh Tea Research Institute, Srimangal, Moulvibazar, an organ of Bangladesh Tea Board. Planters will know the effect of the temperature, humidity, rainfall on the production of tea and how to mitigate the climate change on tea production. In this strategy, judicious selection of suitable lands for new planting or replanting, use of drought and heat tolerant varieties, establishment of water reservoir, irrigation system, mulching, cover crops, soil improvement through compost, intercropping, crop diversification, and establishment and management of shade trees are the most viable adaptation measures to mitigate climate change for sustainable tea cultivation.

The fifth article reports a new species of Black looper in Bangladesh tea. The species of Black looper was identified as *Hyposidra infixaria* Walker. Author studied the biology and seasonal abundance of this new insect pest. It is more virulent than common looper, *Biston suppressaria* Guene. It is a major pest of tea especially in North Bengal region of Bangladesh. But now this caterpillar has become regular pest in many tea gardens of greater Sylhet region, where it was unknown in the recent past. The information generated in this article will help to take decision making in integrated management of the new species of Black looper in Bangladesh tea.

The sixth article is on the clonal adoption by the tea estates of Bangladesh. The use of clones as a improved planting material is increasing day by day in the tea estates which is now studied 41.64% of total plantation area. Appropriate proportion of planted different planting materials is required to be estimated to reach the future goal of sustainable production. A study on clonal adoption and its impact on the growth of production is essential to understand the potentiality of scientific technologies innovated and for overall crop improvement.

The seventh article is on the evaluation of some potential miticides against red spider mite infesting tea in Bangladesh. Good evidence was obtained from the present experiment that among the tested miticide, Hexythiazox and Fenazaquin showed the most toxic effects on red spider mites. Planters may use these chemicals judiciously for the quick management of red spider mites in tea

Final article is on tea tissue culture and this research was carried out in a chinese university lab. In this research, the authors figured out the suitable hormone combinations for cllus induction from different types of tea explants and their further proliferation for future biotechnological works.

(**Dr. Mohammad Ali**)
Chief Editor

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JUDICIOUS APPLICATION OF FUNGICIDES FOR CONTROLLING BLACK ROT DISEASE OF TEA (*CAMELLIA SINENSIS* (L.) KUNTZE)

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Mohammed Syeful Islam was born in a noble Muslim family at Jamalpur in 1976. He passed SSC examination with 1st division in 1991 from Jharkata High School, Madarganj, Jamalpur and HSC examination with 1st division in 1993 from Notre Dame College, Dhaka. Then he obtained B.Sc.Ag degree in 1997 (Held 2000) with 1st class 17th position and MS in Plant Pathology in 2002 with 1st class from Bangladesh Agricultural University, Mymensingh. During his MS course he was awarded with a fellowship under National Science and Information & Communication Technology (NSICT) Programme, Ministry of Science and Information & Communication Technology, Government of People's Republic of Bangladesh. Mr. Islam started his career by joining a college as lecturer in Agricultural Science. In October 2004, Mr. Syeful joined the Plant Pathology Division, Bangladesh Tea Research Institute, Srimangal as Scientific Officer. In 2005 he conferred a three months training on "Basic Course in Tea Plantation Management" from Kothari Agricultural Management Centre, Tamil Nadu, India sponsored by Indian Government. He also completed four months training on "Foundation Training for NARS Scientists" in 2009 sponsored by Bangladesh Agricultural Council. Besides these he is awarded with professional training certificates of Administration and Office Management, Training of Trainers (TOT), Research Methodology, Technical Report Writing & Editing, Statistical analysis by using computer packages, Archives & Records Management, Agroforestry Technology for Professionals and Climate change, Carbon Sequestration & adoption strategies. In 2015, Mr. Islam attended a training course on "2016 Seminar on Pollution free Tea Production Technology for Developing Countries" held in the College of Science and Technology, Zhangzhou, Fujian, China sponsored by Chinese Government. Mr. Syeful was promoted to Senior Scientific Officer in the same division in 2012. Since his joining he is working for developing tea industries by evolving and disseminating technologies regarding tea disease and weed management. He has published more than 35 scientific research papers in many recognized journals of national and international. He also acts as a regular trainer in MTC, PDU, Bangladesh Tea Board, Srimangal. He has experience of co-supervising of master's students of the Sahajalal University of Science & Technology and Sylhet Agricultural University. Mr. Islam is now a PhD fellow under the Department of Food Engineering & Tea Technology, Sahajalal University of Science & Technology, Sylhet. Mr. Syeful is married and father of one son and one daughter in his personal life.

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JUDICIOUS APPLICATION OF FUNGICIDES FOR CONTROLLING BLACK ROT DISEASE OF TEA (*CAMELLIA SINENSIS* (L.) KUNTZE)

M.S. Islam[1*], I. Ahmad[2], M. Ali[3], M. Ahmed[4], M.M.R. Akonda[5] and R.M. Himel[6]

Abstract

A field study was conducted during 2015- 2016 to find out the suitable groups of fungicides for controlling Black rot disease of tea. The experiment was laid out in Randomized Complete Block Design (RCBD) having ten treatments with three replications. The treatments consist of various groups of fungicides with different of mode of actions. The treatments were applied in two times at fortnight interval starting from March- April. Alternate application was done as first and second sprays by two different fungicides of mode of action as per recommendation of Bangladesh Tea Research Institute. Data were recorded on the yield of green leaves expressed by kilogram of made tea per hectare and severity of Black rot disease by Percent Disease Index (PDI). All the treatments conjointly showed about 25% and 5% impact on increasing yield of tea and reducing in PDI respectively. The treatment Carbendazim 50WP @ 750 g/ha showed better performance through highest production of tea (2,268.41 kg/ha) as well as lowest PDI (2.08). Carbendazim 50WP (Systemic) @ 750 g/ha can increase 21.75% yield by reducing PDI 56.57% over the control. Carbendazim 50 WP+ Copperoxychloride 50 WP (Contact) gave second highest values of 20.7% increased yield by reducing PDI 46.76%. Only contact fungicides can cause 11.15 to 15.74% increase of yield and decrease PDI by 7.09 to 11.06%. From the result of the study, it is concluded that two sprays with systemic fungicide after fist monsoon rain at fortnight interval is better for controlling Black rot disease. Alternate spraying with systemic and contact is equally effective for controlling the disease severity.

Keywords: Judicious, Fungicides, Control, Black rot, Tea

Introduction

Tea [*Camellia sinensis* (L.) Kuntze] is one of the oldest, nonalcoholic, beverage yielding perennial crop widely consumed all over the world. It is also one of the largest agro-based

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industries in Bangladesh. 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 and Chen, 1990) viz. foliage, stem and root. Among the diseases, Black rot disease of tea is one of the important diseases in tea. The disease caused by a fungus belonging to the genus *Corticium* (Basidiomycetes). Two species under the genus *Corticium* namely *Corticium theae* Berand and *Corticium invisum* Petch are the causal organisms of the disease (Sana, 1989). In Bangladesh tea, Black rot disease was recorded for the first in 1960 (Ali, 1992). Islam *et al.,* (2016) reported that the disease was seen in the field in the month of February with very negligible amount. Maximum incidence was found in September but severity was found in July. From the month of April to May, an increasing trend of disease development is found. During this time, temperature, relative humidity and rainfall are also high. The disease becomes evident after about four weeks following the onset of rain. Mature leaves and adjacent stems are infected by the disease. Infected leaves do not fall off but remain dangling and attached to the next leaf by means of small pads of mycelium at the point of contact. A white cord of mycelium is found in case of *Corticium theae* which enters into the leaf through under surface of the petiole. The disease reduces the photosynthetic potential of the plant (Sarma, 1960). Tunstall and Sarmath (1947) recorded a loss in the yield up to 50% on a bush attacked by Black rot when left untreated for four seasons consecutively. In 1999 and 2000 27.65% and 27.65% crop loss was recorded due to Black rot disease at BTRI (Anon, 1999-2000). It is assumed that unless any control measures are taken crop loss will gradually increase in case of such type of disease. The disease persists in tea ecosystem and spreads by the Basidiospores, which are air- borne and also by contact through pluckers whereby the diseased leaves and spores get transferred onto the uninfected portions. August- September is the peak season for sporulation of pathogen during warm humid days with low sunshine hours. A resting body called sclerotium is found above 25 cm from the ground level, which is never found on green wood or fresh wood. Most of the diseased parts are removed during pruning and hence there is a great opportunity to reduce the disease intensity during LP operations (Ali, 1992). Over dense shade, improper air circulation, proximity to jungles and bamboo baries are the predisposing factors of the disease occurrence. There is no variety or clone of tea known to be immune to the disease under conditions favourable for the fungus (Satyanarayana, 1973).

For controlling the disease different groups of new generation of fungicides are mostly used. These are also used in various crops as seed treatment, soil drench and foliar application. Due to frequent injudicious use of same fungicides of same groups the pathogens become resistant. On the basis of mode of action of fungicides, environmental factors towards the disease development, growth stage of fungus should be under consideration for successfully control the disease. Besides, available literatures in relation to judicious application of fungicides to control the disease are very limited in Bangladesh. Therefore, this study was under taken to find out and evaluate the efficacy of different groups of fungicides in controlling Black rot disease of tea.

Materials and Methods

A field experiment was conducted during 2015- 2016 at the experimental tea field of Bangladesh Tea Research Institute (BTRI), Srimangal to find out and evaluate the efficacy of different groups of fungicides in controlling Black rot disease of tea. The treatments were T_1= Control, T_2= Carbendazim 50WP, T_3= Copperoxychloride 50WP @ 2.8 kg/ha, T_4= Hexaconazole 5 EC @750 ml/ha, T_5= Propiconazole 25EC @750 ml/ha, T_6= Mancozeb 72WP @ 2.0 kg/ha, T_7= Mancozeb+Metalaxyl 80WP @ 2.0 kg/ha, T_8= COC+ Carbendazim, T_9= Carbendazim+ COC and T_{10}= Azoxystrobin+ Difenoconazole 32.5 SC @750 ml/ha. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The unit plot size was 5 m X 3 m. Number of bushes in each plot was counted. To minimize the variation in per plot bush number, bushes in all unit plots of the experimental field were counted and divided by the total number of unit plots (10×3= 30). The average number of bushes in each plot was considered for data computing (Huq *et al.,* 2010). The treatments were applied two times at fortnight interval starting from March to April. In treatment T_8, first spray was given with contact fungicide followed by a systemic one as second spray and reverse way in T_9. Data were recorded on the yield of green leaves and severity of Black rot disease. Green leaves were harvested at seven days interval maintaining plucking standard. The yield of green leaves in a unit plot was divided by actual number of bushes in that plot and multiplied by average number of bushes as computed before. Considering the plot size, the leaf yield plot^{-1} was converted into kg ha^{-1}. The yield was expressed in kilogram of made tea per hectare based on 23% recovery. To collect data on severity of Black rot, ten bushes were selected in each plot following simple random technique (Cochran, 1953). Data were recorded 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).

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants counted}} \times 100$$

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of plants observed} \times \text{Highest rating}} \times 100$$

Collected data were analyzed statistically by MSTAT-C computer program. Means were compared following LSD test using the same computer program.

Results and Discussion

All treatments caused significant reduction in severity of Black rot in terms of PDI and increase of yield of made tea as compared to control. Among the treatments, T_2 (Carbendazim 50WP @ 750 g/ha) showed better performance in highest production of tea (2,268.41 kg). It is statistically (P= 0.05) similar to T_9 (Carbendazim 50 WP+ COC) and T_4 (Hexaconazole 5 EC @750 ml/ha). First spray was applied with systemic fungicides in these plots. Accordingly disease severity was found in low percent with these treatments (Table 1).

Mancozeb, Mancozeb+ Metalaxyl and Azoxystrobin+ Difenoconazole grouped fungicides gave second highest identical results. As contact fungicide only Copperoxychloride 50 WP was least effective in increasing yield and reducing disease severity (Table 1). About 25% impact on increased yield was expressed in figure 1. With an average increased yield of 17.70%, two times spraying with only systemic fungicides like Carbendazim 50 WP gave 21.75% increased yield followed by one (first) spray with systemic and second with contact having 20.70% increased yield. On the other hand two times spraying with only contact fungicides like COC 50 WP gave least increased yield (11.15%) followed by others contact fungicides (Figure 1). With an impact of about 5% in reducing Percent Disease Index (PDI), the highest reduction of severity was found by 56.57% due to spraying with only systemic fungicides like Carbendazim 50 WP followed by one (first) spray with systemic and second with contact having 46.76% reduced severity. Whereas two times spraying with only contact fungicides like COC 50 WP gave least reduced severity (7.93%) followed by others contact fungicides (Figure 2). The relationship between reduction in disease severity and increase in yield was positive i.e. the higher reduction in disease severity the higher is the increase in yield (Figure 3).

Table 1. Effect of different fungicides on the severity of Black rot disease and yield of tea.

Treatments	Made tea (Kg/ha)	Disease Severity (PDI)
T_1= Control	1,863.15 h	4.79 a
T_2= Carbendazim 50WP @ 750 g/ha	2,268.41 a	2.08 e
T_3= Coperoxychloride 50WP @ 2.8 kg/ha	2,071.07 g	4.41 a
T_4= Hexaconazole 5 EC @750 ml/ha	2,226.09 bc	2.73 d
T_5= Propiconazole 25EC @750 ml/ha	2,190.21de	2.73 d
T_6= Mancozeb 72WP @ 2.0 kg/ha	2,156.48 f	4.26 ab
T_7= Mancozeb+Metalaxyl 80WP @ 2.0 kg/ha	2,194.20 de	3.39 c
T_8= COC+ Carbendazim 50 WP	2,207.54 cd	2.66 d
T_9= Carbendazim 50 WP+ COC	2,248.94 ab	2.55 de
T_{10}= Azoxystrobin+ Difenoconazole 32.5 SC @750 ml/ha	2,175.64 ef	3.73 bc

Same letter (s) followed by values in column is/ are not statistically different from each other.

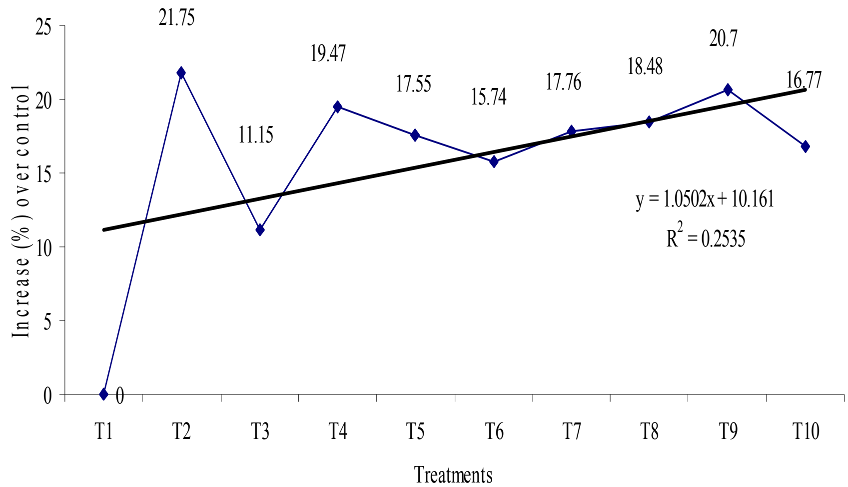

Figure 1. Increase in yield of tea due to application of different fungicides

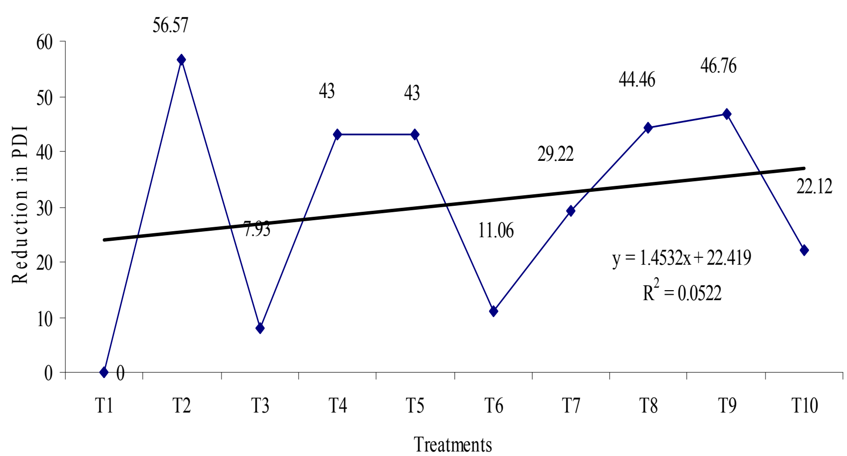

Figure 2. Reduction of PDI of Black rot due to application of different fungicides

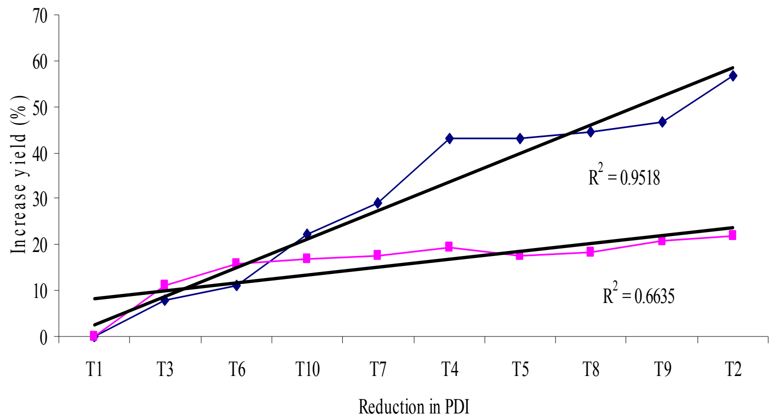

Figure 3. Relationship between reduction in PDI and increase in yield

The fungus of Black rot disease attack maintenance leaves causing gradually detoriation of bush health. The pathogen survives in pruning liters, plant debris and soil also. During first rain, it regenerates and cause new infection. During first rain in season, when the pathogen is ready to regerminate, time is optimum for controlling the pathogen by systemic fungicides. Contact fungicides are suitable in those areas where pathogenic structure is visible on the symptom as sign. After application of systemic fungicides, these are translocated within the plants. The pathogen which entered into the host cells are killed and further spread is stopped. It is reported that spraying with Bavistin 50 WP (Systemic fungicide) followed by Indofil 80 WP gave better response in controlling Cercospora Leaf spot of Mungbean (Mian *et al,* 2000) that is comparable to this study.

From the result of the study, it is concluded that two time spray with systemic fungicide after first monsoon rain at fortnight interval is better for controlling Black rot disease. Alternate spraying with systemic and contact may be applied for minimizing the disease severity which will also prevent fungicide resistance.

References

Alam, A.F.M.B. 1999. Profile of Tea Industry in Bangladesh. *In*: Global Advances in Tea Science. Araval Books Int. (P) Ltd. p. 9.

Ali, M.A. 1992. Black rot disease of tea. Pamphlet no. 14. Bangladesh Tea Research Institute, Srimangal. PP. 5-8.

Anonymous. 1999-2000. Crop loss due to Black rot disease. Annual report. BTRI.

Chen, Z.M. and Chen, X.F. 1990. The diagnosis of tea diseases and their control in China. Shanghai Sci. Tech. publishers, Shanghai, China. pp. 73-88.

Cochran, W.G. 1953. Simple Random Sampling. Sampling Technique. John Wiley & Sons, Inc. New York. PP.11-12

Huq, M., Ali, M. and Islam, M.S. 2010. Efficacy of Muriate of Potash and foliar spray with fungicides to control red rust disease (*Cephaleuros parasiticus*) of tea. *Bangladesh J. Agril. Res.* 35 (2): 273-277.

Islam, M.S. and Ali, M. 2011. Efficacy of Sedomil 72 WP and Recozeb 80 WP in controlling red rust of tea. *Bangladesh J. Agril. Res*. 36 (2): 279-284.

Islam, M.S., Ahmad, I. and Ahmad, M. 2016. Present status of the black rot disease of tea in Bangladesh. *African Journal of Agricultural Research*, 11 (37): 3562- 3568.

Mian, M.S., Hossain, M.D. and Jalaluddin, M. 2000. Effect of fungicides and urea on Cercospora leaf spot of Mungbean. *Bangladesh J of Plant Pathol, 16 (1&2): 23- 26*.

Sana, D.L. 1989. *Tea Science*. Ashrafia Boi Ghar, Dhaka. pp. 34- 35, 204-226.

Sarma, K.C. 1960. Diseases of tea and associated crops in N-E India, *ITA*. *Memorandum* 26. Indian Tea Association.

Satyanarayana, G. 1973. Black rot control. *Two and a bud*, **20**(1): 18-19.

Singh, R.S. 2000. Assessment of disease incidence and loss. *Introduction to Principles of Pathol. 3rd edition*. Offord & IBH Publishing co. pvt. ltd. pp. 328.

Tunstall, A.C. and Sarmah, K.C. 1947. Black rot of tea in N.E. India. *ITA Memorandum* No. 19. Indian Tea Association.

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POLYPHENOL OXIDASE (PPO) RESPONSIBLE FOR TEA PROCESSING EXTRACTION, CHARACTERIZATION AND POTENT INHIBITOR IDENTIFICATION

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Authors Profile

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Mr. Md. Arif Mahmud Howlader was born on 31st January, 1985 in an noble Muslim family at Tongi in the district of Gazipur. He passed his SSC in a local school Tongi Pilot High School in 2000 and HSC from a renowned institute Notredame College in 2002. In Both Exam of SSC and HSC, he obtained star marks. After that he successfully completed his Honor's (2008) and MS (2009) on Biochemistry & Molecular Biology from University of Dhaka. He conducted his MS thesis in collaboration with International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). Before publishing the result of MS, he started his journey of career in a pharmaceutical Biopharma Laboratories Ltd. After publishing result, he joined in International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) as Research Officer. During his service as a Research Officer he gathered a vast knowledge on different sophisticated molecular biology method. Here he got an opportunity to work with world top class scientists. After that, he joined in Incepta Pharmaceuticals Ltd. (second largest pharmaceutical in Bangladesh) as an Officer, R & D in the division of Biotechnology Derived Product Facility (BDPF). Here he received different abroad and in-house trainings. Among them, from Pondicherry, India, he got training on 'Insulin production from bacterial system (*E. coli*) through fermentation process'. After that, he joined in Bangladesh Tea Research Institute (an organ of Bangladesh Tea Board) as a Scientific Officer in the division of Biochemistry from 2nd May, 2016. Here he got a basic training on 'Tea Culture'. He has two international publications. He is trying to enrich tea industry through his research based knowledge. He is married and by the grace of almighty Allah, he has a son. In his personal life, he is honest, punctual, modest, amicable and social.

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POLYPHENOL OXIDASE (PPO) RESPONSIBLE FOR TEA PROCESSING EXTRACTION, CHARACTERIZATION AND POTENT INHIBITOR IDENTIFICATION

A.M. Howlader[1*]

Abstract

Polyphenol oxidase (PPO) responsible for fermentation of tea leaves was extracted by a new method and partially purified through $(NH_4)_2SO_4$ precipitation. The optimum pH for PPO activity was found to be 6.02. The enzyme showed high activity over a broad pH range of 4.00-9.00. The optimum temperature for PPO activity was 30 °C. The enzyme had more than 70% of the maximum activity between 10-80 °C. Storage of the enzymes solutions at 25, 4 and -20°C at pH 6.12 for 3 months indicated that tea PPO possessed the highest stability at -20°C. Of the inhibitors tested, sodium chlorite was the best potent inhibitor which can be used as a control agent for browning inhibition caused by tea PPO.

Keywords: Polyphenol Oxidase, Inhibitor, Activity, Stability.

Introduction

Tea (*Camellia sinensis*) is the most consumed non-alcoholic beverage in the world. It is one of the oldest beverages in the world. Today it is available for consumption in six main formulations (FAO, 2015), based on the oxidization and fermentation technique applied. World tea production (Black, Green and Instant) increased significantly by 6 percent to 5.07 million tonnes in 2013. The internal consumers of the country are presently consuming about 98% of its produce. Consequently, this abrupt increase in internal consumption resulted in the decrease of exportable surplus due to the slow rate of production, which ultimately declined the export of tea (Ahammed, 2012.). It is estimated that about 2.5 million metric tons of dried tea are manufactured annually, of which about 78% is black tea (Unal *et al,* 2011). Thearubigin pigments affect the desirable colour and briskness of the made tea. The theaflavins are formed by the enzymatic oxidation and condensation of catechins with di- and trihydroxylated B rings. The reaction involves the oxidation of the B rings to the quinones followed by a Michael addition of the gallocatechin quinone to the catechin quinone, prior to carbonyl addition across the ring and subsequent decarboxylation (Halder *et al,* 1998 and Subramanian *et al,* 1999).

The oxidative and hydrolytic enzymes (endogenous to tea shoots) are crucial for generation of involving the tea catechins as the substrates, leading to the formation of theaflavin and thearubigins. Polyphenol oxidizes (PPOs) are enzymes, belonging to a group of copper containing metalloproteinase and are members of oxidoreductases, that catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen.

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There are mainly three types of polyphenol oxidases classified according to their substrate specificities and mechanism of actions. These are; tyrosinase, catechol oxidase and laccase (Baker *et al,* 2001 and Boyd and Carlucci*,* 1993). Polyphenol oxidase (monophenol, dihidroxyphenylalanine, oxygen oxidoreductases, E.C. 1.14.18.1) catalyzes two distinct reactions involving molecular oxygen, namely a) the o-hydroxylation of monophenols to odiphenols, or cresolase activity; and b) the subsequent oxidation of o-diphenols to o-quinones, or catecholase activity (Chunhua *et al,* 2000). The catalytic action of PPO is connected to undesirable browning and flavor generation in stored and processed foods of plant origin (Alahmad, *et al*, 2015). Polyphenol oxidases are widely used in several applications. In food industry, they are used for enhancement of flavor in coffee, tea and cocoa production, and determination of food quality. In medicine, they have several uses in treatments of Parkinson's disease, phenlyketonurea and leukemia. In wastewater treatment, they are used for the removal of phenolic pollutants from wastewaters. In pharmaceutical industry, differentiation of morphine from codeine is possible by means of polyphenol oxidase immobilized electrodes (Zaidi a*t el*, 2014). The present study was aimed at purification and characterization of PPO from tea leaves.

Materials and Methods

Fresh and mature leaves of tea (*Camellia sinensis*) were collected from the experimental field of Bangladesh Tea Research Institute (BTRI). Chemicals used such as catechol, sodium metabisulphite, potassium dihydrogen phosphate, dipotassium hydrogen phosphate and acetone, all were of analytical grade.

Preparation of crude PPO extracts

The enzyme was extracted by homogenizing 20 gm of fresh matured tea leaves with 250 ml cold potassium phosphate buffer (0.2 M, pH 7) by homogenizer(model SHM1).The homogenate was filtered through cheese cloth and centrifuged at 5000 g for 10 minutes. The enzyme was precipitated from the supernatant by adding 1.5 volumes of cold acetone (-5^{O}C) with gentle stirring for 60 minutes. The mixture was centrifuged at 10000g for 15 minutes and the precipitate was dissolved in 50 ml of potassium phosphate buffer. This crude enzyme extract was used for the enzyme characterization.

Enzyme activity assay

PPO activity was assayed by measuring the increase in absorbance at 420 nm using a spectrophotometer (Hitachi U-2900). Catechol was used as the substrate following the method described by Shi et al. (2002). One unit of PPO activity is defined as the amount of enzyme that causes an increase in absorbance of 0.001/minute (Mahmood *et al.*, 2009).

Determination of optimum temperature

The activity of PPO was measured at different temperatures ranged from 10 to 90^{O}C. PPO activity was calculated in the form of percent residual PPO activity at the optimum temperature.

Determination of optimum pH

The rate of catechol oxidation by PPO was estimated in various pH ranges between 4.0-8.0 using potassium phosphate buffer (0.2 M).

Thermal stability

The enzyme solution was kept in a water bath at 20, 30 40, 50, 60, 70, 80 and 90^{o}C for 1.5 hours. The residual activity was measured at the optimum temperature.

pH stability

The enzyme solution for pH stability was held at pH values ranged from 4 to 9. PPO activity was measured in a range of pH of 4- 5.5 in Citrate- phosphate buffer, 6-7.5 in potassium phosphate buffer and 8-9 in Tris-HCl. At the end of the incubation period their residual activities were estimated.

Effects of Inhibitors

Ascorbic acid, Sodium metabisulfite and Sodium Chlorite were used as inhibitors. The reaction mixture contained 0.8 mL of catechol at a final concentration of 100 mM in 200 mM phosphate buffer (pH 6.02), 0.1 mL inhibitor at a final concentration of 0.1, 1.0 or 10.0 mM and 0.1 mL enzyme solution. Percentage inhibition was calculated using the following equation: Inhibition (%) = $[(A\text{o} - A\text{i})/A\text{o})].100$, where, *A*o is the initial PPO activity (without inhibitor) and *A*i is the PPO activity with inhibitor.

Storage stability

The enzyme solutions were stored at 25, 4 and -20^{o}C for 3 months. PPO activity was measured every 4 days for refrigeration storage and 8 days for freezing storage.

Results and Discussion

Optimum pH for polyphenol oxidase activity

PPO activity was determined in a pH range of 4.0 -9.00, and the results are depicted in Fig. 2. With the increase of pH from 4.0 to 9.0, the enzyme activity increased, with maximal activity occurring at pH 6.12, after which the activity started to decline. The enzyme exhibited high activity in a broad pH range. In the pH range of 4.5-9.0, the enzyme activity was more than 72%.

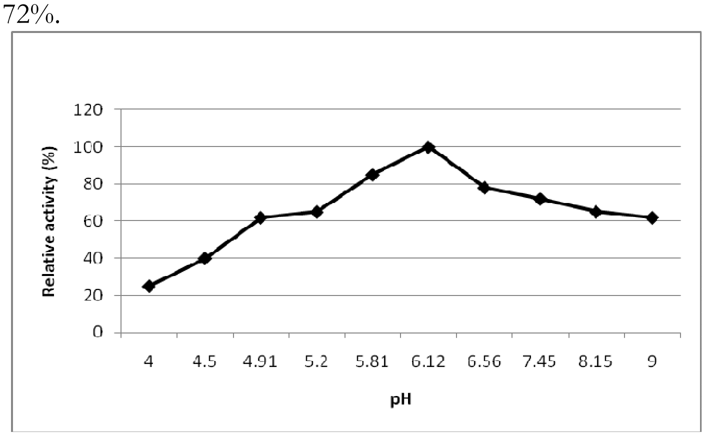

Fig. 1. Relative activity of PPO at different pH

In general, most plant polyphenol oxidases show a maximum activity at neutral pH (Benjamin and Montomery, 1973; Siddiq *et al.*, 1992; Unal, 2007; Yue-Ming, 1999). The optimum pH of PPO activity may vary depending on some factors such as enzyme source, maturity of the leaf, extraction method, temperature, substrate type and concentration of the buffer (Whitaker, 1994; Ziyan and Pekyardimic, 2003). Optimum pH was found to be 7.0 for apricot, apple and eggplant PPO and 6.4 for potato PPO (Mahmood *et al,* 2009). In another experiment of Unal *et al,* 2011, optimum pH of PPO extracted from tea leaf was 6.02 which is similar to our study.

Optimum temperature for polyphenol oxidase activity

Effect of temperature on PPO activity was investigated in a temperature range of 10-90°C and the results are depicted in Fig. 2. As seen in Fig. 2, the optimum temperature for PPO activity was found to be at 30°C. After 30°C, the activity started to decrease gradually. However, the enzyme had a very high activity at a broad temperature range. The enzyme had 70% of the maximal activity even at 80 °C.

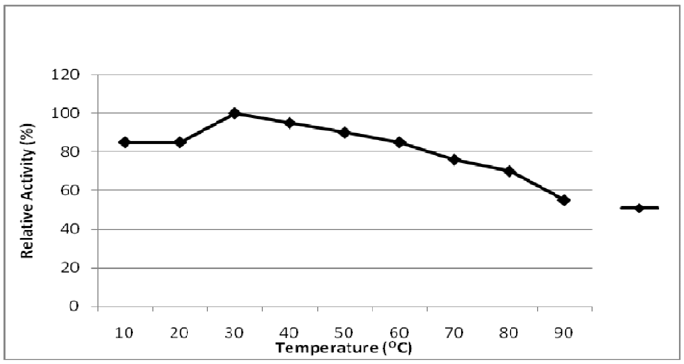

Fig. 2 Relative activity of PPO at temperatures

The enzyme isolated from apricot and apple showed maximum activity at 20 °C; and for eggplant and potato it is 22 °C (Mahmood *et al.*, 2009). Above these temperature values, the activity rapidly decreased. Oktay *et al.* (1995) and Ziyan and Pekyardimic (2003) had reported optimum temperatures of 18 and 20 °C for apple and pear PPOs, respectively. Optimum temperature of polyphenol oxidase may change depending on the type of the substrate used and the source from where it is extracted.

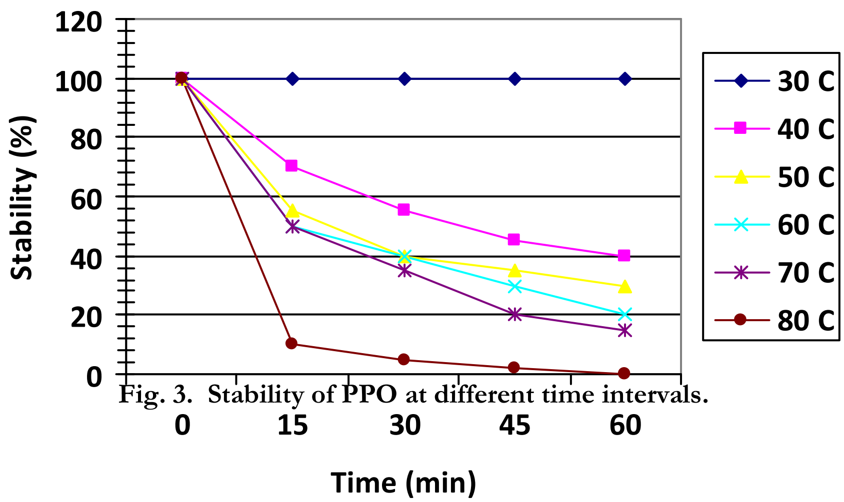

Fig. 3. Stability of PPO at different time intervals.

Thermal stability

Thermal inactivation of PPO was studied at the selected temperatures for various times (0, 15, 30, 45 and 60 min) at 30°C, 40°C, 50°C, 60°C and 80°C. The screw-cap tubes were pre-heated to the selected temperature to prevent temperature lag before the addition of a 300 μL aliquot of enzyme solution. The enzyme samples were removed from water bath after pre-set times and were immediately transferred to ice bath to stop thermal inactivation. After the sample was cooled in ice bath, the residual activity (*A*) was determined spectrophotometrically using the standard reaction mixture. A non-heated enzyme sample was used as blank (*A*o). The percentage residual activity was calculated by comparison with the unheated sample.

Effects of Inhibitors

Effects of ascorbic acid, sodium metabisulfite and sodium chlorite on tea PPO activity were studied at various concentrations using catechol as the substrate. In table 1 result were given as percentage inhibition. From the result it can be said that Sodium Chlorite was the most potent inhibitor.

Table 1. Concentration of different inhibitors for the inhibition of Tea PPO

Inhbitor	Concentration (mM)	Inhibition (%)
Ascorbic acid	0.1	13.8±4.8
	1	15.6±0.8
	10	20.2±1.2
Sodium Metabisulfite	0.1	8.2±0.8
	1	18.1±1.1
	10	24.3±2.2
Sodium Chlorite	0.1	12.2±4.2
	1	24.6±1.3
	10	52.2±0.8

Sodium chlorite (SC) is a strong oxidizing agent, which can generate chlorine dioxide under acidic conditions. These findings suggest that SC might have the potential ability to be used as on agent for browning inhibition caused by tea PPO.

Storage stability

PPO solutions at pH of 6.12 were stored at 25, 4 and -20ºC and the residual activity was measured to estimate the loss of activity during storage period. Results indicated that tea PPO retained 85% of their original activities over 90 days of storage at 4ºC, but at 25ºC it was found 65%. At -20ºC, tea PPO showed 100% activity after the same storage period. Concerning the effect of storage temperature, freezing storage was found to maintain more activity in the storage period as compared to 4ºC and 25ºC.

Conclusion

This is the first study in Bangladesh for the extract of Polyphenol Oxidase from tea leaves. This enzyme has showed very good enzymatic activity which can be used to remove reactive dyes used in different textile industries. Therefore, further study must be conducted in this regard. Fourthermore, that this PPO can also be used in our pharmaceutical industry to synthesize L-Dihydroxy phenylalanine (L-DOPA) which is a naturally occurring dietary supplement and psychoactive drug. PPO is also known to be the key enzyme in melanin biosynthesis.

References

Ahammed, K.M. 2012. Investment for Sustainable Development of Bangladesh Tea Industry - An Empirical Study. Bangladesh Economic Society.

Alahmad, K., Gasmalla, M.A.A., and Alyousef, H. 2015. Efficient Methods for Polyphenol Oxidase Production. International Journal of Nutrition and Food Sciences, 4(6): 656-659.

Baker, G. C., Gaffar, S., Cowan, D. A., and Suharto, A. R. 2001. Bacterial community analysis of Indonesian hot springs. Fems Microbiology Letters, 200 (1):103-109.

Benjamin, N. D. and Montgomery, M. W. 1973. Polyphenol oxidase of Royal Ann cherries. Purification and characterization. J. Food Sci., 38:799.

Boyd, T.J.,and Carlucci, A.F., 1993, Degradation rates of substituted phenols by natural populations of marine bacteria. Aquatic Toxicology, 25:71– 82.

Chunhua, S., Dai, Y., Xia ,B., Xu, X., Xie, Y., and Liu, Q. 2001. "The Purification and Spectral Properties of Polyphenol Oxidase I from Nicotiana tabacum", Plant. Mol. Biol. Reporter.

FAO Intergovernmental Group on Tea A Subsidiary Body of the FAO Committee on Commodity Problems (CCP), Rome, 2015.

Halder J., Tamuli P., and Bhaduri A.N. 1998. Isolation and characterization of polyphenol oxidase from Indian tea leaf (Camellia sinensis). J Nut Biochem, 9:75-80.

Mahmood, W.A, Sultan, S.H., and Hamza, S.R. 2009. Extraction and characterization of polyphenol oxidase from apricot, apple, eggplant and potato. Mesopotamia J. of Agriculture, 37)4):2009.

Oktay, M., Kufrevioglu, O. I., Kocacaliskan, I., and Sakiroglu, H. 1995. Polyphenol oxidase from Amasya Apple. J. Food Sci., 60: 494.

Shi, C., Dai, Y., Xu, X., Xie, Y., and Liu, Q. 2002. The purification of polyphenol oxidase from tobacco. Protein Expression and Purification, 24(1): 51-55.

Siddiq, M., Sinha, N. K., and Cash, J. N.1992. Characterization of polyphenol oxidase from Stanley plums. J. Food Sci., 57: 1177.

Subramanian N., Venkatesh, P., Ganguli, S., and Sinkar, V.P. 1999. Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. J Agric Food Chem, 47:2571-2578.

Unal, M.U., Yabacı,S.N., and Sener, A.2011. Extraction, partial purification and characterization of polyphenol oxidase from tea leaf (Camellia sinensis).GIDA, 36 (3): 137-144.

Unal, M. U. 2007. Properties of polyphenol oxidase from Anamur banana (*Musa cavendishii)*. Food Chemistry, 100: 909–913.

Whitaker, J. R. 1994. Principles of Enzymology for the Food Sciences. New York, Marcel Dekker, INC. New York.

Yue-Ming, J. 1999. Purification and some properties of polyphenol oxidase of longan fruit. Food Chemistry, 66 (1): 75-79.

Zaidi, K.U., Ali, A.S., Ali, S.A., and Naaz, I. 2014. Microbial Tyrosinases: Promising Enzymes for Pharmaceutical, Food Bioprocessing, and Environmental Industry. Biochemistry Research International, 2014: 1-16.

Ziyan, E., and Pekyardimic, S. 2003. Characterization of Polyphenol Oxidase from Jerusalem Artichoke (Helianthus tuberosus). Turk. J. Chem., 27: 217- 225.

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EFFECT OF FOLIAR APPLICATION OF ZINC ON THE YIELD OF TEA

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EFFECT OF FOLIAR APPLICATION OF ZINC ON THE YIELD OF TEA

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Abstract

Zinc deficiency is most widespread micronutrient deficiency in the world. An experiment was conducted at BTRI Farm during 2011 to 2014 to observe the effect of foliar application of zinc on the yield of tea as well as to estimate the proper dose of zinc. Zinc was applied as zinc sulphate in different rates (0, 2, 4, 6 and 8 kg/ha) with the BTRI recommended fertilizer doses. Zinc sulphate was applied three times in a year. Yield of tea was recorded at seven days interval during the harvesting season. Highest yield (1484 kg made tea/ha) was recorded in treatment T_4 where 6 kg/ha zinc sulphate was applied along with BTRI recommended chemical fertilizer. The yield increase was 9.88% over the control. The effect of different treatments on the yield of tea was statistically significant at 1% level.

Keywords: Zinc sulphate, foliar spray, tea

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is grown as a perennial monoculture and fertilizer application plays a vital role for its economic production. Fertilizers improve the nutritional status of plants and soil for the plants. Tea is grown on recommended optimal soil conditions that are described as deep, well aerated soils with pH tending toward acidic (4.5 - 5.6 pH levels). Fertilizer application is an important part of the normal intensive production of tea, and one of the regular field management practices with significant bearing on both yield and quality of tea. Foliar application of fertilizers is becoming increasingly the most effective way to increase yield and plant health. Experiments have shown that foliar feeding can increase yield from 12 to 25 percent when compared to conventional soil fertilizer application (Njogu *et al.*, 2014). Foliar fertilizers widely used in vegetable and fruit crops, contain various macro and micronutrients essential for proper growth and yield. Foliar applications are best suited for new flush as the young leaves readily absorb nutrients. The leaves are green factories where the complex chemical processes of photosynthesis produce the compounds, needed for plant growth. 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 (Njogu *et al.*, 2014).

Zinc, a micro nutrient element, is required for plant growth relatively to a smaller amount but is essential for normal, healthy growth and reproduction of plants. Plant root absorbs zinc in the form of Zn^{++}. Zinc is required as a structural component of a large number of proteins, such as transcription factors and metalloenzymes (Figueiredo *et al.,* 2012).

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It involves in a diverse range of enzymatic activities. The functional role of zinc includes auxin metabolism. It influences the activities of hydrogenase and carbonic anhydrase, synthesis of cytochrome and the stabilization of ribosomal fractions (Tisdale *et al*., 1984). The Zn is required for integrity of cellular membranes to preserve the structural orientation of macromolecules and ion transport systems. Its interaction with phospholipids and sulphydryl groups of membrane proteins contributes for the maintenance of membranes (Alloway, 2004). Zinc plays an important role in photosynthesis and mobilization of assimilates and has been shown to mobilize photosynthates towards pluckable shoots in tea. Leaf chlorophyll content, stomatal conductance and net photosynthesis are adversely affected by inadequate supply of Zn (Barbora *et al*., 1993).

Zinc deficiency is one of the most widespread micronutrient deficiencies in plants and causes severe reductions in crop production (Cakmak, 2000). Deficiency of Zinc in tea was first reported from Srilanka (Tolhurst, 1962). Since then more and more tea growing countries in widely separated parts of the world have recognized the importance of zinc in increasing crop productivity. Zinc has thus been accepted as an essential micro-nutrient for tea. It is possible that Zinc deficiency is becoming more prevalent because of limited Line reserves Zinc in soils and non-replacement of the loss of zinc in crop removal (Sultana *et al*., 1978).

Severe Zn deficiency is characterized by root apex necrosis (dieback), whilst sub lethal Zn deficiency induces spatially heterogeneous or interveinal chlorosis (mottle leaf), the development of reddish-brown or bronze tints (bronzing), and a range of auxin deficiency-like responses such as internode shortening (rosetting), epinasty, inward curling of leaf lamina ('goblet' leaves) and reductions in leaf size ('little leaf') (Marschner, 1995).

The total zinc content of soil ranges from less than 10 to 1000 ppm (Shaheen *et al*., 2007). Zinc is taken up by the plants as Zn^{++} ion or in the form of complex with Chelating agents e.g. EDTA (ethylenediamine tetraacetate). Salts and complexes of zinc can easily be absorbed directly through leaves. Therefore, application of zinc mostly as foliar spray for correcting deficiency has become a routine practices (Barpujari & Dey, 1982). The objective of this study was to evaluate the effect of foliar application of zinc on the yield of mature tea.

Materials and Methods

An experiment was conducted at BTRI Farm during 2011 to 2014 to observe the effect of foliar application of zinc sulphate on the yield of mature tea as well as to estimate the proper dose of zinc sulphate. The experiment was laid out in a randomized block design having five different treatments with three replications. The unit plot size was 36.36 sq. m. The treatment combinations were T_1= Control (No $ZnSO_4$) + BTRI recommended fertilizer dose (N_{130} P_{35} K_{75} kg/ha); T_2= 2 kg/ha $ZnSO_4$ + BTRI recommended fertilizer dose; T_3= 4 kg/ha $ZnSO_4$ + BTRI recommended fertilizer dose; T_4= 6 kg/ha $ZnSO_4$ + BTRI recommended fertilizer dose; T_5= 8 kg/ha $ZnSO_4$ + 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 and the second dose was applied in the first week of August during 2011 to 2014. Zinc sulphate was applied three times (April, July and October) as foliar application in every year from 2011 to

2014. On the basis of treatments 2, 4, 6 and 8 kg zinc sulphate per ha was mixed with 1000 liters water and applied in the experimental plots.

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) 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 was extracted with 1M ammonium acetate (pH 4.8) and determined by AAS.

Statistical Analysis

The analysis of variance (ANOVA) of the data was computed to determine the F-value and the test of significance was computed by Duncan's Multiple Range Test (DMRT), using IBM SPSS Statistics V.23.

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 of low 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 changes of the nutrient content was noticed but statistically the change was insignificant.

Table 1. Soil analytical results

Soil parameters	Before experiment (Initial)	After experiment (Final)
Texture	Sandy loam	Sandy loam
pH	4.55	4.57
Organic carbon (%)	1.17	1.20
Total Nitrogen (%)	0.113	0.110
Available P (ppm)	3.38	4.20
Av. K (ppm)	35	33
Av. Ca (ppm)	97	88
Av. Mg (ppm)	20	16
Av. Zn (ppm)	1.10	1.06

The effect of foliar application of zinc on the yield of tea is presented in Table 2. The result shows that, in every treatments yield was increased over control. Highest yield was recorded in treatment T_4 where 6 kg/ha zinc sulphate was applied. The rate of increase (in %) over control was 9.88 in the case of treatment T_4. The statistical analysis showed that the effect of different treatments on the yield of tea was significant (F=238.294) at 1% level. T_4 is the best treatment and there is no significant difference in yield between treatment T_3 and T_5.

Table 2. Effect of foliar application of zinc on the yield of mature tea

Treatment	Average yield (kg/plot)	Average yield (kg/ha)	Made tea (kg/ha)	Increase over control (%)
T_1	18.18	5000	1150d	…….
T_2	18.64	5126	1179c	2.50
T_3	19.21	5282	1215b	5.70
T_4	**23.46**	**6452**	**1484a**	**9.88**
T_5	19.29	5304	1220b	6.15

This finding indicate that zinc had significant influence on the yield of tea. The rate of increase in yield was 2.50%, 5.70%, 9.88% and 6.15% over control for 2, 4, 6 and 8 kg/ha doses of zinc fertilizer, respectively. This means that the yield of tea was increasing with the increasing dose of zinc fertilizer. But zinc is a heavy metal and several tea consuming countries have imposed restriction on its content in the processed tea. Keeping this in mind, the annual dose of zinc sulphate should be restricted to 6 kg/ha.

Conclusion

Tea soils are acidic in nature and enriched with free iron and aluminum oxides and hydroxides. These oxides fix the available zinc in soil and make it unavailable to the plants. Moreover, leaching of available zinc ions in acid soils may be another factor for which the amount of available zinc is sometimes lower in the acidic soil. In Bangladesh, three nutrients such as N, P and K are usually applied in soil as Urea, TSP and MOP in order to obtain higher tea yield. In recent years, zinc deficiency in tea is reported in some areas of Bangladesh. The results discussed in this experiment reveal that in order to obtain an optimum tea production, application of zinc with other nutrients may be advised.

References

Alam, A.F.M. Badrul. 1999. Profile of Tea Industry in Bangladesh. In: Global Advances in Tea Science (Edited by N. K. Jain), Aravali Books International (P). Ltd, W-30, Okhla Industrial Area, Phase II, New Delhi-110020. pp. 1-22.

Alloway BJ. In Zinc in Soil and Crop Nutrition. International Zinc Association. Brussels, Belgium; 2004

Barbora, B.C.,Rahman,F.and Barman,T.S.(1993). Zinc in the nutrition of the tea plant. Effects of fertilisers on tea yields and quality. A review with special reference to Africa and Sri-lanka. Int J Tea Scie. Available online at http:www.up.ac.za/dspace/handle/2263/8222 accessed on 10 August 2014.

Cakmak I. Role of zinc in protecting plant cells from reactive oxygen species. New Phytol. 2000; 146: 185-205.

Cakmak, Ismail. "Tansley Review No. 111. Possible Roles of Zinc in Protecting Plant Cells from Damage by Reactive Oxygen Species." The New Phytologist 146, no. 2 (2000): 185-205.

Figueiredo, D.D., Barros, P.M., Cordeiro, A.M., Serra, T.S., LourenÃ§o, T., Chander, S., Oliveira, M.M., Saibo, N.J. 2012. Seven zinc-finger transcription factors are novel regulators of the stress responsive gene OsDREB1B. Journal of Experimental Botany. 63, 3643-3656.

Imamul Huq, S.M and Alam, M.D (eds.). 2005. A Handbook on Analyses of Soil, Plant and Water. BACER-DU, University of Dhaka, Bangladesh, xxii+246pp.

M Sultana, N Barpujari & S K Dey. 1978. Studies on the Zinc Content of Tea Soils and Uptake of Zinc by Young Tea, Two and a Bud, Vol 25, No 1, June 1978, pp 21-27.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. 2nd Ed. Academic Press. London. p 889

N Barpujari & S K Dey. 1982. Zinc Requirement of Tea in North East India, Two and a Bud, Vol 29, No 2, December 1982, pp 40-46.

Njogu, Rachael Njeri E., Kariuki, David K., Kamau, David M. and Wachira, Francis N. 2014. Effects of Foliar Fertilizer Application on Quality of Tea (*Camellia sinensis*) Grown in the Kenyan Highlands. American Journal of Plant Sciences, 2014, 5, 2707-2715

Riffat Shaheen, M. K. Samim, R. Mahmud, 2007. Effect of zinc on yield and zinc uptake by wheat on some soils of Bangladesh. J. Soil. Nature. 1(1): 07-14

Tisdale, S.L., Nelson, W.L. and Beaten, J. D. 1984. Zinc In soil Fertility and Fertilizers. Fourth edition, pp. 382-391. Macmillan Publishing Company, New York.

Tolhurst, J.A.H. (1962). Zn deficiency in tea in Ceylon. Tea Q. 33: 134-137.

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IMPACT OF CLIMATE CHANGE ON TEA PRODUCTION IN BANGLADESH: A CASE STUDY ON BILASHCHERRA EXPERIMENTAL FARM

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IMPACT OF CLIMATE CHANGE ON TEA PRODUCTION IN BANGLADESH: A CASE STUDY ON BILASHCHERRA EXPERIMENTAL FARM

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Abstract

A case study was carried out to know the impact of climate change on tea production in Bangladesh with special reference to Bilashcherra Experimental Farm, Bangladesh Tea Research Institute, Srimangal, Moulvibazar, an organ of Bangladesh Tea Board. Meteorological data were collected from the Department of Meteorology, Srimangal station from 1987-2016. Thirty years yield data were also collected from Bilashcherra Experimental Farm, BTRI, Srimangal, Moulvibazar from 1987-2016. Secondary data have been used in this study. In the process of analyses of collected data, various statistical tools like averages, percentages, tables, and diagrams applied in order to make the study worthier, informative, and useful for the purposes. Result revealed that the temperature has a positive impact on the production of tea. The ideal temperature for the growth and development of tea is 20-30°C. Maximum and minimum temperature both hampered the production of tea. In 2003 & 2014, the yield declined but temperature was supportive to tea. In the same time, pests and diseases were severely infested at Bilashcherra Farm. In 2015 & 2016, production increased due to new technology incorporated in the Farm. Relative Humidity is one of the important weather parameter that influences the yield of tea. The ideal humidity range is 70-87% for the growth and development of tea plants. If humidity increase >90% the production decreased. Rainfall is an integral part of weather that also influences the yield of tea. The ideal annual rainfall is 2000 mm for the proper growth and development of tea. Monthly distributional pattern of rainfall is important. Excess water negatively influences the production of tea due to saturation of soil, failure of absorption of water by plants. Physiological growth stunted due to heavy rainfall. At the same time, if sunlight is not sufficient then production reduces. In this strategy, judicious selection of suitable lands for new planting or replanting, use of drought and heat tolerant varieties, establishment of water reservoir, irrigation system, mulching, cover crops, soil improvement through compost, intercropping, crop diversification, and establishment and management of shade trees are the most viable adaptation measures to mitigate climate change for sustainable tea cultivation.

Keywords: Climate Change, Tea Production, Bangladesh

Introduction

Tea, a popular beverage in the world, as it is produced from the leaves of evergreen shrub *Camellia sinensis* (L.) O. Kuntze and it is a long established

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plantation crop of enormous importance to Bangladesh meeting the entire domestic demand of this cheapest health beverage. Now, there are 162 tea estates having about 59,018 hectare of tea plantation producing about 85.05 million kg of finished tea per annum with an average yield of about 1,587 kg per hectare in Bangladesh (BTB, 2017). It is now ranked 8th, 10th & 12th position in the world in respect of area, production and export respectively (ITC, 2015). The production of tea is greatly hindered due to climatic factors. Tea cultivation and production are facing climate-related challenges, which need to be addressed. Climate change affecting local weather conditions (prominently changing rainfall trends resulting in frequent flood and droughts besides increase in temperature, change in relative humidity and sunshine hours) further exacerbates the situation. Tea production is greatly increased or hampered due to climatic factors such as rainfall, temperature, drought, humidity, sunshine hours, wind velocity etc. Tea is a crop of wide adaptability and grows in a range of climates and soils in various parts of the world. Tea ecosystem is an agro-ecosystem comprising tea plant, shade tree and other ancillary crops along with various abiotic elements. The life processes of biotic community are critically balanced with the tea biosphere, which includes climate and soil. The climate of tea area in Bangladesh can be classified regime-wise under four important parameters; rainfall, temperature, humidity and sunshine that are responsible for successful tea culture (Sana, 1989). The yield of tea is influenced by air and soil temperature, air saturation deficits, radiation, soil water deficits, rainfall and evaporation (Stephens *et al.,* 1992).

Baruah and Baghat (2012) stated that minimum temperature rises by two degrees centigrade and there is a reduction in the rainfall by around 200 millimeters in the last 90 years. This is very important and significant from the point of tea as a plantation crop. Scientists say rising temperatures can affect the ability of tea bush to grow. Tea production in the Assam region has declined in recent years, although the area under cultivation has risen. Erratic rainfall patterns are of particular concern to planters because the tea plant is largely dependent on the weather. They point out that last year there were fewer days with sunshine, resulting in humid conditions, which are unfavorable for the growth of the tea plant. Patra *et al.* (2013) conducted a study at Darjeeling Tea Research and Development Centre (DTR & DC), Kurseong to see the effect of climate change on production of tea. Results demonstrate that productivity of green leaf in 2012 has reduced by 41.97 % and 30.90 % as compared to 1993 and 2002, respectively. Highest productivity of 1974.44 kg green leaf ha^{-1} was obtained during 1994, thereafter productivity declined continuously.

Climate change is more than a buzz word. The world is getting warmer, and temperatures throughout the world have risen by approximately 0.8°C since the onset of the Industrial Revolution, increasing by 0.15- 0.20°C per decade since 1975. The World Meteorology Organization ranked 2014 as the warmest year on record since the mid-1800s (IPCC, 2014). The study on impact of climate change on tea production has made steady progress and has experienced a meteoric in the past decade. Unfortunately, reports on basic and applied aspects are scattered and it is impossible to keep oneself up-to-date in all facets of climate changes. Many basic and applied researches on the impact of climate change on tea production have been done in many tea growing countries. There is no significant researches were found on climate change and its relation with tea production in Bangladesh. The possible fallouts of the climate change are already witnessed in the loss of yields and increased management costs for developing coping strategies. Therefore, any spatial and/or temporal changes in the regional climate pattern will directly affect the regional economy and consequently the well being of the region. Most of the tea growing regions are typified by monsoonal climate or an alternate wet or dry season interspersed by temperature changes from mild to severe.

Major tea producing countries in the world like China, India, Kenya and Sri Lanka have been working for decades on different tea cultivation practices under different geo-physical environments existing in the particular countries. However, all these countries, have witnessed a significant production upheavals in the recent past mainly owing to climate extremes causing significant impact on regional economies and livelihood of dependents. It is anticipated that this change will continue even with greater pace in the coming decades. Therefore, development and introduction of management strategies to cope with climate change is urgent in order to reduce the risks and ensure sustainable development of tea industries in the major tea growing countries of the world. Some trends in weather changes suggest that with rising temperatures due to global warming, tea growing areas may be extended to newer areas/ecosystems, and growing/flushing period can be lengthened in subtropical regions. Community involvement and technology extension are necessary to implement government policies and apply climate change mitigation and adaptation measures. Promotion of indigenous knowhow on this subject also needs to be integrated with new scientific mitigation and adaptation technologies.

Statement of the problems

Tea production is greatly either increased or hampered due to climatic factors such as rainfall, temperature, drought, humidity, sunshine hours, wind velocity etc. Major tea producing countries like India, China, and Kenya have a lot of study on it. However, there is very limited or significant study was found on it in Bangladesh. So primarily, it is an initiative to know how the tea production is affected due to climate change in Bangladesh in general and Bilashcherra Experimental Farm, Srimangal, Moulvibazar in particular.

Objectives of the study

General objective:
To study the impact of climate change on tea production in Bangladesh especially at Bilashcherra experimental farm at Srimongal, Moulvibazar.

Specific objectives:

i. To find out possible remedies
ii. To find out suitable technologies that could be adapted for mitigation and adaptation strategies

Methodology

The study covered the impact of climate change on the productivity of tea in Bangladesh especially Bilashcherra Experimental Farm (BEF), a research farm of Bangladesh Tea Research Institute (BTRI), Srimangal, Moulvibazar under Bangladesh Tea Board. The farm was established in 1965. The location is situated at 24.29° North Latitude and 91.73° East longitude 23 m altitude from mean sea level. The area of the farm is 228.36 hectare and about 100 hectare area is under tea. Secondary data has been used in this study. Secondary data have been collected from different published documents like International Tea Committee (ITC) Report, Statistical bulletin on tea, various national and international publications. A questionnaire was prepared and distributed to the interviewee to collect data. In the process of analyses of collected data, various statistical tools like averages, percentages, tables, and diagrams applied in order to make the study worthier, informative, and useful for the purposes. Meteorological data were collected from the Department of Meteorology, Srimangal station from 1987-2016. Thirty years yield data were also collected from Bilashcherra Experimental Farm, BTRI, Srimangal, Moulvibazar from 1987-2016.

Results and Discussion

Effect of climate change on tea production in Bangladesh

Being low elevated area and located at the sub-tropical region, the variation of weather parameters in Bangladesh is extreme. The year begins and ends with dry periods in Bangladesh. Tea zones experience dry season from November to March while the rainy season continues from April to October and above 80% of annual rainfall is obtained during June – September. From the recent Meteorological report of Bangladesh, it is evident that in April-May *i.e* during the summer, temperature reaches up to the maximum of 40^0C whereas during the December or January the minimum temperature drops to the extent of 7.8^0C. Due to the rapid climate change and global warming, the fluctuation of temperatures will be more in future. Like the temperature and rainfall, other parameters which are important for tea production e.g. relative humidity, sunshine hours, day length etc. also fluctuate in similar way. Bangladesh is one of the world's most vulnerable countries to the negative effects of climate change. Each year, roughly a fifth of Bangladesh is flooded and flooding in the country is set to increase by up to 40 per cent at the end of this century as global temperatures rise, the latest climate models suggest. Though the tea industry is located comparatively at the upland area, a serious threat will arise in the near future due to climate change particularly for increasing the temperature and changing the rainfall pattern. Hence, proper strategies should be persuaded by the tea industry to maintain its current level and importance to the economy, employment and the domestic beverage market. However, the rainfall was more irregular in the recent years but it is observed that tea production and yield both are increasing at a slower pace instead of declining. Total production of tea in 2015 (67.38 million kg) was more than double comparing with the production of 1970 (31.38 million kg) showed in Fig. 1. It has happened mainly for using improved technology adopting from Bangladesh Tea Research Institute (BTRI), plantation of high yielding tea clones, gradual expansion of tea area and finally for the better management. Though the yield of tea is increasing, it is almost in plateau during the recent years ranging in between 1200-1300 kg/ha. Tea growing is an increasingly competitive business all over the world. Being the cheapest and safest beverage next to water, domestic consumption of tea in Bangladesh is increasing day by day. But the tea production in Bangladesh is adversely influenced by erratic rainfall, severe drought, flash floods, variation in temperature, high humidity, landslide in hilly zones, extreme solar radiation during summer, heavy fogs during winter, unpredicted cyclones, tropical storms and hail storms, frequent lightening, pest outbreaks etc. All of which are likely to increase because of climate change – the ultimate consequence of global warming.

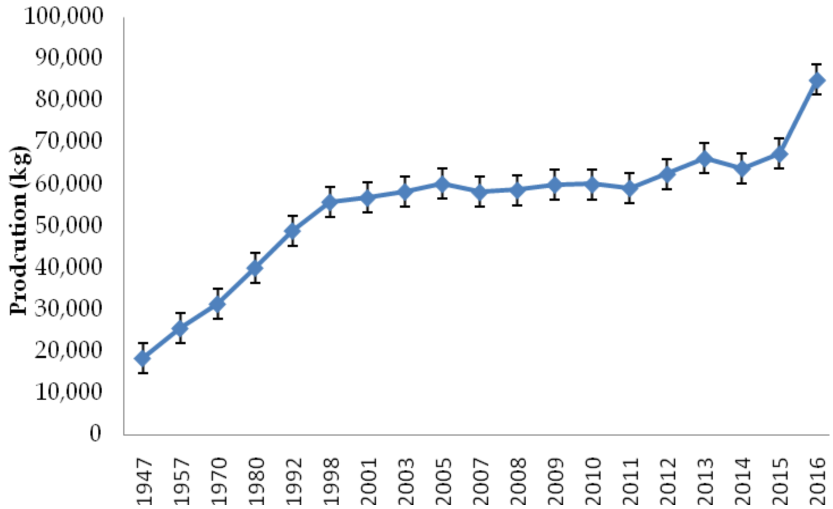

Fig. 1. Trend of tea production in Bangladesh in different periods

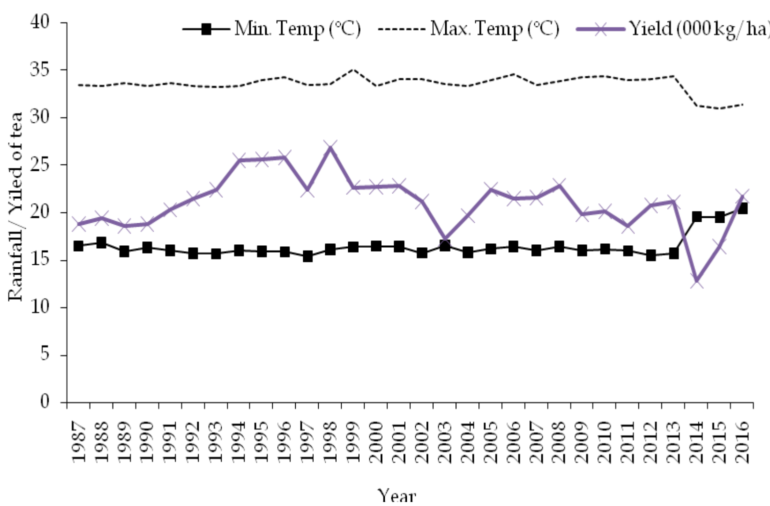

Fig. 2. Relationship between temperature and yield of tea at BEF during 1987 to 2016

Result revealed that temperature has a positive impact on the production of tea. The ideal temperature for the growth and development of tea is 20-30°C. Maximum and minimum temperature both hampered the production of tea. In 2003 & 2014, the yield declined but temperature was supportive to tea (Fig. 2). At that time, pests and diseases were severely infested at Bilashcherra Farm. In 2015 & 2016, production increased due to new technology incorporated in the Farm.

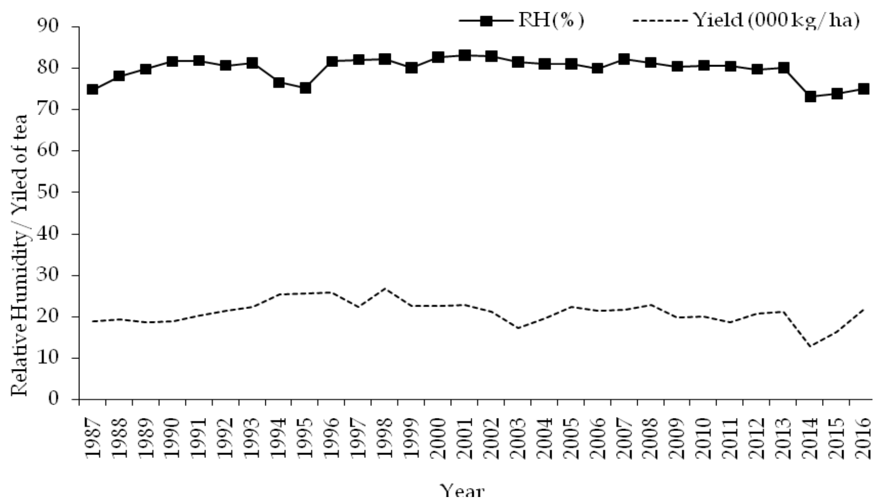

Fig. 3. Relationship between relative humidity and yield of tea at BEF during 1987 to 2016

Relative Humidity is one of the important weather parameter that influences the yield of tea. The ideal humidity range is 70-87% for the growth and development of tea plants. If humidity increase >90% the production decreased (Fig. 3).

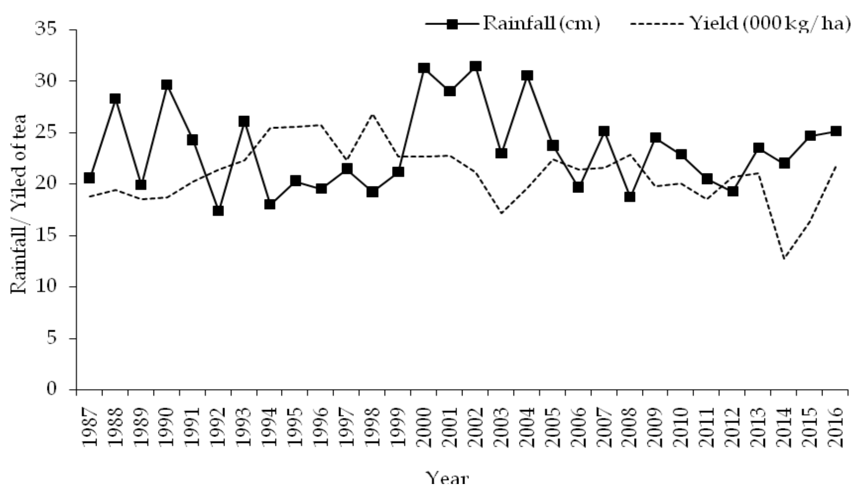

Fig. 4. Relationship between rainfall and yield of tea at BEF during 1987 to 2016

Rainfall is an integral part of weather that also influences the yield of tea. The ideal annual rainfall is 2000 mm for the proper growth and development of tea. Monthly distributional pattern of rainfall is important. Monthly rainfall should be 140-160 mm for optimum growth. However, excess water negatively influences the production of tea due to saturation of soil, failure of absorption of water by plants. Physiological growth stunted due to heavy rainfall. At the same time, if sunlight is not sufficient then production reduced (Fig. 4).

Adaptation Strategies

In tea cultivation, more importance should be given on these factors to minimize the ill effect of climate change. The following strategies can be adopted to combat the impact of climate change (Fig. 5).

1) Planting material: Planting of drought tolerant cultivar is an important adaptation measure. Bangladesh Tea Research Institute has developed 20 tea clones, and 5 biclonal and polyclonal seed stocks. BT clones were characterized based on yield, quality, their preference for manufacture and response to drought. Some of them are tolerant, moderately tolerant, susceptible and highly susceptible to drought. It is evident that BT2, BT4, BT8, BT12, BT18 and BTS1 are found to be drought tolerant. It is believed that seed plants are more climate resilient than the clones varieties. New/replanting of those stress tolerant cultivars in drought prone area can be considered as the major adaptation measure to combat the ill effect of climate change. Emphasis should be given to find out new clones/ biclonal seedlings that are capable to survive against drought and other pest and disease attack in concern of the changing environment.

2) Irrigation: Irrigation is one of the important and integral components in tea husbandry. One of the main reasons for lower yield of tea in Bangladesh is prolonged drought. It is needed to seek for increasing the yield of tea in drier months and to protect the tea plants from drought damage through irrigation. Water reservoir should be made in the gardens so that rain water can harvested in the garden. Many established tea gardens in Bangladesh are applying irrigation through sprinkler system during the drought and getting benefits e.g. Haldavalley Tea Estate, Srigobindapur Tea Estate, Finlays etc. It should be mandatory to have irrigation facility in every tea estate during the dry period.

3) Improving farm management practices: Negative impact of climate change can be reduced by adopting proper management practices as follows:

i) **Reduction of chemical load by integrated nutrient management:** Chemical fertilizer, pesticide and weedicide are the major agro chemical inputs in tea plantation. They are vital for increasing crop production. However, their overuse has been degrading the environment. In most cultivated lands, there is a depletion of organic matter, which affects the water holding capacity of the soil. In such case Integrated Nutrient Management (INM) helps overcome the problem. Integrated nutrient management is a practice where all sources of nutrients namely organic, inorganic and biofertilizer are combined and applied to the soils. It gives optimum crop nutrition, optimum functioning of the soil health and minimum nutrient losses or other adverse effect on the environment. The INM practices improve both physical and chemical fertility of soils, thus making production system more resilient to climate change.

ii) **Organic agriculture practices in tea:** Organic agriculture is highly adaptable to climate change when compared with the conventional agriculture. Organic agriculture preserves inherent soil fertility and maintains organic matter in soils, which can sustain productivity in the event of drought, irregular rainfall with floods, and rising temperatures. Organic agriculture in general requires less fossil fuel per hectare. It has considerable potential for reducing emissions of greenhouse gases. It aims at improving soil fertility and nitrogen supply by using farm yard manure, leguminous crops, crop residues and cover crops Organic matter is a source of cementing substance required for soil aggregate formation and thus protects the soil from erosion. Organic matter increases the water holding and cation exchange capacities of soil and creates favourable conditions for adequate aeration and water movement.

iii) **Mulching with succulent vegetative matter:** In tea, mulching should be done at the time of new plantations, in young tea and in mature tea. Mulching should be done immediately after rainy season so that it can conserve the soil moisture during the dry spell that follows until March and April. Mulching conserves soil moisture by reducing evaporation losses, reduces the impact of falling raindrops, surface run-off and soil erosion, as a result more water can infiltrate into the soil. Mulching lowers the soil temperature in summers. The most preferred mulching material is Guatemala, Napier grass or any cut jungle vegetation such as Eupatorium, Ageratum and Water hyacinth.

iv) **Retaining pruning litter and shade tree droppings:** Tea being a shade loving plant, it is needed to establish optimum shade tree in the plantation area. During the summer in Bangladesh, most of the time temperature prevails above of the congenial temperature for tea production. Optimum shade reduced leaf temperature by 10-12^{0}C at mid-day (Gee *et al.*, 1982). Hence, proper shade tree establishment in the plantation area of Bangladesh can be an effective way to mitigate the affect of climate change. Retaining pruning litter is one of the easiest means of recycling crop residues. Along with pruning litter considerable amount of organic matter is also added through leaf fall and pod droppings from shade trees.

v) **Compost:** Organic residues are piled up, moistened, turned occasionally to aerate and allowed adequate time to decompose partially and bring down the carbon nitrogen ratio to about 30. This process is called composting. In terms of climate change adaptation, composting provides numerous benefits. Adding compost to soil improves soil structure and aeration, which means better moisture-holding capacity, nutrient retention and ultimately, reduced vulnerability to water and wind erosion. It is estimated that 1kg of humus (relatively stable component of compost) can hold up to 6 litres of water. During rains, the addition of compost helps water to infiltrate into the soil rather than running off the surface. The addition of compost also protects against wind erosion as the humus in the compost helps to bind the soil particles together.

vi) **Green Manure and Cover crop:** Green manuring is the growth of a crop, not for the purpose of yielding a harvest, but in order to increase the fertility of land on which other productive crops are also grown. Usage of green leaf manure is advantageous both for crops and soil. In young tea, adequate ground cover is to be provided by providing green manure crop to cover the ground besides improving the microclimate required to support growth of young tea. Therefore, in sloppy topography green crop should be planted before planting tea, whereas in plains it should be grown at the time of planting.

Drought tolerant varieties of tea released by BTRI

Solar Power — Sprinkler Irrigation

Water Reservoir — Shade trees

Compost — Mulching — Cover crops

Fig. 5. Adaptation strategies for climate change in tea plantation in Bangladesh

Conclusion

Climate change has altered the way we view the world. Becoming a world commodity, it has enough space to work together of tea scientists of different countries to mitigate the worst impact of climate change on tea. To save the tea industry of a developing country like Bangladesh, it is needed to share of knowledge, research findings and technologies with the countries, which have higher yield and maximum production of tea. Climate change is due to persistent increase in temperatures over a long period. Depending on proximities, it can either increase or reduce the amount of rainfall received in an area. Temperature variability is evident from the study and it has the greatest impact on tea productivity. From lagged correlations, rainfall increased tea production significantly. This also ascertains the rainfall and especially its distribution is critical for sustainable tea production. In cases where the rainfall distribution is unpredictable as is expected with climate change, other sources of moisture should be considered for instance use of irrigation. At the farm level, adaptive mechanisms for tea include adjustments in planting and harvesting dates, adjustments of dates for different recommended cultural practices, integrated pest management, installation of efficient irrigation facilities and recommending new areas for planting. Considering the above study on climate change on tea production, it is important to implement adaptation measures in tea plantations, aiming at minimizing adverse impacts, and exploit the beneficial effects of climate change without a delay, as it takes a considerable period to bring about changes to a tree crop system such as tea cultivation. In this strategy, judicious selection of suitable lands for new planting or replanting, use of drought and heat tolerant cultivars, soil and soil moisture conservation, soil improvement, intercropping, crop diversification, and establishment and management of shade trees are the most viable adaptation measures proposed for tea cultivations. A competitive environment for tea production and processing safeguarded by governments will create socio-economic and environmental sustainability in the long term that will help mitigate the impact of climate change.

References

Baruah, R.D. and Baghat, R.M. 2012. Climate trends of Northeastern India: a long term pragmatic analysis for tea production. *Two and a bud*, 59(2): 46-49.

BTB. 2017. Monthly Bulletin of Statistics on tea. January 2017. Bangladesh Tea Board, Nasirabad, Chittagong.

Gee, G.W.; Sandanam, S.; Kulasegaram, S. and Anandacoomaraswamy, A. 1982. Effect of shade on leaf water diffusion resistance in clonal tea (*Camellia sinensis*). Tea Quarterly, 51: 12-20.

IPCC. 2014. Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom. https://www.ipcc.ch/

ITC. 2015. Annual Bulletin of Statistics (September 2015). International Tea Committee (ITC). London, U.K. 158p.

Patra, P.S., Bisen, J.S., Kumar, R., Choubey, M., Basu Mazumdar, A., Mahipal Singh and Bera, B. 2013. Effect of climate change on production of darjeeling tea: a case study in darjeeling tea research & development centre, tea board, kurseong**.** *Global Journal of Biology*, *Agriculture and Health Science*, 2(4): 174-180.

Sana, D.L. 1989. *Tea Science*. Ashrafia Boi Ghar, Dhaka, Bangladesh. pp. 184-185.

Stephens, W; Othieno, C O; Carr, M. K. V. 1992. Climate and Weather of Tea Research Foundation of Kenya. Agricultural and Forest Meteorology, 61(3-4): 219-235.

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NEW RECORD ON THE OCCURRENCE OF BLACK LOOPER, *HYPOSIDRA INFIXARIA* WALKER IN BANGLADESH TEA

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Mr. Shovon Kumar Paul was born in 1st January, 1982 at Manirampur Upazila in Jessore district. He got 1st Division both SSC and HSC examination from Port Secondary School and Govt. M.M. City College, Khulna under Jessore Board in 1996 and 1998 respectively. He obtained B.Sc. Ag. (Hons.) in 2003 and MS in Entomology in 2007 from Khulna University and Bangabandhu Sheikh Mujibur Rahman Agricultural University respectively and secured 1st class in both examination. Mr. Paul started his career as IPM Field Organizer at Narsingdi under BARC coordinated project entitled 'Facilitating the Development and Spread of the Integrated Pest Management Collaborative Research Support Programme' funded by USAID. He has been working as a Scientific Officer in Entomology Division of Bangladesh Tea Research Institute since 15 July 2008. He visited foreign Tea Research Institutes named Tocklai Tea Research Institute, Jorhat, Assam and UPASI Tea Research Foundation, Valparai, Coimbatore, Tamil Nadu, India during 2013 and 2015 respectively. He awarded a two months training course on 'Advanced Certificate Programme in Tea Plantation Management' organized by KAMC, Tamilnadu, India under Colombo Plan Scholarship sponsored by the Government of India in 2015. Moreover, he has completed training course on Pesticide Residue Analysis in Tea; Research Methodology; Research Proposal Preparation and Scientific Report Writing; Use, Maintenance and Trouble Shooting of Gas Chromatography; Tea Culture; Post Graduate Certificate Course on Seed Technology and Data Management. Mr. Paul involves the entomological research both in laboratory and field on IPM of major insect pests in tea, standardizing pesticides for tea, analyzing nematode of nursery soils of the tea estates, detection of pesticide residue in made tea. Besides the research works, he renders all sorts of advisory services to the tea estates on the problems arising out of pests in tea. He also imparts training and workshop on tea pest management on annual courses, MTC and in tea estates to the trainees particularly managerial as well as field staff of the tea industry. He has experienced on conducting projects under Research Grant of BARC entitled 'Determination of residue level of different pesticides in black tea at different intervals after spraying' and 'Studies and Development of IPM strategies for plant parasitic nematodes in tea' during 2008-2010 (2007-2010) and 2014-2017 respectively as Co-Principal Investigator. Now he is conducting a BARC funded project under CRG entitled 'Determination of residue level of commonly used pesticides in tea' as Principal Investigator for the year of 2017-2018. He has experience of co-supervising of undergraduate students' from the Department of Food Engineering & Tea Technology of Sahajalal University of Science and Technology, Sylhet. He is a member of Krishibid Institution of Bangladesh, Entomological Society of Bangladesh, Zoological Society of Bangladesh and Weed Science Society of Bangladesh. He has published more than 14 scientific research papers in different national and international journals. Mr. Paul is happily married with Mrs. Keya Paul and blessed with a cute daughter Triparna Paul.

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NEW RECORD ON THE OCCURRENCE OF BLACK LOOPER, *HYPOSIDRA INFIXARIA* WALKER IN BANGLADESH TEA

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Abstract

An experiment was conducted at Entomology laboratory in Bangladesh Tea Research Institute (BTRI), Srimangal, Moulvibazar during 2015-16 to identify new species of black looper. The biology, seasonal abundance and morphometric measurement of this new looper species were also studied. Different larval stages of looper were collected from the main farm and Bilascherra experimental farm of BTRI and reared in the Entomology laboratory. The adult was identified as a new species of black looper, *Hyposidra infixaria* Walker. Eggs were light green in colour. Five instars were found in their larval period. Distinct colour change was observed in different larval instars. The pupa was blackish red in colour and the female pupa was bigger than the male pupa. The wing colour of both male and female moth was brownish with several minute black spots. The whole wings were pointed and designed with three distinct wavy lines of dark brownish colour. A tuft of hairs was found at the abdominal tip of the male moth. This looper completed its life cycle in about 42 days. Incidence of black looper was attained peak in the month of April-May and August-September during the study period. This is the new record on occurrence of *H. infixaria* in Bangladesh tea.

Keywords: Looper caterpillar, Biology, Seasonal abundance, Morphology, *H. infixaria*

Introduction

Looper is one of the emerging pests of tea in Bangladesh. It is a major pest of tea especially in North Bengal region of Bangladesh (Ahmed *et al.,* 2010). It belongs to the family Geometridae under Lepidoptera order. Young caterpillars feed on tender leaves, making punctures along the margin. With the increase in larval age, feeding rate also increases and consume the entire leaves, leaving only the mid ribs. The mature larva prefers older leaves and the bushes are completely stripped of leaves in severe attack. In greater Sylhet region, looper caterpillar was not a major pest of tea in the past (Sana, 1989; Ahmed *et al.,* 2010). But the activity of this looper pest has considerably increased in recent years. This caterpillar has become regular pest in many tea gardens, where it was unknown in the recent past (Ahmed *et al.,* 2010). This insect of tea plantations of North-East India causes heavy crop losses which may go upto 40 per cent (Chutia *et al.,* 2012).

The tea looper was first reported by Cotes (1895) as a tea insect from Nowgong, India in 1890. The incidence of the looper has been recorded to be a major importance in the tea districts of Dooars and Cachar, India since 1900 (Das, 1965). In Bangladesh tea, a severe outbreak occurred in several tea estates of Sylhet in 1963.

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After that a sporadic and localized incidence was observed in lower valley circles of Bangladesh tea (Sana, 1989). Now-a-days, looper caterpillar was recorded as a serious pest at Madhupur, Kaliti, Dinarpur, Brindaban, Junglebari, Zareen and many other tea estates in Greater Sylhet since 2005 (Ahmed *et al.,* 2010).

Globally, looper especially *Hyposidra* spp. is a pest of cocoa, *Theobroma cacao* L. (Sterculiaceae), cinchona, *Cinchona* spp. L. (Rubiaceae) and mangosteen, *Garcinia mangostana* L. (Clusiaceae) in Australasian region and that of dipterocarpacean forests of Southeast Asia (Sinu *et al.,* 2011). In North-East India, it is known as a pest of sal (*Shorea robusta* Roth., Dipterocarpaceae) forests (Sen-Sharma and Thakur, 2008). Shade trees like *Albizzia* spp. (*A. odoratissima, A. chinensis, A. procera, A. lucida, A. lebek and A. moluccana*) and *Indigofera teysmanii* are also the preferred host for oviposition and provide resources for first two instars of the larvae of looper caterpillar (Antony *et al.,* 2012).

Climatic change, habitat loss due to deforestation and anti-predatory behaviour of the pest might be the important reasons for increasing the infestation of black looper in the recent years (Antony *et al.,* 2012). Large scale use of synthetic insecticides against other major pest of tea like *Helopeltis*, red spider mite, thrips etc. might be another vital reason. Indiscriminate use of synthetic pesticides has significant effect on reduction of beneficial insect population resulting pest resurgence of minor pest to major one (Ahmed, 2005; TRA, 2011).

The only looper species is common looper, *Biston suppressaria* Guene so far identified from Bangladesh tea. However, no report on the occurrence of black looper caterpillar, *Hyposidra infixaria* Walker has been documented in Bangladesh tea. So, attempt has been made to identify *H. infixaria,* a new species of looper caterpillar in Bangladesh tea. The detailed biology, morphology and seasonal abundance of this new species were also studied. This knowledge can be helpful to predict where and when infestation will occur, how severe they can be, and how long they will last. Ultimately this information will help to take decision making in integrated pest management in tea.

Materials and Methods

The experiment was conducted during 2015-16 in the Entomology laboratory of BTRI, Srimangal, Moulvibazar at temperature range of 24-32^{0}C and 65-85% RH. Different larval stages of black looper were collected randomly by hand picking from infested mature tea sections at main farm and Bilascherra experimental farm of BTRI and reared in the laboratory (plate 1a) on pesticide free tender shoots. Young tea shoots were inserted in a beaker (5.0 cm diameter) filled with wet sand and the beaker, placed on petridishes (14 cm diameter) containing soil, were kept in the wooden net cage measuring 1' x 1'. Fresh shoots were supplied to the black loopers as and when required till feeding stage was over. Black loopers were checked daily for moulting and mortality. The full grown caterpillars came down from the tea shoots to soil, placed in the petridishes for pupation (plate 1j). The pupae were kept in rearing cage until the emergence of moths. After emergence, the male and female moths were kept for pairing in rearing cage provided with 50% honey solution, soaked in cotton as food. Small pieces of bark of shade tree, *A. odoratissima* and splitted tea stems were provided in petridishes for egg laying. The fertile females laid eggs on the bark of shade tree. Eggs were removed using a camel hairbrush and kept on petridishes at room temperature for hatching. Immediately after hatching the larvae were transferred to young tea shoots placed inside the rearing cage. The pupae and adult were reared according to the method described earlier.

The seasonal abundance of this looper was monitored at main farm of BTRI during 2016. Fields were selected depending on the previous history of looper occurrence. In each field, three randomly selected plots were marked for the study. Each plot comprised of 50 tea bushes and replicated three times. These plots were managed by using recommended agronomic practices except that no pesticides were applied. Sampling for population of looper was carried out at 15 days interval. For population assessment direct count of caterpillars per plant was made for each replication. A single plant was used as the basic sampling unit.

Identification was done according to Browne (1968). It was also compared with the species identified by Department of Entomology, Tocklai Tea Research Institute, Tea Research Association, Assam, India (TRA, 2011). The data were recorded on the morphometric characters and the durations of life stages. The experiment on biological study was replicated three times. Data were statistically analyzed.

Results and Discussion

The black looper is identified as *Hyposidra infixaria* Walker. The systemic position of this species is-

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Geometridae
Genus: *Hyposidra*
Species: *H. infixaria* Walker

Morphological characters of black looper

There were five larval instars of looper recorded in its complete life cycle. The young looper ate out very small holes along the margins of young leaves and then cut off small pieces at the margins. With the progress of growth, the caterpillars voraciously consumed mature tea leaves. Morphology and measurements of different growth parameters are cited in Table 1-3.

Egg

Eggs were light green in colour and oval in shape (plate 1b, 1c). The length and breadth were about 0.05 ± 0.007 and 0.03 ± 0.010 cm, respectively. The incubation period was about 5.78 ± 0.34 days.

Larval stages

First instar: The body colour of 1st instar larva was brownish black with seven transverse white strips (plate 1d). The length and width of larva was about 0.33 ± 0.12 cm and 0.08 ± 0.03 cm, respectively. Duration of this stage was 3.8 ± 0.35 days (Table 1).

Second instar: The larval colour changed to dark brown. At this stage, seven transverse strips and white spots appeared throughout the body and a light cream lateral line became distinct with three pairs of thoracic legs (plate 1e). The larva was 0.56 ± 0.19 cm in length and 0.10 ± 0.07 cm in width. The 2nd instar larva lasted for 3.2 ± 0.46 days (Table 1).

Third instar: The larva was brown ash in colour with distinct seven white transverse strips and white spot on the dorsal side of the body. At this stage the lateral lines became more prominent (plate 1f). The 3rd instar larva was about 1.70 ± 0.19 cm in length and 0.17 ± 0.06 cm in width. This larval stage was 4.1 ± 0.35 days (Table 1).

Fourth instar: The colour of the larva changed into light brown dorsally and blackish brown ventrally. Two oblique black lines were present dorso-laterally at the upper edges of the thoracic legs. At the hind end of the body, one pair of abdominal legs, nine pairs of spiracles and a pair of clasper were present. Anal flap was slightly dark brown in colour, while the colour of the lateral line remained the same (plate 1g). The 4th instar larva was 3.57 ± 0.33 cm long and 0.35 ± 0.04 cm broad. Its duration was 4.4 ± 0.28 days (Table 1).

Fifth instar: The larva of 5th instar was light brown in colour dorsally, with several minute black spots on the upper and lower surface of the body. The larval body is also characterized by 7 paired oblique black and white stripes. Mouthparts were dark brown with light blackish shade and covered with tiny black spots. The anal flap was light brown. Nine pairs of spiracles were prominent on the dorso-lateral surface of the larval body (plate 1h). The 5th instar larva was 5.38 ± 0.33 cm in length and 0.55 ± 0.26 cm in width. The duration of this stage was 7.3 ± 0.16 days (Table 1). Larva was also found on the trunk of shade tree, *A. odoratissima* (plate 1i).

Table 1. Growth parameters of *H. infixaria* at different larval stages

Larval stages	**Measurement**		**Duration (days)**
	Length (cm)	**Width (cm)**	
First instar	0.33 ± 0.12	0.08 ± 0.03	3.8 ± 0.35
Second instar	0.56 ± 0.19	0.10 ± 0.07	3.2 ± 0.46
Third instar	1.70 ± 0.19	0.17 ± 0.06	4.1 ± 0.35
Fourth instar	3.57 ± 0.33	0.35 ± 0.04	4.4 ± 0.28
Fifth instar	5.38 ± 0.33	0.55 ± 0.26	7.3 ± 0.16

*Mean of three replications, ± Standard error of means

Pupa

The pupa was blackish red in colour. The shape of pupa was less slender and cuticle was thin, shiny and glossy (plate 1j, 1k). The female pupa was bigger than the male pupa. The male pupa was 1.46 ± 0.15 cm in length and 0.35 ± 0.05 cm in width whereas the female pupa was 1.84 ± 0.21 cm in length and 0.57 ± 0.08 cm in width. The pupal periods of male and female were 6.9 ± 0.39 days and 7.5 ± 0.45 days, respectively (Table 2). It was also observed that pupation also took place under the bark of shade tree, *A. odoratissima* (plate 1l).

Table 2. Morphometric measurements and duration of pupa of *H. infixaria*.

Types of pupa	**Measurement**		**Duration (days)**
	Length (cm)	**Width (cm)**	
Male pupa	1.46 ± 0.15	0.35 ± 0.05	6.9 ± 0.39
Female pupa	1.84 ± 0.21	0.57 ± 0.08	7.5 ± 0.45

*Mean of three replications, ± Standard error of means

Adult

Male moth: The wing colour of the male moth was brownish with several minute black spots. Two distinct white spots were present at the apical region of the fore wing. Four prominent black spots were also present on both the fore and hind wing. Body was blackish brown in colour dorsally, head dark brown, thorax blackish and abdomen was brownish gray in colour. A tuft of hairs was found at the abdominal tip of the male moth. This is the important identifying character of this species (Browne, 1968). The whole wings were pointed and designed with three distinct wavy lines of dark brownish colour. The upper portion of fore wing slightly light brownish colour and lower portion of fore and hind wings were appeared as dark shades of gray and brownish in colour. The antennae were bi-pectinate and blackish brown in colour (plate 1n). Moth was about 1.46 ± 0.11 cm long and 0.27 ± 0.06 cm wide. Wing span of the moth was about 3.39 ± 0.15 cm. The longevity of the moth was 5.41 ± 0.51 days (Table 3)

Female moth: The wings of female moth were blackish brown in colour. Head was dark brown, thorax light brown and dorsal side of the abdomen was brownish grey with a greenish tinge. The upper portion of ventral abdomen was light brown and the lower portion was greenish, wings were pointed and designed with three distinct wavy lines of dark shades of grey and brown. Two prominent white spots were present at the apical point of the fore wing. The antennae were filiform and slightly brown in colour (plate 1o). Moth was about 1.68 ± 0.21 cm long and 0.44 ± 0.12 cm wide. The wing span of the moth was about 4.51 ± 0.32 cm. The longevity of the moth was 6.57 ± 0.49 days (Table 3)

Table 3: Morphometric measurement, wing span and longevity of *H. infixaria*.

Adult stages	**Measurement**		**Wing span (cm)**	**Longevity (days)**
	Length (cm)	**Width (cm)**		
Male moth	1.46 ± 0.11	0.27 ± 0.06	3.39 ± 0.15	5.41 ± 0.51
Female moth	1.68 ± 0.21	0.44 ± 0.12	4.51 ± 0.32	6.57 ± 0.49

*Mean of three replications, ± Standard error of means

Seasonal abundance

H. infixaria was present throughout the year, nevertheless it was lowest in November to February. The population of *H. infixaria* usually started to build up in the month of March/April reaching a peak in May. Afterwards, their abundance decreased. Again population started to increase from the month of July and reached peak in August-September (Fig. 1).

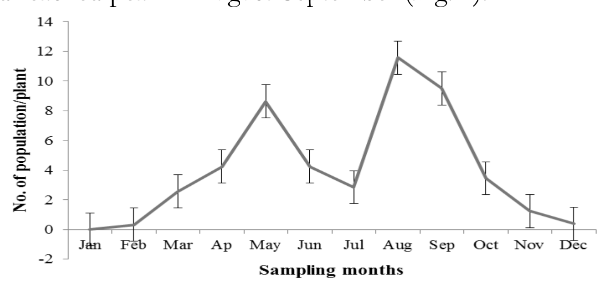

Fig. 1. Seasonal abundance of *H. infixaria* at BTRI main farm during 2016

This is the first report on occurrence of *H. infixaria* in Bangladesh tea. Similar species of black looper has been identified recently in the tea growing areas of North-East India (Das *et al.,* 2010; Majumder *et al.,* 2011; Chutia *et al.,* 2012; Das & Mukhopadhyay, 2014). Chutia *et al.* (2012) also reported that *Hyposidra* spp. is widely distributed in the low land forests of Indo-Australian tropics from North-East Himalayas to Queensland and Solomons, mostly in India, Indonesia, Malaysia, Hong Kong, Taiwan, China, Thailand, Papua New Guinea and Australia. Duration of different developmental stages as well as their seasonal trend varied with the findings of Das *et al.* (2010); Majumder *et al.* (2011) and Chutia *et al.* (2012). Climatic condition, host preference, topography of land etc. may be the reasons of variation.

Conclusion

Tea growing areas of Bangladesh especially Panchagarh and North Sylhet region are very much close to those of Indian border. So, there is a great possibility to advent the new species of black looper in Bangladesh tea. The identification of new species of black looper in Bangladesh tea has already proved it. The infestation of black looper is increasing day by day which is alarming to our tea. Besides, the larva of this insect is polyphagous in nature and reported to feed on a variety of trees, shrubs and weeds. Considering the magnitude of the problem, further studies on detailed bio-ecology of this new looper species are needed to develop an effective integrated management strategy with great importance.

References

Ahmed, M. 2005. *Tea Pest Management*. Evergreen Printing and Packaging, Dhaka. 13p.

Ahmed, M., Mamun, M.S.A. and Paul, S.K. 2010. *Looper caterpillar- A threat to tea and its management*. BTRI Circular no. 132 (Bangla and English Version), Bangladesh Tea Research Institute, Srimangal, Moulvibazar. pp. 1-7.

Antony, B., Sinu, P.A. and Rahman, A. 2012. Looper caterpillar invasion in North-East Indian tea agro-ecosystem: Change of weather and habitat loss may be possible causes. *Journal of Tea Science Research*. 2(1): 1-5.

Browne, F. G. 1968. Pests and diseases of forest plantation trees: An annotated list of the principal species occurring in the British Commonwealth. Oxford: Clarendon press. 251p

Chutia, B.C., Rahman, A., Sarmah, M., Barthakur, B.K. and Borthakur, M. 2012. *Hyposidra talaca* (Walker): A major defoliating pest of tea in North East India. *Two and a Bud*. 59: 17-20.

Cotes, E.C. 1895. An account of the insects and mites which attack the tea plant in India. Govt. of India, Revenue and Agric. Deptt., Calcutta. 71p.

Das, G.M. 1965. Pests of tea in North East India and their control. Memorandum No. 27. Tocklai Tea Research Institute, Tea Research Association, Jorhat, Assam, India. pp. 169-73.

Das, S and Mukhopadhyay, A. 2014. First report on occurrence of melanic forms of two Lepidopteran pests of tea, *Hyposidra talaca* and *Hyposidra infixaria* (Insecta: Geometridae), from Sub-Himalayan West Bengal. *North Bengal University Journal of Animal Sciences*. 8: 33–36.

Das, S., Mukhopadhyay, A. and Roy, S. 2010. Morphological diversity, developmental traits and seasonal occurrence of looper pests (Lepidoptera: Geometridae) of tea crop. *Journal of Biopesticides*. 3(1 Special Issue): 16–19.

Majumder, A.B., Pathak, S.K. and Hath, T.K. 2011. Growth and survival of a geometrid defoliator *Hyposidra infixaria* Walker (Lepidoptera: Geometridae) on four clonal varieties of tea. *Acta Entomologica Sinica*. 54(7): 815-819.

Sana, D.L. 1989. *Tea Science*. Ashrafia Boi Ghar, Dhaka, Bangladesh. pp. 184-185.

Sen-Sharma, P.K. and Thakur, M.L. 2008. Pests of Dipterocarpaceae and their management, pp. 165–187. *In:* Forest Entomology (edited by L. K. Jha & P. K. Sen-Sharma). APH Publishing, New Delhi.

Sinu, P.A., Mandal, P. and Antony, B. 2011. Range expansion of *Hyposidra talaca* (Geometridae: Lepidoptera), a major pest, to North Eastern Indian tea plantations: Change of weather and anti-predatory behaviour of the pest as possible causes. *International Journal of Tropical Insect Science*. 31(4): 242–248.

TRA. 2011. Tea looper caterpillar. Tocklai Tea Research Institute, Tea Research Association, Jorhat, Assam, India. *Bulletin No. PP/02/2011*. pp. 1-9.

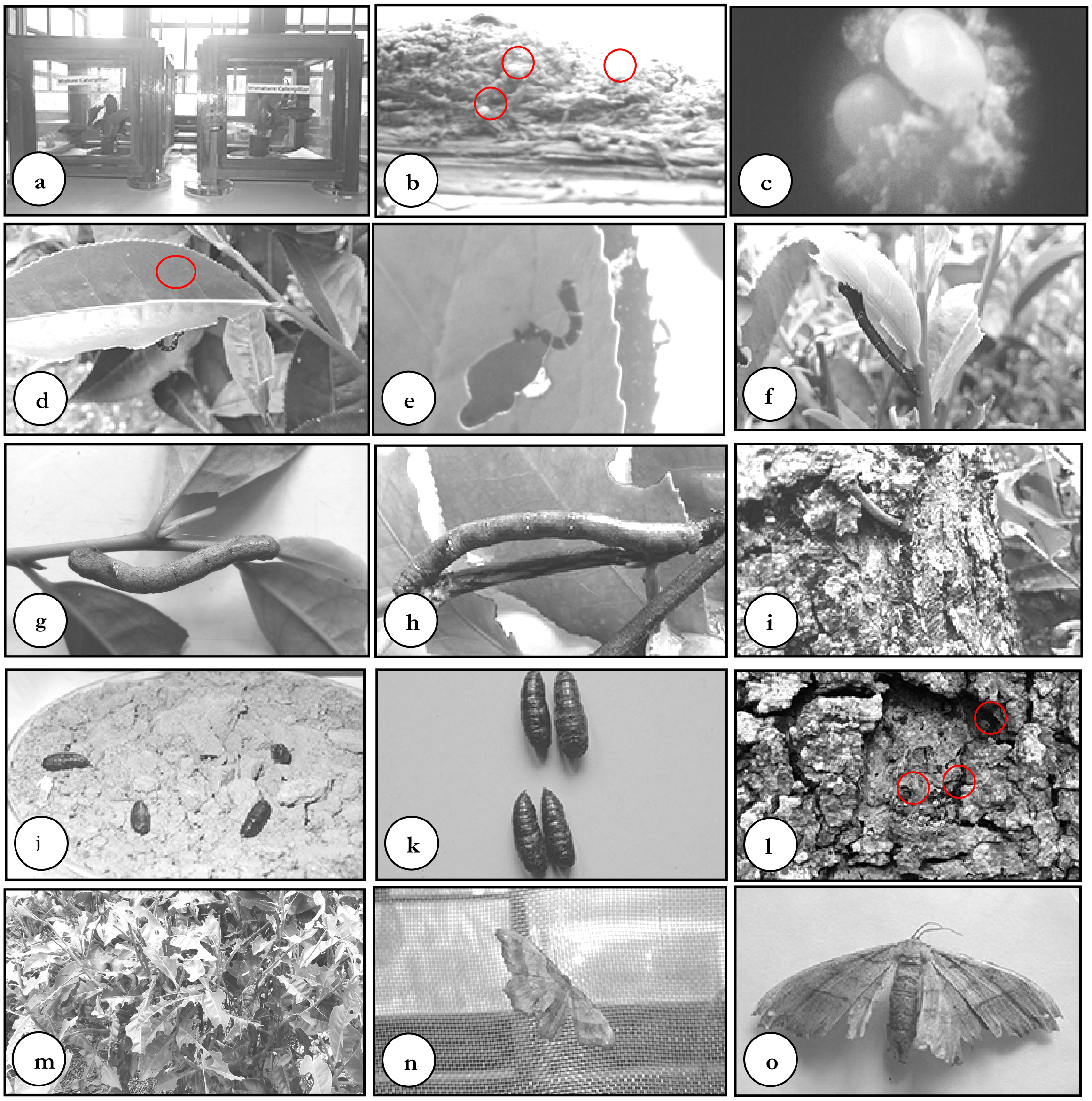

Plate 1: Laboratory study on biology of *H. infixaria* showing the rearing in laboratory (a), egg laying on bark of shade tree (b), microscopic view of egg (c), Different larval stages (d-h): 1st instar (d), 2nd instar (e), 3rd instar (f), 4th instar (g), 5th instar (h), larvae on the trunk of shade tree in field (i), pupation occurs in soil on Petridishes (j), pupa (k), pupation occurs under bark of shade tree in field (l), looper infested tea bush (m) male (n) and female (o) moth.

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A STUDY ON CLONAL ADOPTION AND ITS IMPACT ON TEA CULTIVATION IN BANGLADESH

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A STUDY ON CLONAL ADOPTION AND ITS IMPACT ON TEA CULTIVATION IN BANGLADESH

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Abstract

A complete feature of clone plantation in the tea estates of Bangladesh is needed to estimate the capability of tea estates for further growth of improved production. In this study an attempt has been made to explore the adoption percentages of clones in the tea estates in different valley circles of Bangladesh. The result was tabulated collecting data from 144 tea estates. The study has disclosed that the assimilation of clones for plantation has increased along with the acceptance of BT clones by the gardeners. A remarkable progress in production as well as average yield per hectare of Bangladesh tea is noticed from the last few decades. Increased use of elite clones is significantly showing the positive impact on production. In 2001, the annual production and productivity was 53.15 million kg and 1176 kg/ha whereas in 2016, those were 85.5 mkg and 1587 kg/ha respectively. The clonal plantation was 21% in 2001. The strategic plan of Vision 2002-2021 projected to reach the target of 111 million kg and 1748 kg/ha which was predicted to achieve by increasing plantation with high yielding clones and seed stocks that are adaptive to our environment. The present status of clone plantation is 41.64% of which 45.05% is BT clones and 40.02% is TV clones and others. The average per hectare yield of BT and TV clones of these areas are 1590 kg and 1667 kg respectively which are closer to the current national average yield (1587 kg/ha). Planting of high yielding clones and seed stocks should be intensified along with the proper management practices to reach the targeted production which will reflect on the national average yield. A follow up data regarding clonal adoption in the tea estates will be helpful to study the yield trend and further growth of production. Appropriate proportions of different adequate planting materials also need to be justified. Therefore, the present study was aimed to figure out the impact of clone plantation on the increment of yield comparing the productivity of past and present.

Keywords: BT clones, TV clones, yield trend, adoption.

Introduction

Tea has been historically promoted for having a variety of positive health benefits. Chinese has first used tea as a medicinal drink as early as 3000 B.C. and by the end of sixth century as a beverage. Recent scientific reports also emphasized on beneficiary effects of drinking tea. It is reported that, tea leaves contain more than 700 chemical constituents and most of them have significant importance in human health (Mondal 2004).

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Apart from the medicinal value, the popularity of drinking tea is also due to its mild stimulating effect and low cost. Now it is habituated as a regular drink after water in the world. Thus this primitive sip turned into a worldwide common drink which makes its position in the international trade. The acceptance of natural purity, beautification of variability (like white tea, green tea, black tea etc.), the flavor and refreshment as well as enchanting views of green coverage of the plantation areas make the human mind deep into the tea gulp.

At present about 52 counties are producing tea around the world (Mukhtar and Ahmad 2000). The world tea production in 2015 was 5.3 billion kg (ITC 2016). Tea is a major cash crop as well as a significant export item in Bangladesh. Bangladesh earns a substantial amount of foreign exchanges that contributes 0.11% of the GDP by exporting tea (BTB 2012). The tea industry plays an important role in sustaining the socioeconomic status of Bangladesh. This sector is an employment generating area where about 0.15 million people are employed, which is nearly 3.3% of the country's total industrial employment (Haque *et al*. 2000). There are 162 tea estates having about 62.5 thousand hectare of land under tea cultivation in Bangladesh. From the last few years, the tremendous success in tea production made Bangladesh government to give the tea industry a special weight as a part of important agro-based industry in our country. Breaking the previous all records of annual production, the year 2016 was the year of highest production with 85.05 million kg having an average yield of 1587 kg per hectare (BTB 2017). Though the yield is in increasing trend, the present average yield of our country is far below than that of other leading tea growing countries of the world. The top five tea producing countries are China, India, Kenya, Sri Lanka and Turkey, whose total production occupies about 84.4% of the world tea production. Bangladesh stands in the 10^{th} position among the tea producing countries in the world contributing 1.3% of total world tea production (ITC 2016).

From the last seven years, Bangladesh has started to import tea. In 2011, about 7.0 million kg tea was imported, whereas the export was only 1.45 mkg (ITC 2016). The reason behind the declining export is increased domestic consumption. In 2016, only 0.47 mkg made tea has been exported whereas the internal consumption was 81.64 mkg (BTB 2017). The global tea market is now very much competitive and expected to be higher in the near future due to increased demand of the quality tea. Specific cultivar-based made tea might be the requirement for the future generation of tea consumers. Considering the local demand and export potential, Bangladesh tea 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 of the tea industries of Bangladesh. But, due to unavailability of new land for extension planting, the existing tea plantation areas need to be enriched with high yield and quality clones. By vacancy infilling and new planting in uprooted old areas with improved clones can promote the tea production towards the desired goal along with the old tea estates economically viable. So the tea planters need to give emphasis on the improved cultivars as per the requirement of the industry (Palni *et al*. 1999).

Tea is a highly heterozygous out-crossing crop. High degree of genetic variability is present in the natural population. Wide spread natural hybridization increases the genetic variability in tea which has been usually exploited for developing many new elite clones in different tea producing countries like India, Sri Lanka, Kenya etc. Exploiting the genetic variability present in the natural population and using the conventional hybridization methods, Bangladesh Tea Research Institute (BTRI) has developed 20 recommended elite clones in the past 60 years. BTRI has developed a number of technologies including the elite clones from its beginning and made a great impact on tea crop improvement. Most of the tea plantation areas of Bangladesh are occupied by the exotic and locally developed elite clones. There is a tendency among the planters to plant exotic clones specially the Indian clones in their tea estates for increasing the production. Though the BT (Bangladesh Tea) clones are more adaptable and suitable for the local environmental point of view (BTB 2002), TV (Tocklai Variety) clones are appreciated by the tea planters because of the behavior of early coverage.

However, there is no current information about the percentage of the application and adoption of these clones in the tea plantation areas and its efficiency in implementation. So the current data regarding the disseminated technologies including clonal plantation adopted by the tea estates required to be up-to-dated. This will be helpful to know the efficiency of these technologies in the field and will disclose hinders of dissemination strategies of the BTRI technologies. Therefore, the study has aimed to make an up-to-date statistical database regarding the clonal plantation in the tea estates which is essential for further improvement of production.

Materials and methods

The experiment was conducted by structural interview with a well designed questionnaire. The questionnaire was designed to gather the information about the adoption of the different matured technologies of BTRI in the tea estates of Bangladesh. In the present report the clone adoption percentage in the tea states has highlighted. This questionnaire was sent to all the 162 tea estates and the collected data were tabulated for analyzing the information regarding different aspects of adoption of the BTRI technologies in the tea estates. Among the 162, a total of 87 tea estates responded initially. They have sent their information through the questionnaire. The necessary information of rest of the gardens was collected from the monitoring reports of Project Development Unit (PDU), BTB. The collected data from 144 gardens were then partially summarized according to valley wise adoption of clonal plantation. For collecting the previous yield records and other necessary information, the BTRI Annual reports and Journals, Statistical Bulletins of BTB, ITC reports etc. were reviewed.

Results and Discussion

In the present experiment, the adoption percentages of both BT and TV clones in the tea estates of different valleys were tried to explore. The calculated data on clonal plantation area of the tea estates are presented in the table 1. About 41.64% land of total tea area of the seven valleys were occupied by clonal plantation with the average production of 1607.48 kg/ha. The use of BT and TV clones was 45.05% and 40.20% respectively. Among the valleys, the highest clonal plantation was found in Chittagong valley (60.18%) followed by Luskerpur (48.17%), Juri (44.86%), Monu-Doloi (41.24%), Lungla (40.81%), Balisera (35.63%) and North Sylhet (24.29%). The highest (74.62%) BT clones are adopted by Chittagong valley and the lowest (21.82%) by Balisera valley. Maximum tea plantation area of Balisera valley is occupied by seedlings. Which is 56.41% of the sampled tea area of the present study. Balisera is one of the oldest tea plantation areas of Bangladesh. The most of the plantation has started in this area in eighteenth century when clones were not introduced. After developing Tockli varieties, several TV clones along with some other garden clones were planted in this area. Within the extended clonal plantation area of Balisera valley, 78.39% is TV clones with some mixed garden clones. TV clones are highly adopted in other valley circles also. Only in Chittagong valley planting of TV clones are found less prominent compared to other valleys. About 40-50% of clonal area is planted with TV clones in Juri, Lungla, North Sylhet and Monu- Doloi valley whereas in Chittagong, 28.35% of total clonal area is covered with TV clones (Table 1). The BT clones are being accepted by the gardeners from the beginning of their commercial release. But due to perennial nature, the tea plantation requires long time to establish. The uprooting and new extension depends upon the economic age of the old plantation areas and economic viability of the gardens. The tea estates which were established newly are occupied by BT clones and Bi- clonal seed stocks in large percentage of their tea area. In Chittagong valley, most of the tea estates are planted with BT clones. Now, the new extension tea areas of Chittagong hill tracts and Panchagarh District are expanding tea cultivation specially with BT clones. However, the access of clones completely depends on gardener's choice and availability.

Table 1. Valley wise clone plantation in different tea estate

Valley	Total tea area (ha)	Total seedling area (ha) & (%)	Total clone area (ha)	% of BT clones planted	% of TV clone planted	Av. yield of BT area (kg/ha)	Av. yield of TV area (kg/ha)
Balisera	14537.91	8200.66 (56.41)	5179.3 (35.63)	21.82	78.39	1801.5	1836.37
Monu-Doloi	7410.07	3852.64 (51.99)	3055.71 (41.24)	48.78	46.26	1706.75	2403.00
Lungla	8025.3	4482.29 (55.85)	3274.86 (40.81)	51.28	44.77	1592.78	1715.83
Juri	7634.26	3701.86 (48.49)	3424.56 (44.86)	47.69	50.57	1765.67	1582.5
Luskerpur	8348.23	4332.49 (51.90)	4021.51 (48.17)	68.31	31.10	1591.2	1527.2
North Sylhet	4632.51	3485.06 (73.98)	1125.42 (24.29)	51.79	48.20	994.2	960.6
Chittagong	5326.97	2327.93 (43.70)	3205.99 (60.18)	74.62	28.35	1684.67	1646.43
Total	**55915.25**	**30382.93 (54.34)**	**23287.35 (41.64)**	**45.05 (Av.)**	**40.20 (Av.)**	**1590.97**	**1667.42**

In the parenthesis the percentage has given.

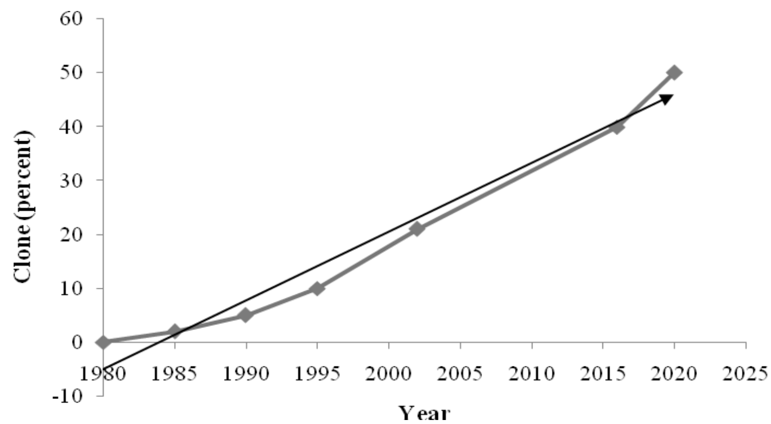

Figure 1. Adoption trend of the clones in the tea estates

It is important for a planter to select improved planting materials to get desire production. Generally the recommended proportion in percentage of clone and seedling plantation is 50:50. In 1994, the study calculated that 90% of total tea area was occupied by seedlings and the rest was clones of which maximum were the garden clones with a little of introduced clones (Alam 1994). In the later study of 2001, an improvement was noticed on clone plantation. In that study 58.72% of total tea area was examined of which 21.02% was absolute clonal with 3.6% bi and polyclonal seedling and rest were general seedlings (Alam 2002). From the present study it is revealed that the average seedling area is 54.34% whereas the clone area is 41.64% of the total tea plantation area. The trend of clone utilization in the tea estates has increased remarkably (Fig. 1). In the strategy of vision 2021, 34 thousand hectare new tea area is expected to plant with 50% improved seedlings and 50% improved clones and about 16 thousand hectare land should be occupied by proven clones suited to our environment (BTB 2002). It is clear that the recommended proportion of clone and seedling plantation is nearly fulfilled by the tea estates. It was noticed that maximum seedling areas are occupied by the general seedlings. General seedling areas are proven for giving lower and un-even yield. In terms of clone plantation, a progressive result was found from the study that a large percentage of total plantation area is adopted by clones. From this, it can be assumed that increased clone plantation was the key factor that enhanced the overall tea productivity of Bangladesh in the recent years.

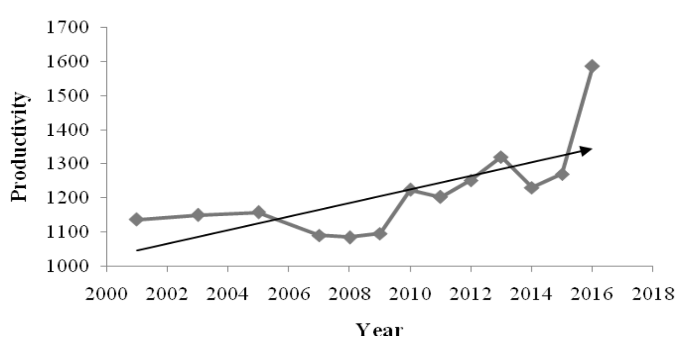

Figure 2. Yield trend of Bangladesh tea

The average yield of Bangladesh tea has moved from 963 kg/ha in 1990 to 1587 kg/ha in 2016. In 2001 the annual production was 53.15 mkg whereas in 2016 the production reached to 85.05 mkg (Table 2). The yield trend curve from 2001 to 2015 increased gradually but surprisingly in 2016 the yield increased sharply breaking previous all records (Fig. 2). The production reached at 85.05 mkg in 2016 which was 67.38 mkg in 2015 and the rate of increase was 26.22 percent. The extra ordinary favorable environment especially the rainfall along with the proper cultural practices and the hard work of the planters might be the reasons behind this sudden improved productivity. The necessary steps taken by BTB at appropriate moments and continuous technical support by BTRI also helped to achieve this success. However, to reach the target of 111.70 mkg of vision 2021, attention should be given in all sectors that are responsible to promote production.

Table 2. Area, production and average yield of Bangladesh tea from 2001-2016

Year	Tea Area (ha)	Production (mkg)	Yield (kg/ha)
2001	50,037	53.15	1136
2002	51,117	53.62	1174
2003	50,700	58.30	1150
2004	52,088	56.00	1219
2005	51,922	60.14	1158
2006	52,820	53.41	1146
2007	53,208	58.19	1090
2008	53,518	58.66	1084
2009	54,000	59.99	1095
2010	55,000	60.04	1224
2011	56,846	59.13	1203
2012	57,210	62.52	1252
2013	58,719	66.26	1320
2014	59,609	63.88	1230
2015	60,179	67.38	1270
2016	62,500	85.05	1587

Source: Statistics on tea, BTB, 2016.

If we compare the leading tea growing countries of the world the average productivity of Bangladesh is still behind. It is required to intensify the existing suitable areas for further extension with high yield clones or seed stocks. The plantation area of BT (45.05%) and TV (40.2%) clones is nearly same (Table 1). The average production is also same with a tiny difference between BT and TV clones. Per hectare yield of BT clones was found 1591 kg whereas for TV clones it was 1667 kg, which is almost near the present national average yield. The poor yield of the sick gardens is the main reason of shrinking down the national average yield of Bangladesh. So, the average yield can be increased by taking the initiatives for proper maintenance and improved cultural practices in the existing tea areas along with the intensive plantation of high yielding clones. The remaining vacancies should be filled up with high yielding adaptive clones or seed stocks. About 7677.87 hectare suitable areas are still available for tea plantations which may be utilized for further extension with improved clones (PDU 2015). BT clones have proven as adaptive and suitable to our environment with appreciable yield and quality. To intensify the production as well as sustainable quality of our tea, these clones in a required percentage need to be utilized in the tea cultivation areas. This way our tea industry might reach towards the desired goal of production and quality.

Conclusion

The final remark is an expectation for constant and sustainable growth of total production of Bangladesh tea. That is possible with the fulfillment of the principle factors i.e. efficiency of management, intensive culture using high yield and quality planting materials in an appropriate proportion as well as adoption of newer scientific concepts and technologies. Increased clonal plantation along with the progressive production is a positive sign for the tea culture of Bangladesh. The data regarding assimilation of improved clones and their yield potential need to follow up to maintain further extension in the tea estates.

References

Alam, A.F.M.B 2002. Researches on varietal improvement of tea and their utilization in the tea industry of Bangladesh. Proceedings of the seminar on "Varietal development of tea in Bangladesh". July 13. Bangladesh Tea Research Institute, Srimangal. pp 7-25.

Alam, A.F.M.B., 1994. Influence of improved genetic material towards higher productivity of tea in Bangladesh. Proceedings of the International Seminar on "Integrated Crop Management in Tea towards higher productivity" April 26-27. Colombo, Sri Lanka. International Potash Institute, CH-4001, Basel, Switzerland. pp 33-49.

BTB. 2002. Vision 2021-Strategic research plan of BTRI for the millennium 2002-2021. Bangladesh Tea Board, 171-172 Baizid Bostami Road, Nasirabad Chittagong. pp: 15-16.

BTB. 2012. Statistics on tea. Bangladesh Tea Board, 171-172 Baizid Bostami Road, Nasirabad, Chittagong.

BTB. 2017. Monthly statistical bulletin for the month of January 2017. Bangladesh Tea Board, 171-172 Baizid Bostami Road, Nasirabad, Chittagong.

Haque, S.K.L, Samad M.A. and Alam A.F.M.B., 2002. Varietal improvement and multiplication of tea plant through tissue culture technique. Final report of contract research project of Bangladesh Tea Research Institute with Bangladesh Agricultural Research Council.

ITC 2012. Annual Bulletin of Statistics. International Tea Committee. London, UK.

ITC 2015. Annual Bulletin of Statistics. International Tea Committee. London, UK.

Mondal T.K., Bhattacharya A., Laxmikumaran M. and Ahuja P.S., 2004. Recent advances of tea (*Camellia sinensis* L.) biotechnology. Review of Plant Biotechnology and Applied genetics. Plant cell, Tissue and Organ Culture, **76**: 195-254.

Mukhtar H. and Ahmad N. 2000. Tea Polyphenols-Prevention of cancer and optimizing health. Amer. J. Clin. Nutr. 71: 1698S-1702S.

Palni L.M.S. Hao C. and Nakamura Y., 1999. Advances in Tea Biotechnology. Global Advances in Tea Science. pp. 449-462.

PDU. 2015. Statistics on Bangladesh tea Industry, Project Development Unit, Bangladesh Tea Board, Srimangal-3210, Moulvibazar.

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EVALUATION OF SOME POTENTIAL MITICIDES AGAINST RED SPIDER MITE INFESTING TEA IN BANGLADESH

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Dr. Mohammad Shameem Al Mamun was born on 1 July 1978 in a noble Muslim family at Ghatail Upazila of Tangail district, Bangladesh. He obtained B.Sc.Ag. (Hons.) and M.S. in Entomology degree (1st class) from Bangladesh Agricultural University (BAU), Mymensingh in 2001 and 2005, respectively. He joined in Bangladesh Tea Research Institute (BTRI) as Scientific Officer (Entomology) in 30 July, 2007. Later he awarded Post Graduate Diploma (PGD) in Tea Plantation Management with Grade A+ from Kothari Agricultural Management Centre (KAMC), Tamil Nadu, India in 2010 as a scholar of Colombo Plan Scholarship sponsored by the Government of India. Dr. Mamun awarded a PhD degree from the Department of Food Engineering & Tea Technology, Shahjalal University of Science & Technology, Sylhet in 2017. Dr. Mamun was promoted to Senior Scientific Officer of the Entomology Division in 20 March 2013. Now he is the In-charge of BTRI Sub Station, Panchagarh. He successfully completed a training course on 'Pollution free Tea Production Technology for Developing Countries' held in 2016 at Zhangzhou College of Science and Technology, Fujian, China as a scholar of China Aid Scholarship sponsored by Chinese Government. Planning, formulating, conducting, guiding entomological researches for tea and Pesticide residue detection and analysis of made tea are the specialized field of research. He also imparts training and workshop on tea pest management on annual courses, MTC and in tea estates to the trainees particularly managerial as well as field staff of the tea industry. He has 37 scientific journals published in national and international indexed journals related to integrated pest management in tea. Moreover, he has two books published by LAP LAMBERT Academic Publishing, Germany related to integrated pest management. He obtained diverse training on Tea Culture, Basic Training on Computer Application, Agricultural Project Management, Pesticide Residue Analysis in Tea, Research Methodology, Biological Control of Agricultural Pests and Diseases, Research Planning and Proposal Writing, Development of Web Portal, Innovation in Public Services, Project Development and Management, Pollution-Free Tea Production Technology from home and abroad. He has experienced in conducting different agricultural research projects (Pesticide Residue Project, Nematode Project & IPM Project) of BARC as Co Principal Investigator as well as Principal Investigator in his service life. He has experience of co-supervising of undergraduate and master's students of the Department of Food Engineering & Tea Technology, Sahajalal University of Science & Technology, Sylhet. Dr. Mamun is a member of different national and international professional organization and societies such as Krishibid Institution Bangladesh, Bangladesh Association for the Advancement of Science, Bangladesh Botanical Society Zoological Society of Bangladesh, Bangladesh Entomological Society, Weed Science Society of Bangladesh, Entomological Society of America, The Acarological Society of Iran and Tea Professional Welfare Society, Bangladesh. Dr. Mamun is married with Shamima Nasrin Happy and blessed with two sweet daughters Sarah & Sabah.

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EVALUATION OF SOME POTENTIAL MITICIDES AGAINST RED SPIDER MITE INFESTING TEA IN BANGLADESH

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Abstract

An experiment was carried out to evaluate the efficacy of some potential miticides such as Abamectin (Abom 1.8EC), Chlorfenapyr (Intrepid 10SC), Fenazaquin (Magister 10EC), Fenpropathrin (Danitol 10EC), Fenpyroxymate (Mitisol 5EC) Hexythiazox (Mite scavenger 10EC), Propargite (Omite 57EC) and Spiromesifen (Oberon 240SC) @ 0.5 mL, 1.0 mL, 1.0 mL, 0.6 mL, 1.0 mL, 0.5 mL, 1.0 mL and 0.4 mL L^{-1} of water, respectively against red spider mite, *Oligonychus coffeae* Nietner infesting tea under both laboratory and field conditions at Bangladesh Tea Research Institute (BTRI), Srimangal, Moulvibazar during January-December 2014. Unsprayed condition was considered as control. Each treatment was replicated thrice. Prepared solutions of miticides in water were sprayed on the red spider mites in both the laboratory and the field conditions. Data were collected at 24, 48, and 72 HAT (hours after treatment) in the laboratory and at weekly in the field experiment. Economic analysis was done by partial budgeting technique. Result revealed that all the tested miticides has satisfactory performance to reduce the infestation of red spider mites significantly ($p>0.05$) in the laboratory and the field conditions. Among the tested miticides, Hexythiazox (75.88%) showed the highest performance in reducing mite population followed by Fenazaquin (74.12%) in the laboratory. The similar trend was observed in case of field condition. The order of performance of the tested pesticides was Hexythiazox> Fenazaquin> Spiromesifen> Fenpyroxymate> Propargite> Fenpropathrin> Chlorfenapyr> Abamectin. Per hectare yield (2115.27 kg) of made tea and net return (Tk 405412.38) were higher in Hexythiazox treated plots. But the highest marginal rate of return (3219.77%) was obtained by spraying Fenazaquin over control compared to other miticides. The most economically acceptable miticide against red spider mite was Fenazaquin @ 0.6 L ha^{-1}. Good evidence was obtained from the present experiment that among the tested miticide, Hexythiazox and Fenazaquin showed the most toxic effects on red spider mites. Planters may use these chemicals judiciously for the quick management of red spider mites in tea.

Keywords: Tea, Red Spider Mite, *Oligonychus coffeae,* Chemical Control, Miticide, Acaricide

Introduction

Tea is a popular beverage in the world, is produced from the leaves of evergreen shrub *Camellia sinensis* L (O Kuntze). It is a perennial crop grown under monoculture on large contiguous areas providing favourable conditions for a variety of pests. Tea plants are subjected the attack of several pests such as insects, mites and nematodes.

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Moreover, a characteristic feature viz. the performance of shade trees, ancillary crops, forests, an uniformity of cultural practices such as sequential pruning cycles, weekly plucking rounds, weeding, mulching etc. have a greater impact on the subsequent colonization, stabilization and distribution of pests (Mamun, 2011). In world tea, 1034 species of arthropods and 82 species of nematodes are associated with tea plants (Chen and Chen, 1989). In Bangladesh tea, so far 25 insects, 4 mites and 10 species of nematodes have been recorded (Ahmed, 2005). Among them, Tea mosquito bug, Red spider mites & Termites are the major pests in mature tea plantation; while Aphids, Jassids, Thrips, Flushworms and Nematodes are the major pests in nursery and young tea plantation (Mamun and Ahmed, 2011).

Mite pests are the notorious pests of many agricultural crops over the world. More than 60,000 species of mites have been described from various regions of the world (Evans, 1992). Red spider mite, *Oligonychus coffeae* Nietner (Acarina: Tetranychidae) is one of the major and serious pests of tea in all most all tea producing countries of south-east Asia and Africa. Hundreds of spider mites are found on the upper and undersurface of every tea leaf, together with thousands of eggs (Ahmed and Sana, 1990). Red spider mites are responsible for depredation of yield and debilitation of tea plants causing considerable crop loss. It is estimated that 9.57% crop loss occurred due to this pest (Ali *et al.*, 1994). The red spider mite has revisited almost every tea estates in Bangladesh. Most of the valley circles reported severe infestation of red spider mites, which are more prevalent and alarming round the year for the tea industry (BTB, 2015). The larvae, nymphs and adult mites cause the damage. When large numbers of mites are present, sucking one leaf cell after another and sucking out the contents, the whole leaf eventually changes to a bronze colour, dries up and drops- especially in hot and dry weather (Sana, 1989; Ahmed *et al.,* 2012). It may also be mentioned here that the red spider mite prefers mature leaves, and young leaves are not normally attacked, but in severe outbreaks when the growth of the bushes checked, particularly under conditions of drought, both young and mature leaves may be equally attacked (Das, 1959). Thus drought accelerate the mite infestation in tea plantation. Now-a-days, drought is a common phenomenon and therefore, infestation of red spider mite is emerging threat to the tea industry of Bangladesh.

Management of tea red spider mites has largely depended on the use of conventional, neurotoxic, broad-spectrum, quick action, synthetic chemical pesticides such as organochlorine, organophosphate, organocarbamates, pyrethroids since 1960 (Mamun *et al.*, 2014). It is established that tea-growing countries of the world are benefited by the chemical control of pests. In this perspective, chemical control of pests is a dominating feature in Bangladesh tea. Pesticides are one of the important components in IPM. Presently red spider mite cannot be satisfactorily managed without the application of acaricides. Several generations of structurally diverse synthetic acaricides, directed against various biochemical and physiological targets, have been launched until now. Until the mid 1970s the second generation of specific acaricides as organotins, formamidines, quinoxalines, and propargite appeared (Jeppson *et al.,* 1975). Mite growth inhibitors (Clofentezine, Hexythiazox), launched during the 1980s, represented the third generation. In addition to specific acaricides, a number of insecticides with considerable acaricidal activity (Pyrethroids, Avermectins, and Benzoylureas) have also been introduced during 1970s and 1980s (Dekeyser and Downer, 1994; Knowles, 1997; Dekeyser, 2005; Pitterna, 2007). With the advent of compounds acting on respiration targets, by the beginning of 1990s, the modern era in development of synthetic acaricides began (Dekeyser, 2005). Different group of pesticides such as Sulphur, Ethion, Quinalphos, Propargite, Abamectin, Dimethoate, Fenvalerate, Fenpropathrin, Fenazaquin, Bifenthrin, Hexythiazox, Spiromesifen and Fenpyroxymate etc. are being used as the commonly used miticides for the control of red spider mite in tea plantation in Bangladesh (Mamun *et al.,* 2014). These chemicals are non systemic acaricide and insecticide properties with rapid knock down and long residual effect showing excellent activity against all developing stages of wide range of phytophagus mites. The chemical method of pest control involves costly inputs like pesticides, labours, spraying equipments etc. It is very important to use those inputs effectively, economically and judiciously at right time and in a proper way in order to minimize production cost and maximize benefits for a particular area (Ahmed *et al*., 1998). Reduction of cost as well as chemical volume is looking for newer generation of miticides which have small volume of dose rate, low cost and long persistent in the tea field. There is a need to evaluate newer chemicals to manage mite pests and replace ineffective ones. In view of this, the present study was undertaken to evaluate the potentiality and economic analysis of some novel miticides against red spider mite infesting tea in Bangladesh.

Materials and Methods

An experiment was carried out to evaluate the efficacy of some potential miticides such as Abamectin (Abom 1.8 EC), Chlorfenapyr (Intrepid 10 SC), Fenazaquin (Magister 10 EC), Fenpropathrin (Danitol 10 EC), Fenpyroxymate (Mitisol 5 EC), Hexythiazox (Mite scavenger 10 EC), Propargite (Omite 57 EC) and Spiromesifen (Oberon 240 SC) against red spider mite, *O. coffeae* infesting tea under both laboratory and field conditions at Bangladesh Tea Research Institute, Srimangal, Moulvibazar during January – December 2014.

Rearing of mites

Red spider mites, *O. coffeae* were collected from infested leaves of tea bushes from the areas where no acaricide was used at the main farm of Bangladesh Tea Research Institute and transferred to caged plant for subsequent culturing with help of fine camel hair brush. One day old adult females were taken from the cultured source and gently transferred onto detached tea leaves (30/leaf/petri plate) on 90 mm petri plates containing a thin layer of 1.5% water agar at the base because the leaves on water agar remain green for more than 10 days and supports the mites for normal development. The petri plates were observed under binocular microscope for mite mortality.

Acaricides used in the experiment

For laboratory and field experiments, the recommended doses of eight acaricides commercially available in the market for the control of red spider mite in tea were selected in this experiment (Table 1). The concentration of active ingredient (a.i.) ha^{-1} of all acaricides for the control of red spider mites were converted to mg a.i. per litre on the base of average total volume of about 1000 litres of water used per hectare.

Table 1. Some potential miticides evaluated against red spider mite infesting tea

Chemical Name	Commercial Name	Manufacturer/suppliers Name
T_1: Abamectin	Abom 1.8 EC	McDonald Bangladesh Ltd.
T_2: Chlorfenapyr	Intrepid 10 SC	BASF, Germany
T_3: Fenazaquin	Magister 10 EC	Petrochem Bangladesh Ltd.
T_4: Fenpropathrin	Danitol 10 EC	Shetu Corporation Ltd.
T_5 : Fenpyroxymate	Mitisol 5 EC	Semco Pesticides Ltd.
T_6 : Hexythiazox	Mite Scavenger 10 EC	Eminence Chemical Ind. Ltd.
T_7: Propargite	Omite 57 EC	Shetu Corporation Ltd.
T_8 : Spiromesifen	Oberon 240 SC	Bayer Crop Science Ltd.

Treatments:

T_1: Abamectin (Abom) @ 0.5 ml L^{-1}
T_2: Chlorfenapyr (Intrepid) @ 1.0 ml L^{-1}
T_3: Fenazaquin (Magister) @ 0.6 ml L^{-1}
T_4: Fenpropathrin (Danitol) @ 1.0 ml L^{-1}
T_5: Fenpyroxymate (Mitisol) @ 1.0 ml L^{-1}
T_6 : Hexythiazox (Mite scavenger) @ 0.5 ml L^{-1}
T_7: Propargite (Omite) @ 1.0 ml L^{-1}
T_8: Spiromesifen (Oberon) @ 0.4 ml L^{-1}

Laboratory bioassay test

The solution of each acaricide with 4 different solutions was prepared in distilled water and each tea leaf disc of 5 cm diameter was dipped in that solution for 5 seconds. After air drying, each leaf disc was placed, lower side up, on a bed of a thin layer of 1.5% water agar at the base in a 90 mm petri dish. The 30 adult female of red spider mites were transferred to each of this petri dish with the help of fine brush. To prevent mites from escaping, a strip of moist cotton wool (approx. 10 mm in width), was placed around the perimeter of the treated leaf. All perti dishes with mites on leaves were then put in a growth chamber at 25 ± 2°C, 70 ± 5% RH and 16:8 L:D. The mortality was assessed after 24, 48 and 72 HAT (hours after treatment) under a binocular microscope in all treated and control petri dishes. Using a fine brush, each female was stimulated and the individuals incapable of crawling or without any body movement were recorded as dead. The percent corrected mortality was calculated in all the treatments by Abbot's (1987) formula as described earlier.

Field experiment

The field experiment was conducted to determine the field efficacy of some potential miticides against red spider mite infesting tea at the A2 section (mixed clones) of BTRI main farm, Srimangal, Moulvibazar. Ten different groups of acaricides and one untreated control were considered as treatments. The acaricides were applied at recommended doses against red spider mites in tea according to Mamun *et al.* (2014). The experiment was laid out in RCBD with eleven treatments including control each having three replications. The plot size was kept as 5 x 5 m^2 having 30 tea bushes. Three rounds of chemical were sprayed during the study period. The spraying was done with CP-15 hand operated Knapsack sprayer fitted with hollow cone nozzle. The water volume was used 1000 L. of water per hectare. Other routine operations such as pruning, plucking, weeding, application of fertilizers etc. were done by the farm management section. The acaricides were sprayed in the early morning before noon. The row of each side of each treatment was not treated and considered as buffer. Mite population was counted just prior to application of acaricides. Post treatment observations were taken at weekly interval for twelve weeks after treatment. To assess the number of mites, a sample of 10 leaves per plot was collected weekly and mites were brushed off from both surfaces of the leaves with a mite brushing machine. The numbers of all motile stages of mites were counted under a stereoscopic microscope. Subsequently, yield was recorded at each plucking before, during and after red spider mite season. Green leaf was converted to made tea per hectare by multiplying green leaf yield ha^{-1} by a factor of 0.23. Yield difference was determined between treated and untreated (control) plots. Effectiveness of miticides was calculated by using Henderson and Tilton's formula (Henderson and Tilton, 1955) as described earlier.

Economic analysis

The economic analysis was carried out by using partial budgeting technique (CIMMYT, 1988) to determine the economically viable miticide against red spider mite. Only variable costs (costs of miticides per hectare) of different miticides were considered for these treatments and the rest of the costs were considered to be constant. Variable costs are those cost which vary proportionately with saleable produce.

Data analysis

The experimental data were statistically analysed by Completely Randomized Design (factorial CRD) and Randomized Complete Block Design (RCBD) 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).

Results and Discussion

Laboratory bioassay test

Results revealed that all the tested chemicals showed the toxic effect on red spider mite in tea and reduced mite population significantly in laboratory conditions ($p<0.05$). Among the tested chemicals, the highest average mortality of red spider mites was observed in Hexythiazox (75.88%) followed by Fenazaquin (74.12%) and Spiromesifen (72.46%). The lowest average mortality was observed in Chlorfenapyr (64.17%) and Abamectin (64.50%) (Table 2). Similar trend was also observed at 24 HAT, 48 HAT and 72 HAT.

Table 2. Laboratory evaluation of some potential miticides against red spider mites in tea

Treatments	Dosage L^{-1} water (mL)	Percent Mortality*			Mean (%)
		24 HAT	48 HAT	72 HAT	
T_1 : Abamectin	0.5	55.28±1.28d	63.88±1.72e	74.34±1.86g	64.50±1.62f
T_2 : Chlorfenapyr	1.0	53.85±1.07e	64.68±1.86d	73.98±1.92g	64.17±1.61f
T_3 : Fenazaquin	0.6	62.24±2.32b	73.68±2.53b	86.45±2.86b	74.12±2.57b
T_4 : Fenpropathrin	1.0	58.04±1.86c	66.52±1.94d	78.96±2.47f	67.84±2.09e
T_5: Fenpyroxymate	1.0	60.08±1.98bc	70.08±2.28c	82.64±2.66d	70.93±2.30d
T_6 : Hexythiazox	0.5	63.45±2.53a	75.48±2.69a	88.72±3.01a	75.88±2.74a
T_7 : Propargite	1.0	58.48±1.92c	69.30±2.05c	80.38±2.58e	69.39±2.18d
T_8: Spiromesifen	0.4	61.82±2.24b	71.26±2.66b	84.30±2.84c	72.46±2.58c
T_9 : Control	-	6.67±0.24	10.33±0.46	10.33±0.46	9.11±0.38
Probability level		0.05	0.05	0.05	0.05
Coefficient of variance (CV%)		7.16	7.56	4.37	5.62

*Mean of three observations (30 adults/observation); HAT= Hours after treatment.
Within column values followed by different letter(s) are significantly different by DMRT ($p>0.05$)

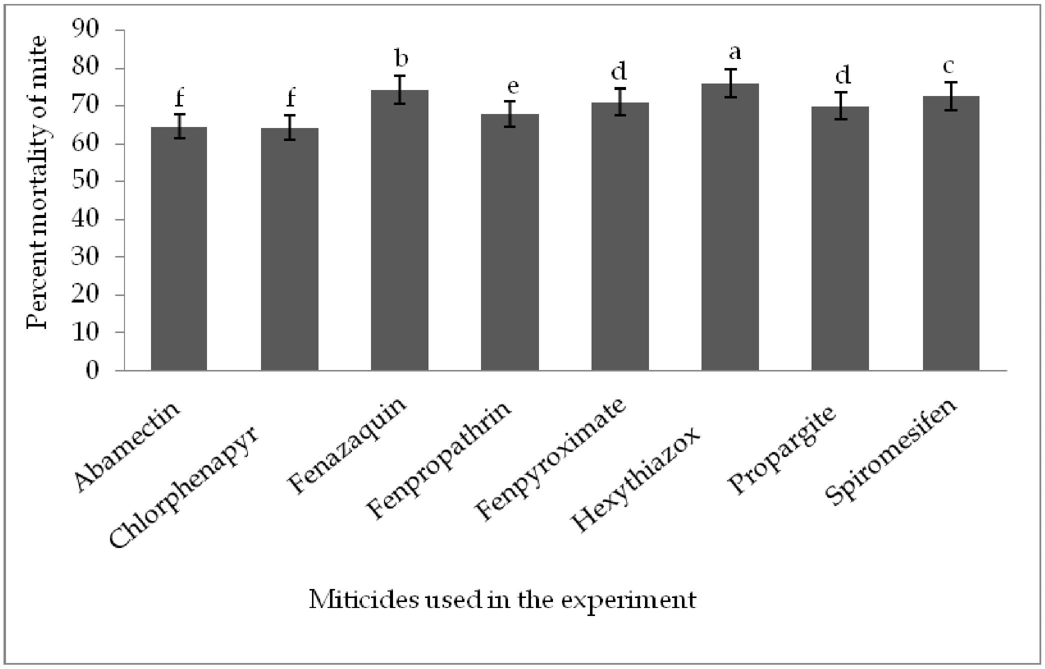

Fig. 1. Average mortality (%) of mites using some potential miticides in the laboratory

The study also showed that mortality of RSM was in a linear trend i.e. increased with the increasing time. The order of toxicity of the tested chemicals on red spider mite was: Hexythiazox>Fenazaquin>Spiromesifen>Fenpyroxymate>Propargite>Fenpropathrin>Abamectin>Chlorfenapyr (Fig. 1). The present findings support to the findings of Amjad *et al.* (2012) on mites and Mehmood *et al.* (2012) on thrips.

Field evaluation

Results revealed from the field evaluation of different tested miticides against red spider mites that all the tested chemicals also have potent acaricidal effect to reduce the infestation of red spider mite infesting tea in the field. All the potential miticides significantly reduced infestation of red spider mite. Application of Hexythiazox @ 500 mL ha^{-1} was found significantly superior (91.43%) in reducing the infestation of tea red spider mite over rest of the treatments after 1st week of 1st application of miticides (Table 3). This was followed by Fenazaquin @ 600 mL ha^{-1} (90.45%), which was at par with Spiromesifen @ 400 mL ha^{-1} (90.06%). The trend was similar after the 2nd and the 3rd spray. From the observation of after twelve weeks, the results indicated that out of 8 treatments selected, the treatment of Hexythiazox @ 500 mL ha^{-1} (T_6) was observed significantly superior (85.08%) based on over all mean than the other treatments followed by Fenazaquin @ 600 mL ha^{-1} (84.30%) and Spiromesifen @ 400 mL ha^{-1} (84.26%). The order of performance of the tested pesticides wasHexythiazox>Fenazaquin>Spiromesifen>Fenpyroxymate>Propargite>Fenpropathrin>Chlorfenapyr>Abamectin (Fig. 2).

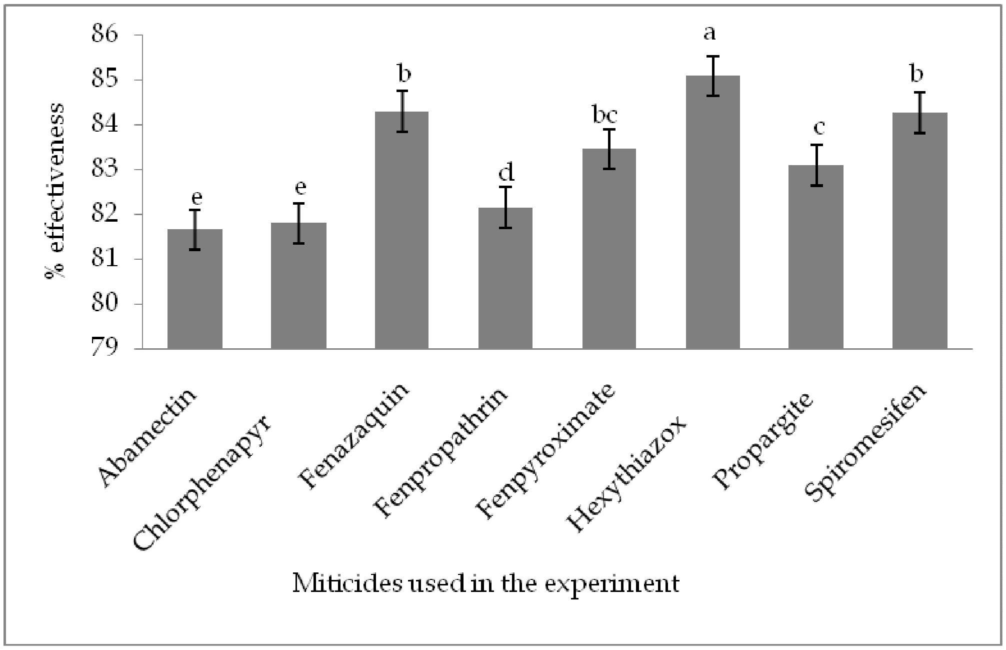

Fig. 2. Effectivenes of some potential miticides against red spider mite in the field

The findings of the present study were similar to that of Roy *et al.* (2009). They found that the new molecules like Propargite, Fenpropathrin and Fenazaquin recorded on an average of 96.60, 99.90 and 99.90% mortality, respectively against red spider mite on tea. Radhakrishnan *et al.* (1999) evaluated the field performance of Fenpropathrin (Danitol 10 EC) against red spider mites of tea. Result indicated that spraying of Fenpropathrin 10 EC @ 500, 750 and 1000 ml ha^{-1} significantly reduced the number of red spider mites. Ahmed *et al.* (2011) standardized the minimum acceptable level of effectiveness of a pesticide as 80%. The miticidal treatments of the present study proved the mite population reduction over control within the acceptable limit. The present findings support the findings of Dutta *et al.* (2012). As pesticide is one of the components of IPM, the judicious use of pesticides may reduce the infestation of red spider mite in tea.

Economic analysis

The yield of tea crop also increased significantly in all the treated plots in comparison with the untreated plots. The identical yield from 1670.32 to 2115.27 kg ha^{-1} was produced in the miticides treated plot. The percent increase of yield due to various treatments against red spider mite over untreated control ranged from 14.74 to 45.30% (Table 4). The present findings are similar to that of Ahmed *et al.* (2011).

Table 3. Field evaluation of some potential miticides against red spider mite in tea during January-December 2014.

Treatments	Doses ha^{-1} (mL)	Pre-treatment obs. (no. of mites)	% Effectiveness of chemicals after application of miticides*												Overall mean
			After 1st application			After 2nd application			After 3rd application						
			1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	8th wk	9th wk	10th wk	11th wk	12th wk	
T_1: Abamectin	500	49	86.35±1.92	82.81±1.68	77.68±1.54	89.46±2.46	82.14±1.66	78.18±1.50	90.73±2.46	82.04±1.66	81.38±1.54	78.34±1.50	76.28±1.42	74.46±1.36	81.65±1.54e
T_2: Chlorfenapyr	1000	45	86.38±1.94	81.96±1.56	78.04±1.54	88.58±2.20	82.37±1.66	79.74±1.54	90.88±2.46	83.06±1.92	80.36±1.52	78.44±1.50	77.27±1.46	74.54±1.36	81.80±1.56e
T_3: Fenazaquin	600	56	91.24±2.48	84.82±1.80	79.74±1.58	92.12±2.58	84.56±1.82	81.66±1.56	93.38±2.62	85.88±1.80	82.96±1.66	81.76±1.57	79.08±1.50	76.24±1.40	84.45±1.82b
T_4: Fenpropathrin	1000	51	88.08±2.18	83.01±1.72	78.22±1.53	89.98±2.40	81.48±1.54	79.34±1.54	91.28±2.53	83.25±1.74	81.82±1.58	78.33±1.50	76.29±1.42	74.66±1.36	82.15±1.66d
T_5:Fenpyroxymate	1000	47	88.32±2.26	84.12±1.76	78.01±1.53	90.78±2.46	83.84±1.74	80.98±1.57	92.24±2.60	86.04±1.92	82.92±1.66	80.84±1.53	78.10±1.50	75.24±1.38	83.45±1.74bc
T_6: Hexythiazox	500	48	91.43±2.52	85.37±1.82	80.23±1.56	91.38±2.52	86.73±1.93	82.82±1.68	94.46±2.88	86.63±1.94	82.73±1.64	81.48±1.54	79.42±1.51	78.26±1.50	85.08±1.83a
T_7: Propargite	1000	46	89.74±2.40	83.29±1.82	78.31±1.54	90.36±2.44	83.08±1.72	79.77±1.54	91.66±2.52	85.32±1.83	82.22±1.64	80.39±1.52	77.84±1.48	75.06±1.38	83.09±1.72c
T_8: Spiromesifen	400	50	90.06±2.44	84.36±1.78	79.58±1.58	92.93±2.60	84.38±1.78	81.25±1.52	93.24±2.62	86.29±1.94	83.44±1.74	81.53±1.54	78.32±1.50	75.78±1.38	84.26±1.80b
T_9: Control (no. of mites)	-	42	46	51	55	59	63	68	72	79	83	88	92	97	105
Probability level	NS														0.05
CV%															8.42

*Mean of three observations.

± Standard error of means at a given concentration.

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

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Table 4. Yield of tea crop due to different miticides used against red spider mite in BTRI main farm during January to December, 2014

Treatments	Doses used (L ha^{-1})	Average yield (kg ha^{-1})	Increase of yield over control (%)
T_1: Abamectin	0.5	1670.32d	14.74d
T_2: Chlorfenapyr	1.0	2020.45b	38.79b
T_3: Fenazaquin	0.6	1980.34c	36.03c
T_4: Fenpropathrin	1.0	2005.67b	37.77b
T_5:Fenpyroxymate	1.0	2050.78b	40.87b
T_6: Hexythiazox	0.5	2115.27a	45.30a
T_7: Propargite	1.0	2025.39b	39.13b
T_8: Spiromesifen	0.4	1980.89c	36.07c
T_9: Control	-	1455.78e	-

Mean of 3 replications in a column having the same letter did not differ significantly by DMRT ($p>0.05$).

The parameters of partial budgeting technique i.e. average yield, variable cost, gross return and net return of different miticides were calculated. The highest net return of Tk. 405412.38 ha^{-1} was obtained in Mite scavenger 10 EC treated plots followed by Mitisol 5 EC (393351.32 Tk ha^{-1}), Omite 57 EC (388725.66 Tk ha^{-1}), Intrepid 10 SC (386567.30 Tk ha^{-1}), Danitol 10 EC (385199.98 Tk ha^{-1}), Magister 10 EC (380585.96 Tk ha^{-1}), Oberon 240 SC (379252.66 Tk ha^{-1}) and Abom 1.8 EC (322630.08 Tk ha^{-1}) (Table 5). The result obtained by Ahmed *et al.* (2011) supports present findings.

Table 5. Partial budget of different miticidal treatments applied for controlling red spider mite in tea

Treatments	Dose used (L ha^{-1})	Average yield (Kg ha^{-1})	Variable cost (Tk ha^{-1})	Gross return[1] (Tk ha^{-1})	Net return[2] (Tk ha^{-1})
Abom 1.8 EC	0.5	1670.32d	1800.00	324430.08e	322630.08e
Intrepid 10 SC	1.0	2020.45b	5400.00	391967.30c	386567.30c
Magister 10 EC	0.6	1980.34c	3600.00	384185.96d	380585.96d
Danitol 10 EC	1.0	2005.67b	3900.00	389099.98c	385199.98c
Mitisol 5 EC	1.0	2050.78b	4500.00	397851.32b	393351.32b
Mite scavenger 10 EC	0.5	2115.27a	4950.00	410362.38a	405412.38a
Omite 57 EC	1.0	2025.39b	4200.00	392925.66c	388725.66c
Oberon 240 SC	0.4	1980.89c	5040.00	384292.66d	379252.66d
Control	-	1455.78e	-	282421.32f	282421.32f

Cost of miticides: Abom 1.8 EC @ Tk 1200 L^{-1}, Intrepid 10 SC @ Tk 1800 L^{-1}, Magister 10 EC @ Tk 200 L^{-1}, Danitol 10 EC @ Tk 1300 L^{-1}, Mitisol 5 EC @ Tk 1500 L^{-1}, Mite scavenger 10 EC @ Tk 3300 L^{-1}, Omite 57 EC @ Tk 1400 L^{-1} and Oberon 240 SC @ Tk 4200 L^{-1}.

Auction price of made tea (BTRI) in 2014 @ 194.00 Tk kg^{-1}; [1]Gross return: Yield x price of a particular product; [2]Net return: Gross return - Variable cost.

According to CIMMYT (1988), a treatment is said to be dominated when there is at least one option that offers a greater net return at an equal or lesser cost and a treatment is undominated when no other options exist offering a greater net return at an equal or lesser cost. The treatments of Oberon 240 SC and Intrepid 10 SC with variable cost of Tk. 5040 ha^{-1} and Tk. 5400 ha^{-1}, respectively, were cost dominated due to its higher cost compared to lower net return (Fig. 3). So these two treatments were eliminated for further analysis.

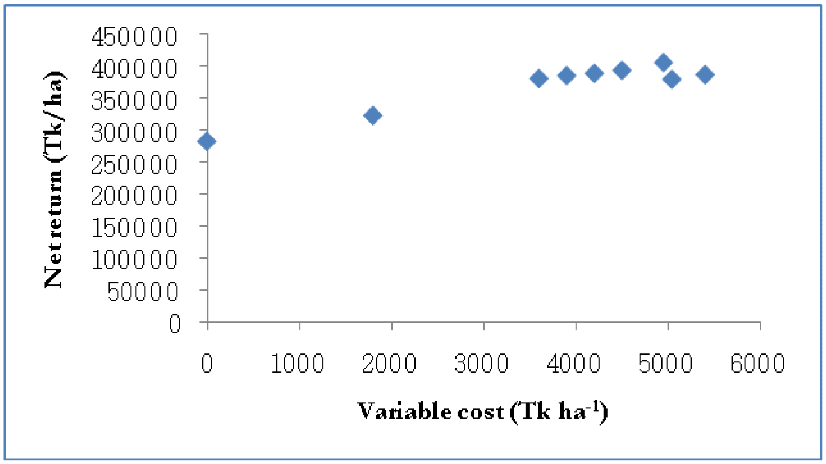

Fig. 3. Dominance analysis of different miticides used for controlling red spider mite in tea

The performances of cost-undominated treatments have been shown through marginal analysis in Table 6. The purpose of marginal analysis was to reveal how the net return from investment increased as the amount of investment increased (CIMMYT, 1988). It was observed that Magister 10 EC showed the highest marginal rate of return (3219.77%) followed by Mite scavenger 10 EC (2680.24%), Abom 1.8 EC (2233.82%), Mitisol 5 EC (1541.89%), Danitol 10 EC (1538.01%) and Omite 57 EC (1175.23%). It indicated that if the planters would spend an additional one hundred taka more by applying Magister 10 EC (Fenazaquin) they could get an extra income of Tk. 3219.77 over the control.

Table 6. Marginal analysis of different miticidal treatments applied for controlling red spider mite in tea.

Treatments	Net return (Tk ha^{-1})	Variable cost (Tk ha^{-1})	[1]Marginal net return (Tk ha^{-1}) (a)	[2]Marginal variable cost (Tk ha^{-1}) (b)	Marginal rate of return (%) (a/b x 100)
Mite scavenger 10 EC	405412.38a	4950.00	12061.06	450.00	2680.24b
Mitisol 5 EC	393351.32b	4500.00	4625.66	300.00	1541.89d
Omite 57 EC	388725.66c	4200.00	3525.68	300.00	1175.23e
Danitol 10 EC	385199.98c	3900.00	4614.02	300.00	1538.01d
Magister 10 EC	380585.96d	3600.00	57955.88	1800.00	3219.77a
Abom 1.8 EC	322630.08e	1800.00	40208.76	1800.00	2233.82c
Control	282421.32f	0.0	0.0	0.0	0.0

[1]Marginal Net Return: The increase in revenue of a farm caused by increasing one extra unit of inputs.
[2]Marginal Variable Cost: The increase in the variable cost of farm caused by increased output by one extra unit.

Effectiveness and tea yield were significantly higher in Hexythiazox (Mite scavenger 10 EC) treated plots @ 0.5 L ha^{-1}. From the economic point of view, Fenazaquin (Magister 10 EC) showed the highest marginal rate of return compared to all other miticides. So, Magister 10 EC @ 0.6 L ha^{-1} is the most economically acceptable miticide for controlling red spider mite in tea.

Conclusion

Many experts promote IPM as the best approach to make plant protection more sustainable. Recognizing general IPM principles, Hoy (2011) points out that effective IPM programs require multiple tactics such as monitoring of plant-feeding mites and their natural enemies (in order to determine if the pest population exceeds the economic injury level), cultural controls (all modification to agronomic practices that are intended to reduce pest damage), host plant resistance, biological control (mostly release of commercially produced phytoseiid mites and other natural enemies), and regulatory methods. Chemical control (using intrinsically selective or operationally compatible acaricides) is considered as the last resort, i.e., if other applied measures are not sufficient in keeping the pest population below the economic threshold. Pesticide is one of the fastest and popular pest control method of IPM. At present insecticide spraying is the principal method of insect pest control for tea in Bangladesh. To avoid resistance of pest pesticide should be sprayed minimal. Therefore, insecticide spraying is essential for control of pests in the bud opening period. On the other hand, for the pests infesting other parts of the tea plant, the effects of natural enemies are substantial. Insecticide spraying is restricted only when the pest density exceeds EIL, and the effects on the natural enemies are considerable for the selection of chemicals. Good evidence was obtained from the present experiment that among the tested miticide, Hexythiazox, Fenazaquin and Spiromesifen showed the most toxic effects on red spider mites. Planters may use these chemicals judiciously for the quick management of red spider mites in tea.

References

Abbott, W.S. 1987. A method of computing the effectiveness of an insecticide. *Journal of American Mosquito Control Association.* 3: 302-303.

Ahmed, M. 2005. Tea Pest Management. Evergreen Printing and Packaging. Dhaka. 118p.

Ahmed, M. and Sana, D.L. 1990. Biological aspects of red spider mite, *Oligonychus coffeae* (Nietner) in tea. *Bangladesh Journal of Zoology.* 18(1): 75-78.

Ahmed, M., Mamun, M.S.A., Hoque, M.M. and Chowdhury, R.S. 2012. Influence of weather parameters on red spider mite – a major pest of tea in Bangladesh. *SUST Journal of Science and Technology.* 19(5): 47-53.

Ahmed, M., Paul, S.K. and Mamun, M.S.A. 2011. Field performance and economic analysis of some commonly used insecticides against tea mosquito bug (*Helopeltis theivora* Waterhouse). *Bangladesh Journal of Agricultural Research.* 36(3): 449-454.

Ahmed, M., Saha, J.K. and Gazi, S.U. 1998. Economics of spraying Endosulfan and synthetic pyrethroids against tea mosquito bug (*Helopeltis theivora* Waterhouse). *Two and a Bud.* 45(2): 11-14.

Ali, M.A., Ahmed, M. and Haq, M.A. 1994. Crop loss due to red spider mites in tea. *Bangladesh Journal of Zoology.* 22(2): 197-202.

Amjad, M., Bashir, M.H., Gogi, M.D., Aslam, M., Zia, K. and Khan, M.A. 2012. Evaluation of some acaricides against two spotted spider mites, *Tetranychus urticae* Koch (Acarina: Tetranychidae) on cotton crop under laboratory and field conditions. *Pakistan Entomologist.* 34(2): 125-129.

BTB. 2015. Statistics on Tea. Bangladesh Tea Board, Nasirabad, Chittagong. pp. 1-12.

Chen, Z.M. and Chen, X.F. 1989. An analysis of world tea pest fauna. *Journal of Tea Science.* 9: 13-22.

CIMMYT. 1988. From agronomic data to farmer recommendations: An economics workbook. International Maize and Wheat Improvement Center (CIMMYT), Mexico. 79p.

Das, G.M. 1959. Bionomics of the tea red spider mite, *Oligonychus coffeae* (Nietner). *Bulletin of Entomological Research.* 50: 265-274.

Dekeyser, M.A. 2005. Acaricide mode of action. *Pest Management Science.* 61: 103–110.

Dekeyser, M.A. and Downer, R.G.H. 1994. Biochemical and physiological targets for miticides. *Pesticide Science.* 40: 85–101.

Dutta, N.K., Alam, S.N., Uddin, M.K., Mahmudunnabi, M. and Khatun, M.F. 2012. Population abundance of red spider mite in different vegetables along with its spatial distribution and chemical control in brinjal (*Solanum melongena* L.). *Bangladesh Journal of Agricultural Research.* 37(3): 399-404.

Evans, G.O. 1992. Principles of Acarology. Willingford: CAB International, Cambridge, U.K. 563p.

Henderson, C.F. and Tilton, E.W. 1955. Tests with acaricides against brown wheat mite. *Journal of Economic Entomology.* 48: 157–161.

Jeppson, L.R., Keifer, H.H. and Baker, E.W. 1975. Mites injurious to Economic plants. University of California Press, London. pp. 307-315.

Knowles, C.O. 1997. Mechanisms of resistance to acaricides. pp. 58–78. *In:* Molecular mechanisms of resistance to agrochemicals. Sjut V, Butters JA (eds). Springer, Berlin.

Mamun, M.S.A. 2011. Integrated approaches to tea pest management in South India: Away of sustainable tea cultivation. (ISBN: 978-3-8465-5088-5). LAP LAMBERT Academic Publishing GmbH & Co. KG DudweilerLandstr. 99, 66123 Saarbrücken, Germany. 68p.

Mamun, M.S.A. and Ahmed, M. 2011. Integrated pest management in tea: Prospects and future strategies in Bangladesh. *J. Plant Protection Sciences,* 3(2): 1-13.

Mamun, M.S.A., Paul, S.K. and Ahmed, M. 2014. Approved insecticides, miticides and nematicides for tea (Revised and Updated). BTRI Circular no. 137, Bangladesh Tea Research Institute, Srimangal, Moulvibazar. pp. 1-7.

Mehmood, M.A., Shah, F., Karar, H. and Ahmad, N. 2012. Performance of different insecticides against flower thrips (Thysanoptera: Thripidae) on rose cultivar 'Wisky Mac'. *Pakistan Entomologist.* 34(2): 173-177.

Pitterna, T. 2007. Chloride channel activators/new natural products (avermectins and milbemycins). pp. 1069–1088. *In:* W. Kramer and U. Schirmer (eds). Modern crop protection compounds, Volume 3. Wiley-VCH Verlag GmbH and Co., Weinheim.

Radhakrishnan, B., Selvasundaram, R. and Muraleedharan, N. 1999. Effect of Fenpropathrin on red spider mite. *Newsletter of UPASI Tea Research Institute.* 9(2): 3.

Roy, S., Gurusubramanian, G. and Mukhopadhyay, A. 2009. Detection of acaricide susceptibility in field populations of tea red spider mite, *Oligonychus coffeae* in North Bengal through in situ bioassay method. *Current Biotica.* 2: 251-254.

Sana, D. L. 1989. Tea Science. Ashrafia Boi Ghar, 36, Bangla Bazar, Dhaka, Bangladesh. pp. 106-200.

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EFFECTS OF EXPLANT TYPE AND PLANT GROWTH REGULATORS ON CALLOGENESIS IN SEEDLING DERIVED TEA EXPLANTS

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EFFECTS OF EXPLANT TYPE AND PLANT GROWTH REGULATORS ON CALLOGENESIS IN SEEDLING DERIVED TEA EXPLANTS

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Abstract

An experiment was conducted at the State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, Anhui, China to study the effects of explant type and plant growth regulators on callogenesis in seedling derived tea explants. Different types of induced calli were also further tested for proliferation. Results suggested that cotyledon explants significantly induced the highest amount of calli (mg·explant^{-1}), whereas callus induction efficiency (mg·mg^{-1} DW of explant) was highest in the leaf explants. Among the supplements tested, 2 mg·L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) in combination with 1 mg·L^{-1} benzyl adenine (BA) was most effective at inducing calli irrespective of explant type. Results suggested that to obtain the highest amount (mg·explant^{-1}) of callus from a specific type of explant, the cotyledon and root explants can be cultured on the medium containing 2,4-D (2 mg·L^{-1}) and kinetin (1 mg·L^{-1}); the leaf explants on the medium containing naphthalene acetic acid (NAA) (2 mg·L^{-1}) and BA (1 mg·L^{-1}); and the stem and hypocotyl explants on the medium containing 2,4-D (2 mg·L^{-1}) and BA (1 mg·L^{-1}). Results suggested that cotyledon derived calli could be a suitable choice for further long term proliferation on the B5 medium supplemented with 2,4-D (0.25 mg·L^{-1}) and kinetin (0.1 mg·L^{-1}).

Keywords: Callus, Induction, Proliferation, Dry weight.

Introduction

Plant tissue culture, or the aseptic culture of cells, tissues, organs, and their components under defined physical and chemical conditions *in vitro*, is an important tool in both basic and applied studies as well as for commercial applications (Thorpe, 1990). Tea plants synthesize many medicinally important secondary metabolites (Li, 2015; Muthaiya et al., 2013), and their commercial production through cell or tissue culture can be a valuable tool. The tea plant synthesizes more than 700 chemical constituents, among which flavonoids, amino acids, vitamins (C, E, and K), caffeine and polysaccharides are required for health (Mondal et al., 2004). Billions of people consume the tea beverage daily due to its pleasant flavour and health benefits against chronic pathologies such as cancer (Butt et al., 2015) and cardiovascular diseases (Stangl et al., 2007). The beverage is also known to have anti-aging, anti-inflammatory, antibacterial and anti-fungal properties (Friedman, 2007; Yang et al., 2006). The commercial utilization of some of these secondary metabolites from tea plant as

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important raw materials for the pharmaceutical (and food) industries shows great potential (Mondal et al., 2004), and could be of great benefit to humankind. One basic requirement for their utilization at the commercial level is constant availability with standardized quality. However, inconsistent environmental factors result in variable metabolite production, and genetic factors such as high heterozygosity further compound this problem (Mishra and Rakhi, 2008). Biotechnology offers an opportunity to exploit the cells, tissues or organs by growing them *in vitro* in a controlled environment to obtain the desired compounds at a constant rate by selecting suitable best cell lines. Secondary metabolites in the *in vitro* cultured plant cells and tissues can also be synthesized at higher level than in the primary explants (Dubravina et al., 2005).

As the initial step in the culture of plant cells and tissues *in vitro*, it is necessary to induce calli from the primary explant. A callus is a proliferation of cells in response to injury from the wounded or cut region of an explant (Smith, 2012). Callus tissue cultured *in vitro* provides a relatively simple system of predominantly undifferentiated cells, and when cultured on suitable media, it may be maintained indefinitely by periodic subculturing. Such callus cultures may be grown on media of known composition and subjected to a range of controlled environmental conditions, alleviating the difficulties of rigidly controlling the environment of an intact plant (Forrest, 1969). Once established, callus cultures may be used for a variety of experiments such as studying protoplast isolation, cell suspension culture, secondary product production, somatic embryogenesis, organogenesis and genetic transformation. In addition, callus culture can provide the opportunity to develop large-scale and continuous production of target tissues for genetic transformation of a recalcitrant species like tea, where a large amount of initial explant material is required for the transformation attempt.

The explant for callus induction may be the different parts of an aseptically germinated seedling or surface-sterilized roots, stems, leaves, or reproductive structures; not all types of explants may be suitable for callus induction, and not all cells in an explant contribute to the formation of the callus (Smith, 2012). The level of plant growth regulators (auxins, cytokinins, gibberellins, etc.) in the culture media are also important for the formation of callus from explants. Concentrations of the plant growth regulators for callus induction may vary for different species and genotypes, as well as for different explant types. Thus, this study was conducted to identify the most appropriate explant source, and to test different combinations of pant growth regulators for effective callogenesis on the seedling derived tea explants of *Camellia sinensis* var. *sinensis* (cv. Nong Kangzao). In addition, the generated calli from different tea explants were also further cultured to monitor their phenotypic changes and proliferation efficiency to identify the best one for long term proliferation.

Materials and Methods

This research was conducted in the Sate Key Laboratory of Tea Plant Biology & Utilization, 210 Biological Science Building, Anhui Agricultural University, Hefei, Anhui, China. Different medium components and chemicals used for the research are purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China) and from Sigma-Aldrich (St. Louis, USA). The Gamborg (B5) medium (Gamborg et al., 1968), also used in this study, was purchased from Qingdao Hope Bio-Technology Co. Ltd (Henan, China).

Treatments

In this study, different tea explants and media were tested step by step for callus induction and proliferation. Based on the result of one experiment, the next experiment was conducted with selective treatments which are summarized below.

For callus induction, two sets of treatments were included together in the same experiment, which are as follows:

A. Explant: 5

i)	Leaf	– (E1)
ii)	Stem	– (E2)
iii)	Cotyledon	– (E3)
iv)	Hypocotyl	– (E4)
v)	Root	– (E5)

B. Media: 4

i)	½ MS + 2,4-D (2 mg·L^{-1}) + Kinetin (1 mg·L^{-1})	– (M1)
ii)	½ MS + 2,4-D (2 mg·L^{-1}) + BA (1 mg·L^{-1})	– (M2)
iii)	½ MS + NAA (2 mg·L^{-1}) + Kinetin (1 mg·L^{-1})	– (M3)
iv)	½ MS + NAA (2 mg·L^{-1}) + BA (1 mg·L^{-1})	– (M4)

Callus proliferation experiment was conducted in two steps. Initially, calli generated from five different types of tea explants were used as five different treatments of the experiment which are as follows:

i) Leaf calli
ii) Stem calli
iii) Cotyledon calli
iv) Hypocotyl calli
v) Root calli

In the second step, one group of calli (cotyledon derived) that were expected to have the potential to be used for continuous proliferation based on phenotypic appearances were further tested into two different types of media for long term proliferation, which are as follows:

i)	½ MS + 2,4-D (0.25 mg·L^{-1}) + Kinetin (0.1 mg·L^{-1})	– (MS)
ii)	B5 + 2,4-D (0.25 mg·L^{-1}) + Kinetin (0.1 mg·L^{-1})	– (B5)

The above two media were also supplemented with 1 mL·L^{-1} Gamborg's vitamin solution (1000×).

Experimental design

The callus induction experiment was carried out in a two factor factorial (5×4) arrangement with three replications. Proliferation experiments with five different types of calli were carried out in a completely randomized design with three replications. For these experiments, each Petri plate containing 6 explants was considered as a single replication. Another proliferation experiment with cotyledon derived calli was carried out in a completely randomized design with ten replications, and each Petri plate containing of 10 explants was considered as a single replication.

Tea seed sterilization and raising aseptic seedling

Mature seeds were collected from a seven-year-old tea plant (*Camellia sinensis* var. *sinensis*) cv. "Nong Kangzao" grown at the experimental tea farm of Anhui Agricultural University, Hefei, China. The seeds with shells (seed coats) were surface disinfected with 70% (*v*/*v*) ethanol for 2 min and then with 1.5% (*v*/*v*) sodium hypochlorite for 10 min, followed by three washes with sterile distilled water. Surface sterilized seeds were blot-dried and de-shelled under aseptic conditions. For germination, the seeds were then placed on half-strength Murashige and Skoog (MS) basal salts medium (Murashige and Skoog, 1962) supplemented with 30 $g \cdot L^{-1}$ sucrose and 7 $g \cdot L^{-1}$ agar (pH 5.7) and maintained at 25 ± 1 °C with a 16 h photoperiod.

Preparation of explants and incubation for callus induction

For callus induction, eight-week-old *in vitro* grown tea seedlings were used in this study. Different types of tea explants (leaf, stem, cotyledon, hypocotyl, and root) were excised from the seedlings (Figure 1) and they were cut into smaller pieces. The stem, hypocotyl and root explants were cut into 2–3 cm pieces, the leaf explants into about 1.2×1.8 cm rectangular pieces, and the cotyledon explants into 0.4–0.5 cm slices. The leaf, stem, hypocotyl and root explants were wounded with a sterile scalpel and then placed onto the media with different hormone supplements following randomization. The kinetin containing cultures were incubated in the dark with a 16 h photoperiod at 25±1 °C for the induction of calli. They were sub-cultured at four-week intervals until data collection.

Preparation of media with different supplements

In this study, media were prepared with different hormone supplements according to the treatments. Except B5 medium, all the media were also supplemented with 30 $g \cdot L^{-1}$ sucrose and solidified with 7 $g \cdot L^{-1}$ agar. The pH of the media were adjusted to 5.5 before autoclaving at 121 °C for 20 min. Hormones were filter-sterilized and added when the autoclaved media was cooled to about 50 °C.

Determination of dry weight to fresh weight ratio of different tea explants

To ease the process of determining dry weight of induced callus with higher accuracy, dry weight (DW) to fresh weight (FW) ratio (DW/FW) of different tea explants were determined. For this purpose, different types of tea explants (leaf, stem, cotyledon, hypocotyl, and root) were prepared from the eight-week-old *in vitro* grown tea seedlings as per the preparation method for callus induction. The fresh weight of 30 explants of each were measured. Then the explants were dried into an electric oven at 50 °C until a constant weight is achieved to measure their dry weight. Then the ratio of dry weight to fresh weight (DW/FW) of each explant type was determined.

$$\text{Dry weight to fresh weight ratio (DW/FW)} = \frac{\text{Dry weight (DW)}}{\text{Fresh weight (FW)}}$$

Determination of dry weight of induced callus

The fresh weights of different tea explants were first measured before incubation to the callus induction medium. These fresh weights were then converted to dry weights by multiplying with DW/FW ratios of specific explant type to get the initial dry weights of explants. After 10 weeks of incubation in the callus induction medium, the explants together with the generated callus were dried into an electric oven at 50°C until a constant weight is achieved to get the final dry weights. Then the dry weights of the generated calli from different tea explants were obtained from the differences of initial and final dry weights.

$$\text{Initial dry weight of an explant} = \text{Fresh weight} \times (\text{DW/FW}) \text{ ratio}$$

$$\text{Dry weight of induced callus} = \text{Final dry weight - Initial dry weight}$$

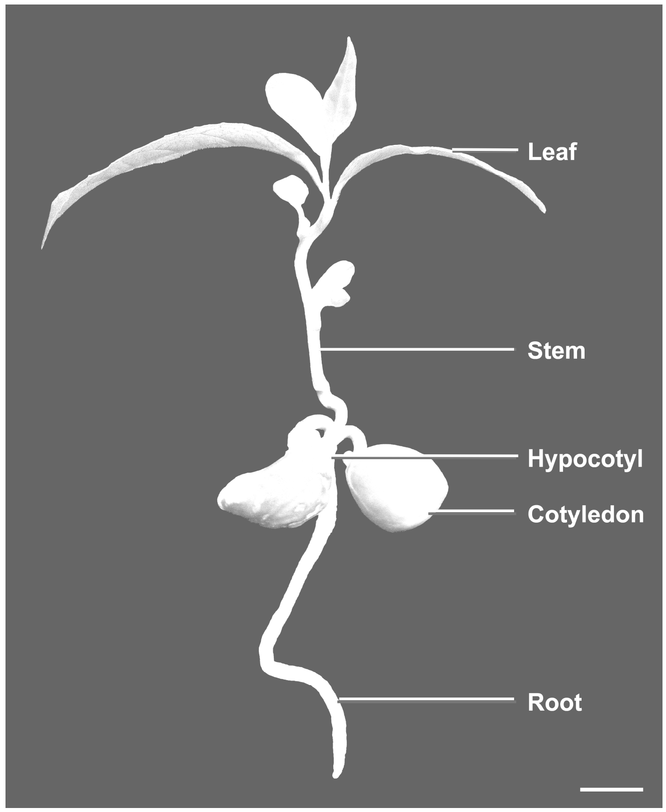

Figure 1. An eight-week-old *in vitro* grown tea seedling showing different plant parts. Scale bar = 1 cm.

Data collection on callus proliferation

To observe the proliferation efficiency of the calli generated from different tea explants, another group of explants were also incubated in the same culture conditions. After 10 weeks of incubation, the generated calli were aseptically separated from the explants. Treatment wise, almost equal amounts (weights) of five different types of calli were separately incubated into the MS (half-strength) medium supplemented with 2 $mg \cdot L^{-1}$ 2,4-D and 1 $mg \cdot L^{-1}$ BA at 25 ± 1 °C in the dark for proliferation. After 8 weeks of proliferation, the dry weights of each type of calli were determined. For the long term proliferation study with cotyledon derived calli, the calli were maintained for about a year with frequent sub-culturing at four-week intervals. During this period, their growth and phenotypic appearance were monitored; and the growth data of one of their respective early stage cultures was compared and presented.

Statistical analysis

All the experiments were carried out with three independent biological replicates. The results were expressed as mean value ± either standard error (SE) or standard deviation (SE). Data were analyzed using ANOVA, using the statistical package SPSS (Version 15.0), and statistical differences between means were compared by Duncan's multiple range test (DMRT).

Results and Discussion

Dry weight to fresh weight ratio of different tea explants

From the results, differences were observed dry weight to fresh weight ratios (DW/FW) of different tea explant (Table 1). The highest DW/FW ratio was obtained from stem explants, followed by leaf, hypocotyl and root explants. The lowest ratio was obtained in the cotyledon explants. Park and Kim (1993) used the ratio of dry weight to fresh weight (DW/FW) as an index of the cell water content or cell size. Thus, the result of this study suggests that cells of stem explants of tea seedling possess the lowest water content while the cotyledon explants possess the highest water content.

Table 1. Dry weight to fresh weight ratio of different tea explants

Type of explant	No. of explants	Fresh weight (g)	Dry weight (g)	Ratio (DW/FW)
Leaf	30	0.572	0.175	0.306
Stem	30	1.875	0.608	0.324
Cotyledon	30	14.742	2.390	0.162
Hypocotyl	30	5.347	1.230	0.230
Root	30	2.532	0.498	0.197

Effect of explant type on callus induction

After 10 weeks of incubation, it was observed that different types of explant induced callus with varying phenotypes and to different extents (Figure 2). Colour of the calli varied from greenish to yellowish, and textures were from loose to compact (Figure 2a). The textures of stem and leaf calluses were comparatively loose, whereas others were compact (Figure 2b). Khaliq et al. (2002) also reported variation in callus phenotype obtained from different tea explants. In their study with stem, leaf, and anther culture, they observed soft to compact texture and greenish yellow to yellow colour of the calli. In this study, callus induction rate and efficiency by different types of explants also varied significantly ($p < 0.05$). Cotyledon explants induced significantly higher amounts of callus (~ 42.658 $mg \cdot explant^{-1}$) followed by hypocotyl (~ 29.200 $mg \cdot explant^{-1}$), leaf (~ 26.428 $mg \cdot explant^{-1}$), and stem (~ 15.272 $mg \cdot explant^{-1}$) explants, and the lowest was obtained from the root (~ 13.044 $mg \cdot explant^{-1}$) explants (Figure 3). The variations in induction between hypocotyl and leaf explants, and between stem and root explants were not statistically different ($p > 0.05$).

Considering callus induction efficiency (per mg DW of explant), a different trend was observed. The leaf explants induced calli with extraordinarily high efficiency (~ 2.739 $mg \cdot mg^{-1}$ DW of explant), followed by hypocotyl (~ 1.098 $mg \cdot mg^{-1}$ DW of explant), root (~ 0.802 $mg \cdot mg^{-1}$ DW of explant) and stem (~ 0.755 $mg \cdot mg^{-1}$ DW of explant) explants (Figure 3). Interestingly, the cotyledon explants that induced highest amount of callus per explant showed the lowest induction efficiency (~ 0.699 $mg \cdot mg^{-1}$ DW of explant). However, the induction efficiency of root and stem explants did not differ statistically ($p > 0.05$) with the cotyledon explants.

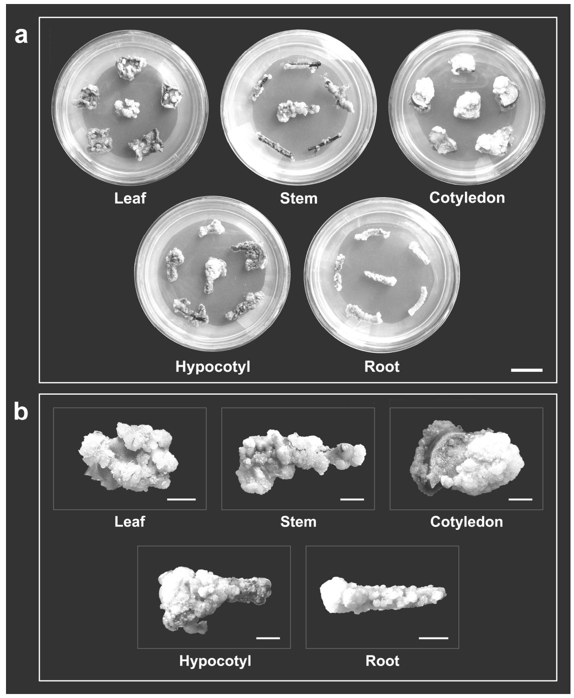

Figure 2. Induced callus on different tea explants after ten weeks of culture. (a) Plates showing the induced calli from different tea explants. Scale bar = 2 cm; (b) Close up view of five different types of tea explants along with the induced callus. Scale bar = 5 mm.

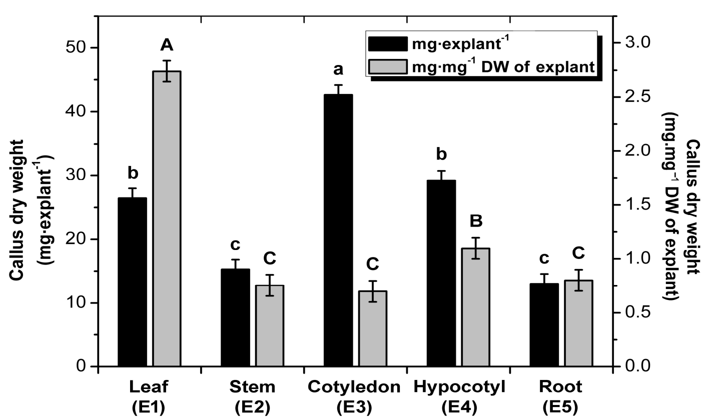

Figure 3. Effect of explant type on the induction of callus from tea explants after ten weeks of culture. Data show the mean ± standard error (SE) (n = 12). Statistical significances were analyzed using ANOVA. Means followed by the same letter are not significantly different ($p > 0.05$); capital and lowercase letters are used to distinguish two different groups of means.

Effect of media on callus induction

Callus induction rate and efficiency from the tea explants varied significantly ($p < 0.05$) by different media (Figure 4). Among the four types of media tested, significantly highest ($p < 0.05$) amount of callus per explant was obtained in the M2 (~ 33.376 mg·explant^{-1}) followed by M4 (~ 25.702 mg·explant^{-1}) and M1 (~ 25.476 mg·explant^{-1}), and the lowest was obtained from the M3 (~ 16.727 mg·explant^{-1}). The effects of the media M4 and M1 on the callus induction per explant were found statistically identical. Considering callus induction efficiency (per mg DW of explant), almost same trend was obtained, and it was expected. Since the callus induction rate (mg·explant^{-1}) and efficiency (mg·mg^{-1} DW of explant) by different types of explants may show different trend because of their competency to callus induction, but in a specific type of media the amount of callus induced per explant would be correlated with the induction efficiency of the explant. Thus, similar with the induction rate, callus induction efficiency was highest in the M2 (~ 1.747 mg·mg^{-1} DW of explant) followed by M4 (~ 1.504 mg·mg^{-1} DW of explant), M1 (~ 0.994 mg·mg^{-1} DW of explant), and the lowest was obtained from the M3 (~ 0.630 mg·mg^{-1} DW of explant). The induction efficiency by the media M2 and M4 were not statistically different ($p > 0.05$). Results suggested that in combination with a cytokinin, supplementation of 2,4-D was better than NAA in inducing callus from tea explants. Seran et al. (1999) also reported variation in callus induction from tea anther cultures due to different medium supplements. In addition, they mentioned that non-phenoxy auxins like NAA, IAA, or IBA are better to promote root initiation whereas phenoxy-auxin like 2,4-D promotes callus growth, which is in accordance with the result of this study. Again, it was also noticed that induction was always higher in the M2 and M4 media (supplemented with BA in combination with any of the auxin) compared to their counterparts in the M1 and M3 media (supplemented with kinetin in combination with any of the auxin). These suggests that BA was more effective over kinetin in inducing callus from tea explants irrespective of explant type. This result is in agreement with the result of Mishra and Rakhi (2008) who also noticed that when BAP was replaced with kinetin, only 52.5% cultures responded for callusing which was 76.6% with BAP, in tea anther cultures.

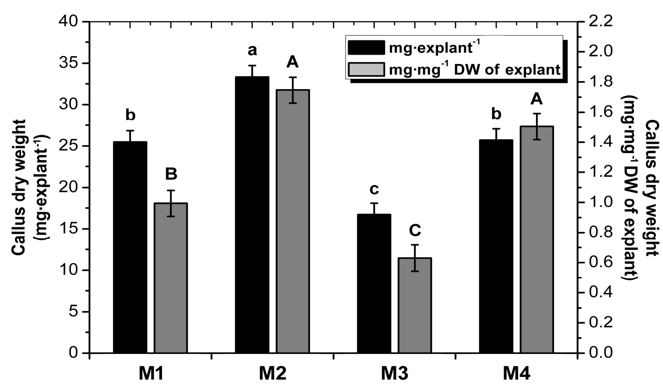

Figure 4. Effect of media on the induction of callus from tea explants after ten weeks of culture. Data show the mean ± SE (n = 15). Statistical significance was analyzed using ANOVA. Means followed by the same letter are not significantly different ($p > 0.05$); and capital and lowercase letters are used to distinguish two different groups of means.

Proliferation efficiency of different types of tea calli

Phenotypic appearances of different types of tea calli varied greatly after eight weeks of proliferation (Figure 5a). Colours of the cotyledon calli were whitish to yellowish, hypocotyl calli were from yellowish to brownish, and the others were in between. Leaf, stem, and root calli showed comparatively hard and compact textures, while the hypocotyl callus showed quite granular friable texture and the cotyledon callus was in between.

In this study, different tea calli showed significantly different ($p < 0.01$) proliferation rates (Figure 5b). Dry weight increment per callus was highest in the stem calli (57.234 ± 9.811 mg·callus^{-1}), followed by root calli (44.137 ± 10.362 mg·callus^{-1}), leaf calli (42.982 ± 9.140 mg·callus^{-1}), and cotyledon calli (27.498 ± 4.316 mg·callus^{-1}). Significantly lowest was obtained from the hypocotyl calli (19.189 ± 3.643 mg·callus^{-1}). However, the differences in the dry weight increment between stem, root, and leaf calli and in between hypocotyl and cotyledon calli were not statistically significant. Dry weight increment rate (mg·mg^{-1} DW of callus) also followed a similar trend. The highest rate was obtained in the stem calli (1.197 ±

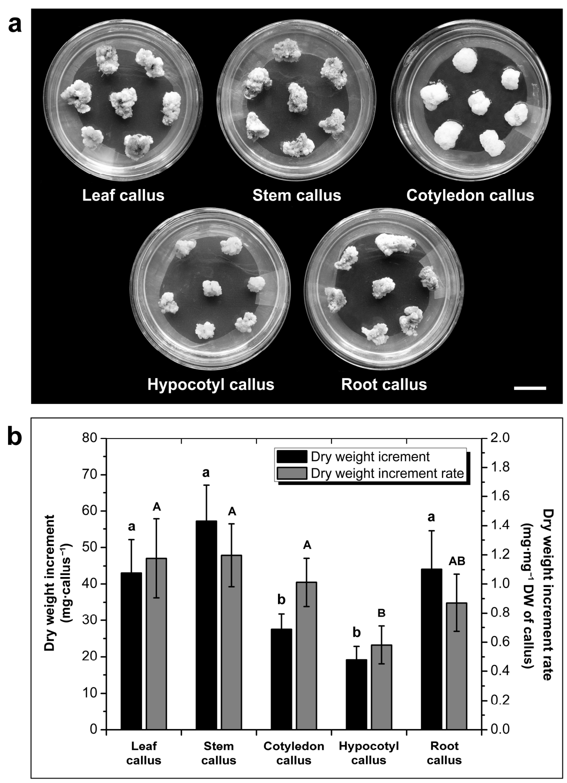

Figure 5. Proliferation efficiency of different types of tea calli (eight weeks of proliferation after induction). (**a**) Plates showing the comparative phenotypic appearances of different types of tea calli. Scale bar = 2 cm; (**b**) Comparative growth performances of different types of tea calli. Data show the mean ± SD (n = 3). Statistical significance was analyzed using ANOVA. Means followed by the same letter are not significantly different ($p > 0.05$); capital and lowercase letters are used to distinguish two different groups of means.

0.216 mg·mg⁻¹ DW of callus) followed by leaf calli (1.176 ± 0.271 mg·mg⁻¹ DW of callus), cotyledon calli (1.011 ± 0.165 mg·mg⁻¹ DW of callus) and root calli (0.870 ± 0.197 mg·mg⁻¹ DW of callus); the lowest was obtained in the hypocotyl calli (0.580 ± 0.130 mg·mg⁻¹ DW of callus). However, the differences in the increment rate between stem, leaf, cotyledon and root calli, and in between hypocotyl and root calli were not statistically different.

It is important to note that although the stem, root, and leaf calli showed higher dry weight increment after eight weeks of initial proliferation, their textures had become hard and non-friable. On the other hand, though the hypocotyl calli was somewhat friable, their growth was very slow. Thus, comparatively soft, somewhat friable and compact cotyledon calli were selected as a suitable candidate for further long term proliferation study. It is worth mentioning that due to the faster growth of the stem, root, and leaf calli, nutrients from the media may have been depleted early, and this could be the reason for their hard texture at the end of the culture period. It is possible that instead of four-week-interval, sub-culturing at 2−3 week-intervals could have resulted in better performance than the cotyledon calli. This requires further investigation for confirmation.

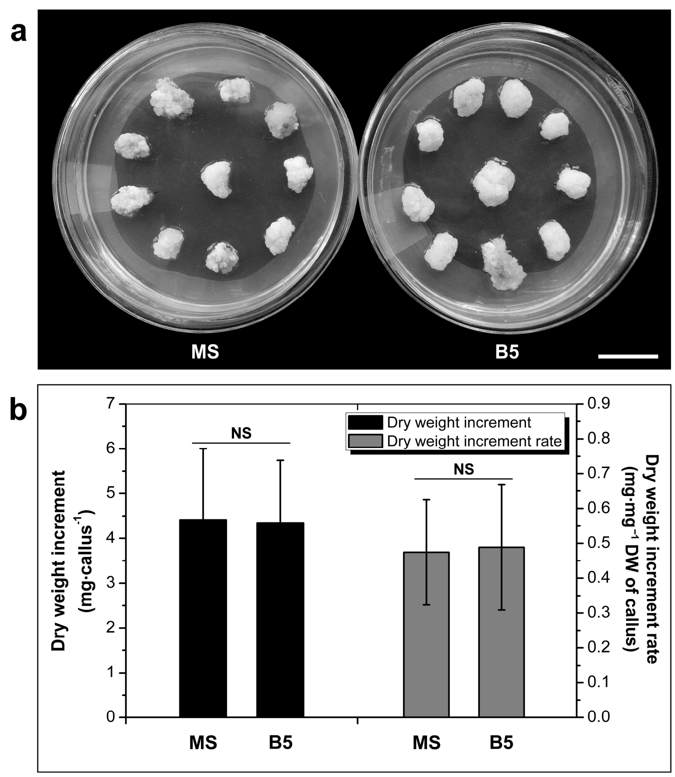

Figure 6. Proliferation efficiency of cotyledon derived tea calli into two different types of media (MS and B5). (**a**) Plates showing the comparative phenotypic appearances of the calli after four weeks of proliferation. Scale bar = 2 cm; (**b**) Comparative growth performances into two different media. Data show the mean ± SD (n = 10). Statistical significance was analyzed using ANOVA. NS, Not significant ($p > 0.05$).

Proliferation efficiency of tea cotyledon calli into different media

No great variations were observed in the phenotypic appearances of the cotyledon derived calli in the early subcultures in the two tested media supplemented with 2,4-D and kinetin (Figure 6a). Only a little browning appeared in the calli cultured in the MS media, compared to calli cultured into the B5 media. Colour of the calli were whitish in both media. Seran et al. (1999) also obtained whitish calli from tea anther culture in the medium supplemented with 2,4-D and kinetin. No significant differences (p > 0.05) were observed either by dry weight increment ($mg \cdot callus^{-1}$) or dry weight increment rate ($mg \cdot mg^{-1}$ DW of callus) for early subcultures of calli in the two tested media (Figure 6b). However, in later subcultures, the texture of the MS cultured calli became progressively harder. Conversely, the majority of the B5 cultured calli were soft, friable and compact. From these results it can be seen that in B5 media, dry weight increment ($mg \cdot callus^{-1}$) of the calli was tiny low, whereas dry weight increment rate ($mg \cdot mg^{-1}$ DW of callus) was slightly higher compared to the MS media (Figure 6b). As their sizes were similar, this result is indicating that calli cells on B5 media were slightly larger and contained more water, which likely contributed to their softer texture relative to the calli cultured on the MS media. The calli in the B5 media continued to grow and showed vigorous growth even after about eight months of culture (Figure 7). Therefore, the cotyledon derived tea calli in this study have been identified as having the potential for long term proliferation with satisfactory growth in B5 medium supplemented with 0.25 $mg \cdot L^{-1}$ 2,4-D and 0.1 $mg \cdot L^{-1}$ kinetin.

Figure 7. A vigorously growing line of the cotyledon derived calli after about eight months of culture. Scale bar = 5 mm.

Conclusion

In conclusion, it can be said that to induce highest amount ($mg \cdot explant^{-1}$) of callus from tea explants, the cotyledon explants can be cultured on medium containing 2,4-D (2 $mg \cdot L^{-1}$) and kinetin (1 $mg \cdot L^{-1}$); the leaf explants on medium containing NAA (2 $mg \cdot L^{-1}$) and BA (1 $mg \cdot L^{-1}$); the hypocotyl explants on the medium containing 2,4-D (2 $mg \cdot L^{-1}$) and BA (1 $mg \cdot L^{-1}$). For long term proliferation, cotyledon derived calli can be a suitable choice; and this calli can be cultured on B5 medium supplemented with 2,4-D (0.25 $mg \cdot L^{-1}$) and kinetin (0.1 $mg \cdot L^{-1}$) in combination with a vitamin (1 $mL \cdot L^{-1}$ Gamborg's vitamin solution) for vigorous growth along with a soft, friable and compact phenotype.

References

Butt, M.S., Ahmad, R.S., Sultan, M.T., Qayyum, M.M. and Naz, A. 2015. Green tea and anticancer perspectives: updates from last decade. Critical Reviews in Food Science and Nutrition, 55(6): 792–805. http://doi.org/10.1080/10408398.2012.680205

Dubravina, G.A., Zaitseva, S.M. and Zagoskina, N.V. 2005. Changes in formation and localization of phenolic compounds in the tissues of European and Canadian yew during dedifferentiation *in vitro*. Russian Journal of Plant Physiology, 52: 672–678.

Forrest, G.I. 1969. Studies on the Polyphenol Metabolism of Tissue Cultures Derived from the Tea Plant (*Camellia sinensis* L.). Biochemical Journal, 113: 765–772.

Friedman, M. 2007. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Molecular Nutrition & Food Research, 51: 116−134.

Gamborg, O.L., Miller, R.A. and Ojima, K. 1968. Nutrient requirements of suspension culture of soybean root cells. Experimental Cell Research, 50: 151–158.

Khaliq, A., Rashid, H. and Quraishi, A. 2002. Tissue culture studies of tea (*Camellia sinensis* L.). Pakistan Journal of Agricultural Research, 17(3): 297–301.

Li, C.F., Zhu, Y., Yu, Y., Zhao, Q.Y., Wang, S.J., Wang, X.C., et al. 2015. Global transcriptome and gene regulation network for secondary metabolite biosynthesis of tea plant (*Camellia sinensis*). BMC Genomics, 16: 560. DOI:10.1186/s12864-015-1773-0.

Mishra, V.K. and Rakhi, C. 2008. Evaluation of chemical and physical parameters for callus induction from anther cultures of tea (*Camellia sinensis* (L.) O. Kuntze). Research Journal of Biotechnology, Special Issue, 270–273.

Mondal, T.K., Bhattacharya, A., Laxmikumaran, M. and Ahuja, P.S. 2004. Recent advances of tea (*Camellia sinensis*) biotechnology. Plant Cell Tissue and Organ Culture, 76: 195–254.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15: 473–497.

Muthaiya, M.J., Nagella, P., Thiruvengadam, M. and Mandal, A.K.A. 2013. Enhancement of the productivity of tea (Camellia sinensis) secondary metabolites in cell suspension cultures using pathway inducers. Journal of Crop Science and Biotechnology, 16(2): 143–149. DOI:10.1007/s12892-012-0124-9.

Park, I.S. and Kim, D.I. 1993. Significance of fresh weight to dry cell weight ratio in plant cell suspension cultures. Biotechnology Techniques, 7: 627−630.

Seran, T.H., Hirimburegama, K., Hirimburegama, W.K. and Shanmugarajah, V. 1999. Callus formation in anther culture of tea clones, *Camellia sinensis* (L.) O.Kuntze. Journal of the National Science Foundation of Sri Lanka, 27(3): 165–175.

Smith, R.H. 2012. Callus induction. *In:* Plant Tissue Culture: techniques and experiments, 3rd Edition, Academic press, pp. 63–79. DOI:10.1016/B978-0-12-415920-4.00006-2.

Stangl, V., Dreger, H., Stangl, K. and Lorenz, M. 2007. Molecular targets of tea polyphenols in the cardiovascular system. Cardiovascular Research, 73(2): 348–358. http://doi.org/10.1016/j.cardiores.2006.08.022.

Thorpe, T.A. 1990. The current status of plant tissue culture. *In:* Bhojwani SS (ed), Plant tissue culture: applications and limitations. Elsevier, Amsterdam, pp. 1–33.

Yang, C.S., Lambert, J.D., Ju, J., Lu, G. and Sang, S. 2006. Tea and cancer prevention: Molecular mechanisms and human relevance. Toxicology and Applied Pharmacology. DOI:10.1016/j.taap.2006.11.024.

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