Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2016.16.25

Research Article
Physiological and Biochemical Changes in Tea Leaves and Made Tea Due to Red Spider Mite Infestation

[1,2]Mohammad Shameem Al Mamun, [1]Md. Mozammel Hoque, [2]Mainuddin Ahmed and [1]Md. Yasin

[1]Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet, Bangladesh
[2]Entomology Division, Bangladesh Tea Research Institute, Srimangal, Moulvibazar, Bangladesh

Abstract
Tea is a popular health beverage in the world made from the leaves of evergreen shrub *Camellia sinensis*. Red spider mite is one of the important mite pests of tea causing damage in respect of both quantity and quality. A study was undertaken to determine the effect of red spider mite infestation on the physiological and biochemical changes in tea leaves as well as made tea. Physio-biochemical parameters of green leaves and made tea were analyzed with standard methods using UV-visible spectrophotometer in the laboratory. Result revealed that the physiological and biochemical contents were significantly ($p>0.05$) reduced with the increase of infestation (from low to high) of red spider mites. Photosynthetic pigments such as chlorophyll a (2.0 mg g^{-1}) and b (1.05 mg g^{-1}), carotenoids (0.55 mg g^{-1}) and biochemical components such as polyphenols (45.56 ppm), catechins (10.40 ppm) and reducing sugar (34.57 ppm) in severely infested green leaves were found lower than the fresh leaves. Black tea manufactured from severely infested leaves had the lower amount of theaflavin (TF) (0.43%), high polymerized substances (6.16%), total liquor colour (2.20%), colour index (3.27), lipid (3.77%), caffeine (54.68 ppm), moisture content (0.63%) and water extract (22.66%) but it is reverse in case of thearubigin (TR) (7.00%), total ash (5.70%) and dry matter (99.37%). Made tea manufactured from mite infested shoots was valued inferior (31.65-32.90) when subjected to organoleptic evaluation. It is concluded that the infestation of red spider mites deteriorate the physiological and biochemical contents of tea leaves as well as quality of made tea.

Key words: Tea, *Camellia sinensis*, red spider mite, infestation, physiology, biochemistry, quality

Received: November 23, 2015 Accepted: January 30, 2016 Published: March 15, 2016

Citation: Mohammad Shameem Al Mamun, Md. Mozammel Hoque, Mainuddin Ahmed and Md. Yasin, 2016. Physiological and biochemical changes in tea leaves and made tea due to red spider mite infestation. Asian J. Plant Sci., 15: 16-25.

Corresponding Author: Mohammad Shameem Al Mamun, Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet, Bangladesh

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tea is a popular health beverage in the world made from the leaves of evergreen shrub *Camellia sinensis*, under the family Theaceae. Tea plant is subjected to the attack of insects, mites, nematodes and some plant pathogenic diseases. 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 and 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).

Mites 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, 1982). Four species of mites are recorded from Bangladesh tea (Ahmed *et al*., 2012). Red spider mite, *Oligonychus coffeae* Nietner (Acarina: Tetranychidae) is one of the major and serious pests of tea in almost all tea producing countries of South-East Asia and Africa (Sana, 1989). 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 (RSM) are responsible for depredation of yield and debilitation of tea plants causing considerable crop loss. Normally red spider mite attacks the upper surface of the mature leaves in which the sap is not flowing freely. The larvae, nymphs and adult mites cause the damage. Infestation starts along midrib, veins and gradually spreads to the entire upper surface of leaves. Red spider mites feed on the chlorophyll in the cells of leaves, damaging their ability to use photosynthesis. Because of feeding, the maintenance foliage turns ruddy bronze, making red spider infested fields distinct even from a distance. Severe infestation by this mite ultimately leads to defoliation (Ahmed, 2005).

Pest infestation deteriorates the quality of food, fruits and beverages including tea. The pest of tea also causes damage in relation to physio-biochemical properties in tea leaves as well as quality of made tea. Damage by sucking pests like thrips and mites resulted in dull appearance of tea. The tea leaves severe infested by flushworm, mites, thrips and tea mosquito bug adversely affected flavor and decreased the polyphenolic content (Murthy and Chandrasekaran, 1979; Sudhakaran *et al*., 2000). Tea made prepared from flushworm infested shoots had low levels of extractable solids and high crude fiber content (Murthy and Chandrasekaran, 1979). Liquors obtained from such tea were flat and the presence of excreta of the larvae was perhaps responsible for the deterioration in quality (Das, 1957). However, tea manufactured from cicadellid (*Empoasca flavescens* Fabr.) infested leaves had higher amount of pigments. An increase in polyphenol oxidase activity and total polyphenols were associated with the high pigment formation in teas (Mahanta *et al*., 1992).

From the economic point of view, the red spider mite is considered the major one causing both qualitative and quantitative damage to tea. The quantity of crop loss in respect of yield due mite infestation was assessed in different regions of the world (Ali *et al*., 1994; Cranham *et al*., 1962; Rattan, 1992). But there is very little information on the physiological and biochemical as well as quality deterioration of made tea caused by the infestation of red spider mite. The present study is aimed to determine the effect of red spider mite infestation on the physiological and biochemical changes in green leaves as well as quality of made tea.

MATERIALS AND METHODS

A study was undertaken to determine the physiological and biochemical changes in tea leaves and made tea due to the attack of red spider mites on tea. The study was conducted at Bangladesh Tea Research Institute (BTRI), Srimangal, Moulvibazar and the laboratory of Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh, during the period of January-December, 2013.

Sample collection: The pluckable green leaves from the tea bushes comprising of "Two leaves and a bud" were collected from BTRI main farm (D2 section) where the section was planted with red spider mite susceptible clone BT2. The experimental plots were divided into four categories such as T_1: Fresh leaves (no mites per leaf), T_2: Low infested leaves (<10 mites per leaf), T_3: Medium infested leaves (10-20 mites per leaf) and T_4: Highly infested leaves (>20 mites per leaf) and considered them as treatments (Fig. 1). The categorization was made according to the number of mites in the infested leaves. Each treatment was replicated thrice. Besides, the mature fresh and infested leaves from selected bushes were also collected for the analysis of chlorophyll a, b and carotenoid contents.

Laboratory analysis: Physiological parameters i.e., chlorophyll contents, carotenoids, polyphenols, catechins and reducing

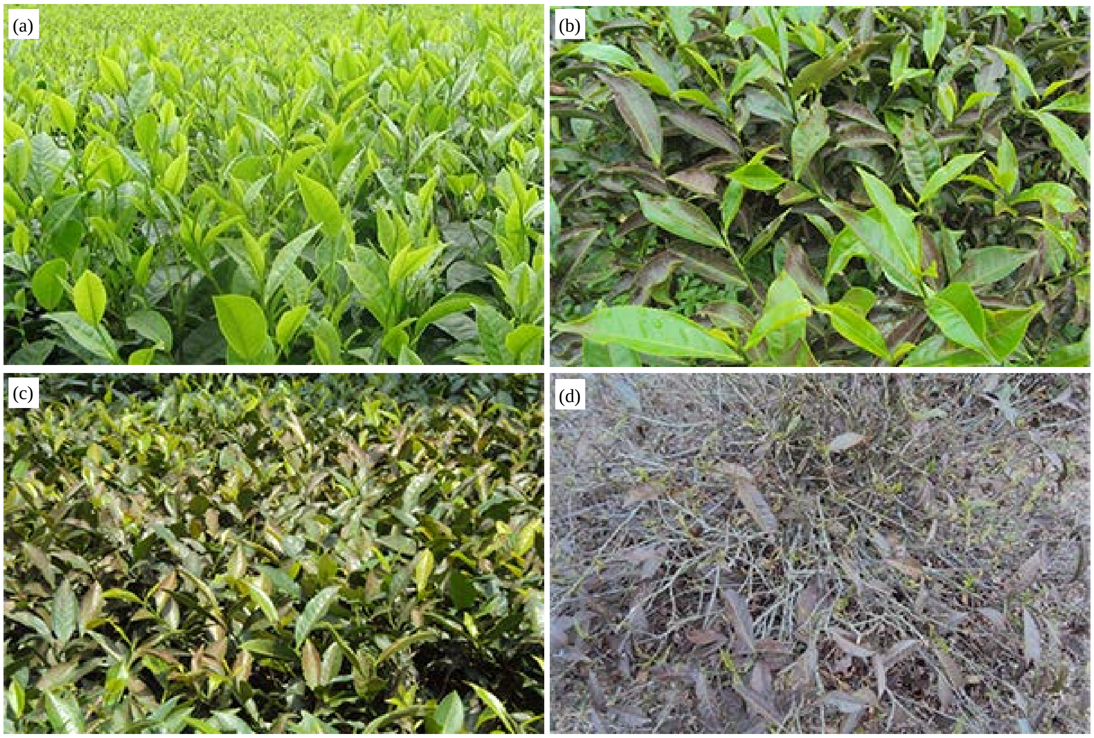

Fig. 1(a-d): Different categories of tea shoots, (a) Fresh tea leaves, (b) Low infested leaves, (c) Medium infested leaves and (d) Highly infested leaves by red spider mites

sugar in green leaves of tea and biochemical parameters i.e., theaflavin, thearubigin, highly polymerized substances, total liquor colour, lipids, caffeine, total ash, water extract, dry matter content and moisture content in made tea were analyzed with standard methods in the laboratory of the Department of Food Engineering and Tea Technology, SUST. The analytical procedure of the physio-biochemcial parameters in laboratory is enumerated.

Estimation of chlorophyll content and carotenoids: Chlorophyll contents of tea shoots were measured using the method suggested by Wellburn (1994). A tea shoot was weighed in table top balance and ground with a mortar and pestle with a sufficient quantity of prechilled methanol (100%). Finely ground sample was filtered and made up to 50 mL in a standard volumetric measuring flask using methanol and further diluted five times with methanol. Absorbance of the diluted methanolic extract was read at 470, 653 and 666 nm using UV-visible spectrophotometer against methanol as blank. Photosynthetic pigments i.e., chlorophyll a, b and carotenoids were calculated using the following formulae and the chlorophyll and carotenoids values were expressed as mg g^{-1}.

Chlorophyll a = (15.65 A_{666}-7.34 A_{653}), chlorophyll b = (27.05 A_{653}-11.21 A_{666}), carotenoids = (1000 A_{470}-2.86 chlorophyll a-129.2 chlorophyll b)/221.

Preparation of alcoholic extract: Tea shoot comprising two leaves and a bud (about 1.0 g) was grinded well with 100% ethyl alcohol. The contents were filtered and the filtrate was made up to 50 mL with ethyl alcohol. The alcoholic extract was used for estimation of polyphenols, catechins and reducing sugars. The total polyphenols, catechins and reducing sugar content were determined with using spectrophotometer with gallic acid, pure catechin and dextrose standard curve (Fig. 2).

Estimation of polyphenols: One milliliter of the alcoholic extract was diluted to 50 mL with distilled water. Two milliliters of diluted extract was added with 4 mL of 1:1 Folin-Ciocalteu's reagent and water mixture and 2 mL of 35% sodium carbonate. The contents were further made up to 10 mL with distilled water and the mixture was shaken thoroughly and allowed to stand still for 30 min. Absorbance of the blue color developed was read at 700 nm against the reagent as blank using UV-visible spectrophotometer. Quantum of polyphenols present in tea leaves was computed using the standard calibration curve derived from known

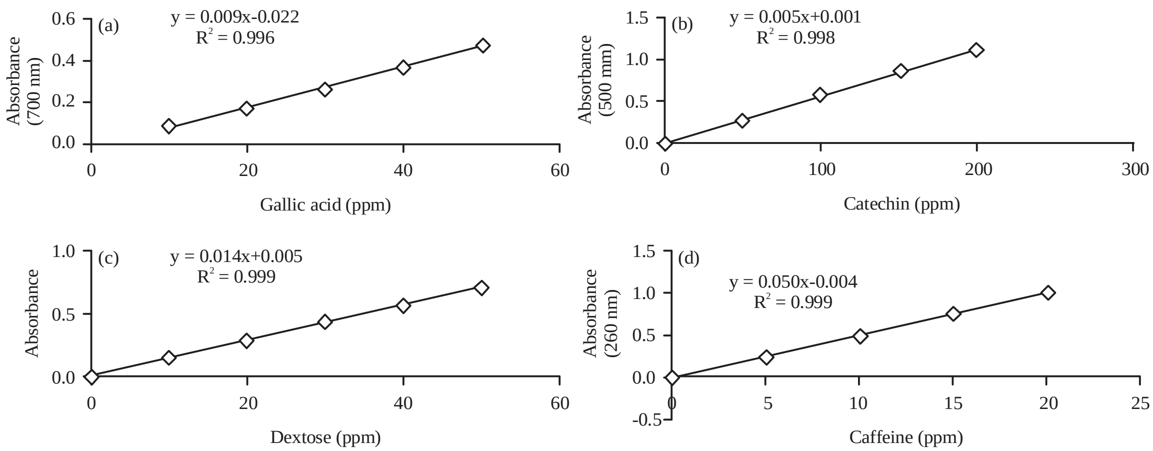

Fig. 2(a-d): Standard curve of, (a) Gallic acid, (b) Pure catechin, (c) Dextrose and (d) Caffeine

concentrations (10-50 ppm) of gallic acid and the results were expressed as percent gallic acid equivalents (Choudhary and Goswami, 1983).

Determination of catechins: One milliliter of the alcoholic extract was diluted to 50 mL with distilled water. Two milliliters of the diluted extract, 6.5 mL of ice cold vanillin (1% vanillin in 70% sulphuric acid) was added slowly to avoid immediate color development. The contents were made up to 10 mL with distilled water, shaken well and allowed to stand still for 15 min for completion of reaction. Absorbance of the orange color developed was read at 510 nm against the reagent as blank using UV-visible spectrophotometer. Amount of catechins present in tea leaves was calculated using the standard calibration curve computed with the values obtained against known concentrations (10-50 ppm) of (+) catechin and the results were expressed as percent catechin equivalents (Swain and Hillis, 1959).

Estimation of reducing sugar: Two milliliters of the alcoholic extract was diluted to 10 mL with distilled water. One milliliter of dilutent taken in a test tube was incubated under ice cold conditions; 4 mL of ice cold acidified anthrone reagent (0.2%, w/v in concentrated sulphuric acid) was added to it. Contents were then incubated in a boiling water bath for 8 min and cooled down to room temperature under running water. Absorbance of the green color developed was read at 630 nm against the reagent blank in a UV-visible spectrophotometer. Percent reducing sugars (dextrose equivalents) present in tea samples was computed using the standard calibration curve where known concentrations of (+) dextrose were used to derive calibration curve (Hedge and Hofreiter, 1962).

Determination of quality parameters of made tea: About 1.0 kg of each infested and fresh tea shoots were collected separately from the field and black tea was manufactured by using miniature Crush, Tear and Curl (CTC) unit, as reported by Thanaraj and Seshadari (1990).

Black tea manufacture: Tea green leaves were spread on a miniature withering trough. Ambient air was passed with a velocity of 45 CFU for a period of 16 h to reduce the moisture content and to bring about adequate physical and chemical wither. Withered leaves were passed through miniature CTC machine five times to get adequate maceration. Crushed sample was kept in a cabinet fermenter at 25°C and 95% relative humidity. Optimum fermentation time was assessed adopting the method as reported by Ullah (1977). Fermented dhool was dried in a drier at 120°C for 2 h, which reduce the moisture content to around 3%. Thus tea samples were manufactured and stored for analysis (Kumar, 2004).

Estimation of theaflavin (TF), thearubigin (TR), Highly Polymerized Substances (HPS) and Total Liquor Colour (TLC): Two grams of black tea sample were weighed and transferred in a 250 mL conical flask. To the tea sample, 100 mL of boiled water was added and the contents were infused over the boiling water bath for 10 min with intermittent shaking. It was then filtered through cotton wool and the analysis was carried out as per the scheme furnished. Solvent extraction of tea extract was carried out in separating funnels with adequate shaking at every stage. Contents of TF, TR, HPS and TLC were calculated from the absorbance values as follows as Fig. 3:

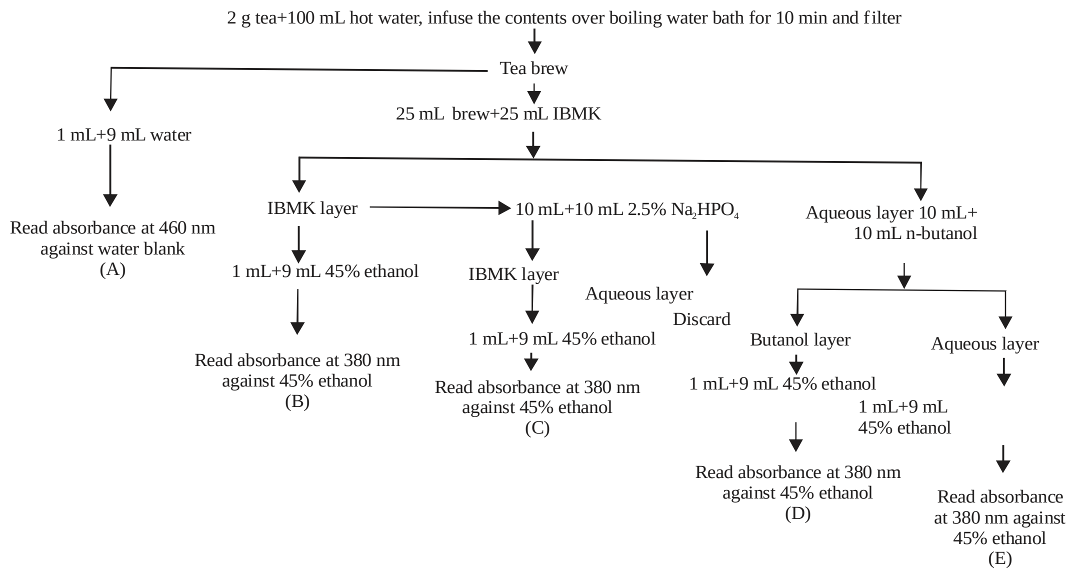

Fig. 3: Estimation of TF, TR, HPS and TLS

Where:

TF (%) = (4.313×C×2×100)/(Sample weight×DMC)
TR (%) = (13.643×(B+D-C)×2×100)/(Sample weight×DMC)
HPS (%) = (13.643×E×2×100)/(Sample weight×DMC)
TLC (%) = (10×A×2×100)/(Sample weight×DMC)

Multiplication factors of TF and TR were derived from molar extinction coefficients of pure compounds and dilution factor (Roberts and Smith, 1963). In the case of TLC, value 10 is the dilution factor (Thanaraj and Seshadari, 1990).

Colour index: Colour index was derived using the equation, as reported by Ramaswamy (1986):

$$CI = \frac{TF \times 100}{TR + HPS}$$

Quantification of lipids: Tea sample (1.0 g) was added to 25 mL of chloroform and methanol mixture (2:1) in a separating funnel. Five milliliters of 0.9% sodium chloride was added and the mixture was shaken well. The mixture was allowed to stand till there is separation of layers and the chloroform layer was carefully transferred to a pre-weighed china dish. Entire extraction procedure was repeated twice and extractants were pooled together. Pooled chloroform extract was evaporated to dryness on a boiling water bath. China dish with lipids was dried and weighed again. From the difference in weights, the percentage of lipids present in the leaf material was calculated gravimetrically and expressed as percent according to Ravichandran and Parthiban (2000).

Estimation of caffeine: For determining caffeine, 50 mL distilled water was heated at 40°C and then 100 mg tea sample was added to the hot water and stirrer for 30 min with magnetic stirrer. Then it was filtered and cooled to room temperature. Fifty milliliters of chloroform was poured into the tea infusion and stirred for 10 min with magnetic stirrer. The water phase was separated from the organic phase (chloroform) with a separating funnel. The organic solution was poured into quartz of UV cell and take absorbance in 260 nm. The sample was measured with precision and accuracy. Quantum of caffeine present in tea leaves was computed using the standard calibration curve derived from known concentrations (0-20 ppm) of caffeine and the results were expressed as percent caffeine equivalents (Maidon *et al*., 2012). Pure caffeine standard curve was needed to determine caffeine contents (Fig. 2).

Determination of total ash: Total ash was estimated by directly incineration of sample taken in a crucible according to the official methods of AACC (2000). About 2.0 g of sample were taken in a crucible and the material was charged carefully in a Bunsen burner to burn fatty substance. Then the sample was transferred to a muffle furnace and ash at a temperature of 550°C for 3 h. The dish was heated again at 550±10°C for 30 min and cooled in a desiccator and the weight was measured. This process of heating was repeated for 30 min, cooling in a desiccator and weighing until the difference between two successive weighing is less than 1.0 mg. The lowest weight was recorded. Finally, ash was calculated as:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Moisture and dry matter content: The moisture content was measured according to the official methods of AACC (2000). About 1.0 g of sample was taken and placed it in an oven set at 105°C for 3 h. The sample was allowed to cool. Then the weight of the cooled sample was measured. The sample was heated again at 105°C for 30 min and cooled in a desiccator and the weight was measured. This process of heating was repeated for 30 min, cooling in a desiccator and weighing until the difference between two successive weighing was less than 1.0 mg. The lowest weight was recorded. The moisture content of the sample was calculated using the following equation:

$$\text{Dry matter (\%)} = \frac{\text{Weight of dried sample (g)} \times 100}{\text{Weight of fresh sample (g)}}$$

$$\text{Moisture (\%)} = \text{100-Dry matter}$$

Water extract: About two grams of the sample were taken in a beaker and 200 mL water was added and boiled in hot plate at 110°C for 1 h. Then it was cooled and filtered into 500 mL volumetric flask and made up 500 mL with water. Fifty milliliters diluents were taken in a 100 mL beaker, which was pre-weighted and placed in a hot plate or water bath near about to dryness. Then it was transferred to oven at 105°C up to drying. Then it was allowed to cool and weight was taken:

$$\text{Water extract (\%)} = \frac{\text{Weight of extract} \times 100 \times 100 \times 5}{\text{Sample weight} \times (100\text{-moisture})}$$

Organoleptic test: Made tea samples prepared from different categories of mite infested shoots were forwarded to a professional tea taster for assessment of taint as positive or negative and for organoleptic test. All observations were replicated thrice. Leaf infusion, liquor colour, briskness, strength and creaming down parameters were considered for organoleptic test and standard score was as >34 being Excellent (E), 32-34 being Above Average (AA), 30-32 being Average (A) and <30 being Below Average (BA) out of 50 (Hossain *et al*., 2011).

Statistical analysis: The experimental data were statistically analyzed by Randomized Complete Block Design (factorial RCBD) using MSTAT statistical software in a microcomputer. The results were 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

Chlorophyll and carotenoid contents: Results revealed that the photosynthesis in green leaves of tea was badly affected due to the attack of red spider mite by reducing the photosynthetic pigments i.e., chlorophyll a, b and carotenoids. In case of tender shoots, lower amount of chlorophyll and carotenoid contents were found in the infested tender shoots than the fresh leaves (Fig. 4). Low infested tea leaves contain 2.5 mg g^{-1} chlorophyll a, 1.2 mg g^{-1} chlorophyll b and 1.0 mg g^{-1} carotenoids. Medium infested tea leaves contain 2.3 mg g^{-1} chlorophyll a, 1.1 mg g^{-1} chlorophyll b and 0.67 mg g^{-1} carotenoids. High infested tea leaves contain

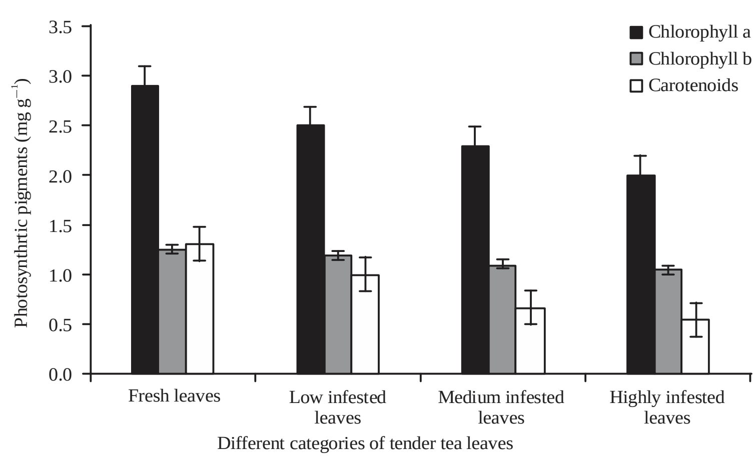

Fig. 4: Chlorophyll and carotenoid contents (mg g^{-1}) of different categories of tender tea shoots infested by red spider mites, error bars are Means±SE

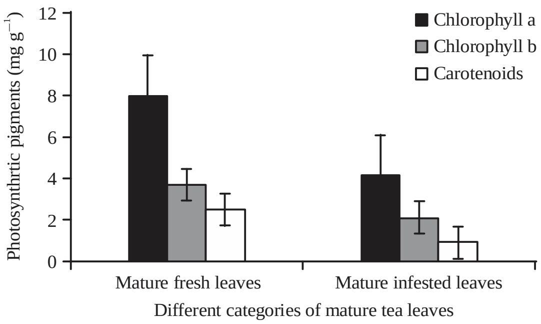

Fig. 5: Chlorophyll and carotenoid contents (mg g^{-1}) in mature tea leaves infested by red spider mites, error bars are Means±SE

2.0 mg g^{-1} chlorophyll a, 1.05 mg g^{-1} chlorophyll b and 0.55 mg g^{-1} carotenoids where fresh tea leaves contain 2.91 mg g^{-1} chlorophyll a, 1.25 mg g^{-1} chlorophyll b and 1.31 mg g^{-1} carotenoids. Photosynthetic pigments gradually reduced with the increase of the level of infestation. Similar trend was also observed in mature tea leaves infested by mites compare with fresh mature tea leaves (Fig. 5).

Polyphenols, catechins and reducing sugar content: Polyphenol content in fresh tender leaves was found 76.22 ppm and gradually decreased in low, medium and highly infested leaves 57.44, 46.00 and 45.56 ppm, respectively (Table 1). The catechin level in fresh leaves was found 21.20 ppm and the catechin content was gradually decreased in low infested leaves (20.60 ppm), medium infested leaves (19.60 ppm) and highly infested leaves (10.40 ppm). The reducing sugar level in also found to be lower in the three categories of infested leaves (low, medium and high as 53.64, 40.07 and 34.57 ppm, respectively) than the fresh leaves (55.57 ppm). Reduced level of polyphenols, catechins and reducing sugar content of the infested leaves may be attributed to the hypersensitivity of plant to feeding injury of red spider mites.

Quality parameters of made tea

Estimation of TR, TF, HPS, TLC, CI, TR:TF, lipid, caffeine, total ash, moisture content, dry matter content and water extract: Results revealed that black tea manufactured from red spider mite infested leaves had lower amount of theaflavin (TF), High Polymerized Substances (HPS), Total Liquor Colour (TLC), colour index, lipid, caffeine, moisture content and water extract than that of fresh leaves; but it is reverse in case of thearubigin (TR) (Table 2). It is established that the ratio between TR and TF must be 1:10 for better quality of tea. It

Table 1: Effect of red spider mite infestation on the biochemical components in green leaves

Different grades	Polyphenol (ppm)	Catechin (ppm)	Reducing sugar (ppm)
T1: Fresh leaves	76.22±1.76^{c}	21.20±0.76^{bc}	55.57±1.24^{c}
T2: Low infested leaves	57.44±1.38^{b}	20.60±0.68^{b}	53.64±1.32^{c}
T3: Medium infested leaves	46.00±1.24^{a}	19.60±0.72^{b}	40.07±0.86^{b}
T4: Highly infested leaves	45.56±0.98^{a}	10.40±0.60^{a}	34.57±0.44^{a}
CD	3.58	1.24	2.36

Within the column value means followed by a different letter (s) are significantly different by DMRT ($p<0.05$)

Table 2: Influence of red spider mite infestation on the quality parameters in made tea

Grades	TF (%)	TR (%)	TF:TR	HPS (%)	TLC (%)	Color index	Lipid (%)	Caffeine (ppm)	Total ash (%)	Moisture (%)	Dry matter (%)	Water extract (%)
Fresh leaves	0.53±0.01^{b}	5.31±0.18^{a}	1:10^{a}	9.81±0.40^{d}	4.25±0.17^{d}	3.50±0.12^{c}	4.70±0.20^{d}	62.04±1.74^{c}	5.21±0.31^{a}	3.45±1.12^{d}	96.55±2.48^{a}	36.44±1.78^{d}
Low infested leaves	0.52±0.03^{b}	5.78±0.22^{b}	1:11^{b}	8.73±0.28^{c}	4.17±0.22^{c}	3.58±0.16^{c}	4.49±0.16^{c}	56.82±1.18^{b}	5.36±0.24^{b}	3.08±1.02^{c}	96.92±2.62^{b}	31.04±1.52^{c}
Medium infested leaves	0.49±0.01^{a}	7.40±0.26^{c}	1:15^{c}	6.86±0.32^{b}	2.38±0.11^{b}	3.44±0.21^{b}	3.94±0.11^{b}	56.68±1.30^{b}	5.53±0.16^{c}	2.88±0.08^{b}	97.12±2.20^{b}	25.71±1.64^{b}
Highly infested leaves	0.43±0.04^{a}	7.00±0.14^{d}	1:16^{d}	6.16±0.24^{a}	2.20±0.98^{a}	3.27±0.28^{a}	3.77±0.94^{a}	54.68±1.26^{a}	5.70±0.22^{d}	0.63±0.02^{a}	99.37±3.08^{c}	22.66±1.06^{a}
CD	0.06	0.10	0.90	0.54	0.16	0.12	0.15	0.18	0.13	0.26	0.30	2.36

Within the column value means followed by a different letter (s) are significantly different by DMRT ($p<0.05$), TF: Thea flavin, TR: Thearubigin, HPS: High polymerized substance, TLC: Total liquor colour

Table 3: Taster's score of different categories of made tea produced from infested leaves due to red spider mites

Category	Dry leaf	Creaming down	Strength	Briskness	Liquor colour	Infusion	Taster's score*	Grade
Fresh leaves	Blackish	Creamy (3.2)	Strong (7.6)	Brisky round (7.6)	Colory (7.6)	Coppery (7.8)	33.80[a]	AA
Low infested leaves	Blackish brown	Creamy (2.9)	Strong (7.5)	Have briskness (7.5)	Bright (7.5)	Coppery (7.5)	32.90[b]	AA
Medium infested leaves	Brownish	Quite creamy (2.7)	Strong (7.4)	Have briskness (7.3)	Bright (7.2)	Dull mixed (7.2)	31.80[c]	A
Highly infested leaves	Brownish	Quite creamy (2.6)	Useful (7.2)	Not flat (7.15)	Bright (7.5)	Dull mixed (7.2)	31.65[c]	A

*Quality score including leaf infusion, liquor colour, briskness, strength and creaming down, within the column value means of taster scores followed by a different letter(s) are significantly different by DMRT ($p<0.05$)

was also observed that the ratio is standard in fresh leaves but gradually increased in the infested leaves that deteriorate the quality of made tea. The biochemical contents were gradually reduced significantly ($p>0.05$) in different categories of infested leaves than fresh leaves. In case of total ash and dry matter/crude fibre content, the lower value was found in low infested leaves and higher value was found in highly infested leaves. It is evident that higher values of those parameters are not acceptable for good quality made tea. Reduction levels of polyphenols, catechins exhibited direct impact on made tea constituents like theaflavin and thearubigin.

Organoleptic evaluation in respect of quality: Made tea manufactured from infested shoots was valued inferior when subjected to organoleptic evaluation. Made tea manufactured from the red spider mite infested leaves scored significantly lower values than fresh leaves. Organoleptic evaluation also confirmed the analytical results. Organoleptic test revealed leaf infusions of made teas prepared from mite infested leaves as brownish dry leaf, quite creaming down, dull mixed infusion and bright liquor strength, scoring 31.65-32.90 on a 50-point scale (Table 3).

From the findings of the present study, it was found that the physiological parameters and biochemical components were reduced significantly with the rate of infestation mite increased from low to high level. Made tea manufactured from the green leaves infested by red spider mite was found inferior quality than the fresh leaves. Since no study has been made on physio-biochemical changes in tea leaves due to this phytophagous mites either in Bangladesh or elsewhere, it is not possible to compare the present results with those published earlier. However, few early workers reported that infestation of mites caused various physio-biochemical changes including changes in minerals, inorganic and organic compounds in plants leading to their physiological and morphological changes (Shree and Nataraja, 1993).

The infested tea shoots by red spider mites turn bronze colour due to reduction of chlorophyll content. As a result, the active photosynthetic rate was also reduced. Increased polyphenol oxidase activity was attributed to the conversion of polyphenols to quinines as reported earlier in eriophyid mite infested citrus leaves (Ishaaya and Sternlicht, 1971). Reduction level of polyphenols, catechins and browning of infested region by mites may be attributed to the hypersensitivity of plant to feeding injury.

Similar observation towards increase of phenolic compounds was also reported by Kielkiewiez (1981). Sanjib *et al*. (2005) reported increasement of phenol compounds in case of jute (*Corchorus capsularis* Linn.) due to the infestation of mite *Polyphagotarsonemus latus.* Livinali *et al*. (2014) also reported that strawberry fruit from two spotted spider mite infested plants present the highest levels of acidity and exhibit low levels of anthocyanin and phenolic compounds.

Sanjay and Baby (2007) studied the physiological and biochemical changes of tea leaves due to Pestalotiopsis infection under green house conditions. Physiological parameters such as photosynthetic rate, stomatal conductance, water use efficiency and chlorophyll fluorescence were reduced in the infected leaves, while stomatal resistance had an increase. Biochemical constituents were higher in healthy leaves compared to that of healthy tissue of infected leaves, except for polyphenol and catechin contents.

Kumaravadivelu *et al*. (1996) also reported a reduction in biochemical constituents in tea leaves due to flushworm infestation. The percentage of polyphenols was higher inleaves of south Indian tea clones i.e., UPASI 17 when compared to UPASI 1. The results also support the present findings in respect of biochemical contents in green leaves and made tea. The present findings also showed a significant reduction in chlorophyll, carotenoid and other biochemical contents in mite infested tea leaves. Similar observations were reported earlier in pink mite infested tea leaves in South India (Loganathan, 1992).

Sudhakaran *et al*. (2000) studied on the influence of Tea Mosquito Bug (TMB) *Helopeltis theivora* infestation on the photosynthetic rate (Pn), biochemical constituents of green leaf and quality parameters of black tea. Biochemical constituents such as chlorophyll, carotenoids, polyphenols, catechins, amino acids and total sugars were very much reduced when the shoots were severely infested. Black tea prepared from infested tea shoots contain significantly lesser amount of theaflavins, thearubigins, high polymerized substances and total liquor color when compared to normal tea.

CONCLUSION

It can be concluded that the infestation of red spider mites in tea affects on the physiological and biochemical contents of tea leaves as well as quality of made tea. This study conveys the message to the planters, researchers and brokers that the quality of green leaves and made tea are deteriorated due to red spider mites infesting tea. As tea consumption is gaining popularity all over the world, quality tea has to be produced with standard biochemical compounds as well as improved agronomical characteristics which are for successful cultivation and commercialization. Hence, further research is needed to minimize the biochemical losses in green leaves and made tea due to red spider mites infesting tea.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Md. Ismail Hossain, Principal Scientific Officer, Botany Division and Mr. Md. Imrul Hasan Choudhury, Scientific Officer, Biochemistry Division, Bangladesh Tea Research Institute for assisting in organoleptic test and biochemical analysis, respectively.

REFERENCES

AACC., 2000. Approved Methods of the American Association of Cereal Chemists. 10th Edn., American Association of Cereal Chemists Press, St. Paul, MN., USA.

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

Ahmed, M., 2005. Tea pest management. Evergreen Printing and Packaging, Dhaka, Bangladesh, pp: 95.

Ahmed, M., M.S.A. Mamun, M.M. Hoque and R.S. Chowdhury, 2012. Influence of weather parameters on red spider mite-a major pest of tea in Bangladesh. SUST J. Sci. Technol., 19: 47-53.

Ali, M.A., M. Ahmed and M.I. Haq, 1994. Crop loss due to red spider mites in tea. Bangladesh J. Zool., 22: 197-202.

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

Choudhary, M.N.D. and M.R. Goswami, 1983. A rapid method for determination of total polyphenolic matter in tea *Camellia sinensis* L. O Kuntze. Two Bud, 30: 59-61.

Cranham, J.E., D.J.W. Ranaweera and G.B. Rajapakse, 1962. The mite pests of Ceylon tea: Recognition and control. Tea Quart., 33: 189-195.

Das, G.M., 1957. *Enarmonia (Cydia) leucostoma* Meyr. (The flush worm). Two Bud, 4: 3-4.

Evans, G.O., 1982. Principles of Acarology. CAB International, Wallingford, UK., Pages: 563.

Hedge, J.E. and B.T. Hofreiter, 1962. Carbohydrate Chemistry. 17th Edn., Academic Press, New York, USA.

Hossain, M.I., M.J. Dutta, A.Q. Khan, M.S.A. Mamun and M.M. Rana, 2011. Yield performances and cup quality of some BTRI test clones. Int. J. Agricult. Res., Innov. Technol., 1: 24-28.

Ishaaya, I. and M. Sternlicht, 1971. Oxidative enzymes, ribonuclease and amylase in lemon buds infested with *Aceria sheldoni* (Ewing) (Acarina: Eriophyidae). J. Exp. Bot., 22: 146-152.

Kielkiewiez, M., 1981. Physiological, anatomical and cytological changes in leaves of two strawberry varieties resulting from feeding by two spider mites. Ph.D. Thesis, University in Warsaw, Poland.

Kumar, R.S.S., 2004. Studies on improving quality of South Indian black teas. Final Technical Report Submitted to Tea Board, Kolkata, India, pp: 1-71.

Kumaravadivelu, P., B. Radhakrishnan and N. Muraleedharan, 1996. Studies on flush worm (*Cydia leucostoma* Meyrick) tea plant interactions. J. Plant. Crops, 24: 309-318.

Livinali, E., R.A. Sperotto, N.J. Ferla and C.F.V. de Souza, 2014. Physicochemical and nutritional alterations induced by two-spotted spider mite infestation on strawberry plants. Electron. J. Biotechnol., 17: 193-198.

Loganathan, S., 1992. Studies on the pink mite, *Acaphylla theae* (Watt) (Acarina: Erophyoidea: Eriophuidae) infesting tea in Southern India. Ph.D. Thesis, Barathiar University, Coimbatore, India.

Mahanta, P.K., S.K. Barua, H.K. Baruah and L.C. Bora, 1992. Pigment profile as the quality criteria in various categories of black teas. Proceedings of the 31st Tocklai Conference, (TC'92), TRA, Jorhat, India, pp: 173-185.

Maidon, A.B.M.A., A.O. Mansoer and H. Sulistyarti, 2012. Study of various solvents for caffeine determination using UV spectrophotometer. J. Applied Sci. Res., 8: 2439-2442.

Mamun, M.S.A. and M. Ahmed, 2011. Prospect of indigenous plant extracts in tea pest management. Int. J. Agric. Res. Innov. Technol., 1: 16-23.

Murthy, R.L.N. and R. Chandrasekaran, 1979. Effect of Pest Damage on the Quality of Made Tea. In: Plant Protection (Entomology, Microbiology, Nematology, Plant Pathology and Rodentology), Venkataram, C.S. (Ed.). Indian Society for Plantation Crops, Kerala, India, pp: 375-283.

Ramaswamy, S., 1986. Improving tea quality in South India. UPASI Tea Sci. Dept. Bull., 41: 12-24.

Rattan, P.S., 1992. Pests and Diseases Control in Africa. In: Tea: Cultivation to Consumption, Willson, K.C. and M.N. Cliford (Eds.). Chapman and Hall, London, UK., ISBN: 9780412338502, pp: 331-352.

Ravichandran, R. and R. Parthiban, 2000. Lipid occurrence, distribution and degradation to flavour volatiles during tea processing. Food Chem., 68: 7-13.

Roberts, E.A.H. and R.F. Smith, 1963. The phenolic substances of manufactured tea. IX.-The spectrophotometric evaluation of tea liquors. J. Sci. Food Agric., 14: 689-700.

Sana, D.L., 1989. Tea science. Ashrafia Boi Ghar, Bangla Bazar, Dhaka, Bangladesh.

Sanjay, R. and U.I. Baby, 2007. Physiological and biochemical changes in tea leaves due to *Pestalotiopsis* infection. J. Plant. Crops, 35: 15-18.

Sanjib, G., S.K. Gupta and B. Mukherjee, 2005. Depletion of minerals, inorganic and organic compounds in the leaves of Jute, *Corchorus capsularis* Linn., due to infestation of the mite, *Polyphagotarsonemus latus* (Banks). Proc. Zool. Soc., 58: 39-41.

Shree, M.P. and S. Nataraja, 1993. Post-infectional biochemical and physiological changes in mulberry. Curr. Sci., 65: 337-341.

Sudhakaran, R., R. Selvasundaram and N. Muraleedharan, 2000. Physiological and Biochemical Changes in tea Leaves due to Tea Mosquito Bug Infestation. In: Recent Advances in Plantation Crops Research, Muraleedharan, N. and R. Rajkumar (Eds.). Allied Publishers Ltd., Chennai, India, pp: 289-292.

Swain, T. and W.E. Hillis, 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric., 10: 63-68.

Thanaraj, S.N.S. and R. Seshadari, 1990. Influence of polyphenol oxidase activity and polyphenol content of tea shoot on quality of black tea. J. Sci. Food Agric., 51: 57-69.

Ullah, M.R., 1977. Alternative test for assessment of fermentation. Proceedings of the 29th Tocklai Conference, (TC'77), Assam, India, pp: 75-77.

Wellburn, A.R., 1994. The spectral determination of chlorophyll a and chlorophyll b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol., 144: 307-313.