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Editorial

The present publication (vol. 43) of Tea Journal of Bangladesh contains five articles on tea husbandry.

The first article is on the use of growth promoter Gibberelic Acid (GA₃) on tea for increasing its ultimate yield. In the early days of Gibberelin discovery, the institute in the mid-nineteen sixties first worked with GA on nursery tea plants and in nineteen seventies on plucking tea. The first one found manifest effect on nursery plant but in the second one it could break the dormancy of plucking tea in advance but found no net gain in the summed up total crop of the year. Now this experiment with new generation of GA reveals a positive development that GA₃ can now be used to promote growth of tea plants and higher yield to tea could also be achieved by using GA₃ in appropriate dose (200 ppm).

The second paper is about monitoring termite population by using food traps. Termite is a major pest of tea in Bangladesh. The experimental findings show that monitoring of termite population can effectively be done by using dried tender bamboo sleeves, soft timber, tissue paper as well as jute sticks. The findings help controlling termites in tea.

The third article discusses about the use of biopesticides for controlling parasitic nematodes in tea. Environmental degradation is a burning question now a days all over the world. So, the use of organic pesticides which are less harmful to the environment is increasing day by day. The authors elaborately discuss usage, advantages and limitations of biopesticides in this article. One can include bio pesticides as a component of IPM for controlling plant parasitic nematodes in tea.

The fourth paper reports a new disease of tea found in nursery. The causal organism was identified to be a *Cylindrocladium* sp. which causes rot in cuttings planted in primary bed of a tea nursery. Early identification of the causal organism of the disease will help choose right control measure.

The last article is on the use of some entomopathogens for controlling red spider mite. Authors opined that commercially formulated entomopathogens can be used to control red spider mite in tea. It is a new area of research in Bangladesh tea. These eco-friendly entomopathogens can be considered as safer bio pesticides and one can be included as a component of IPM.



(Dr. Mainuddin Ahmed)
Chief Editor

EFFECTS OF DIFFERENT RATES OF GIBBERELIC ACID (GA₃) ON SHOOT GROWTH DYNAMICS AND YIELD OF MATURE CLONAL TEA (*CAMELLIA SINENSIS* L.)

T. Ahmed^{1*}, W.A.J.M. De Costa² and M.A. Wijeratne³

Abstract

Yield of tea (*Camellia sinensis* L.) consists of tender shoots which are produced on axillary buds of tea bushes on the plucking table. In the present study, it was hypothesized that foliar application of Gibberellic acid (GA₃) could influence shoot growth dynamics and yield components of tea and ultimately affected on its yield. Therefore, the above hypotheses were investigated on a mature clonal tea (cv. TRI2025) in a field experiment at the Tea Research Institute, Low-Country Station, Ratnapura, Sri Lanka. Different rates of GA₃ were used as treatments and sprayed on the foliage at monthly intervals which continued up to six months. GA₃ showed significant effect on length of shoot replacement cycle (L_{src}), shoot extension rate, average shoot fresh weight, formation of banji shoots, harvested shoot density and ultimately affected on the harvested yield of tea. Higher made tea yield was observed in GA₃ @ 500ppm which was similar with GA @ 200ppm but significantly different with GA₃ @ 100ppm and the control, respectively.

Keywords: GA₃, Shoot growth dynamics, Shoot density, Tea yield

Introduction

The average yield of tea (*Camellia sinensis* L.) in Bangladesh is very low in comparison to other major tea producing countries e.g. India, Sri Lanka and Kenya. One of the main reasons for such yield difference is the length of the harvesting season. Being in the sub-tropics, tea is harvested about 8-9 months per year in Bangladesh, where as being in the tropics South India, Sri Lanka and Kenya it is harvested all the year round due to prevailing favorable climatic conditions e.g. day length, temperature and rainfall (De Costa *et al.*, 2007).

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Barua (1969) found that there is a greater tendency for tea shoots to become dormant (banji) when the photoperiod is less than 11.16 h and during the winter it becomes 10.6 h in Bangladesh (Alam, 1999). From an observation, Palni (2001) reported that if the winter dormancy can be reduced by just one month in North-East India, the annual tea production would enhance by as much as 40 million kg which is located similar geographical area to Bangladesh. Although the reasons for banji formation in tea have not been clearly understood, it has been reported to be the result of either hormonal interaction (Pethiyagoda, 1964; Ahmed *et al.*, 1965; Kulasegaram, 1969; Ranganathan *et al.*, 1983) or lack of nutrient supply to actively growing apices (Bond, 1945).

Shoot growth is the major physiological process which determines the tea yield. Yield components of tea are the number of plucked shoots per unit land area and the mean weight per shoot (Rahman, 1977) and of these two components, shoot density is the major factor that determines tea yield and more than 80 per cent of the variation in tea yield is accounted for by the shoot density (Wijeratne, 2001). For obtaining higher yield from a tea area, it is must to harvest a large number of heavier shoots. These parameters are largely depended on the quality of cultivar, plant density (spacing), pruning and plucking policies, fertilizer management, pest and disease control and climatic and soil conditions. Wijeratne (2001) reported that changes of weather pattern and application of fertilizers and other growth promoters contribute to the production of several shoots per harvested shoot, thereby increasing the shoot replacement ratio and shoot population density.

Plant growth regulators (PGRs) are considered as potential tools of crop management in plantation and horticultural crops. Since the economic yield in tea depends on the number, size and weight of harvested shoots, it is important from the commercial point of view that the plant shows vigorous vegetative growth and produces more shoots over the entire period. Attempts have been made in different countries to increase vegetative growth and yields of tea by applying certain growth regulators. Gibberellins and cytokinins promote the growth of dormant buds, although auxins have no effect in stimulating them to grow (Mohotti *et al.*, 2003). Manivel (1986) reported that gibberellic acid stimulates the growth of dormant buds, thereby reducing the dormant phase and in a separate study Manivel *et al.* (1998) stated that response to growth regulators depends on the rate and time of application. Nandi *et al.* (1995) stated that GA₃ induced bud-break earlier by two weeks while ABA and NAA were found to be inhibitory to shoot growth. Therefore, this study was undertaken to investigate the effects of different rates of Gibberellic acid (GA₃) on shoot growth dynamics, yield components and yield of tea.

Materials and Methods

The experiment was carried out at the Tea Research Institute, Low Country Station, Ratnapura (6°40'N, 80°25'E and 30 m amsl) and continued over a period of six months (from May 2012 to November 2012). It was a single factor experiment having four treatments of different rates of Gibberellic acid (GA₃) namely, no application of GA₃ (control); GA₃ @ 100ppm (a.i.); GA₃ @ 200ppm (a.i.) and GA₃ @ 500ppm (a.i.). The experimental design was a completely randomized design with four replications and all together there were 16 experimental plots. 10 bushes were demarcated in each plot. A standard tea cultivar TRI2025 was used and tea bushes were 12-year old with double-hedge spacing (0.6m x 0.9m x 1.5m) and were in the first year of the third pruning cycle. Application of fertilizers and cultural practices were carried out according to the recommendations of TRI, Sri Lanka. The GA₃ were sprayed at monthly intervals and on every occasion control tea plants were sprayed with water.

Plucking was done in the morning on weekly rounds for estimation of the plot yield in g per plot. After taking the fresh weight of harvested leaf, counting was done for each plot to get the number of active shoots, banji shoots and then total number of harvested shoots. The number of shoots for each plot was then converted into the number of harvested shoots per bush and m².

To know the effects of treatments on shoot growth dynamics, six shoots each having three leaves with an active bud were randomly selected from the centre of the bush from each plot at two months intervals. Selected shoots were plucked and tagged for future identification. Three of the tagged shoots were plucked when they reached at the standard of two leaves with active bud. The following measurements were made on these harvested shoots e.g. fresh weight per shoot and length of shoot replacement cycle (L_{SRC}) for attaining the standard of two leaves with a bud. The other three tagged shoots were left without harvesting until they become dormant. The following measurements were made on these shoots e.g. days required for the shoot to become dormant and the number of leaves that were produced before becoming dormant. To get the shoot extension rate, after completing the harvesting operation in weekly round five (5) remaining shoots (*arimbu*) from the centre of the plucking table were tagged from each plot which were almost similar in size. At the time of tagging the length of each shoot was measured by a ruler and recorded. After a week, at the time of harvesting the length of each tagged shoot was measured again and the difference between the two readings of a particular shoot gave the value of shoot extension rate (mm week⁻¹) of that shoot.

Results and Discussion

Shoot growth dynamics

Length of shoot replacement cycle (L_{SRC}) for being the two leaves with an active bud stage was significantly different for treatments. L_{SRC} was longer in the control (32.9 ± 0.2 days) which was similar with $GA_3 @ 100\text{ppm}$ but significantly different with $GA_3 @ 200\text{ppm}$ and $GA_3 @ 500\text{ppm}$, respectively (Table 1).

Shoot extension rate (mm week^{-1}) was significantly different for treatments. The highest mean shoot extension rate was observed in the treatment of $GA_3 @ 500\text{ppm}$ ($60.9 \pm 1.1 \text{ mm week}^{-1}$) which was significantly different from other treatments and followed by $GA_3 @ 200\text{ppm}$, $GA_3 @ 100\text{ppm}$ and the control (Table 1). Consequently, average shoot fresh weight (two leaves with an active bud stage) was significantly different for treatments. Greater shoot fresh weight was observed in $500\text{ppm } GA_3$ ($0.74 \pm 0.01 \text{ g}$) which was significantly different from all other treatments and followed by $200\text{ppm } GA_3$, $100\text{ppm } GA_3$ and the control (Table 1).

Days required for banji formation of a pluckable shoot was non-significant for treatments, however number of leaves produced at banji stage of a pluckable shoot were significantly different for treatments. Greater number of leaves at banji formation stage were observed in the treatment of $500\text{ppm } GA_3$ (4.4 ± 0.1) which was similar with $200\text{ppm } GA_3$ but significantly different with others (Table 1).

The time taken by an axillary bud to become a harvestable shoot is termed as shoot replacement cycle. The length of shoot replacement cycle (L_{SRC}) has a remarkable effect on yield. L_{SRC} was shorter in the $500\text{ppm } GA_3$ applied treatment. Moreover, after plucking and weighing of that shoot greater fresh weight per shoot was observed in the treatment of $500\text{ppm } GA_3$ and the lowest was in the control. Similarly in a study on application of PGSSs, Nandi *et al.* (1995) found that the greater mean fresh weight of shoot was in higher dosages of GA_3 applied treatment which was significantly different with others. The variation of shoot fresh weight was due to the difference of shoot extension rate which was also significantly higher in $GA_3 @ 500\text{ppm}$ than others. In supporting with this observation, Wijeratne (2001) stated that if the rate of shoot growth is fast, it may become a harvestable shoot within short period of time and also secure more weight. GA_3 is known for causing cell elongation and utilization of food material, increased internodal and shoot length in comparison to control. From a field study, Kulasegaram (1969) reported that higher concentrations of GA_3 (800ppm) promoted shoot growth earlier, supported by present findings. Harvestable tea shoots turned into banji earlier in the control than treatments treated with GA_3 , though it was not statistically different. However, number of leaves at the time of banji formation in the harvestable shoot was significantly greater at the higher rates of GA_3 applied treatments. Ali-Zade (1963) in the Soviet Union observed the similar result that GA_3 increased growth by increasing shoot length and number of leaves.

Table 1. Effect of different rates of GA₃ on length of shoot replacement cycle (L_{SRC}) and fresh weight per shoot (two leaves with active bud stage), shoot extension rate, days required for banji formation of a pluckable shoot and number of leaves at the time of banji

Treatments	L _{SRC} (Days)	Shoot extension rate (mm/week)	Fresh weight per shoot (g)	Days required for banji formation	Leaves at banji (No.)
Control	32.9 (±0.2) ^a	40.3 (±0.8) ^d	0.49 (±0.01) ^d	40.1 (±0.4)	3.9 (±0.1) ^b
GA ₃ @ 100ppm	32.6 (±0.2) ^{a b}	46.3 (±0.8) ^c	0.58 (±0.01) ^c	40.3 (±0.4)	4.0 (±0.1) ^b
GA ₃ @ 200ppm	31.9 (±0.3) ^{b c}	52.8 (±1.2) ^b	0.65 (±0.01) ^b	41.0 (±0.4)	4.3 (±0.1) ^a
GA ₃ @ 500ppm	31.6 (±0.3) ^c	60.9 (±1.1) ^a	0.74 (±0.01) ^a	41.0 (±0.4)	4.4 (±0.1) ^a
Mean	32.3	50.1	0.61	40.6	4.2
Level of significance 0.05					
LSD	0.72	2.53	0.02	NS	0.27
CV (%)	6	14	9	7	16

Harvested shoot density and banji shoots in the harvested leaf

Harvested shoot population density (No. m⁻²) was significantly different for treatments. Greater number of harvested shoots (m⁻²) observed in the treatment of GA₃ @ 500ppm (38.6±1.0) which was similar with GA₃ @ 200ppm but significantly different with GA₃ @ 100ppm and the control (Table 2). Having the greater number of harvested shoot density in the higher rates of GA₃ applied treatment, greater number of active shoots and banji shoots were also followed the similar trend.

Table 2. Effect of treatments on harvested shoots (active shoots + banji shoots) (No. m⁻²)

Treatment	Harvested shoots	Active shoots	Banji shoots
Control	32.6 (±0.8) ^b	8.0 (±0.3) ^c	24.6 (±0.6) ^b
GA ₃ @ 100ppm	33.3 (±0.6) ^b	7.7 (±0.2) ^c	25.6 (±0.6) ^b
GA ₃ @ 200ppm	36.6 (±0.9) ^a	8.9 (±0.4) ^b	27.6 (±0.8) ^a
GA ₃ @ 500ppm	38.6 (±1.0) ^a	9.7 (±0.4) ^a	28.8 (±0.8) ^a
Mean	35.3	8.6	26.7
Level of significance 0.05			
LSD	2.42	0.87	1.97
CV (%)	19	16	20

When the harvested shoots for different treatments expressed in percentage, it was observed that formation of banji shoots (%) was significantly different for treatments (Figure 1). Greater percentage of banji shoots observed in the control (76%) which was similar with GA₃ @ 100ppm but significantly different with others.

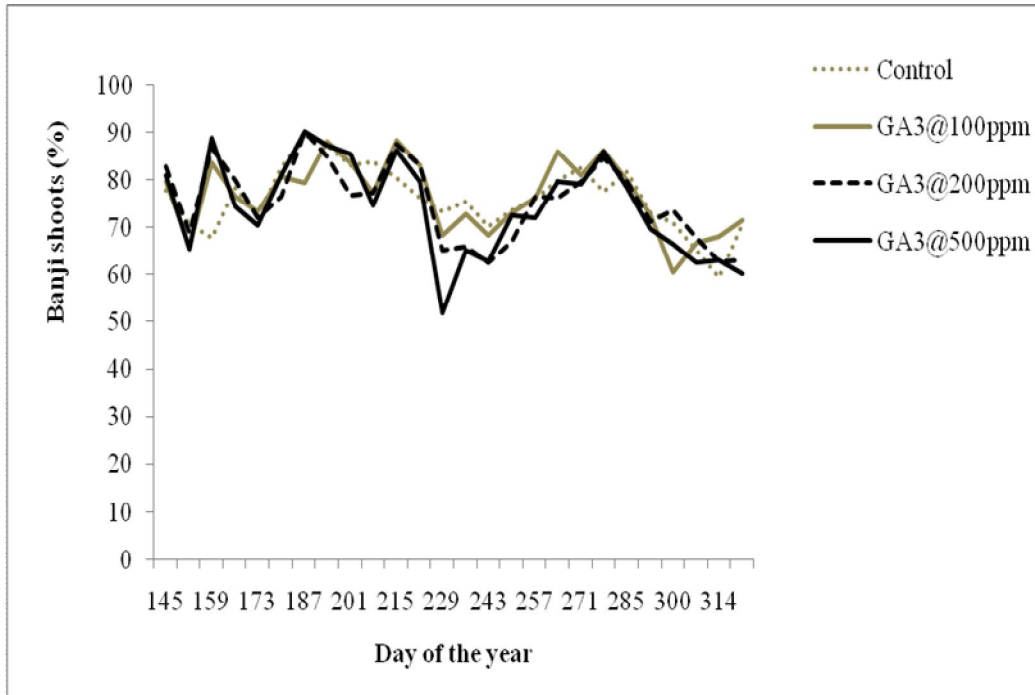


Figure 1. Variation of banji shoots (%) in different treatments at different DOYs

Increase in the number of shoots in higher rates of GA₃ applied treatment was due to shorter L_{SRC} and greater shoot extension rate. GA₃ has a wide range of activity on various aspects of growth of almost any plant organ (Paley, 1965) and hence, plants treated with higher rate of GA₃ recorded the higher number of shoots per unit area. When harvested shoots of each treatment calculated as percentage, less banji shoots observed in higher rates of GA₃ applied treatments which supported by Ng'etich *et al.* (2000) who stated that GA₃ would increase the yield by reducing the level of banji shoots during cold and dry periods.

Harvested yield records

Harvested leaf fresh weight and dry weight (g m⁻²) both were highly significant for treatments. Greater harvested leaf fresh weight and dry weight (g m⁻²) were observed in the treatment of GA₃ @ 500ppm which was similar with GA₃ @ 200ppm but significantly different with GA₃ @ 100ppm and the control (Table 3).

Table 3. Effect of treatments on harvested leaf fresh weight and dry weight of tea

Treatments	Fresh weight (g m ⁻²)	Dry weight (g m ⁻²)
Control	28.0 (±0.9) ^c	6.9 (±0.2) ^b
GA ₃ @ 100ppm	30.1 (±0.9) ^b	7.2 (±0.2) ^b
GA ₃ @ 200ppm	33.9 (±0.9) ^a	7.8 (±0.2) ^a
GA ₃ @ 500ppm	35.5 (±0.9) ^a	7.9 (±0.2) ^a
Mean	31.8	7.4
Level of significance	0.05	
LSD	2.08	0.49
CV (%)	24	24

Accordingly, greater made tea yield (kg ha⁻¹ yr⁻¹) was observed in the treatment of GA₃ @ 500ppm (4,120±436) which was similar with GA₃ @ 200ppm (4,045±429), however significantly different with GA₃ @ 100ppm (3,727±193) and control (3,562±280) (Figure 2).

The significant yield improvement under GA₃ applied treatments were due to producing higher number of harvested shoots (m⁻²), greater average shoot fresh weight (g) and less banjji shoots (%). All these parameters were significant and positively correlated with harvested leaf yield, described by many researchers (Rahman, 1977; Tanton, 1992; Wijeratne, 2001; De Costa *et al.*, 2007). In agreement with present findings, Deka (2003) stated that the highest green leaf yield was obtained from those plants that had received GA₃ foliar spray. From this study, it is clear that higher rate of GA₃ application produced more yield which is partially supported by Manivel *et al.* (1998) and Kumar *et al.* (1999) who reported that response of growth regulators depend on its rates of application. In comparison with control, 500ppm GA₃ application produced about 16% and 200ppm GA₃ application produced about 14% more made tea yield (kg/ha), though these two treatments were statistically similar. Similar findings described by Ng'etich *et al.* (2000) that GA₃ @ 1000ppm gave the highest yield though it was not different from 200ppm and 500ppm. From the present findings it is evident that application of GA₃ above 200ppm is impractical as the yield is not increasing significantly when GA₃ is applied at the rate of above 200ppm.

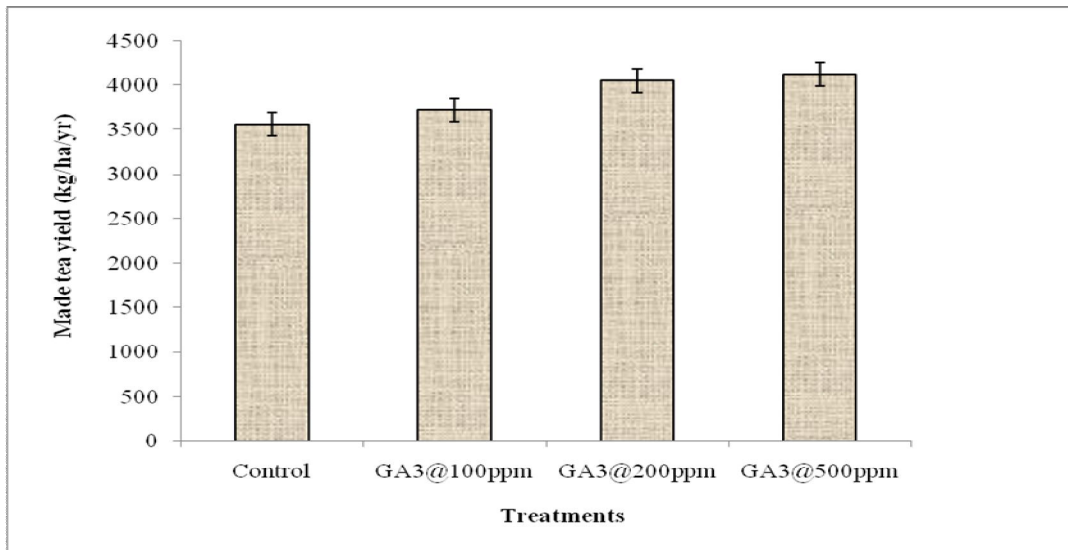


Figure 2. Variation of made tea yield due to treatments

Conclusion

Shoot growth dynamics and yield related parameters of tea were significantly affected by the foliar application of GA_3 . Having the greater shoot extension rate, greater shoot weight, shorter L_{SRC} , higher harvested shoot density and less banji shoots the highest yield of tea was reported in $GA_3 @ 500ppm$ which was similar with 200ppm but significantly different with others. Hence, to maximize the tea yield, it may be suggested to apply $GA_3 @ 200ppm$ for a few rounds during the dry period of the year.

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USE OF FOOD TRAPS FOR MONITORING TERMITE POPULATION IN TEA

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Abstract

An experiment was conducted to monitor termite population in tea area. The experiment was laid out with a randomized completely block design (RCBD) at Bangladesh Tea Research Institute main farm. Five types of food traps, namely, 1) Saw dust @ 100g, 2) Tissue paper @ 100 cm long, 3) Dried tender bamboo sleeves @ 100 sleeves, 4) Jute sticks @ 100 sticks and 5) Susceptible soft timber @ 100 pieces were used as treatments in this experiment. In the control plots, nothing was used. Each treatment was replicated thrice. The intensity of termite damage was recorded at 30 days interval during the study. Results revealed that the proneness of invasion of termite to different food traps differed significantly from each other. The damage matrix indicates that the dried tender bamboo sleeves (73.50%) are more susceptible to termite damage than that of soft timber (67.50%) and jute sticks (56.75%). On the other hand, saw dust (27.50%) and tissue paper (28.00%) are less attacked or damaged by termite activity. So, food traps like bamboo sleeves and soft timber are considered to be a suitable tool for destruction and management of termite.

Keywords: Food traps, IPM, termites, tea

Introduction

Termites are soil pests and invade all types of woody materials such as tea plants, shade trees, cover crops, bamboos, grasses and mulches used in tea culture (Ahmed, 1997). The great majority of termites live in tropical and subtropical regions. They are polymorphic social insects which live in nests (termitaria) of their own construction. Soil particles are frequently used for their nest construction. They are highly organized, relying on chemical and sensory messages for communication and defence, enabling them to exist in total darkness. Termites abound throughout the tropics and also occur in most warm and temperate countries in the world. They extend to about 45°N to 50°N (Metcalf, 1951; Emersion, 1955; Haris, 1961; Araujo, 1970). Many termite species are responsible for considerable damage to tea bushes and shade trees. Among the termite castes, worker caste is very dangerous to tea (Ahmed, 2010).

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According to Ali and Ahmed (1990) termites that are responsible for damage to tea bushes may be classified into two groups- *Live wood termites*: These attack living tissues of tea bushes and are considered to be primary pests of tea. They excavate galleries within the live wood of healthy tea plants.

Tea plantation of Bangladesh is primarily invaded by four species of live wood termite, namely *Coptotermes heimi* Wasmann of Rhinotermitidae family; *Microcerotermes championi* Snyder, *Microtermes obesi* Holmgren and *Macrotermes aleemi* Holmgren of Termitidae family (Ahmed, 1999). *Scavenging termites* generally attack dead and dying tissues and are regarded as secondary pests of tea. Three species of scavenger termites, namely *Odontotermes feae* Wasmann, *Odontotermes horni* Wasmann and *Odontotermes parvidens* Holmgren & Holmgren are common in Bangladesh tea (Ahmed, 1996).

Many control measures have been adopted to combat this problem. Use of resistant agrotypes is the major component of IPM approaches. Some BTRI released clones and agrotypes of tea have been screened out against termites (Ahmed *et al.*, 1994; Ahmed *et al.*, 1999; Ahmed *et al.*, 2010). Based on the findings, it may be concluded that BT6, BT8, BT7, BT9, BT13, BT15, BT4, BT12, BT14 and BT16 were found to be the most suitable while BT1, BT2 and BT3 may be the second preference for cultivation in Bangladesh. Special caution has to be made for cultivation of the clones BT5, BT10 and BT11 as they are more susceptible to termite attack. Chemical control has been exercised since tea cultivation. Different groups of chemical pesticides are being used for the control of termites in tea plantation in Bangladesh (Ahmed, 1996; Ahmed, 2000; Mamun and Ahmed, 2012). Use of food traps is one of the outstanding components of integrated pest management (IPM) system (Ahmed, 2011). Food baits based on protein solutions, fermenting sugar solutions, fruit juices, and vinegar have been used since 1918 for the capture of females of several species. Little research has been done on food trap for the management of termite in tea. In order to monitor termite population and determine the damage matrix on those variable food materials in plantation areas or in rehabilitation areas and subsequently to control the invading termites, simple field testing devices using food traps were constructed at Bangladesh Tea Research Institute main farm for the following purposes:

- i. Detection survey: To determine if species are present in an area.
- ii. Delimiting survey: To determine the boundaries of an area considered to be infested or free from termite pest.
- iii. Monitoring survey: To verify the characteristics of a pest population including seasonal population fluctuation, relative abundance, host sequence and others.

Materials and Methods

An experiment was conducted to monitor termite population in tea area at BTRI main farm. Five types of food traps, such as, 1) Saw dust, 2) Tissue paper, 3) Dried tender bamboo sleeves, 4) Jute sticks and 5) Susceptible soft timber were used as food traps in this experiment. Untreated control treatment was also included.

A randomized complete block design (RCBD) was followed with the following six treatments:

T1: Saw dust
T2: Tissue Paper
T3: Dried tender bamboo sleeves
T4: Jute sticks
T5: Susceptible soft timber
T6: Control

Each treatment was replicated thrice. Saw dust @ 100g/replication was put in lump on the bare soil. Similarly tissue paper @ 100 cm long/replication was spreaded. On the other hand, dried tender bamboo sleeves (each 50 cm long) @ 100 sleeves/replication, jute sticks (each 50 cm long) @ 100 sticks/replication and susceptible soft timber (50 cm long) @ 100 pieces/replication were scatteredly placed over the soil surface. In the control plots, nothing was used. The intensity of termite damage was recorded at 30 days interval during the study.

Results and Discussion

Results revealed that the proneness of invasion of termite to different food traps differed significantly from each other. The damage matrix indicates that the dried tender bamboo sleeves are more affiniated or susceptible to termite damage than that of soft timber and jute sticks. On the other hand, saw dust and tissue paper are less attacked or damaged by termite activity (Table 1). The observed value of F is greater than the theoretical value of F for 4 and 19 degrees of freedom both at 5% and 1% level of significance.

Table 1. Damage matrix of termite in relation to food traps

Treatments/ Food Traps	Damage Matrix (%)
T1: Saw dust @ 100g	27.50d
T2: Tissue Paper @ 100 cm	28.00d
T3: Dried tender bamboo sleeves @ 100 sleeves	73.50a
T4: Jute sticks @ 100 sticks	56.75c
T5: Susceptible soft timber @ 100 pcs	67.50b

Within column values different letters indicates significantly different at $p > 0.005$ & > 0.001

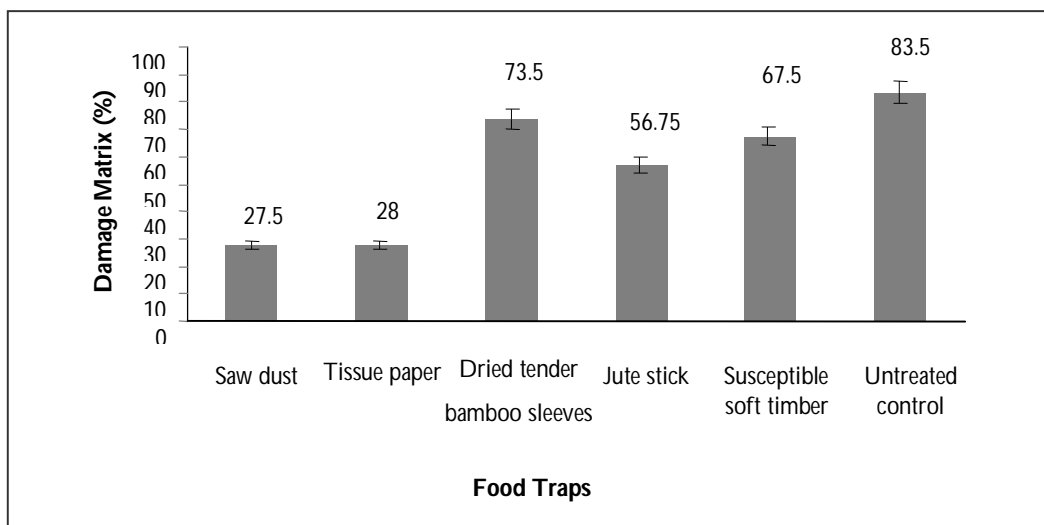


Fig. 1. Graphical presentation showing damage matrix of termites using food traps

It was observed in the practical field that the use of food traps especially bamboo sleeves and or soft timber induce termite infestation in derelict redundant tea sections and subsequent use of proper pesticide to control them might provide an environmentally sustainable control method for termite. So, food traps like bamboo sleeves and soft timber are considered to be a suitable tool for destruction and management of termite.

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FIRST REPORT OF CYLINDROCLADIUM CUTTING ROT ON BANGLADESH TEA

M. M. R. Akonda^{1*}, R. M. Himel¹ and M. Ali²

Abstract

An experiment was conducted to find out the causes of dying of nodal leaf-cuttings of tea planted on primary bed at Bangladesh Tea Research Institute (BTRI) farm in 2014. The aboveground symptoms of the diseased cuttings were identified as blighting, drying, defoliation of leaves and weak development of dormant axillary buds. The belowground symptoms were recorded as brown to blackish necrotic lesion on the stem that failed to callusing and rooting. Cutting rot symptom was developed at later stage in the primary beds. The fungus isolated from the infected cuttings composed of septate mycelia ($157.40\mu \times 18.15\mu$) bearing regularly and repeatedly dichotomously branched conidiophores. Conidia ($40.75 \times 6.65\mu$) were straight, cylindrical or rod shaped having 1-2 septation. Vesicle ($39.69\mu \times 22.85\mu$) was clavate in shape. Brown and roundish Chlamydo-spores were formed in chain and measured as 9.59μ in diameter. The disease was identified and confirmed after pathogenicity test as cutting rot of tea caused by *Cylindrocladium* spp.

Keywords: cutting, *Cylindrocladium*, pathogenecity, tea.

Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze, is regarded as the most popular temperance drink made from the young shoots of tea plant. Different agro-techniques are involved in tea plantation which may be put under two management components, viz. nursery management i.e. methods of propagation and multiplication of tea seeds, cuttings, saplings, etc. and crop management embraces various fields techniques of bringing up tea and ancillary crops that are intended to exploit the highest possible productivity. In both nursery and field, tea plants suffer from different biotic and abiotic stresses. The most prevailing stresses are microbial, entomological, hydrological and edaphic that often leads to death of tea plants. In Bangladesh, pest prevalence in tea causes 10-15% yield loss year after year. Several microflora like *Colletotricum gloeosporioides*, *C. camelliae*, *Corticium theae*, *Fusarium oxysporum*, *Macrophoma theicola*, *Pestalotia theae*, *Ustilina zonata*, *Phomopsis theae* etc. harbors in tea ecosystem and infect plants when predisposing factors become favorable (Sana, 1989; Alam, 2003).

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The fungus, *Cylindrocladium* (Teleomorphs: *Calonectria*) described by Morgan in 1892, is an important plant pathogen on plants in 66 genera of 31 families recorded in North America (USA, Canada), South America (Brazil, Argentina), West Indies (Jamaica), Australia and New Zealand, Asia (India, Japan, Malayasia) and Africa. It has been reported as a pathogen in a wide range of host viz. eucalyptus, pine, tea, blueberry, yellow poplar, sweetgum, crotalaria and other hardwood seedlings. *Cylindrocladium* is also reported as a pest of conifers, beet, strawberry, watermelon, legume weeds like patridgepea, sicklepod, and Florida beggarweed. This fungus survives and overwinters as microsclerotia in infected plant tissues and infested soil. When seedling roots come in contact with the microsclerotia, they germinate and infection occurs. During the periods of high humidity and rainfall, foliage and stem infection may also develop by airborne conidia or ascospores. It has the unique characteristic of tolerating a wide range of soil pH for fungus growth and host infection. *C. scoparium* is known as a nursery pathogen, where it can cause damping-off in young seedlings, root rot, seedling blight, stem canker at ground level and leaf spot in older stock, but rarely causes loss to field plantation and crops (Bell and Sobers, 1966; Thies and Patton, 1970; Anon, 1973; Porter *et al.*, 1991; Crous *et al.*, 1993; Padgett *et al.*, 1995 and Crous, 2002). At present 52 *Cylindrocladium* spp. and 37 *Calonectria* spp. are recognized based on sexual compatibility, morphology and phylogenetic inference (Lombrad *et al.*, 2010).

Under favorable environmental conditions, conidiophores bearing conidia may be produced on infected plant parts or in culture over the surface of the agar. Conidiophores arising from septate mycelia are regularly and repeatedly dichotomously and trichotomously branched, each branch terminating in two or three phialides which are ovoid to dolliform. Conidia of all species of *Cylindrocladium* are cylindrical with rounded ends. However, they vary in size and number of septations among species. Conidia of *C. scoparium* and *C. floridan* have one septum, and are $50-60 \times 4.5-6.0$ and $36-57 \times 2.6-4.6$ microns, respectively. Conidia of *C. crotalariae* have two or more septa and are $58-107 \times 4.8-7.1$ microns. Species of *Cylindrocladium* also can be separated on the basis of vesicle shape. Vesicles of *C. scoparium* are primarily ellipsoid; those of the other two species are globose. Chlamydospore develops in abundance at later stage of infection as well as in older cultures, and form in chains or clumps. At this stage the cultures appear almost black from below. These clumps of chlamydospores are referred to as microsclerotia (Barnett, 1960; Thies and Patton, 1970; Anon, 1973; Cordel and Skilling, 1975; Cordell, 1976 and Barnard, 1984). In tea, nodal leaf cuttings planted in the primary bed die due to various reasons. The present research was undertaken to describe the disease and its cause found in Bangladesh tea.

Materials and Methods

The experiment was carried out at Bangladesh Tea Research Institute farm during June-December, 2014. Soil of the farm was well drained, sandy loam in texture containing sufficient organic matter. The soil p^H of the farm was ranging from 4.43-4.5. Data on temperature, rainfall and relative humidity were collected from BTRI weather office and presented in the table-1.

Planting of Nodal leaf cuttings

The nodal leaf cuttings bearing a dormant axillary bud were planted on raised primary beds on 15 July, 2014. The primary beds were prepared in June, 2014. The size of each primary bed was 30m × 1.5m. Proper spacing, shading and other intercultural operations were done timely.

Isolation of the pathogen from rotted cuttings

The planted cuttings bearing rot symptom were taken in the plant pathology laboratory, Bangladesh Tea Research Institute. Rotted stems were washed with sterile water to remove the surface soil. After washing, infected stems were cut into 1.5-2 mm pieces containing both healthy and diseased part. The inocula were then surface-sterilized by using 30% ethanol for 2 minutes and washed three times with consecutive changes of sterile water. Excess water remained onto the inocula was removed by sterilized blotting paper. Four inocula were plated in each of the five petridishes containing PDA media. The whole operation was done in a laminar air flow cabinet. The petri plates were incubated at 29±1⁰ C and kept under observation for 2 weeks.

Pathogenicity test

Inoculum for pathogenicity test was prepared by growing the fungus at 29±1⁰ C for 3 weeks on potato dextrose broth amended with 2 g / L of east extract. Mycelial mats were removed from five growth media and shredded in a conical flask with sterile water. The mycelial suspension was made upto 500 ml. 10 test nodal leaf cuttings were transplanted separately in 10 plastic bags (25cm × 15cm) containing nutrients rich sterilized soil. 50 ml of mycelial suspension was poured in each polybag and then the treated cuttings were kept in a controlled environment. Ten cuttings were kept as untreated control.

Results and Discussion

Aboveground symptoms

The initial symptoms on aboveground parts were observed as blighting of leaves and weak development of dormant axillary buds. Drying and defoliation of leaves were observed at later stages that lead to death of the cuttings within 4-6 weeks. Severity of cutting rot was high during September, 2014 (Plate 1).

Belowground symptoms

The cuttings (2.5-3 cm) that planted in primary beds showed necrotic, brown to blackish lesions on stem. The affected stems failed callusing and rooting. In most cases, the bark of the affected cuttings peeled off easily. Water and nutrients conducting tissues i.e. xylem and phloem were damaged severely. Black rot symptom of the belowground stem was noticed at later stage of disease development (Plate 2).

Results for pathogenicity test

Symptoms developed on the leaf at 70 days after inoculation. The symptoms were similar to those observed under natural conditions. Treated cuttings died after 85-90 days, but all cuttings in control remained healthy. Stems of dead cuttings had brown, discolored patches. The pathogen was reisolated from the diseased tissues, which was characteristically similar to previously isolated pathogen.

Characterization of the pathogen

Primarily, white mycelial extension was observed from the test inocula after 24 hours of incubation. The white extension became reddish-brown which started from 7 days after incubation (Plate 3). The older cultures appeared almost black from below. The isolated pathogen was observed under compound microscope ((Primostar Trinocular microscope of Carl Zeiss Microscopy)) and images were taken at 100X magnification.

Mycelia: The older mycelia were brown in color, septate, having the average diameter 18.15μ . The average distance between two septa was measured as 157.40μ (Plate 4).

Conidiophore: Conidiophores regularly and repeatedly dichotomously branched, each branch terminating in two or three phalides which are ovoid to dolliform. The primary cells of conidiophore were measured as 35.48μ in length and 10.13μ in diameter (Plate 5).

Conidia: Conidia were straight, cylindrical or rod shaped and having 1-2 septation. The average size of the conidium was measured as $40.75 \times 6.65\mu$ (Plate 6).

Vesicle: Vesicles were clavate in shape and measured as 39.69 μ in length and 22.85 μ in diameter (Plate 7 and Plate 8).

Microsclerotia: Chlamydospores developed in abundance in older cultures and formed in chains. These chains of chlamydospores are known as microsclerotia. The average diameter of the brownish and roundish microsclerotia was measured as 9.59 μ (Plate 9).

Identification and confirmation

The pathogen was identified as *Cylindrocladium* spp. (more probably *C. camelliae*/*C. scoparium*) and confirmed following the literature described in CMI-Description of pathogenic Fungi and Bacteria, No. 362, Set 37, published in 1973. Further confirmation was made following the literature described in Illustrated Genera of Imperfect Fungi by H. L. Barnett, published in 1960.

Taxonomy

Kingdom: Plantae

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae

Genus: *Cylindrocladium* (Anamorphs/Mitotic morphs),

Calonectria (Teleomorphs/Meiotic morphs)

Disease name: *Cylindrocladium* cutting rot of tea

Cylindrocladium cutting rot of tea, a new nursery disease has been recorded at Bangladesh Tea Research Institute farm in 2014. In the present findings, it has been shown that the aboveground symptoms of the diseased cuttings were identified as blighting, drying, defoliation of leaves, and weak development of dormant axillary buds. Underground symptoms were recorded as brown to blackish, necrotic lesion on the stem that failed to callusing and rooting. Earlier studies indicated that *Cylindrocladium* spp. might cause a variety of symptoms including damping off, foliage blight, stem lesions, root rot etc. on both conifers and hardwood species such as yellow-poplar, black walnut, and sweetgum (Thies and Patton, 1970; Cordell, 1976; Cordell and Skilling, 1975 and Barnard, 1984). *Cylindrocladium* species was reported to cause majority of the diseases found in forest nurseries including leaf diseases and shoot blight resulting in defoliation of trees leading to loss of vigor. Similar Cutting rot symptoms caused by *Cylindrocladium* spp. was also noted in *Vallea stipolaris* and *Eucalyptus* species (Hodges & May, 1972; Sharma *et al.*, 1985; Crous *et al.*, 1991; Booth *et al.*, 2000; Park *et al.*, 2000; Crous & Kang, 2001; Crous, 2002; Old *et al.*, 2003; Rodas *et al.*, 2005 and Lombard *et al.*, 2009, 2010).

In the present findings, it has been noted that the fungus isolated from infected tea cuttings composed of septate mycelia bearing regularly and repeatedly dichotomously branched conidiophores. Conidia were straight, cylindrical or rod shaped ($40.75 \times 6.65\mu$) having 1-2 septation. Vesicle was clavate in shape. Brown and roundish Chlamydospores were formed in chain and measured as 9.59μ in diameter. Comparatively similar result was found in the previous investigation. The previous study had shown that the sporogenous part of the conidiophore was formed of two or more bifurcate lateral branches to the main stripe composed of septate, cylindrical conidia of different size, ellipsoid or globose vesicle and chained microsclerotia (Barnett, 1960; Thies and Patton, 1970; Anon, 1973; Cordell, 1976; Cordell and Skilling, 1975 and Barnard, 1984).

Conclusion

Cutting rot in primary bed is hitherto a common problem in tea culture. The present finding of the research will provide a preliminary message to the tea associates about the identified causal agent i.e. *Cylindrocladium* spp., and the assigned disease *Cylindrocladium* cutting rot of tea. Further research based on environment friendly control strategies will be continued to save the cuttings from fungal infection in primary beds.

Acknowledgement





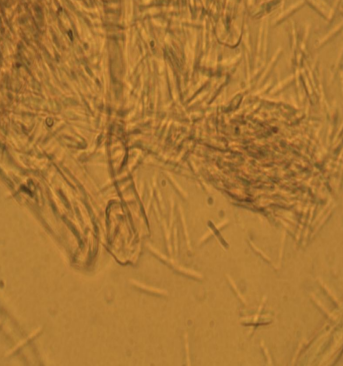

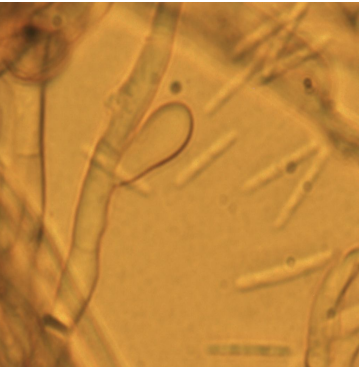
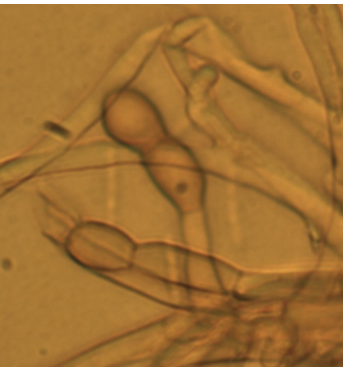
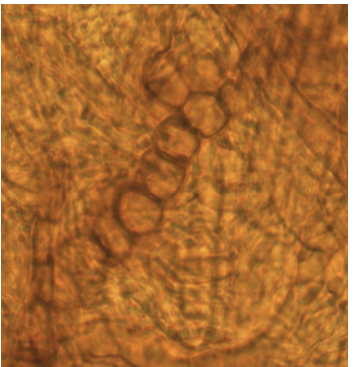
We are thankful to the honorable Chairman, Bangladesh Tea Board and the Director, BTRI for their continuous inspiration in doing new research for the betterment of tea industry. We are also indebted to the division of entomology for cordial support in keeping microscopic images of the identified fungus.

Table 1. Monthly rainfall, temperature and relative humidity report (June-December, 2014)

Months	Rainfall (mm)	Temperature ($^{\circ}\text{C}$)		Relative Humidity (%)
		Max ^m	Min ^m	
June	457	32.91	25.28	82.33
July	310	34.0	25.8	77.4
August	399	32.6	25.5	82.0
September	487	32.8	24.6	81.2
October	74	32.2	21.8	77.9
November	00	30.7	16.3	71.8
December	00	26.4	12.2	75.5

Source: BTRI weather office, Srimangal, Moulvibazar, Bangladesh.

Different Vegetative and reproductive structure of *Cylindrocladium* spp.

		
<p>Plate 1. Infected primary bed</p>	<p>Plate 2. Healthy & diseased stem</p>	<p>Plate 3. Culture of inocula on PDA</p>
		
<p>Plate 4. Mycelia of <i>Cylindrocladium</i></p>	<p>Plate 5. Branched conidiophores</p>	<p>Plate 6. Conidia of <i>Cylindrocladium</i></p>
		
<p>Plate 7. Immature vesicle</p>	<p>Plate 8. Mature vesicle</p>	<p>Plate 9. Microsclerotial chain</p>

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BIOPESTICIDES: A POTENTIAL TOOL FOR THE MANAGEMENT OF PLANT PARASITIC NEMATODES IN TEA

S.K. Paul^{1*}, M. Ahmed² and M.S.A. Mamun³

Abstract

Several species of plant parasitic nematodes have been encountered in tea soils and cause a significant economic damage to tea plants both in the nursery and in new plantation. Nematodes mainly destroy or cease to function of the root system of tea seedlings resulting stagnation of growth, yellowing and wilting of leaves even die-back and death may occur in severe cases. Chemical pesticides are only the widely useful remedy to reduce nematode population from the tea soil world wide. Continuous application of inorganic pesticides imparts resistance to the pests, deteriorate soil health, kill the beneficial microorganism as well as damage human health and environment. So, the development of alternative control strategies and tactics to replace or complement chemical nematicides for the management of plant parasitic nematodes is urgently needed. In this context, biopesticides like botanicals (plant extracts) and nematopathogenic microorganisms (bacteria and fungi) can be a potential tool for controlling plant parasitic nematodes because they are considered as environmentally safe, biodegradable, economical and renewable. This review article discusses the potential uses, advantages and limitations of biopesticides. It also encourages the use of biopesticides as a component of integrated pest management of plant parasitic nematodes in tea.

Keywords: Plant parasitic nematodes, Biopesticides, Potential tool, Tea.

Introduction

Tea (*Camellia sinensis* L.) is the most popular and cheapest beverage in the world. The global production of tea is about 4,299 million kg produced in about 40 countries of the world (ITC, 2013). Tea plants are subjected to the attack of several insects, mites, nematodes, fungal pathogens and weeds causing important economic losses. Amongst the various constraints to tea production, plant parasitic nematodes have a significant economic importance (Muraleedhran, 1993; Ahmed, 2005; Mamun *et al.*, 2011). It is an important soil pest of tea in the nursery and in new clearing which invades tea seedlings, upto 1 year old, in young plantation (Sana, 1989). But the species of root-knot nematode, *Meloidogyne brevicauda* is the only one that attacks mature tea and has been so far recorded in Sri Lanka, North East India and South India (Sivapalan, 1972).

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The crop loss due to nematode infestation is estimated to be about 15-20% plant injury and 350-500 kg of made tea per hectare per year in Sri Lanka (Sivapalan, 1972).

More than 40 species of plant parasitic nematodes, belonging to 20 genera have been recorded in different tea growing countries of the world (Sivapalan, 1972) while Chen and Chen (1989) reported 82 species of nematodes are associated with tea plants. The most frequently occurring nematodes associated with tea plants in different countries are species of *Pratylenchus*, *Meloidogyne*, *Aphelenchoides*, *Helicotylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Ditylenchus*, *Hemicriconemoides*, *Longidorus*, *Paratylenchus*, *Radopholus*, *Rotylenchulus*, *Tylenchus*, *Rotylenchus* and *Xiphinema* (Nalini *et al.*, 2005). In Bangladesh tea, 10 species of nematodes have been recorded. Among them the species of *Meloidogyne*, *Pratylenchus*, *Hoplolaimus* and *Tylenchus* are predominant in Bangladesh tea (Ahmed, 2005). The root system is reduced or eradicated by nematode causing gall or lesion of the roots. The above ground symptoms of such infected plants are stunting, wilting and yellowing of leaves (Sana, 1989).

The management of nematodes is more difficult than that of other pests because nematodes mostly inhabit the soil and usually attack the underground parts of the plants (Mian, 1998). Use of chemical nematicides has been one of the primary means of controlling plant parasitic nematodes for the past five decades. However, the repeated application of various chemicals under intensive cultivation has not only contaminated the ground and surface water but has also disturbed the harmony existing among the soil, plant and microorganisms (Elyousr *et al.*, 2010). There has been growing public concern about the negative impact of pesticides on the environment as well as on the safety and quality of food. Due to increasing awareness of pesticidal hazards and contamination of biosphere, biopesticides such as botanicals (plant extracts) and nematopathogenic microorganisms (bacteria and fungi) have created worldwide interest in pest control methods, which offer an environmentally safe and ecologically feasible option for plant protection with great potential for promoting sustainable agriculture (Radhakrishnan, 2010; Pendse *et al.*, 2013). The beneficial effects of certain types of plant derived materials and microorganisms in soil have been attributed to a decrease in the population densities of plant parasitic nematodes in different crops world wide (Chitwood, 2002; Elyousr *et al.*, 2010; Rahanandeh *et al.*, 2012; Taye *et al.*, 2012; Mamun *et al.*, 2014). In this review, the potential uses of botanicals and nematopathogenic microorganisms have been discussed in reducing populations of nematodes to facilitate future research on the management of plant parasitic nematodes in tea.

Advantages of biopesticides and their mode of action

As an alternative to chemical pesticides biopesticides are being increasingly used. Biopesticides are mainly derived from plant extracts (botanicals) and nematopathogenic organism (microbials). Use of botanicals maintains the health of the soil and sustains its life by increasing soil organic matter content. They are biodegradable, economical and can be locally prepared (Radhakrishna, 2010; Akphekhai *et al.*, 2012; Taye *et al.*, 2012). Microbial pesticides rely upon the potential biochemicals synthesized by the microbes and it requires in small quantities often decomposing rapidly (Tian *et al.*, 2007; Elyousr *et al.*, 2010; Khan and Haque, 2011). Biopesticides are less toxic than chemical pesticides and safer to the beneficial microorganism, human and environment. In a number of instances, they have been proved as effective control agents in managing plant parasitic nematodes. In general, they have specific mode of action with a narrow range of targets. Since, they are slow acting upon the target organisms, they need relative critical application time which results in suppression of populations rather than their elimination (Elyousr *et al.*, 2010).

(a) Plant extracts

Plants contain many bioactive organic chemicals in the form of metabolites which act against pests as insecticidal, repellent, antifeedant, bacteriocidal, fungicidal, nematicidal and also as stomach poison. As many as 2121 plant species have been reported to possess pest control properties. It has been estimated that the plants contain as many as 4,00,000 secondary metabolites (Radhakrishnan, 2010). Over 90% of the aqueous extracts from 153 Chinese herbal remedies representing 71 plant families were nematicidal or nematostatic to *Pratylenchus* spp or *Meloidogyne* spp (Chitwood, 2002). In recent years, a variety of plants have been evaluated for their nematicidal properties and efficacy in the management of plant parasitic nematodes (Isman, 2006; Radhakrishnan, 2010; Taye *et al.*, 2012).

The mechanisms of action of plant extracts may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Elyousr *et al.*, 2010; Taye *et al.*, 2012). Extracts from leaves, seeds, flowers, stems, cloves, rhizomes, roots etc. of different plants reduce nematode population of *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Helicotylenchus*, *Tylenchorhynchus*, *Radopholus*, *Tylenchulus*, *Hoplolaimus*, *Heterodera*, *Xiphinema* etc. (Wiratno *et al.*, 2009; Radhakrishnan, 2010; Taye *et al.*, 2012; Akphekhai *et al.*, 2012). Botanicals reduce root gall formation, inhibit the hatching of juveniles and kill nematode (Akphekhai *et al.*, 2012). This inhibitory effect of extracts might be due to the chemicals, terpenes (mono and sesquiterpenes) and lactones present in extracts that possess ovicidal or larvicidal properties resulting in inhibition of nematode multiplication (Taye *et al.*, 2012). These chemicals either affected the embryonic

development or killed the eggs. Three types of plant extracts are generally used in controlling nematodes i.e. aqueous, methanolic and ethanolic. In most of the cases the effective concentration of plant extracts ranges from 5% to 10% (Radhakrishnan, 2010). In broad-spectrum, plant species used as biopesticides should be perennial in nature and should not become a weed or a host to insect pests or plant pathogens. They may have subsequent economic uses but should not be harmful to non-target organisms, wildlife, human beings or to the environment. It must be easy to cultivate, harvest, extract preparation should be simple, not to be laborious/ time-consuming or require high technical input. Plant species identified as nematicides along with their family, the plant parts used and active ingredients are cited in Table 1.

Table 1. Potential biopesticidal plants for controlling plant parasitic nematodes

Common name	Botanical name	Family	Plant parts used	Active ingredients
Adathoda	<i>Adhatoda vasica</i>	Acanthaceae	Leaves	Vasicine
Bankalmi	<i>Ipomoea sepiaria</i>	Convolvulaceae	Leaves	-
Betel vine	<i>Piper betle</i>	Piperaceae	Leaves	Monoterpenes
Bhat	<i>Clerodendron infortunatum</i>	Verbenaceae	Leaves	Saponins
Black nightshade	<i>Solanum nigrum</i> <i>S. khasianum</i>	Solanaceae	Leaves, berries, root	Solanine
Black pepper	<i>Piper nigrum</i>	Piperaceae	Berries	Piperine
Bur weed	<i>Xanthium strumarium</i>	Asteraceae	Leaves	-
Calotrope	<i>Calotropis procera</i>	Asclepiadaceae	Leaves, fruits	Calotropain
Castor bean	<i>Ricinus communis</i>	Euphorbiaceae	Seeds	Ricin
Chinaberry	<i>Melia azedarach</i>	Meliaceae	Seeds	Terpenes
Citronella	<i>Cymbopogon nardus</i>	Poaceae	Leaves, stem	Citronellal
Custard apple	<i>Annona squamosa</i>	Annonaceae	Leaves, seeds	Squamocin
Datura	<i>Datura metel</i> <i>D. stramonium</i>	Solanaceae	Leaves, fruits	Tropane
Dholkalmi	<i>Ipomoea fistulosa</i>	Convolvulaceae	Leaves	Tarprnes
Garlic	<i>Allium sativum</i>	Amaryllidaceae	Cloves	Allicin
Ginger	<i>Zingiber officinale</i>	Zingiberaceae	Rhizome	Zingerone
Green amaranth	<i>Amaranthus viridis</i>	Amaranthaceae	shoot	Phenolic acid
Karanja	<i>Pongamia pinnata</i>	Fabaceae	Seeds	Karanjin
Lantana	<i>Lantana camara</i>	Verbenaceae	Leaves	Lantanilic acid
Lemongrass	<i>Cymbopogon citrates</i>	Poaceae	Leaves, stem	Limonene
Mahogany	<i>Swietenia mahagoni</i> <i>S. macrophylla</i>	Meliaceae	Leaves, seeds	Saponins

Marigold	<i>Tagetes</i> spp	Asteraceae	Leaves	α - therthienyl
Neem	<i>Azadirachta indica</i>	Meliaceae	Leaves, seeds	Azadiractin,
Nishinda	<i>Vitex negundo</i>	Lamiaceae	Leaves	terpenes
Papaya	<i>Carica papaya</i>	Caricaceae	Leaves, seeds	Benzyl isothiocyanate
Pigweed	<i>Chenopodium album</i>	Amaranthaceae	Shoot, root	Saponins
Pyrethrum	<i>Chrysanthemum cinerariaefolium</i> , <i>C. coronarium</i>	Compositae	Flowers	Stachydrine
Sweet flag	<i>Acorus calamus</i>	Acoraceae	Rhizomes	β -asarone
Tamarind	<i>Tamarindus indica</i>	Fabaceae	Leaves, seeds	-
Tobacco	<i>Nicotiana tabacum</i>	Solanaceae	Leaves	Nicotine
Tree marigold	<i>Tithonia diversifolia</i>	Asteraceae	leaves	Alkaloids
Turmeric	<i>Curcuma longa</i>	Zingiberaceae	Rhizome	Curcumin
Water pepper	<i>Polygonum hydropiper</i>	Polygonaceae	Whole plant	Sesquiterpens

(b) Microbials

Plant-associated microorganisms have important roles in natural and induced suppressiveness of plant parasitic nematodes. Many researchers have reported on the effectiveness of several culturable microorganisms *viz.* bacteria and fungi as biocontrol agent against plant parasitic nematodes in different crops (Rahanandeh *et al.*, 2012; Mukhtar *et al.*, 2013). They produce and excrete metabolites that are inhibitory to nematodes and induce systemic resistance against nematodes (Elyousr *et al.*, 2010).

Bacteria: Bacteria are numerically the most abundant organisms in soil, and some of them, for example members of the genera *Pasteuria*, *Pseudomonas* and *Bacillus* (Tian *et al.*, 2007), have shown great potential for the biological control of nematodes. Nematopathogenic bacteria are distributed broadly, possess diverse modes of action, and have broad host ranges. A variety of nematopathogenic bacterial groups have been isolated from soil, host-plant tissues, and nematodes as well as their eggs and cysts (Tian *et al.*, 2007). They affect nematodes by a variety of modes: for example parasitizing; producing toxins, antibiotics, or enzymes; interfering with nematode-plant-host recognition; competing for nutrients; inducing systemic resistance of plants; and promoting plant health (Tian *et al.*, 2007; Rahanandeh *et al.*, 2012). These bacteria have a wide range of suppressive activities on different plant parasitic nematodes *viz.* *Pratylenchus* spp., *Meloidogyne* spp., *Radopholus similis*, *Rotylenchulus reniformis*, *Helicotylenchus dihystra*, *Heterodera* spp. (Tian *et al.*, 2007; Elyousr *et al.*, 2010; Khan and Haque, 2011; Rahanandeh *et al.*, 2012). They form a network with complex interactions among bacteria, nematodes, plants and the environment to control populations of plant parasitic nematodes in natural conditions (Tian *et al.*,

2007; Rahanandeh *et al.*, 2012). Commonly used nematopathogenic bacteria and their pathogenic effect are given in Table 2.

Table 2. Available bacterial groups with pathogenic activity against plant parasitic nematodes

Bacteria	Pathogenic effects on nematodes
<i>Bacillus subtilis</i>	Penetrate the nematode cuticles and eventually digest the target organism.
<i>Bacillus thuringiensis</i>	Produce cry proteins that show toxicity to larval stages of parasitic nematodes
<i>Pasteuria penetrans</i> ; <i>P. thornei</i> ; <i>P. nishizawae</i> ; <i>P. usage</i>	Major economically important plant parasitic nematodes have been observed to be parasitized by <i>Pasteuria</i>
<i>Pseudomonas fluorescens</i>	Colonizes the rhizosphere, which facilitates the infection of egg masses protruding from female root knot nematodes on infected roots

Fungi: Nematopathogenic fungi are carnivorous fungal species that use their spores or mycelial structures to capture vermiform nematodes, or use their hyphal tips to parasitize the eggs and cysts of nematodes or produce toxins to attack nematodes (Khan and Haque, 2011). They are the natural enemies of nematodes and have developed very sophisticated strategies to either infect or capture these small animals. Nematopathogenic fungi are a diverse group of microorganisms, and their nematopathogenic habit is generally considered to have evolved independently in different fungal classes. Nematopathogenic fungi or fungi destructive to nematodes can infect, kill and digest nematodes in each of three phases (eggs, larvae and adults). These soil fungi are present in most parts of the world and are found in all types of weather. Over 200 species from 6 different classes of fungi were reported to parasitize on nematode eggs, juveniles, adult and cysts (Mukhtar *et al.*, 2013). Many of the nematopathogenic fungi are facultative parasites and they can survive in soil as a saprophyte. If there is a host plant, they can change from a saprophytic to a parasitic stage and produce an infection form structures, e.g. trapping organs, hyphal coils or appressoria. These infection structures vary depending on the type of host nematode, fungus or plant. Some potential fungi and their pathogenic effect are described in Table 3.

Table 3. Common fungi groups with pathogenic activity against plant parasitic nematodes

Fungi	Pathogenic effects on nematodes
<i>Aspergillus fumigatus</i> , <i>A. niger</i>	Kill J2 larvae of root knot nematode
<i>Beauveria bassiana</i>	Produce secondary metabolites bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein
<i>Metarhizium anisopliae</i>	Produce cyclic peptides and destruxins toxin that parasitize J2 larvae of target nematodes
<i>Paecilomyces lilacinus</i>	Produce serine protease toxin which parasitize egg and egg mass of target nematodes.
<i>Pochonia chlamydosporia</i>	infects nematode eggs and sedentary females of cyst nematodes by hyphae produced on actively growing mycelium
<i>Trichoderma harzianum</i> , <i>T. viride</i> ,	Produce nematode cuticle degrading enzymes which are toxic to juveniles of root-knot nematodes and inhibit egg hatch.
<i>Verticillium lecanii</i>	Infect eggs and kills J2 larvae of target nematodes

Prospects of biopesticides for controlling plant parasitic nematodes in tea

There is a great scope to use the aforesaid biopesticides in controlling plant parasitic nematodes in tea. The indigenous plants like *Adhatoda vasica*, *Clerodendron infortunatum*, *Piper betel*, *Piper nigrum*, *Melia azedarach*, *Cymbopogon nardus*, *Ipomoea fistulosa*, *Lantana camara*, *Cymbopogon citrates*, *Swietenia mahagoni*, *Azadirachta indica*, *Polygonum hydropiper* are available in or surroundings of tea estates. Besides, some indigenous plants such as *Xanthium strumarium*, *Calotropis procera*, *Ricinus communis*, *Annona squamosa*, *Datura metel*, *Zingiber officinale*, *Pongamia pinnata*, *Tagetes* spp., *Vitex negundo*, *Carica papaya*, *Curcuma longa*, *Allium sativum* can be easily grown in abundant or fallow land of tea garden. The planters can also establish such herbal plots by growing those indigenous plants in tea estates. Moreover, *Azadirachta indica*, *Nicotiana tabacum*, *Chrysanthemum cinerariaefolium*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Ricinus communis*, *Pongamia pinnata*, *Tagetes* spp. etc. based herbal products are commercially available. These commercial products can be used for controlling tea nematodes. Now-a-days, different microbials like *Bacillus thuringiensis*, *Pseudomonas fluorescens*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus*, *Trichoderma viride*, *Verticillium lecanii* and that based commercial formulations are offered to control phytonematodes. Generally, tea nursery is established in a small area of 0.5-1.0 ha which divided into a cluster of mini beds. So, biopesticides can be applied easily in tea nursery. It can be used in pit during plantation in nematode infested area. In case of existing plantation biopesticides may be applied in collar region. Thus it will reduce the chemical load and environmental hazards.

Limitations of biopesticides

Use of biopesticides against nematodes has some limitations. Exploitation of biopesticides in nematode management requires skill, labour intensive, non-systemic and subject to rapid inactivation by varied environmental factors in comparison with other synthetic products. The composition and concentration of active phytochemical can vary with the plant source and the extraction process. Active compounds in biologically-derived products are rarely identified. Activity may not be broad-spectrum, so response of nematode genera to a phytochemical may differ (Chitwood, 2002). In view of low persistence, more frequent sprays of botanicals may be necessary increasing the cost of control.

Inconsistent performance of applied microbes has been reported as a primary obstacle in exploring this mode of management. This inconsistency is due to abiotic and biotic factors. Biotic factors include interactions with non-target organisms, damage caused by non-target pathogens and pests, degree of rhizosphere and/or soil colonization by a biocontrol agent, initial population levels of the target organisms, susceptibility of the host plant species and host plant cultivar. Abiotic factors include climate as well as physical and chemical composition of the rhizosphere (Pendse *et al.*, 2013). These factors mitigating the performance of beneficial microbes explain their differential performance in various soil environments. Formulations that will allow for adequate shelf-life and infectivity in the field are a problem. Microbial pesticides are generally slow-acting, lacking persistence and systemic ability in the field. They are readily inactivated by environmental factors such as sunlight, rain and wind and, therefore, remain infective for a short while.

Conclusion and Recommendations

Biopesticides is eco-friendly option for the management of plant parasitic nematodes. Some plant extracts, bacteria and fungi have been shown potentiality in reducing plant parasitic nematodes population in soil. These potential biopesticides should be incorporated in IPM programme of nematode management in tea. It is important to survey and identify the common plants available in tea growing areas and study the mode of action of the active ingredients. It is also important to develop a simple user friendly technique for the indigenous preparations and to fix the correct dosage of the biopreparations for effective nematode control. Study should also be made to determine the effect of botanicals on other beneficial microorganisms in soil.

Isolation and identification of different species of nematopathogenic fungi and bacteria from tea soil is required. *In vitro* and *in vivo* studies should be made to find out the most effective microbials against tea nematodes. Mass production technique of those effective microorganisms should be developed for large scale use. Finally,

microbials using selected strain(s) of nematopathogenic fungi and bacteria in carrier based dry, powder as well as in liquid form must be developed for convenience of soil application and longer shelf life. Research on adaptation of nematopathogenic microorganisms to unfavorable condition such as temperature, humidity, sunlight or moisture stress should be done further. Assessment of the compatibility of nematopathogenic microorganisms to the chemical pesticides can offer improved scope for their integration. Efforts should be taken to improve efficiency in quality control of potential microbials. In case of commercially available botanicals and microbials, their active ingredient and shelf life is an important factor to be considered. Their efficacy should be tested before use. Research and development on biopesticidal management of plant parasitic nematodes in tea should be intensified.

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IN VITRO AND IN VIVO SCREENING OF SOME ENTOMOPATHOGENS AGAINST RED SPIDER MITE, *OLIGONYCHUS COFFEA* NIETNER (ACARINA: TETRANYCHIDAE) IN TEA

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Abstract

An experiment was carried out to evaluate the bioefficacy of some microbial biopesticides i.e. entomopathogens against red spider mite, *Oligonychus coffea* infesting tea under both in the Entomology Laboratory and main farm of Bangladesh Tea Research Institute (BTRI), Srimangal, Moulvibazar during the period from March 2013 to December 2014. Entomopathogens viz., *Beauveria bassiana*, *Metarhizium anisopliae*, *Streptomyces avermitilis*, *Paecilomyces fumosoroseus*, *Verticillium lecanii*, and *Pseudomonas fluorescens* @ 5.0 g/L, 5.0 g/L, 2.0 ml/L, 5.0 g/L, 5.0 g/L, 4.0 g/L concentration, respectively are considered as treatments. Effect of the entomopathogens on mortality of *Stethorus gilvifrons* and *Oxyopes* sp. the potent predator of red spider mite was also studied. Data were collected at 24 HAT, 48HAT, 72HAT in the laboratory and at weekly interval in field condition. Results indicated that all the biopesticides showed the toxic effect on red spider mite in tea and significantly reduced mite population both in laboratory and field condition. In laboratory condition, among the biopesticides, the highest mortality of red spider mites was observed in *Verticillium lecanii* (64.38%) followed by *Streptomyces avermitilis* (58.46%) and *Paecilomyces fumosoroseus* (56.67%) at 24 HAT. Similar trend was also found at 48 HAT & 72 HAT after spraying of biopesticides. From the field evaluation it was found that the application of *Verticillium lecanii* @ 4.0 kg/ha significantly reduced the mite incidence to the tune of 83.44-98.32% followed by *Streptomyces avermitilis* @ 2.0 L/ha to the tune of 81.83-97.24%. The order of toxicity of the tested biopesticides on adult red spider mite was *V. lecanii*>*S. avermitilis*>*P. fumosoroseus*>*P. fluorescens*>*M. anisopliae*>*B. bassiana* both *in vitro* and *in vivo*. Application of the tested biopesticides did not affect the non-target organisms such as *Stethorus gilvifrons* and *Oxyopes* sp. So, these commercially formulated entomopathogens may be utilized potentially in the tea fields as eco-friendly and safer biopesticides as well as one of the major components of integrated pest management (IPM) strategy to minimize the pesticide load in the tea ecosystem.

Keywords: Tea, Red Spider Mite, *Oligonychus coffea*, Biopesticides, Entomopathogens

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Introduction

Red spider mite, *Oligonychus coffeae* Nietner (Acarina: Tetranychidae) is one of the major and serious pests of tea in 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, 2014). 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 (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 accelerates 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.

To combat these problems different groups of pesticides like organochlorine, organophosphate, pyrethroids, carbamates and some unclassified group have been used in the tea fields since 1960. Different group of pesticides such as Sulphur, Ethion, Quinalphos, Propargite, Abamectin, Dimethoate, Fenvalerate, Fenpropathrin, Fenazaquin, Bifenthrin, Hexythiazox, Spiromesifen and Fenpyroximate 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). Their prolonged and extensive uses cause destruction of beneficial organism including natural predators, parasitoids and pollinators. It may further lead to development of resistance in insect pests, phytotoxicity, residue in tea dust and last but not the least the environmental pollution. Chemical pesticides have been used for a long time, which have serious drawbacks, such as direct toxicity to beneficial insects, fishes and human being, pesticide induced resistance, health hazard and increased environmental and social costs (Pimental *et al.* 1980).

Many tactics are used in IPM strategy in tea plantation, including cultural practices, biological control agents, chemical pesticides, pest-resistant varieties, and physical barriers (Mamun and Ahmed, 2011). The various components of the IPM practices in tea with a few specific examples are described Mamun *et al.* (2014), since the success stories with the use of IPM practices are numerous and increasing day by day.

Use of entomopathogenic fungi is a new arena of research for integrated pest control in tea. Several microbes are pathogenic to tea pests. The microbial biocides as *Beauveria bassiana* (Gurusubramanian *et al.*, 1999), *Verticillium leucanii* (Ghosh Hajra, 2002), *Paecilomyces fumosoroseus* (Barua, 1983), *P. lilacinus* (Gurusubramanian, 2005), *Metarhizium anisopliae* (Debnath, 2004) are very effective and have been used widely especially in organic gardens of Darjeeling against for the management of tea mosquito bug, tea mites, tea thrips. Formulations of the bacterial insecticides, *Bacillus thuringiensis* have been effectively used for the control of looper caterpillars, cutworms, flushworms and other lepidopteran pests (Muraleedharan and Radhakrishnan, 1989). Certain entomopathogenic fungi, *Verticillium lecanii*, *Paecilomyces fumosoroseus* and *Hirsutella thompsonii* were evaluated and found effective against pink, purple and red spider mites (Babu *et al.*, 2008). *Cladosporium* sp., *Aspergillus niger*, *A. flavus* found to be the potential entomopathogenic fungi for the management of *Helopeltis* in tea (Bordoloi *et al.*, 2011). *Metarhizium anisopliae* is the commonest entomopathogenic fungi that reduced the population of red spider mites, thrips and live wood termites in tea (Ahmed and Mamun, 2013).

Unfortunately, a little research work has been done on the use of entomopathogens as IPM approaches against red spider mite infesting tea in Bangladesh. Attempts have been made to evaluate some entomopathogens against red spider mite of tea and its' effect on natural enemies in tea ecosystem in Bangladesh.

Materials and Methods

An experiment was carried out to evaluate the bioefficacy of some microbial pesticides (entomopathogens) viz., *Beauveria bassiana*, *Metarhizium anisopliae*, *Streptomyces avermitilis*, *Paecilomyces fumosoroseus*, *Verticillium lecanii*, and *Pseudomonas fluorescens* against red spider mite, *Oligonychus coffeae* infesting tea under both in the Entomology Laboratory and main farm of Bangladesh Tea Research Institute (BTRI), Srimangal, Moulvibazar during the period from March 2013 to December 2014. The Red spider mite was collected from different sections of BTRI main farm and reared in the Entomology Laboratory, BTRI, at 27-30°C temperature and 70-80% relative humidity on a susceptible tea clone, BT2 by following detached leaf culture method of Helle and Sabelis (1985) with slight modifications. The mite pests were reared on tea leaves in rectangular jars (9.5 cm x 7.5 cm x 20 cm).

The test materials

Formulations of six commercial microbial pesticides (entomopathogens) i.e. *Beauveria bassiana*, *Metarhizium anisopliae*, *Streptomyces avermitilis*, *Paecilomyces fumosoroseus*, *Pseudomonas fluorescens* *Verticillium lecanii* were collected from different multinational companies (Table 1).

Table 1. Entomopathogens evaluated for miticidal activities against red spider mites in tea

Technical Name	Commercial Name	Manufacturer/Supplier Name
<i>Beauveria bassiana</i>	BB-Power	Bioscience India (Pvt.) Ltd.
<i>Metarhizium anisopliae</i>	Bio Terminator	Shakti Biotech Ltd., India
<i>Streptomyces avermitilis</i>	Biotin M	Russell IPM, UK
<i>Paecilomyces fumosoroseus</i>	Shakti Paecil	Shakti Biotech Ltd., India
<i>Pseudomonas fluorescens</i>	Phasal Rakshak	International Panaacea Ltd., India
<i>Verticillium lecanii</i>	Vertifire-L	International Panaacea Ltd., India



Short descriptions of the selected entomopathogens are enumerated below.

Beauveria bassiana

Beauveria bassiana is an insect pathogenic fungus that grows naturally in soils throughout the world. When spore of this fungus come in contact with the cuticle (skin) of susceptible insects, they germinate and grow directly through the cuticle to the inner body of their host eventually killing it. It is being used as a biological insecticide to control a number of pests such as termites, thrips, whiteflies, aphids, different beetles and mites. The product contains WP 2×10^8 CFU/gm spores. It is target specific and do not destroy beneficial organisms. It does not leave any harmful residues in the finished products.

Metarhizium anisopliae

Metarhizium anisopliae, formerly known as *Entomophthora anisopliae*, is a fungus that grows naturally in soil throughout the world. The fungus was first isolated by a Russian scientist Ilya I. Mechnikov from the diseased beetle *Anisoplia austriaca* in 1878. The formulation of *M. anisopliae* is highly effective and naturally parasitizes several insect pests such as aphids, jassids, termites, white grubs and mites of different agricultural crops. When sprayed on the plants it generally enters into the insects through spiracles and pores in the sense organs and kills the insect. The product contains WP 2 x 10⁸ CFU/gm spores.

Streptomyces avermitilis

Streptomyces avermitilis is a bacterium species in the genus *Streptomyces*. The first complete genome sequence of *S. avermitilis* was completed in 2003. *S. avermitilis* is responsible for the production avermectins, of which one of the most widely employed drugs against nematode and arthropod infestations is ivermectin, as well as abamectin. Avermectins are novel family of natural compounds produced by the soil actinomycete, *S. avermitilis*. These compounds are endowed with nematicidal, acaricidal and insecticidal properties. It has shown excellent initial and residual control of immature and adult mites on a number of crops.

Paecilomyces fumosoroseus

Paecilomyces fumosoroseus is a microscopic fungus that infects and kills insects. It shows promise as a biological pesticide with an extensive host range. Among mites, susceptible species include the spotted spider mite (*Tetranychus urticae*), the European red mite (*Panonychus ulmi*), the brown mite (*Byrobia rubrioculus*) and the apple rust mite (*Aculus schlectendali*). The product contains WP 2 x 10⁸ CFU/gm spores. The fungus neither grows nor develops at temperatures above 32 °C and is not thought to be pathogenic to human beings. It has not been found to be toxic to rats, birds, honeybees, bumblebees or a wide range of non-target arthropods.

Pseudomonas fluorescens

Pseudomonas fluorescens is a common gram-negative, rod-shaped bacterium. Some *P. fluorescens* strains present biocontrol properties, protecting the roots of some plant species against parasitic fungi such as *Fusarium* or *Pythium*, as well as some phytophagous nematodes. *Pseudomonas aeruginosa* (Poinar and Poinar, 1998) and *Pseudomonas putida* (Aksoy *et al.*, 2008) have been reported to cause disease in spider mite, *Tetranychus urticae*. Bacterial chitinases have been reported to be effective in controlling the insects and mites by hydrolyzing chitinous exoskeleton (Kramer and Muthukrishnan, 1997). The product contains WP 2 x 10⁸ CFU/gm spores.

Verticillium lecanii

Verticillium lecanii (which was previously known as *Cephalosporum lecanii*) is now an approved name of an entomopathogenic fungus species. Insects are infected when they come into contact with the sticky fungal spores, which then grow and invade the body, thus the internal organs are consumed, leading to their death. The practical application of biopesticides made on the base of *V. lecanii* was searched on different species of phytophages especially on aphids, mealy bugs, thrips, mites and nematodes. The product contains WP 1×10^8 CFU/gm spores. The dosage of the product is 4.0 kg/ha for the control of mites in tea.

Laboratory test for acaricidal activity of entomopathogens

Commercial formulations of certain biopesticides were prepared with distilled water in the required concentrations for bioassay in the laboratory experiment. The required concentrations were *Beauveria bassiana* @ 5.0 g/L, *Metarhizium anisopliae* @ 5.0 g/L, *Streptomyces avermitilis* @ 2.0 ml/L, *Paecilomyces fumosoroseus* @ 5.0 g/L, *Pseudomonas fluorescens* @ 5.0 g/L and *Verticillium lecanii* @ 4.0 g/L. For laboratory evaluation of biopesticides, 30 healthy adult red spider mites were released on a healthy detached tea leaf of BT2 clone in the laboratory. The experiment was designed in Completely Randomized Design (CRD) with three replications. Each concentration of selected biopesticides was sprayed on both surfaces of leaf using fine atomizer. Unsprayed discs were kept as control. The number of live red spider mite was counted by a magnifying glass at 24, 48 and 72 hours after treatment. Each treatment was replicated thrice. Original data were corrected by Abbott's (1987) formula.

Field evaluation of entomopathogens against red spider mite

A field trial was conducted to evaluate the bioefficacy of different entomopathogens (biopesticides) against red spider mite in tea fields at BTRI main farm. The treatments were *Beauveria bassiana* @ 5.0 kg/ha, *Metarhizium anisopliae* @ 5.0 kg/ha, *Streptomyces avermitilis* @ 2.0 L/ha, *Paecilomyces fumosoroseus* @ 5.0 kg/ha, *Pseudomonas fluorescens* @ 5.0 kg/ha and *Verticillium lecanii* @ 4.0 kg/ha and untreated control. The experiment was set up in randomized complete block design (RCBD) with three replications. After selection of the plots, pre-treatment count was taken in the respective plots and two rounds of spray were given at an interval of two weeks with hand operated knapsack sprayer with water volume of 1000 L/ha. Mite populations were assessed at weekly interval by collecting 10 mature leaves at random from each block and from each leaf; mites were counted using mite brushing machine (Model-Leedom Engineering, USA) and a compound microscope. Field performance of selected biopesticides against red spider mites in tea was calculated by using Henderson & Tilton (1955) formula.

Effect of entomopathogens on Stethorus gilviforns and Oxyopes sp.

Entomopathogens was also tested for its effect on the mortality of two important predators of red spider mite i.e. *Stethorus gilviforns* and *Oxyopes* sp. Approved concentrations of biopesticides were sprayed directly onto adults of *Stethorus gilviforns* and *Oxyopes* sp. Spraying was performed as described for the laboratory bioassay and observed everyday for mortality.

Statistical analysis

The experimental data were statistically analyzed 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 \pm 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

The bioefficacy of different entomopathogens against red spider mite, *Oligonychus coffeae* infesting tea under laboratory and field condition are presented in Tables 2-3.

Laboratory test for acaricidal activity of entomopathogens

Data from the laboratory on the pathogenicity of the fungi and other entomopathogens are given in Table 2. The study revealed that there was a distinct significant difference in the susceptibility of red spider mites in tea to certain biopesticides.

Table 2. Laboratory evaluation of certain entomopathogens against red spider mites of tea

Treatment	Dosage	Percent Mortality*			Mean
		24 HAT	48 HAT	72 HAT	
T ₁ - <i>Beauveria bassiana</i>	5.0 g/L	37.78 \pm 1.09f	44.44 \pm 0.84f	52.22 \pm 2.38f	44.81 \pm 1.92e
T ₂ - <i>Metarhizium anisopliae</i>	5.0 g/L	45.55 \pm 2.34e	51.11 \pm 1.12c	56.67 \pm 1.42e	51.11 \pm 1.46d
T ₃ - <i>Streptomyces avermitilis</i>	2.0 ml/L	58.46 \pm 1.62b	63.33 \pm 2.07b	71.11 \pm 0.96b	64.30 \pm 2.36b
T ₄ - <i>Paecilomyces fumosoroseus</i>	5.0 g/L	52.08 \pm 2.03d	61.32 \pm 1.68c	68.72 \pm 2.28c	60.71 \pm 1.66c
T ₅ - <i>Pseudomonas fluorescens</i>	5.0 g/L	56.67 \pm 1.87c	60.96 \pm 1.82d	64.46 \pm 1.64d	60.70 \pm 2.20c
T ₆ - <i>Verticillium lecanii</i>	4.0 g/L	64.38 \pm 1.46a	68.42 \pm 1.54a	73.26 \pm 1.58a	68.69 \pm 1.74a
CV (%)		7.16	7.56	4.37	6.42

*Mean of three observations (30 adults/observation)

HAT= Hours after treatment

\pm Standard error of means at a given concentration

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

Among the biopesticides, the highest mortality of red spider mites was observed in *Verticillium lecanii* (64.38%) followed by *Streptomyces avermitilis* (58.46%) and *Paecilomyces fumosoroseus* (56.67%) at 24 HAT. Similar trend was also found at 48 HAT & 72 HAT after spraying of biopesticides (Table 2). The order of toxicity of the tested biopesticides on adult red spider mite was *V. lecanii*>*S. avermitilis*>*P. fumosoroseus*>*P. fluorescens*>*M. anisopliae*>*B. bassiana* (Fig. 1).

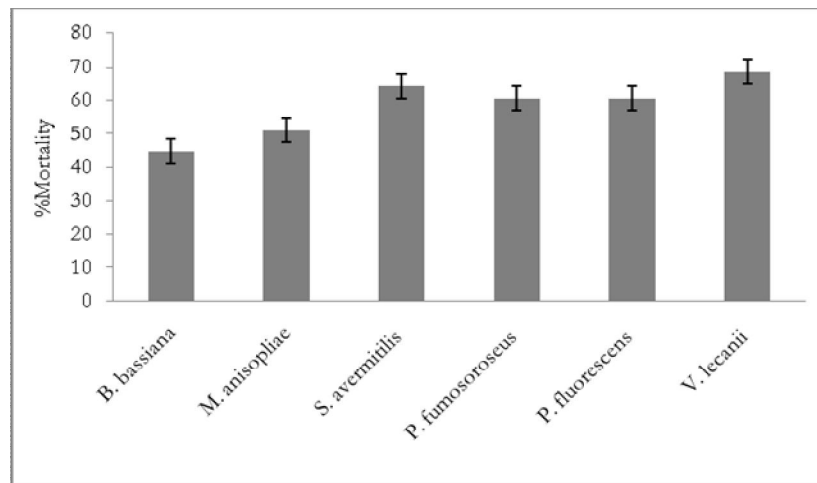


Fig. 1. Laboratory evaluation of some entomopathogens against red spider mite in tea
Field evaluation of entomopathogens against red spider mite

Results revealed from the field evaluation of different entomopathogens against red spider mites that all the biopesticides have the acaricidal value to reduce the infestation significantly of red spider mite in tea.

Table 3. Field evaluation of certain entomopathogens against red spider mites of tea

Treatment	Dosage /ha	Pre-treatment (No. of mites)	% effectiveness of biopesticides*				Mean
			After 1 st spray		After 2 nd spray		
			1 st wk	2 nd wk	3 rd wk	4 th wk	
T ₁ - <i>Beauveria bassiana</i>	5.0 kg	38	60.51f	76.84f	86.20f	90.48e	78.51f
T ₂ - <i>Metarhizium anisopliae</i>	5.0 kg	42	66.53e	80.91e	89.43e	93.45d	82.58e
T ₃ - <i>Streptomyces avermitilis</i>	2.0 L	36	81.83b	91.14b	96.34b	97.24b	91.64b
T ₄ - <i>Paecilomyces fumosoroseus</i>	5.0 kg	40	80.16c	88.36c	94.28c	96.37c	89.79c
T ₅ - <i>Pseudomonas fluorescens</i>	5.0 kg	43	78.28d	84.34d	91.43d	95.99d	87.51d
T ₆ - <i>Verticillium lecanii</i>	4.0 kg	39	83.44a	93.48a	97.76a	98.32a	93.25a
CV (%)			4.09	2.00	1.55	1.48	1.39

*Mean of three replications

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

Analysis of data from the field trial revealed that the application of *Verticillium lecanii* @ 4.0 kg/ha reduced the mite incidence significantly to the tune of 83.44-98.32% followed by *Streptomyces avermitilis* @ 2.0 L/ha to the tune of 81.83-97.24% (Table 3). The order of toxicity of the tested biopesticides on red spider mite was similar to that of laboratory bioassay (Fig.2).

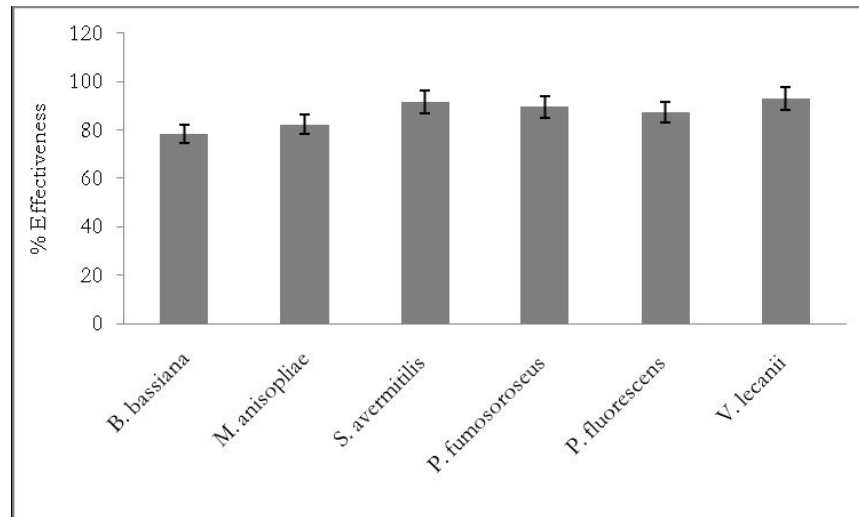


Fig. 2. Field evaluation of some entomopathogens against red spider mite in tea

Effect of entomopathogens on *Stethorus gilvifrons* and *Oxyopes* sp.

Application of the tested biopesticides did not affect the non-target organisms such as *Stethorus gilvifrons* and *Oxyopes* sp. (natural enemies of red spider mite). Therefore, bio-rational pesticide based integrated pest management should be emphasized so that the indigenous predators, parasites and pathogens that exist in tea ecosystem could be preserved for sustainable crop protection and also gives inkling for their better use under IPM program ensuring a healthier pesticide-free tea beverage.

The results of this study revealed the potential of entomopathogens as a microbial biocontrol agent by causing significant mortality of *O. coffeae*. Selvasundaram *et al.* (2001) observed the similar trend of acaricidal activity using entomopathogenic fungi for pink mite control in tea. Selvasundaram and Sudhakaran (1998) found that the commercial formulation of *Beauveria bassiana* @ 0.5% concentration was effective against flushworm in tea. Roobakkumar *et al.* (2011) also found *Pseudomonas fluorescens* as an efficient entomopathogen against *Oligonychus coffeae* infesting tea in South India.

Conclusion

Use of entomopathogens is a new arena of IPM strategy in tea. All the tested entomopathogens are significantly reduced the population of red spider mite infesting tea in Bangladesh. The tested products are also commercially available in the market. These eco-friendly entomopathogens may be considered for the use in tea fields as safer biopesticides as well as one of the major components of IPM to reduce the pesticides load in tea ecosystem.

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