1
Introduction

1. Introduction

1.a Tea plantation of Darjeeling Terai and the Dooars

Tea is the foliage crop harvested from the monocultures of *Camellia sinensis* O' Kuntz. All over the world infusions of tea leaves is taken as a popular invigorating and refreshing drink having excellent medicinal properties (Chakravartee, 1995).

According to Muraleedharan (1991) the tea plant in India originated from the triangular region formed by Naga, Manipur and Lushai extending along the border between Assam and Burma. The tea in Asian mainland stretches to China in North and to the hilly areas of Burma, Bangladesh, Thailand and Viet Nam in the South East. In summary the tea plant originally grew in mountainous forest areas, and then gradually moved to lower elevations where the ecological conditions were different from its original sources, causing changes in its growth forms along with a change in pests and diseases structure.

There are more than 1,500 varieties of tea grown in the world in more than 25 different countries. It is cultivated as a plantation crop, in acidic soil, warm climate with at least 150 mm of rain per annum (Anonymous, 1973). The tea was first used in China as a medicinal drink and later became a popular beverage. Nowadays, it has gained popularity world wide (www.communityipm.org/docs/Tea_Eco-Guide/01_History.PDF).

Tea in India is produced only in certain places. The three principal tea growing regions are Darjeeling, Assam and Nilgiri. Darjeeling and Assam are in the North-East while the Nilgiri regions are in South India. In Darjeeling foothills and plains, the main tea growing regions are Terai and the Dooars. The Terai and the Dooars region is located between 26°16'and 27°13' north latitude and between 87°59' and 89°53' east longitude and are divided by the mighty river Teesta. The Terai is situated 91m above mean sea level with an average rainfall of 350 cm and an average maximum temperature of 35°C and a minimum of 12°C. The soil is moderately acidic, rich in organic material and is suitable for tea plantation. Besides heavy rainfall, Terai and its adjacent Dooars regions are well watered by a number of major rivers and a host of rivulets. The major rivers from east to west of Terai and the Dooars are: Siltorsha, Jaldhaka, Teesta, Mahananda, Balason and Mechi. Some of the tea estates in Terai region easily accessible by road, namely are: Atol, Kamalpur, Maruti, Sanyashi, Gungaram, Dagapur, Simulbari, Haskhoya, Nischintapur, Longview and a few more.

The Dooars or *Duars* (=Doors) area comprises flood plains and the foothills of the eastern Himalayas in North-East India and is the gateway to Bhutan. The altitude of the Dooars region ranges from 90 m to 1750 m. Most of the rivers mark the borders of the Dooars tea districts mainly spread over the political district boundaries of Jalpaiguri. Depending on geophysical and climatic conditions, the Dooars region was divided in to six tea growing sub districts namely Damdim, Chulsa, Nagrakata, Binnaguri, Dalgaon and Kalchini. Monsoon generally starts from the middle of May and continues till the end of September. Winters are cold with foggy mornings and nights. Summer is mild and constitutes a short period of the year. According to the statistics of Tea Board (India), there are 158 big tea estates in the Dooars and total area under tea is 92095 hectares. Total production of made tea is 167 million kg with an average yield of 1816 kgs per ha. Besides these 1,67,245 number of small tea gardens are also recorded in the Dooars and its adjoining areas in West Bengal (Anonymous, 2003).

It was observed that about 78% of the countries total area of tea plantation is located in North-India. Out of the total, 53% is existing in Assam and 22% in West Bengal. (http://www.nabard.org/roles/ms/ph/tea.htm).

1.b Pest problem in tea with emphasis on lepidopteran pests

Various problems of tea estates such as illiteracy, poverty, lock out of tea gardens, diseases and efficacy of tea workers hamper the tea production and processing a lot. But one of the major causes of crop loss is due to pest attack, mainly the insect tea pests.

Annual production of tea in Terai has fallen in 2009 to 76 million kgs as compared to 80 million kgs produced in 2008, mainly due to pest attack and climate change (48^{th} Annual General Meeting of Terai Branch of Indian Tea Association-report, 2010). It was estimated that 1034 species of arthropods infest tea plant (Chen and Chen, 1989). The most common pests of tea are mites, thrips, tea mosquito bug, tea eating caterpillars, mirids, scolytid beetles and termites (Ghosh, 2001). Since the magnitude of pest infestation varies seasonally depending on altitude, climate and cultural practices, an exact assessment of crop loss in tea is anywhere between 6 and 14% (Banerjee, 1976a).

There are approximately, 300 species of insects, mites and nematodes infesting tea, in India (Banerjee, 1993; Muraleedharan *et al.*, 2001) out of which about 25 species have already been recognized as serious pests. Most of them appear to have accepted tea under cultivation as an ideal host plant (Mukherjee and Singh, 1993). It has been found that about 6-14% of tea is lost due to insects, mites and weeds (Banerjee, 1976a).

A steady loss of 10% due to over all pest attack is a generally accepted figure though it could be 40% in devastating attacks by lepidopteran defoliators (Banerjee, 1993). Lepidoptera form the largest order of the pest species followed by Hemiptera (Chen and Chen, 1989). Earlier study of tea pests was by Green, (1890), Watt and Mann, (1903). Information on biology of tea pests of North-East India are available from the works of Hainsworth (1952), Das (1965), Banerjee (1983a, b) and that of south India and Srilanka from the studies of Muraleedharan (1983).

Among the lepidopteran tea pests, looper caterpillar (*Buzura suppressaria* Guenee) is one of the major defoliating pests of tea (Fig. 1, Fig. 2). This pest was first collected from Nowgong district of Assam (Cotes, 1895). It is one of the common pests of tea (Antram, 1911). The looper is an active defoliator of tea (Hill, 1983) and its infestation may become devastating within a short period. Therefore, the time factor for determination of economic injury level becomes very critical (Chakravartee, 1995). On the other hand a new species of looper has emerged in the Dooars and Terai region, by the name *Hyposidra talaca* (Walker), also called black inch worm (Basumajumdar and Ghosh, 2004; Das and Mukhopadhyay, 2008) (Fig. 3, Fig. 4). It has, in recent years, become more dominant than *B. suppressaria* population in many tea estates. The species is polyphagous and is found to infest a large number of weeds and forest trees along with the young leaves of tea bushes, causing a havoc loss in the tea plantation.

Fig. 1: Typical looper (*Buzura suppressaria*) caterpillar

Fig. 2: Leaf damaged by *Buzura suppressaria* caterpillar

Fig. 3: Mature *Hyposidra talaca* larva (5th instar)

Fig. 4: Earlier stage of black-inch worm *Hyposidra talaca* (Walker)

Red slug caterpillar (*Eterusis magnifica* (Butl.)) is one of the occasional pests of tea of regional importance in North-East India (Fig. 5, Fig. 6). The approximate time of occurrence of several discrete broods of red slug was recorded by Mann and Antram, (1906). The occurrence of a different red slug species, *E. virescens* (Butl.). on tea is reported from south India by Rau, (1952). Even sporadic populations of the defoliating pest, red slug should not be allowed to grow to an epidemic stage and control measures should be taken once the pest is seen in tea plantation was suggested by Chakravartee (1995).

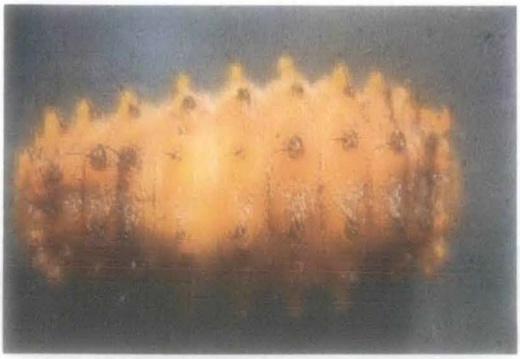


Fig. 5: Fifth (5th) instar caterpillar of *E*terusia *magnifica*

Fig. 6: Injury of matured leaves of tea bushes by *E*terusia *magnifica*

The tea leaf roller (*Caloptilia theivora* Walsingham) has been known to infest tea from the dawn of plantation in North-East India (Fig. 7, Fig 8). Watt and Mann (1903) considered it to be a troublesome pest. It had never been serious in Assam and Bengal but occasionally caused considerable damage to tea in Dehra Dun. During 1988 the intensity of attack of this insect was found to be quite high in a few estates of Jorhat Assam circle when about 40-60% of the shoots in young and mature tea were found to be badly affected. In Darjeeling hill slopes and Terai seasonal occurrence of leaf rollers some times in large number has been witnessed. More than the quantitative loss caused by this pest, the quality of made tea deteriorates due to its faecal contamination.

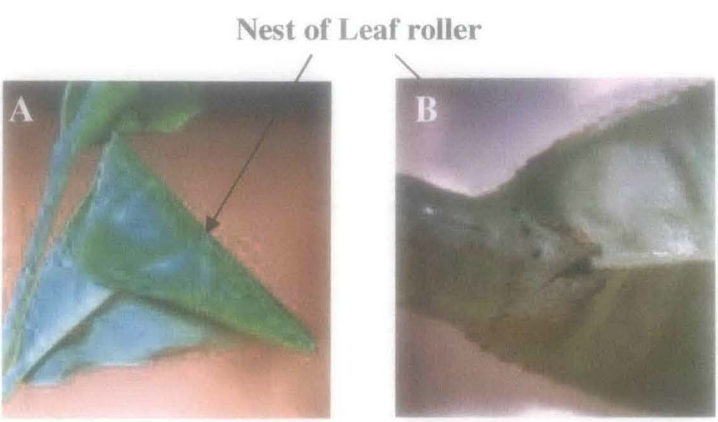


Fig. 7: A. and B. Nest of *Caloptilia theivora*

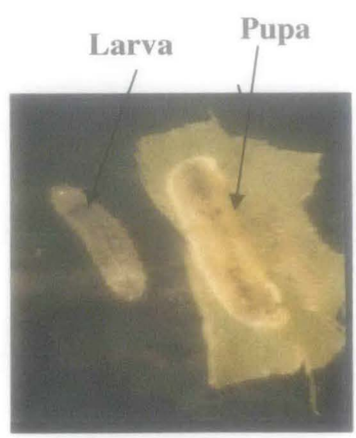


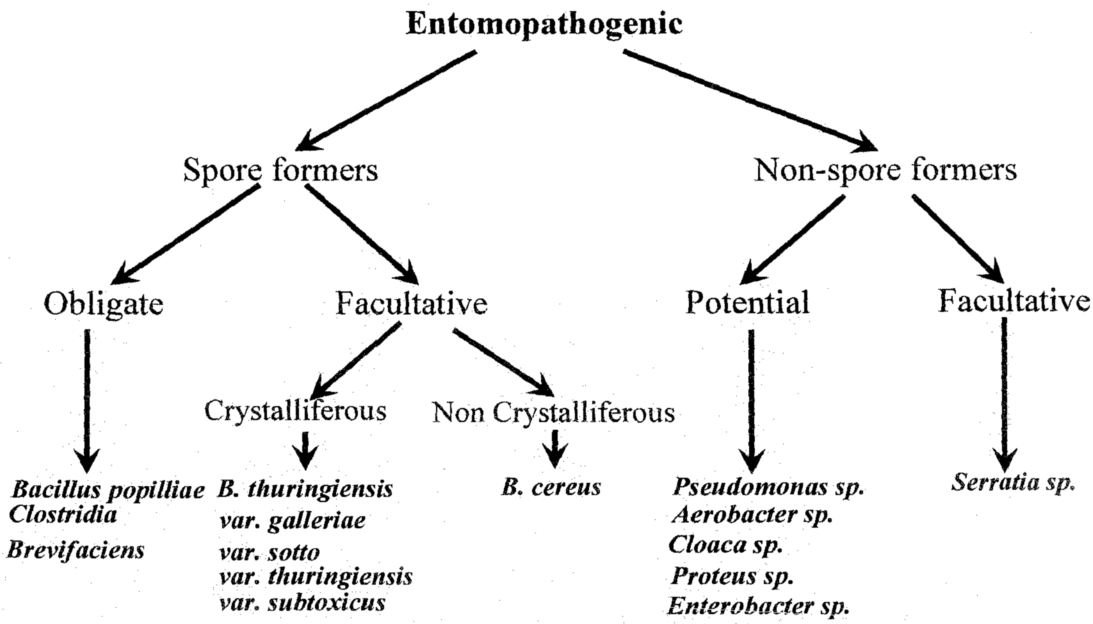
Fig. 8C Larva and pupa of *Caloptilia theivora*

1.c Prospects of research with entomopathogenic bacteria as Biopesticide

To control pest problem in tea most conventional plantation use synthetic insecticides. Various problems concerning target, non-target and environment are associated with these chemical pesticides. Many synthetic pesticides are non-biodegradable, others degrade very slowly and persist in the environment. Insecticides cause pollution of soil and ground water and have harmful effects on a wide range of nontarget organisms (beneficial insects, mammals and human) (Chattopadhyay *et al.*, 2004). With long exposures to pesticides, resistant strains of insect emerge, requiring increased doses of insecticides and introduction of new insecticides. Different chemical pesticides (organophosphate & synthetic pyrethroids) have been found to be less effective against this defoliator in recent time (Sannigrahi & Talukdar, 2003; Sarker and Mukhopadhyay, 2006). Because of their harmful effects, application of a number of these insecticides has been banned by the EPA of the USA and even by the tea research organizations of India, and registration of many others are being reviewed (Chattopadhyay *et al.*, 2004). To minimize chemical residue in tea, European union and German law imposed stringent measures for the application of chemicals in tea and fixed MRL values at < or = 0. 1 mgkg(-1) for the most commonly used pesticides. This is seldom realized in the real practice and therefore, residue problem has become a major constraint for exporting tea to foreign countries especially to UK, Germany and the USA. In order to regulate the situation of the Indian market at global level, central insecticide board and prevention of food adulteration regulation committee have reviewed the MRL position for Indian tea and has recommended 10 insecticides, 5 acaricides, 9 herbicides and 5 fungicides for use in tea and issued the tea distribution and export control order 2005 which will help the country to limit the presence of undesirable substances in tea (Gurusubramanium *et al.*, 2008). Pesticide residues of organophosphorus (e.g. ethion and chlorpyrifos), organochlorine (e.g. heptachlor, dicofol, alpha-endosulfan, beta-endosulfan, endosulfan sulfate) and synthetic pyrethroid (e.g. cypermethrin and deltamethrin) in made tea, fresh tea leaves, soils and water bodies from selected tea gardens in the Dooars and Hill regions of West Bengal, India had already been determined. The organophosphorus (OP) pesticide residues were detected in 100% substrate samples of made tea, fresh tea leaves and soil in the Dooars region. In the Hill region, 20% to 40% of the substrate samples contained residues of organophosphorus (OP) pesticides. The organochlorine (OC) pesticide residues were detected in 33% to 100% of the substrate samples, excluding the water bodies in the Dooars region and 0% to 40% in the Hill region. Sixteen percent and 20% of the made tea samples exceeded the MRL level of chlorpyrifos in Dooars and Hill regions respectively. The residues of heptachlor exceeded the MRL in 33% (April) and 100% (November) in the Dooars and 40% (April) and 20% (November) in the Hill region. Thus it was revealed that the residues of banned insecticides like heptachlor and chlorpyrifos in made tea are still present and may pose health hazards to the consumers (Bishnu *et al.*, 2008).

In view of this and also due to a greater acceptance of organic tea (as compared to chemically managed conventional tea) by health conscious consumers, the future protection and production of tea appear to depend largely on non-conventional control methods. One of the ecofriendly approaches of biological control of insect pests is conservation of the microbial bio-agents or application of some of the effective bacterial and viral control agents in the tea ecosystem. Biopesticides are becoming one of the most promising tools for the control of agricultural pests all over the world. Biopesticide of bacterial origin has already showed prospects to replace chemical pesticide to a great extent (Chakravartee, 1995). The pesticide formulations in which entomopathogenic bacteria are the active components are known as bacterial pesticides (Borthakur, 1986).

Entomopathogenic bacteria are classified as spore formers and non-sporeformers, which are further subdivided into obligate and facultative. Again spore formers may be crystal forming (crystalliferous) or non-crystalliferous. The examples of these different groups are presented below in a schematic diagram (Srivastava, 2004):



The spore forming or sporulating bacteria form endospore which are highly resistant to environmental changes (temperature, humidity etc.) and allow them to persist in a dormant condition outside the intended host to develop into a bacterial cell on being ingested. The spore forming bacteria are, therefore more promising in insect control than the non-sporeforming ones. Among the spore formers, again, it is the crystalliferous ones which are better than non-crystalliferous ones because of the toxic nature of the crystals they produce. One of the best studied species amongst the crystalliferous bacteria is *Bacillus thuringiensis* which was first to be regarded as a potential microbial control agent by Steinhaus in 1956. This *Bacillus* is effective against the caterpillars of many Lepidoptera and exists in many strains or varieties which differ in their pathogenicity for different species of Lepidoptera. Numerous *Bacillus thuringiensis* subspecies had been isolated from dead and dying insect larvae and in most cases the isolate had toxic activity to the insect from which it had been isolated (Goldberg and Margalit, 1977; de Barjac, 1981; Hansen *et al.*, 1996). The bioactivity of *Bt* serotypes 3a, 3b (Dipel R) and one of the components, Cry1Ab, was studied against the larvae of *Plutella xylostella*. *Bt* is also effective in controlling *Spodoptera litura* (Datta and Sharma, 1997) and *Crocidolomia binotalis* (Facknath, 1999). *Bt* accounts for more than 90% of the biopesticides used today (Feitelson *et al.*, 1992). Evaluation of various sub species for their toxicity was carried out against the two lepidopteran pests, viz. *Spodoptera litura* (F.) and *Phthorimaea operculella* (Z.) (Putambekar *et al.*, 1997). The *Bt* strain which exhibited the highest activity against lepidopteran pests under laboratory conditions was evaluated for field efficacy on the pod boring pest complex of *Cajanus cajan* (Putambekar *et al.*, 1997).

Different formulations of *Bacillus thuringiensis* (*Bt*) has already been applied and tested earlier against many tea pests (Muraleedharan and Radhakrishna, 1989). The formulations of *Bt* were effective against leaf rollers. *B. thuringiensis* had already been used against tea pests *Caloptilia theivora* and *Cydia leucostoma* in South India (Unnamalai and Sekar, 1995). Many commercial formulations of *Bt* are used for controlling many important plant pests mainly caterpillars of Lepidoptera (butterflies and moths). The control effects of TA-BR combined with *Bt* on main tea pests *Euproctis pseudoconspersa*, *Ectropis obliqua* and *Andraca bipunctata* reached above 90%, and is safe for natural enemies (LingLing *et al.*, 2004). Various *Bt*-pesticides (from different strains of *B. thuringiensis*) were tested in Japan for their effectiveness on major lepidopteran insect pests of tea. *Bt*-pesticides appeared to be effective in controlling tea tortrix (*Homona magnanima*), smaller tea tortrix (*Adoxophyes* sp.), tea leaf miner (*Caloptilia theivora*) and other lepidopteran pests (Kariya, 1977).

Though different commercial formulations of *Bt* have already been used in controlling different types of insect pests all over the world the main and the foremost problem in using *Bt* as commercial formulation was the development of resistance among the insect pests against *Bt*. The wide spread appearance of resistance to *Bt* presents a cautionary tale for the way of using *Bt* and *Bt* toxin genes in pest management (Schnepf *et al.*, 1998). Other than this resistance problem, two problems are associated with commercialized *Bt* formulation. These include: (i) the longer period necessary to obtain high levels of mortality of pest larvae with *Bt* compared to chemical pesticides, (ii) a particular toxin is usually restricted to only one host insect so, there is a need to isolate more varieties of *Bt* with different and more toxic activity.

In the present research, study of naturally occurring bacterial bio-agents occurring in the lepidopteran (moth) pests of tea were done. There are chances that the bacterial bioagents occurring inside the host body may be a well established novel bacterial entomopathogen, with the potentiality to kill the host than already available commercialized *Bt* formulation used against lepidopteran pests. Therefore the entomopathogenic bacteria of the caterpillar pest of tea were surveyed, isolated, characterized, then bioassayed in laboratory and testing in field for their killing efficacy. Expectedly such bacteria in future with proper biosafety testing may be developed in to microbial pesticides, and the same may be integrated in biocontrol and IPM programs developed to manage lepidopteran tea pests.