1. INTRODUCTION

1.1 Tea growing areas of the world and India

Tea, *Camellia sinensis* (L.) O. Kuntze, is an intensively managed perennial monoculture crop cultivated on large and small-scale between latitudes 41ºN and 16ºS. It is grown as plantation on over 2.71 million hectares (ha) in more than 34 countries across Asia, Africa, Latin America, and Oceania (Hazarika et al. 2009, Roy et al. 2015) (Fig. 1.1) to produce 3.22 million metric tons of made tea annually (FAO 2005). Due to its increasing demand, tea is considered to be one of the major beverages in the global market. As per the research study, in 2013, the global tea market was of US$38.84 bn and its dimension is projected to reach US$47.20 bn by the end of 2020. The market is expected to exhibit a 2.80% compound annual growth rate (CAGR) between 2014 and 2020 (Anonymous 2016a). The Indian tea industry flourished after a British national named Robert Bruce discovered indigenous tea plants for the first time growing in the upper Brahmaputra valley in Assam and adjoining areas in 1823 (Sivanesan, 2013). Since independence, tea production has grown over 250%, while land area has just grown by 40%. However, most of the tea bushes in Darjeeling have their origin from China "smuggled" out by a Scot botanist and adventurer Robert Fortune (Lama, 2013). In India, the crop is grown in the certain districts located in Assam, West Bengal, Kerala, Karnataka and Tamil Nadu and in a limited area of Tripura, Uttarakhand, Uttar Pradesh and Himachal Pradesh. (Fig. 1.2). The total area under tea in our country is about 5,63000 ha which produces 1208.78 million kg made tea annually (Annonymous 2014a). 76.3% of Indian tea is harvested from North East India In 2010, 43% of total country's tea production found place in the international export market (Bordoloi 2012)..

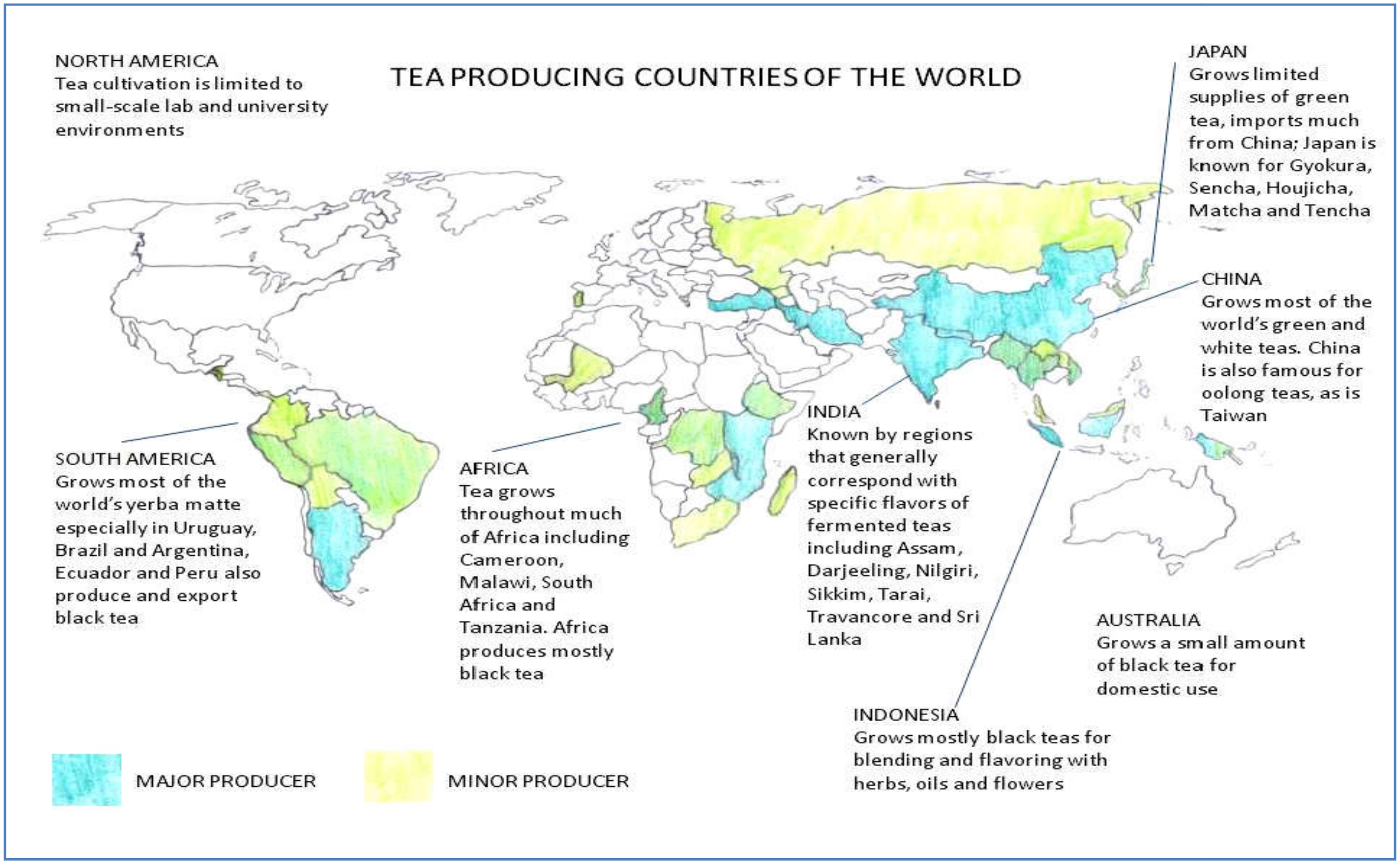

Fig. 1.1: Tea producing countries across the globe.

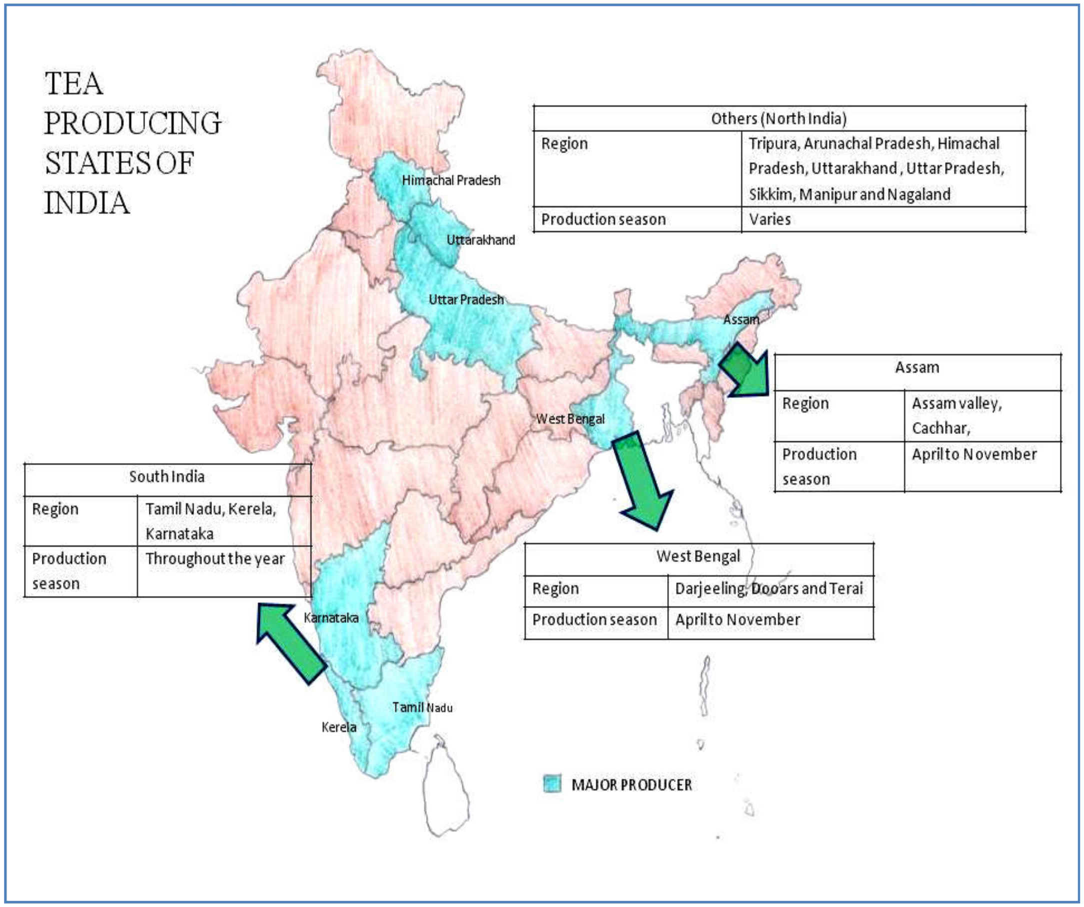

Fig. 1.2: Major tea producing states of India.

India is second largest producer contributing 24.46% to the world production and fourth largest exporter of tea (Annonymous 2014a, 2015).

1.2 Tea producing districts of West Bengal: Darjeeling foothills, Terai (Darjeeling) and the Dooars (Jalpaiguri, Alipurduar)

West Bengal offers teas from Darjeeling, Dooars and Terai and the Darjeeling is known as "The Champagne of Teas", cultivated on the slope of the Himalayas, have unique, delicate flavor and character (Arya, 2013). The tea growing regions of Darjeeling districts are located between 26°31′ and 27°13′North latitude and 87°59′ to 88°53′ East longitude. The northern part of the districts has the distribution of eastern Himalayas while the southern part consists of a stretch of alluvial plain at the base of the hills known as the Terai-Dooars. Darjeeling terai is situated at 91amsl with an average temperature of maximum 35°C and minimum 12°C. The Dooars or *Duars* (=Doors) area comprises flood plains and the foothills of the eastern Himalayas that continues in North East India and is the gateway to Bhutan. The altitude of the Dooars region ranges from 90 amsl to 1750 amsl. There are 308 big and 1232 small tea gardens in North Bengal region and the total area under tea is 122620 ha (Fig. 1.3). North Bengal region of Terai and the Dooars produces 25% of total tea of India (retrieved from www.teaboard.gov.in). In 2003, total production of made tea was 200 million kg (Anonymous 2003a). There are 87 functional tea gardens on a total area of 17,820 ha producing famous 'Darjeeling tea'. The total annual production is 8.91 million kgs. The Terai regions have an area of 49700 ha and in the Dooars region 72,920 hectares under tea plantations producing 125.34 and 177.84 million kgs of tea, respectively (Anonymous 2014a). As per the Tea Board data, the total tea production

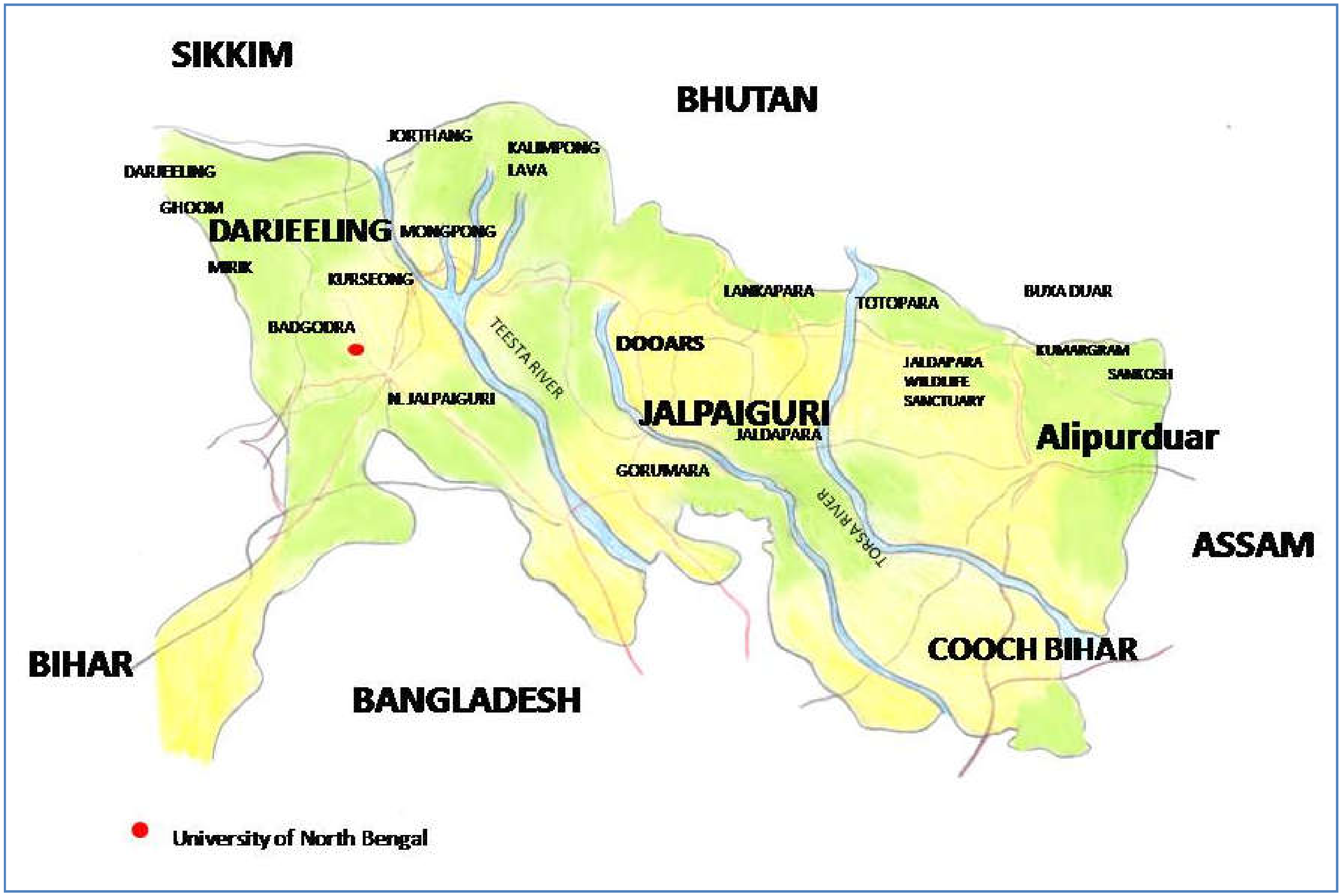

Fig. 1.3: Tea producing districts in Northern regions of West Bengal (North Bengal).

of the country was 1,233.14 million kg in 2016 against 1,197.18 million kg in 2015, 3% rise mainly because of good production in North India (Anonymous 2016b).

1.3 Pest Attack and Crop Losses

Tea plantation as monoculture is a permanent ecosystem which provides habitat continuity. Such an inexhaustible food resource can be colonized by for 1031 species of arthropods and 82 species of nematodes as reported from different parts of the world (Chen and Chen 1989). In Asia, 230 species of insects and mite pests attack tea (Muraleedharan 1992). However, 173 arthropods and 16 nematodes were reported to be major and minor pests of tea in North East India (Hazarika 1994) including Assam, which occupies a prominent position in Indian tea production (Sahewalla and Borthakur 1996). Among the insect pests, order Lepidoptera consists of highest number of pest species (32%) followed by order Hemiptera (27%) (Muraleedharan and Chen 1997). As insects have the dynamic ability to adapt; they can feed on various parts of the tea plant such as leaf, stem, root, flower and seed causing about 10 % loss in yield, which is generally accepted but, it could go up to 40% in case of devastating attacks by defoliating lepidopterans (Banerjee 1983). To control these pests, huge amount of pesticides is needed. Therefore, per hectare consumption of pesticides is often excessively high and also expensive (Chakravartee and Hazarika, 1995).

Looper caterpillars of, *Biston* (=*Buzura*) *suppresseria*, *Hyposidra talaca*, *Hyposidra infixaria*, *Ascotis* sp., *Ectropis* sp. (Lepidoptera: Geometridae) are found throughout the year and are the most destructive pest of tea plantations (Anonymous 1994).

B. suppressaria Guen was reported as a major tea pest in 1900 (Das 1965). They feed on the young leaves. Red slug caterpillar (*Eterusia aedea*) (Lepidoptera: Zygaenidae) feed on the mature leaves of the tea bushes and the bark of young stem (Anonymous 1994). *Euproctis latisfascia* (Lepidoptera: Lymantriidae) feed on the mature and senescent leaves of tea bushes (Anonymous 1994). Flush worm (*Lespeyrasia leucostoma*) (Lepidoptera: Tortricidae) attacks on the pluck-able shoots and leads to the formation of a nest like structure due to folding of leaves. Sporadic infestation of defoliating Bunch caterpillar (*Andraca bipunctata*) (Lepidoptera: Bombycidae), mainly occurs during March to November in Tea plantations of North East India (Das 1965). Sporadic pests are defined as a species whose numbers are usually controlled by biotic and abiotic factors which occasionally break down, allowing the pest to exceed its economic injury threshold (Cherrett and Sagar, 1977, Hill 1983). Another sporadic pest, *Arctonis submarginata* (Lepidoptera: Lymantridae) has been reported relatively recently from the Darjeeling hills and Terai as an emerging tea pest feeding on the mature leaves of the bushes (Mukhopadhyay 2007). Sporadic and occasional attack by *Orgyia* sp. is also well known in the tea gardens of Darjeeling foothills and Terai. However, this pest was first recorded in a number of tea estates of central and western Dooars and Darjeeling during February- September (Das and Roy 1982). Another defoliator, *Orgyia postica* (Walker) (Lepidoptera: Lymantridae) was recorded attacking tea in North East India by Watt and Mann in 1903.

1.4 Brief description of the sporadic pests of tea

- **Hairy caterpillar (*Arctornis submarginata*):**

The hairy caterpillar of *Arctornis submarginata* (Walker) (Lepidoptera: Lymantriidae) (Fig. 1.4A) is found to attack and defoliate mature tea in the foothills and Terai of Darjeeling Himalaya. The geographic distribution of this insect includes North East Himalaya, Borneo and Sumatra (Schintlmeister 1994). Swarms of caterpillars consume and defoliate the mature and maintenance leaves of the tea bushes (Fig.

1.4B), adversely affecting the tea yield in Darjeeling foothill region (Terai) (Mukhopadhyay 2007). They have also been found in good numbers in the Dooars tea plantations. There are six instars in the life cycle of *A. submarginata*. Moths are white with two black spots on the forewing (Fig. 1.4C & 1.4D). They are found mostly in the winter season from November to January.

- **Bunch caterpillar (*Andraca bipunctata*):**

The bunch caterpillar *Andraca bipunctata* Walker (Lepidoptera: Bombycidae) is the earliest known tea pest repoted from Taiwan in 1820. It was recorded as a very widely distributed pest occurring over almost all the districts in North East India (Watt and Mann 1903) including Sub-Himalayan tea gardens of Darjeeling in West Bengal (Andrews 1921, 1931, Hainsworth 1952, Das 1956, Banerjee 1983, Ghorai 1992, Panigrahi 1998, 1999). The bioecology of *A. bipunctata* greatly varies depending upon climatic conditions (Das 1965, Ghorai 1992). Its conspicuous colour and peculiar habit of congregation on the branches and fast pace of defoliating the tea bushes can hardly escape notice. The young caterpillars remain congregated on the under surface of the leaf. They feed on the epidermal tissues, then at the leaf margin and ultimately the entire leaf. From the 3rd instar, they form typical clusters (Fig. 1.5A) on the branch and feed on the leaves. After eating all the leaves of a bush they migrate to the next plant. As a result of stunted growth, these infested plants remain unproductive for nearly 2 years (Ghorai et al. 2010). When present in large numbers they cause heavy loss by defoliating the leaves and even stripping the bark (Fig. 1.5B) of the tea bush. The moths are brown and dull (Fig. 1.5C & 1.5D).

- **Red hairy (*Orgyia postica*):**

The genus *Orgyia* comprises of 65-70 species. Overall, they have spider like appearance and are highly polyphagous in nature. The Lymantrid moth *Orgyia* sp. has a distribution in high altitude tea plantations usually above 500 m in the Darjeeling hills. The sporadic occurrence, but havoc depredation by caterpillars of the moth has been reported from tea plantations of Darjeeling foothills (Pathak 2003). In North East India, *Orgyia postica* was identified as the common defoliator of tea leaf since, 1930s by Watt and Mann.

The larvae of *Orgyia postica* (Walker) (Lepidoptera: Lymantriidae), are covered with bristles and can irritate human skin. Although the larvae sporadically occur, they rapidly defoliate plants causing considerable damage. The female is silky brown having profuse hairs all over the body surface with vestigial wings. The male moths have normal wings (Fig. 1.6C). The female moths being wingless, release a sex pheromone in order to attract males, copulate and lay eggs on the cocoon from which they emerged. Eggs are laid in clusters, loosely covered with hairs on the lower surface of the leaves, at the axil and stems and sometimes on the outer surface of the cocoon (Fig. 1.6D). The newly hatched caterpillars are gregarious in habit and feed on the epidermal tissue of the lower surface of mature leaves. As a result of damage small holes are formed on the leaf surface (Fig. 1.6B). The full-grown caterpillars (Fig. 1.6A) and other immature stages of caterpillars start feeding from the margin of the leaf and are mostly found on the top hamper on the under-surface of leaves and also the branches (Anonymous 1994).

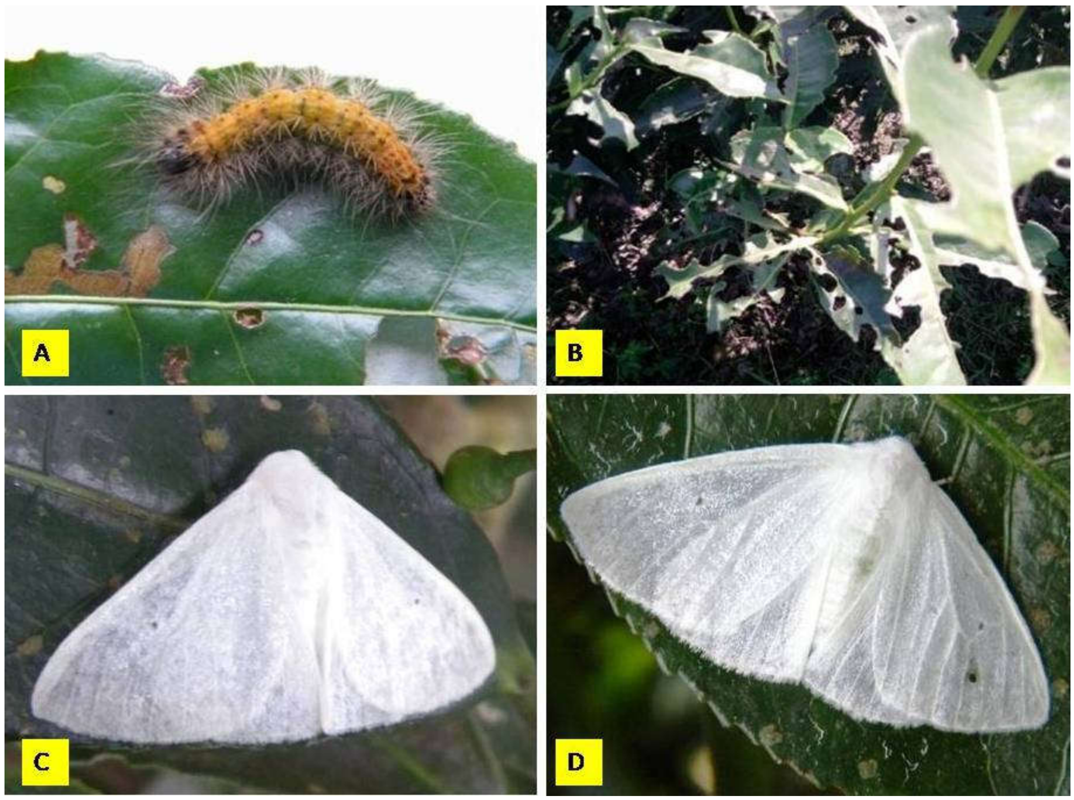

Fig. 1.4: A. Final stage caterpillar of *A. submarginata*; B. Damage symptom; C. Adult Male moth; D. Adult female moth.

Fig. 1.5A: Final stage caterpillar of *A. bipunctata*; B. Damage symptom; C. Adult Male moth; D. Adult female moth.

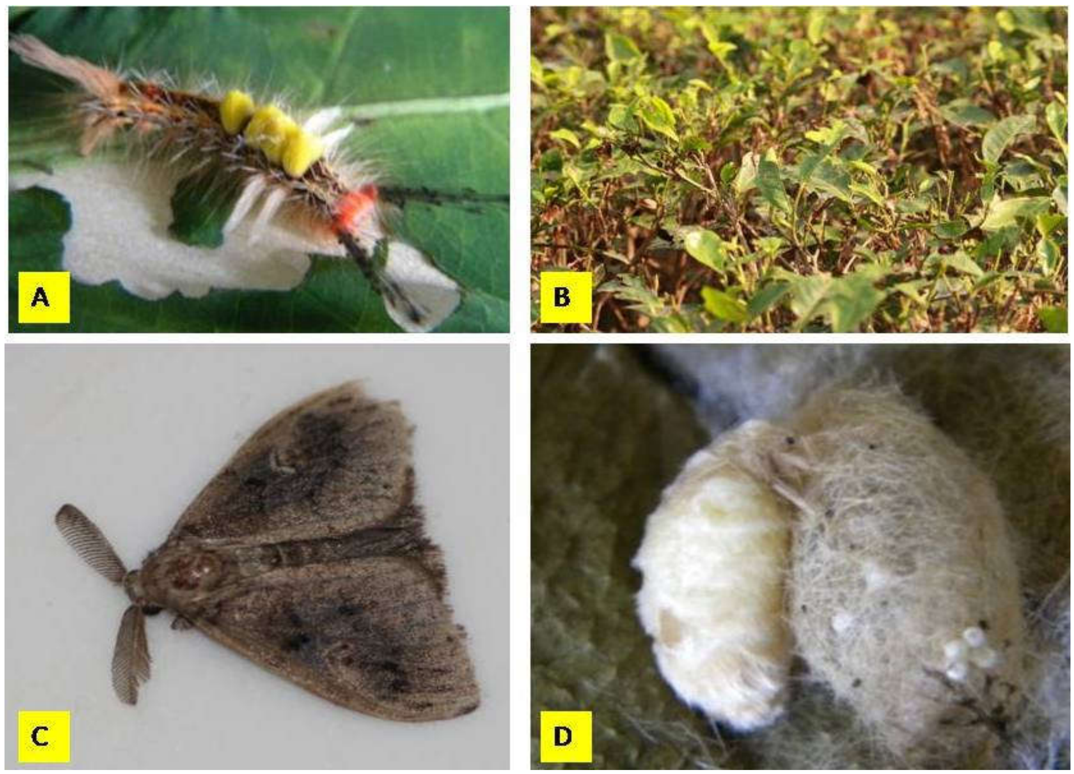

Fig. 1.6:A. Final stage caterpillar of *O. postica*; B. Damage symptom; C. Adult Male moth; D. Adult female moth (wingless).

1.5 Conventional methods of controlling the pest population

Pests, pathogens and weeds cause severe constraints to the productivity and quality of tea. In order to combat these, the tea planters use a wide range of pesticides to ensure high yield and economic returns. Because of the residual toxicity of pesticide in food products (Nagayama 1995, Neidert and Saschenbreker 1996) including made tea (Singh and Agnihotri 1984, Bishnu et al. 2009) there is an increasing public concern, of late, regarding the pesticide residues also in tea. Indiscriminate use of pesticides has caused serious concerns such as insect resistance to pesticides, the resurgence of pests, outbreak of secondary pests, harmful effects on human health and the environment (Muraleedharan and Selvasundaram 2005). Evidences also show that extensive use of chemical pesticide has had many well documented adverse consequences like death of natural enemy from the ecosystem (Ghosh Hajra 1994, Hajra 2002, Obeidat et al. 2004), resistant strains of insect pests, requiring increased doses of insecticides and introduction of new insecticides. Different chemical pesticides (Organophosphates and synthetic pyrithroids) have been found to be less effective against these defoliators (Sannigrahi and Talukdar 2003, Sarker and Mukhopadhyay 2006). Newer pesticides are highly expensive due to stringent safety standard national and International (Cooper and Dobson 2007). Moreover, health conscious consumers prefer organic tea to those of chemically managed conventional tea. Though broad spectrum plant protection chemicals offer powerful incentives for application, yet they have serious drawbacks including harmful effects on human health and the environment at large due to the presence of undesirable residues (Song et al. 1998).

1.6 Need of alternative methods to control these pests

The future protection and production of tea appear to depend largely on non-conventional control methods. For this reason biological pesticides are becoming key components in integrated pest management strategies (IPM) (Obeidat et al. 2004). In many instances, alternative methods of insect management offer adequate levels of pest control and pose fewer hazards. One such alternative eco-friendly approach is the use of microbial insecticides that include bioagents such as bacteria, viruses, fungi, protozoa, and nematodes. The organisms used in microbial insecticides should essentially be non-toxic and non-pathogenic to wildlife, humans and other beneficial/industrial organisms as these are not closely related to the target pest. The safety offered by microbial insecticides is their greatest strength. Its use has been largely increasing due to its high target specificity without harmful side effects (Fadel and Sabour 2002). There is a great diversity of these microorganisms infecting various insect pests. The entomopathogenic bacteria include species which can infect insects and multiply rapidly in the insect hosts following the infection (Aronson et al. 1986). The overuse or misuse of chemical pesticides and their negative impacts are increasingly becoming causes for concern, underlining the need for development of alternative pest control methods (Meadows 1993).

1.7 Importance of Microbial insecticides in controlling the pest population

Despite more than 10 fold increase in insecticide use since 1940 (Lysansky 1994), crop losses due to insects has nearly doubled in the same period. Therefore, microbial insecticides are especially valuable because their toxicity to non- target animals and humans is extremely low and moreover they do not pose adverse health effects even if crops exposed to these microbial pesticides are consumed. Compared to other chemical insecticides, they are safe for both the pesticide user and consumers of treated crops (Mc Coy 1987). Microbial insecticides comprise of microscopic living organisms (viruses, bacteria, fungi, protozoan or nematodes) or the toxins produced. These bioagents function as pathogens of target insects causing infection leading to death of host insects (Charnley 1991). Of the various pathogens that attack insects and other invertebrates, insecticidal bacteria proved to be the easiest and most cost effective for mass production and thus were the first commercially successful microbial insecticides. The modes of action of these microbial agents to the given hosts differ depending on the organisms. Entomopathogenic viruses and bacteria must be ingested by the host insect along with their food for infection. In contrary entomopathogenic fungi may produce infection on contact by the reproductive propagules (spores or conidia) and also through ingestion by the host insect (Burges 1981). Microbial insecticides are relatively host specific, being adapted through co-evolution, to specific groups of insects, and will not cause infection in other groups of animals such as birds or mammals. But these microbes are very sensitive to biotic and abiotic factors of the environment (Lacey and Goettel 1995). Several decades of extensive investigations has resulted in broad use of microbial control in pest management (Burges 1981, Leggett 1995). It was in Japan in the year 1914 that the earliest efforts in microbial control of tea pest began (Hotta 1914). Microbial control measures are gradually being popularized in different tea-growing areas under integrated pest management for the production of chemical pesticide contamination free tea (Aizawa 1971, Kodomari 1993, Hazarika 2009, Ye et al. 2014). Various bacteria having the potential of biocontrol belonging to genus *Bacillus, Paenibacillus, Streptomyces, Pseudomonas* etc. were registered and approved as biopesticides

(Hynes and Boyetchko 2006). The tremendous success in microbial pesticides has come from the use of *B. thuringiensis* (Obeidat et al. 2004). *B. thuringiensis* strains show specific insecticidal activity against insects of different orders such as Lepidoptera, Coleoptera, Diptera, Hymenoptera, Homoptera, Orthoptera and Mallophaga (Schnepf et al. 1998). The most promising biological control agent *Bacillus thuringiensis* is the leading organism used as commercial microbial pesticides (Lambert 1992, Meadows 1993, Lysansky 1994). As of today, it is estimated that 95 percent of the market share of the pesticides is by bacteria in general and *Bacillus thuringiensis* Berliner (*Bt*) (Procaryotae: Firmicutes: Bacillaceae) in particular. It was reported that there was about 30 kinds of commercialized biopesticides globally during past decade (Xu 2008). The popularity of *Bacillus thuringiensis* product increased ever since 1990s. The sales were projected to more than double by the year 2000s (Bernhard 1993). In 1997, the global sales of *Bt* products was $ 984 million and in 2005 it went up to $ 3.6 billion (Wang 2006). Commercial *Bt* insecticides are Generally Regarded as Safe (GRAS) by the EPA, and are approved for most organic certification programs. Commercial preparations of *B. thuringiensis* (spore crystal mixture) have been registered as insecticides since 1961, and a significant quantity of toxicological data has been collected over recent years (Baum et al. 1999). However, *Serratia* sp. has been associated with insect disease (Klein and Jackson 1992) but commercial production of these bacteria was achieved only recently.

In the last few years, 59 pathogenic bacterial species have been developed as pesticides across the world. These various bacterial pathogens of insects are being used successfully in the biological control of insect pests (Thiery and Frachon 1997, Sezen and Demirbağ 1999, Sezen et al. 2001). The microbial control of insect pests is of crucial importance in developing countries (González 1981).

Hence, the need for exploring and discovering entomopathogenic bacteria for management of pests has become essential, especially in organic farming to develop microbial pesticides from them in future. Integrated Pest Management in tea is greatly required (Barbora 1994) in NE India. One of the eco-friendly approaches of microbial control is conservation of the microbial bio-agents or their application for bringing in bacterial control of tea pests.