ABSTRACT

Tea, *Camellia sinensis* (L.) O. Kuntze, has earned the reputation of being a cheap and readily available beverage. This great popularity of tea draws attention of almost all tea producing countries from the economic point of view. Tea is an intensively managed perennial monoculture crop cultivated on large and small-scale. It is the chief plantation crop of the Darjeeling Hills and its adjoining plains (Terai and the Dooars). It is world famous for its quality (leaf tea) and excellent aroma. Districts of North Bengal provide a typical agro-climate for cultivation of tea on which several species of insect pests subsists. In Asia, 230 species of insects and mite pests are reported from tea plantations (Muraleedharan 1992). However, 173 arthropods and 16 nematodes are reported to be major and minor pests of tea in North East India (Hazarika 1994) including the plantations of Assam, which occupies a prominent position in Indian tea production (Sahewalla and Borthakur 1996). Among the insect pests, order Lepidoptera consists highest number of pest species (32%) followed by the order Hemiptera (27%) (Muraleedharan and Chen 1997). These pests are generally managed in conventional way by applying chemical pesticides. Extensive use of chemical pesticide has led to many well documented adverse consequences like environmental contamination, health hazard, pest resistance and death of natural enemy from the ecosystem (Ghosh Hajra 1994, Hajra 2002, Chattopadhyay et al. 2004, Obeidat et al. 2004). With long exposure to pesticides, resistant strains of insect emerge, requiring increased doses of insecticides and introduction of new insecticides. Therefore, 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 of integrated pest management strategies (IPM) (Obeidat et al. 2004).

In many instances, alternative methods of pest control offer adequate levels of plant protection and management with fewer hazards.

In the present work an attempt to isolate and characterize naturally occucrring entomopathogenic bacteria from cadavers of three sporadic lepidopteran pests viz. *Arctornis submarginata*, *Andraca bipunctata* and *Orgyia postica* has been made. These pests occasionally attack the tea plantations of Darjeeling foothills, Terai and the Dooars as minor pests but sometimes the infestation crosses economic injury level (EIL). For collection of sporadic pest specimens (lepidopteran) and their cadavers, different tea plantations of the said regions were extensively surveyed. Isolation of bacteria, from dead larvae was done following techniques recommended by Lacey and Brooks (1997). After proving the Koch's postulates (Koch 1876, Fredericks and Relman 1996) the bacterial viability was checked by inoculating in new agar media. A total of nine *Bacillus* isolate were selected from three sporadic lepidopteran pest species (Arc01, Arc02, Arc03 from *A. submarginata*; Ab01, Ab02, Ab03, Ab04 from *A. bipunctata* and Org2A, Org6A from *O. postica*). Initial identification of the bacterial strain included microscopic examination like Gram's staining, crystal staining and endospore staining which established these bacterial isolates as *Bacillus* sp. Doubling time and biochemical tests were done to characterize the isolates. Sodium dodecyle sulphate Poly acralamide gel electrophoresis (SDS-PAGE) was done for the analysis of crystal protein and whole cell protein. The gels when analysed, Arc01, Arc03 and Ab04 showed 128, 122.7 and 129.8 kDa bands respectively, corresponding to ~130 kDa band *cry*1 gene. Result also showed that the strains Arc01, Arc03 had 64 and 56.3 kDa bands, respectively, while Ab01, Ab03, Ab04 and Org6A had bands of 56.7, 56.2, 56.5 and 57.7 kDa, respectively. All these bands correspond to ~60 kDa of *cry*2 gene. Plasmid profiles of all the nine isolates were done. All the tests showed these newly isolated bacterial strains as comparable to the commercially available *Bacillus thuringiensis kurstaki* used as a standard but with a few differences which suggested that they may be novel isolates from this area. Next bioassay tests were done to evaluate virulence of these isolates against host insects. Here, three strains (Arc03, Ab04 and Org6A) showed highest toxicity to their respective host insects with LC_{50} values of 398.1, 486.6, 354.8 µg/ml, respectively. These most virulent strains were then sequenced for 16S rRNA gene, which in turn revealed a 99% similarity with the *Bacillus thuringiensis* strains available in the NCBI Genebank. When amplified for *cry* gene Arc03 showed presence of *cry*1 and *cry*9 genes, Org6A harbored only *cry*2 gene but Ab04 and *Btk* possessed all three *cry* genes i.e *cry*1, *cry*2 and *cry*9 genes. All these *cry* genes make them toxic to lepidopteran pests. Therefore it can be presumed that these isolates were infact, stains of *Bacillus thuringiensis* found naturally in these sporadic lepidopteran species of tea pests in the tea plantations of Darjeeling foothills, Terai and the Dooars. Further, counting on their virulence it may be taken for granted that these locally available strains of *Bacillus thuringiensis* may be developed as biopesticides in future.

Although importance of biopesticides as alternative pest management method is increasing day by day yet several constrains such as standardization and stable formulations, appropriate dispensing, ease of registration procedure of the new isolates and enhanced cost of application/ha etc. come in the way, specially in developing countries like India (Srinivasan 2012). It is hoped, that these newly reported strains of *Bacillus* with their appreciable entomopathogenicity would find their ways to get established as future biopesticides after meeting the said requirements.