Exploring the biocontrol potential of naturally occurring bacterial and viral entomopathogens of defoliating lepidopteran pests of tea plantations

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ABSTRACT

The foliar crop tea (*Camellia sinensis* O'Kuntz), that yields the cheapest beverage in India is largely produced in North-East India including the Darjeeling Himalayan slope and its Terai (foothills and plains). Naturally occurring entomopathogenic bacteria could be isolated from two species of loopers, *Buzura suppressaria* and *Hyposidra talaca* and also from a slow but steady leaf feeder, the red slug caterpillar (*Eterusia magnifica*) and a new invading hairy caterpillar (*Arctornis submarginata*) that strip tea bushes of their mature and maintenance leaves. Infected larvae of *B. suppressaria* and *H. talaca* yielded spore forming bacteria with crystal proteins and appeared to share many features in common with *Bacillus thuringiensis kurstaki* (*Btk*), but differed from *Btk* in shape of their crystal, biochemical tests, growth phase, molecular weight of crystal protein, and major whole body proteins. A lower LC_{50} and reduced LT_{50} value than *Btk* were also evident. All the *Bacillus* strains i.e. BS01 from *B. suppressaria*, HT01 and HT02 from *H. talaca* were found mutually cross infective to both the looper species but were not pathogenic to *Bombyx mori* (multi voltine strain of silkworm). Field application in RBD of most pathogenic strain of HT01 proved alone to be quite effective at the field dose of 5000 µg/ml concentration. Mean live larvae of *H. talaca* recovered in field after 7 days of spraying the bacterial formulation, was 3.93% . So, the present study revealed that naturally occurring highly pathogenic *Bacillus* strains could be made effective in looper control through a process of isolation, identification, testing, formulation and application especially in biorational or bio-organic tea plantations. Bacterial strain close to *Btk* but with a slightly higher LC_{50} value and a much reduced LT_{50} values could be isolated from red slug caterpillars of *E. magnifica*. Its laboratory based evaluation proved its killing efficacy in the early stage red slug caterpillars. So, this strain of *Bacillus* also hold a substantive promise of being used in biocontrol of the concerned pest. The bacterial strain isolated from hairy caterpillars of *Arctornis submarginata* was tested to be a *Bacillus* with typical spore. The strain showed a longer doubling time and difference in biochemical tests from *Btk*. Moreover, this strain (Arc 01), showed a lower LC_{50} value and a shorter LT_{50} values as compared to *Btk* when tested against early instars of *A. submarginata*. Thus proving that the strain had a definite killing efficacy against the concerned pest when tried in laboratory conditions. Importance of nucleopolyhedrovirus (NPV) extracted from cadavers of looper caterpillars (*B. suppressaria* and *H. talaca*) could be well realized due to their high infectivity and low LC_{50} values. Field application of NPV (1×10^7 OBs/ml) proved efficacious in bringing down the looper population in Terai tea estate. Besides the *Bacillus* strain of *A. submarginata*, a granulovirus was also isolated from naturally infected population. The GV was found to have an LC_{50} value, 4.46×10^4OBs/ml and LT_{50} values, 3.87 days for 1×10^7 OBs/ml concentrate. This GV added an effective bioagent to the biopesticide arsenal for future control of this emerging pest, especially in the organic and biorational tea plantations of Himalayan Terai and foothills.

Key words: *Camellia sinensis, Arctornis submarginata, Buzura suppressaria, Eterusia magnifica, Hyposidra talaca*

INTRODUCTION

Tea plantation (*Camellia sinensis* O'Kuntz), spreads over the hill slopes of Darjeeling Himalaya and its adjoining foothills and plains, known as Terai and the Dooars. After Assam, Darjeeling plantation including Terai and the Dooars areas provide the second largest yield of made tea from North-East India with a high export potential as "Darjeeling brand" with a certification trade mark (GI). Most quality tea clones, marked by flavor are grown at 500 to 2000 mt. altitude in Darjeeling hill while the high yielding clones are mostly planted in Terai and the Dooars foothills and their adjoining plains with a total yield of about 175 million kg/yr.

Distribution of insect pests also showed a pattern with dominance of sucking pest such as the jassid (*Empoasea*

flaviscence Fabr.) and thrips (*Mycterothrips setiventris* Bagnall) in the hill slopes (Pathak and Mukhopadhyaya, 2005) and with more of chewing pests specially the lepidopterans such as loopers, red slugs, black hairy, leaf rollers, bunch caterpillars commonly appearing at the foothills (below 1000 m) and the plains of Terai and the Dooars. Owing to a lesser pest load in tea at the higher reaches of Darjeeling hills, maintaining pesticide-free organic plantation is possible but the situation changes with the high-yield clones at lower elevation and plains. In tropical and subtropical agro-climate, pest attack increases forcing the planters and managers to apply more of conven tional chemical pesticides due to lack of any good alternative biopesticide/non conventional method of pest management.

Three guilds of arthropod pests that ravage the plantation in the foothills, Terai and the Dooars are mainly the mite (*Oligonychus coffeae* Nietner) and the mirid bug (*Helopeltis theivora* Waterhouse) and the defoliating caterpillars of moths. Species of moths that mainly attack foliage are the looper caterpillars [*Buzura suppressaria* Guenee, *Hyposidra talaca* (Walker), *Hyposidra infexaria* (Walker)] found on young leaves, red slug [*Eterusia magnifica* (Butl.)] mainly feeding on mature leaves, leaf rollers (*Caloptilia theivora* Wasingham) folding and nibbling the tender tea leaves, the hairy caterpillars [*Euproctis latifascia* (Walker) and *Arctonis submar ginata* (Walker)) eating up the mature and senescent leaves, bunch caterpillars (*Andraca bipunctata* Walker) and Sungma caterpillars (*Orgyia* sp.) devouring leaves indiscriminantly thus causing rapid denudation of bushes. Among the moth species that have become the main cause of heavy crop loss in recent years in Terai and the Dooars (40% and above) are the loopers, *B. suppressaria*, *H. talaca* and *H. infexaria*. A substantial loss through indirect damage to non-harvested (mature) leaves is caused by sporadic but large scale infestation of *E. magnifica*, *A. submarginata* and *E. latifascia* from the plantation of the Darjeeling foothills and Terai. Successful insect pest management using viral and bacterial pesticide formulations has been reported in many crops. Many commercial formulations of *Bt* have been used for controlling many important plant pests mainly lepidopteran caterpillars (butterflies and moths). *Helicoverpa armigera* (Hubner) (Lepidoptera:Noctuidae), a polyphagous pest, due to its extensive damage caused to several crops in the recent years has attained the status of cosmopolitan pest and the management of this pest has become difficult due to development of its resistance to different insecticides (Lande and Sarode, 1995). As an alternative *Bt*-based biopesticide has proved effective in controlling this pest *(H. armigera*) and another severe pest, *Spodoptera litura* (Datta and Sharma, 1997).

In the 1950s, nucleopolyhedrovirus (NPV) killed caterpillars of the cabbage looper, (*Trichoplusia ni*) were collected by farmers in California. They extracted and sprayed the virus on cotton, potato, *Brassica* and other crops to get adequate control (Falcon, 1975). Granulovirus of the cabbage worm, *Pieris rapae*, has been mass produ ced and used as biocontrol agent in PR China as PrGV since 1978 (Yi and Li, 1989). GVs of *Plutella xylostella* (cabbage) has been used in large scale as biopes ticide in Brazil . NPV of *Mamestra brassicae*, a pest of cabbage, has been registered and used as viral pesticide in France.

Screening and isolation of Entomopathogens

Since most of the lepidopteran tea pests have developed higher tolerance to conventionally used synthetic pesticides (Sarker and Mukhopadhyay, 2006) chemical based management of these pests is increasingly becoming difficult. As an alternative entomopathogenic microbials, that are the principal causative agents of natural death in lepidopteran pest populations, appeared to be promising as biopesticides. Such a possibility led to the exploration, screening and isolation of bacterial and viral pathogens from the infected cadavers of pest caterpillars collected from plantations of Darjeeling foothills and plains.

Pathogenic bacteria of loopers

Out of three major species of loopers bacterial pathogens causing natural death to *B. suppressaria* could be isolated from Terai population. Characterization of the isolated bacterium through polyphasic approach such as morphological, biochemical and physiological characteri sation and close examination of the pattern of colony formation, shape of bacterial vegetative body, spore and crystal protein, along with PAGE analysis of this protein led to the finding of a new strain of *Bacillus* (BS01) (De and Mukhopadhayay, 2008). The new strain was close but all the same different from *Bacillus thuringiensis kurstaki* (*Btk*), often used as a commercial biopesticide against lepidopterans. The pathogenicity of this strain BS01 when tested in laboratory without any adjuvant was appreciable. Its LC_{50} value was 446.7 µg/ml and LT_{50} value was 6.19 days for 1000 µg/ml, 6.5 days for 750 µg/ml and 8.92 days for 500 µg/ml concentrations which were in general lower than that of *Btk*.

In a similar finding ten different strains of spore forming *Bacillus* could be isolated from the other dominant looper species, *H. talaca* (Fig 1 A and B). Two of the strains HT01 and HT02, that were different from *Btk*, more frequently infected the Terai population of *H. talaca*. These strains appeared to be the main bacterial pathogens causing natural

death of the caterpillars. Determination of LC_{50} value of HT01 on second instar stage of *H. talaca* showed it to be significantly lower than that of the other strain HT02, which was again almost half to that of *Btk*. The LT_{50} values for these newly isolated strains were also shorter than *Btk*. These low values indicate a better killing efficacy of HT01 and HT02 strains than *Btk* when used against the early stage caterpillars of *H. talaca*.

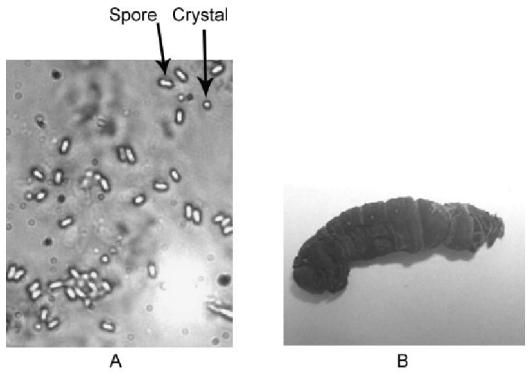

Figure 1. Spore and crystal of *Bacillus* sp. HT01 isolated from *Hyposidra talaca* (A) and dead cadaver of *H. talaca* (B)

A dual advantage of the newly reported *Bacillus* strains (BS01, HT01 and HT02) of the loopers was that they had the capacity to cross-infect the looper species and as such appeared to be effective in killing more than one looper species through formulation of any one bacterial strain or by cocktailing the two strains to be more effective at field level applications. The second advantage is that the strains were not infective to the multivoltine stock of silk worm (*Bombyx mori* nistari, that is commonly cultured in the Terai and the Dooars region (De and Mukhopadhyay, 2008). At plantation, HT01 strain of bacterium when applied in Random Block Design, (RBD) against early stage loopers proved its effective controlling power at 5000 µg/ml concentration. The mean live larvae recovered was 3.93% on 7th day after spraying this concentration.

Pathogenic bacteria of red slug and hairy caterpillars

Mortality of red slug caterpillars in tea plantation with typical symptoms of bacterial infection was evident. The blackened cadavers of larvae when screened for bacterial pathogen yielded a strain close to *Bt*. This new strain (RS01) when tested on early instar red slug caterpillars in laboratory showed a marginally higher LC_{50} value 458.1 µg/ml but a much shorter LT_{50} value than *Btk* (De and Mukhopadhayay, 2008). The discovery of the strain 'RS01' as a natural source adds a new microbial agent besides *Btk*, to be developed in future as a biopesticide after effective formulation, field trials, checking the safety aspects.

Naturally infecting bacterial strains isolated from caterpillars of *Arctornis submarginata* , the emerging defoliating pest of tea showed typical characteristic features of a *Bacillus*. The new strain (Arc01) satisfied all the Koch and biochemical tests and differed from *Btk* in ONPG test, and in utilization of citrate, arabinose, xylose, cellobios, melibiose and saccharose. Its doubling time was recorded as 84 mins. The banding pattern of vegetative protein on PAGE was found to be similar with *Btk*. Calculation of LC_{50} and LT_{50} values for this new strain (Arc01) were 398.1 µg/ml and 7.28 days for 1000 µg/ml respectively which substantially differed from those of *Btk* when tested on *A. submarginata*. Although isolated from the natural population of *A. submarginata*, the bacterial strain (Arc01) showed superiority in being more effective in inducing mortality at a lower dose and shorter time than *Btk* at least in laboratory tests. Given the above properties and efficacies, there is every possibility to develop the new strain (Arc01) into an efficacious biopesticide through more field level trials and bio-safety tests.

Baculoviruses (NPV and GV) of the defoliators

Natural mortality of loopers, *B. suppressaria* and *H. talaca* was not only because of bacterial infectivity but also due to the nucleopolyhedroviruses (Fig 2 A and B). The NPV isolated from the two looper species were found to cause effective mortality to the respective host species (*B. suppressaria* and *H. talaca*).

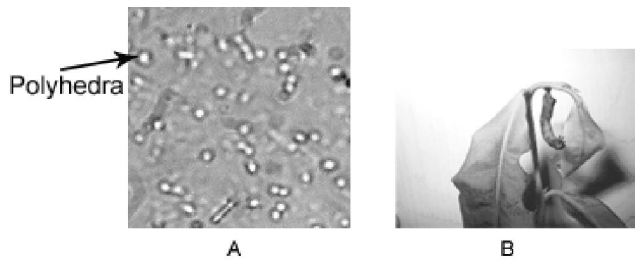

Figure 2. Polyhedra under phase contrast microscope (A) and dead *B. suppressaria* hang head down from a tea twig (B)

Characterization of the NPVs and their occlusion bodies, isolated from the two looper species, through phase and electron microscopy and biochemical determination of the molecular weight of polyhedron protein of OBs and DNA of virions, shared a striking similarities in most features. Further, the NPVs showed cross infectivity. When tested in laboratory *Bs*NPV inflicted a high mortality to early instars of *B. suppressaria* (LC_{50} of $1.23x10^6$ OBs/ml) and in a similar finding the NPV of *H. talaca* (*Ht*NPV) also showed high mortality of its host both in laboratory tests and field level application with LC_{50} of $7.1450x10^4$ OBs/ml and LT_{50} value 4.105 days for $1x10^7$ OBs/ml, 4.86 days for $1x10^6$ OBs/ml, 6 days for $1x10^5$

OBs/ml and 7.62 days for $1x10^5$ OBs/ml concentrations respectively.

The discovery of NPVs from loopers and their killing efficacy (against early stage loopers) with the special character of cross infectivity open up a distinct possibility of developing the viral pathogen into a bio-pesticide after toxicological test and safety clearance. Besides the *Bacillus* isolated from hairy caterpillars of *A. submarginata*, discovery of granulovirus in the natural population of the pest appears to be a great help as an additional biocontrol tool in pest management. The isolated baculoviruses from the cadavers showed the characteristic feature of granulovirus OBs under phase and electron microscope and also in the molecular weight of its polyhedral protein (Mukhopadhaya and De, 2009). Its potential as biopesticide against early stage caterpillars of *A. submarginata* was evident due to its low LC_{50} value $4.46x10^4$ OBs/ml and short LT_{50} period 6.6 days for $1x10^4$ OBs/ml, 5.09 days for $1x10^5$ OBs/ml, 4.45 days for $1x10^6$ OBs/ml and 3.87 days for $1x10^7$ OBs/ml concentrations., which is on a par with other GV formulations used in management of other crop pests.

The use of tea as a beverage is widespread in the domestic front and in most of the European and some American countries. Darjeeling area of North East India being declared an "agri export zone" requires much caution in applying the harsh chemicals including the synthetic pesticides to avoid residual problems (Bishnu *et al*. 2009).

Most tea estates refrain from taking risk of crop loss due to pest attack, hence liberally use acari- and insecticides under 'no threshold category of pest management' (Pedigo, 2002). But there are some tea gardens of the Darjeeling hills and even Terai and the Dooars regions that practice either strictly organic or biorational farming in order to make tea free from pesticide residue, and also avoid contamination of soil and water bodies in and around tea plantations. The proposition of organic farming would be more acceptable to the planters and managers if alternative non-conventional pest management measures can be suggested. Use of biopesticides, specially the microbials such as viruses (NPV/GV), spore forming bacteria (*Bacillus* strains) and fungi (*Beuveria* and *Metarrhizium*) hold a great possibility. So the discovery of newer bacterial strains and baculoviruses that have been found effective in inducing fair mortality in the defoliators of tea need to be further developed into biopestcides through better formulation, toxicity and biosafety testing. Early registering and patenting of the pesticidal properties of these entomo pathogens would ensure future development, improvement and applications of these potential biopesticides that comprise a part of the rich microbial diversity of North East India and in particular that of the tea plantations of Darjeeling foothills and plains.

REFERENCES

Bishnu, A., Chakrabarti, K., Chakraborty, A. and Sah, T. 2009. Pesticide residue level in tea ecosystems of Hill and Dooars regions of West Bengal, India. *Environ Monit Assess*, **149**: 457-464.

Datta, P. K. and Sharma, D. 1997. Effect of *Bacillus thuringiensis Berliner* in combination with chemical insecticides against *Spodoptera litura* Fabricius on cabbage, *Crop Research*,**14**: 481-486

De, D. and Mukhopadhyay, A. 2008. Pathogenicity of two strains of *Bacillus* infecting the lepidopteran tea pests *Buzura suppressaria* and *Eterusia magnifica* in Darjeeling foothill region. *Journal of Plantation Crops*, **36**(3), 408-413.

Falcon L. A. 1975. Use of field-collected insect viruses for pest control. **In:** *Baculoviruses for Insect Pest Control:Safety Considerations*, (Engler, M., Falcon, L., and Vail, P, eds.), Washington, DC: *American Society of Microbiology*, 161 **PP**.

Lande, S. S. and S. V. Sarode. 1995. Response of *Helicoverpa armigera* to pyrethroids. *Pesticides Research Journal*, **7:** 92-94.

Mukhopadhyay, A., De, D. 2009. Pathogenicity of a baculovirus isolated from *Arctornis submarginata* (Walker) (Lepidoptera:Lymantriidae), a potential pest of tea growing in The Darjeeling foothills of India. *Journal of Invertebrate Pathology*, **100:** 57-60.

Pathak, S. K. and Mukhopadhyay, A. 2005. Population variation in common thrips-*Mycterothrips setiventris* Bagnall (Thripidae:Thysanoptera) of Darjeeling tea. *Journal of Plantation Crops*, **33**(3): 210-215.

Pedigo, L. P. 2002. Entomology and Pest Management, 4th edn. Pearson (Education)), Singapore, 742 **PP**.

Sarker, M. and Mukhopadhyay, A. 2006. A preliminary study on host plant related changes of general esterase in *Buzura suppressaria* (Lepidoptera: Geometridae), a major defoliator of tea in the Darjeeling foothills and adjoining plains in India. *Sri Lanka Journal of Tea Science*, **71** (1), 1-7.

Yi, P. and Li , Z. 1989. Microbial pest control in China: viruses and fungi. *Symposium* on *Sino- American Biocontrol Research, San Antonio*.

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