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Review of literature

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2.a Pesticide use pattern in tea cultivation of Darjeeling Terai and the Dooars and its associated problems

Tea (*Camellia sinensis* L.) is one of the most important cash crops in India due to its tremendous export potentialities and demand in the domestic market. It is grown in the Northern region of the state of West Bengal covering an area of about 1.03 million hectare in Darjeeling slopes, Terai and the Dooars regions and contributes about 21% of the total tea production of the country (Bishnu *et al.*, 2008). Pests, pathogens and weeds are the severe constraints of the productivity and quality of tea. As a result, the tea planters use a wide range of pesticides to combat these problems for high yield and economic returns. Though broad spectrum chemicals offer powerful incentives, they have serious draw backs such as resistance to pesticides, pest resurgence, outbreak of secondary pests, harmful effects on human health and environment due to the presence of undesirable residues. Because of the known toxicity of pesticide in food products (Nagayama *et al.*, 1995; Neidert and Saschenbreker, 1996) there is an increasing public concern regarding the pesticide residues in tea (Singh, 1984; Bishnu *et al.*, 2008). In the second half of the 20th century the use of pesticides in tea have been in the rise (Anonymous, 1976) which reached a mark of 1,850,000 litres in seventies (Banerjee, 1976b). A survey was conducted on pesticide use patterns in the tea plantations of Dooars during period 1998 to 2004. The study revealed that on an average 7.499l/kg of insecticides was used per hectare per year of which the organo-chlorine, organo-phosphate and carbamate (non-pyrethroid) accounted 73.5% and pyrethroid represent 36.6% during the survey period. The requirement of synthetic pyrethroid gradually increased with every passing year in all sub districts in Dooars (Roy *et al.*, 2008).Use of such a huge quantity has naturally created problems about their residues.

Samples taken from tea gardens in Darjeeling contained varying levels of residues in made tea. About 28 percent of 182 first flush samples and 31.5 percent of 89 second flush samples were found to carry residues above the maximum residual limits (MRLs). In another set of 65 samples of made tea, 43 per cent contained ethion residues with a maximum of 8.43 ppm while 18 percent of the samples contained dicofol residues of 6.4

ppm (Barooah, 1994) that were all above the MRL standards prescribed by international agencies such as EPA, CODEX, EU etc.

DDT and synthetic pyrethroids (SPs) have been found to induce quick insect resistance. Their common resistance mechanism is referred to as "knockdown resistance" or target site insensitivity (Beilschmidt, 1990).

Germany had rejected the Darjeeling Gold brand of tea from market leader Teekane because it contained 0.24 milligrams of tetradifon-a pesticide used against mites in tea. This was 24 times the MRL fixed by Germany (Singh, 2002).

During the last several decades, the control of pests, diseases and weeds in tea fields is predominantly by the use of synthetic chemicals. They have developed serious effects such as insect resistance to pesticides, resurgence of pests, outbreak of secondary pests, harmful effects on human health and environment (Muraleedharan and Selvasundaram, 2005). Extensive use of chemical pesticide has had many well documented adverse consequences (Ghosh *et al.*, 1994; Hajra, 2002).

Indiscriminate and excessive use of pesticides creates environmental pollution, bio-amplification and cumulation leading to health problem of top carnivores and humans, and resurgence of pests. At present it is a global concern, and to minimizing chemical residue in food staff including beverages, fruits and vegetables comes as a mandate. Some countries have specified very low residues for certain chemicals in tea. Germany has specified 2 ppm levels for Ethion and Dicofol against 10 ppm and 45 ppm respectively by Environmental Protection Agency (EPA) of USA (Barbora *et al.*, 1994).

General observations od available reports reveal that some of the tea gardens of foothills, Terai and the Dooars receive indiscriminate input of pesticides, to boost its production at the cost of undesirable residue levels in the environment (Sannigrahi and Talukdar, 2005; Roy *et al.*, 2007). Heptachlor and Chlorpyrifos pesticides despite being banned are prevalent in made tea at higher concentration than their respective MRLs (Bishnu *et al.*, 2008). This may pose serious health hazards to the consumers apart from losing export credentialities.

2.b Microbials as natural control agents

Tea plantation is severely affected by attacks of different insects of which lepidopteran caterpillars are the major defoliating pests. A non-conventional approach for combating the insect pests is largely based on microbial entomopathogens (Barbora *et al.*, 1994). An important benefit of microbial control agents is that they can be used to replace, at least in part, some more hazardous chemical pesticides. More selective and biodegradable biocontrol agents can provide important ecological benefits. One of the ecological advantages of microbial control agents is that they tend to be highly selective, infecting or killing a very narrow range of target pests (Joung and Cote, 2000). Entomopathogenic micro-organisms like fungi, bacteria and viruses have tremendous potentials in pest management. In tea also several bacteria have been identified as entomopathogenic to mite and insect pests as per records of the recent past (Chakravartee, 1995).

Microbial insecticides are composed of microscopic living organisms (viruses, bacteria, fungi, protozoa or nematodes) or the toxins produced by these organisms. They are formulated to be applied as or with conventional insecticidal sprays, dusts, liquid concentrates, wettable powders or granules (Weinzierl *et al.*, 2005).

The groups of pathogenic bacteria in insects are varied, and include species with the ability to infect uncompromised healthy insects and also a large number of opportunistic pathogens which multiply rapidly if they gain access to the haemocoel of stressed insect hosts through wounds or following infection (Aronson *et al.*, 1986).

Among spore forming *Bacilli* of lepidopterans, the use of *Bacillus thuringiensis kurstaki* (*Btk*) is well established as microbial biocontrol agent. The formulations of *Bacillus thuringiensis* were effective against leaf rollers and against many other tea pests (Muraleedharan and Radhakrishna, 1989). *Bt* products represent about 1% of the total 'agrochemical' market across the world.

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) a polyphagous pest has attained the status of Cosmopolitan pest in the recent years due to severe damage caused to several crops. The management of this pest becomes difficult as it has developed resistance to different chemical insecticides (Mehrotra and Pholeka, 1992; Lande and

Sarode, 1995). In view of this efforts are being made to evolve alternate strategies for the management of this pest. Investigations around the world have indicated the usefulness of *Bt* to combat the menace of insect pests. *Btk* has been found to be the most effective against early second instar larvae of *H. armigera* (Brownbridge and Onyango, 1992).

The use of entomopathogenic bacteria, *Bt* and *Bacillus sphaericus* as biolarvicides are viable alternative for insect control (Reis *et al.*, 2001). The toxins produced by *Bt* are highly specific and are harmless to humans, vertebrates and plants, and are completely biodegradable, so no residual toxic products accumulate in the environment (Schnepf *et al.*, 1998).

Btk is an agriculturally important organism because it kills lepidopteran insects. Another potentially useful strain, *Bacillus thuringiensis isralaensis* had been isolated that produced a toxin effective against mosquitoes and black flies (Yoshishu, 1993). Toxicity of spore crystal complexes and commercial formulations of *Bt* was evaluated against second instar larvae of the diamond back moth, *Plutella xylostella*. Different formulations of *Bt* namely Delfin WG, Dipel 8L, Halt WP etc. were studied against the larvae of *Plutella xylostella* (Pokharkar *et al.*, 2002). HD-1 strain of *Btk*, produces two kinds of entomocidal protein inclusions (delta-endotoxin), bipyramidal and cuboidal. The toxicity of this bacterium against lepidopteran insects is associated with bipyramidal toxins (Tojo, 1986). *Bt* is also effective in controlling *Spodoptera litura* (Datta and Sharma, 1997) and *Crocidolomia binotalis* (Facknath, 1999).

Btk kills the caterpillar stage of a wide array of butterfly and moths. In contrast, *B. popillae* kills only Japanese beetle larvae (Weinzierl *et al.*, 2005).

The microbial insecticides most widely used in the United States since 1960s are preparations of the bacterium *Bt* (Weinzierl *et al.*, 2005).

Bt accounts for more than 90% of the biopesticides used today (Feitelson *et al.*, 1992). Evaluation of various subspecies of *Bt* for their toxicity has been carried out against the two lepidopteran pests, viz. *Spodoptera litura* (F.) and *Phthorimaea operculella* (Z.) (Putambekar *et al.*, 1997).

Cajanus cajan (Pigeaonpea) is one of the major legume (pulse) crops of the tropics and subtropics (Nene and Sheila, 1990). In India, the major insect pests of this crop are the pod boring lepidopteran pests damaging about 24-36% crops in the central and southern states of India (Lateef and Reed, 1983; Reed and Lateef, 1990). In order to check the pest menace 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). Pathogens of insects have been under evaluation as biological control agents for more than a century. With few exceptions, they are not effective as classical biological control agents. Moreover, even as insecticides, only *Bacillus thuringiensis* (*Bt*) has been a commercial success (Federici, 2006).

BLB1 is a new *Bacillus thuringiensis kurstaki* strain effective against third instar *Ephestia kuehniella* (Saadaoui *et al.*, 2009). An isolate of *Bacillus thuringiensis* has been identified with toxic activity against coleopteran insects but not against lepidopteran or dipteran species. Toxin crystals from this strain contain a polypeptide of approximately 64,000 molecular weight possessing insecticidal activity (Herrnstadt *et al.*, 1986).

A novel isolate of *Bacillus thuringiensis* subsp. *thuringiensis* produces a quasicuboidal crystal of Cry1Ab21 which is toxic to larvae of *Trichoplusia ni* (Swiecicka *et al.*, 2008).

Native *Bacillus thuringiensis* strains were isolated, characterized and tested for bioactivity against *Ephestia Kuehniella* (Lepidoptera: Pyralidae) larvae (Öztürk *et al.*, 2009). Mexican strains of *Bacillus thuringiensis* were selected, characterized and their activity was determined against four major lepidopteran maize pests (Bohorova *et al.*, 1996).

Cry1 genes was detected in *Bacillus thuringiensis* isolates from South of Brazil and activity was determined against *Anticarsia gemmatalis* (Lepidoptera:Noctuidae) (Bobrowski *et al.*, 2001). Brazilian strains of *Bacillus thuringiensis*, namely S701, S764 and S1265 were analysed regarding their cry gene and protein contents, crystal type, and activity against larvae of the lepidopteran fall armyworm (*Spodoptera frugiperda*), the velvet caterpillar (*Anticarsia gemmatalis*), the dipterans (*Culex quinquefasciatus* and *Aedes aegypti*) and the coleopteran (*Tenebrio molitor*) (Silva *et al.*, 2004).

The larva of *Scrobipalpuloides absoluta*, a South American moth, is the most devastating insect pest of tomato in Chile. The potential for using bacterial insecticides was studied analysing the relative toxicity of native *Bacillus thuringiensis* (*Bt*) isolates (Theoduloz *et al.*, 1997).

Other than *Bacillus thuringiensis* strains which were found to be entompathogenic to different insect pests of different family, few non-spore forming gram negative bacteria were found to be effective against insect pests. The larvae of scarab beetles, known as "white grubs" and belonging to the genera *Phyllophaga* and *Anomala* (Coleoptera: Scarabaeidae), are regarded as soil-dwelling pests in Mexico. During a survey conducted to find pathogenic bacteria with the potential to control scarab larvae, a native *Serratia* sp. (strain Mor4.1) was isolated from a dead third-instar *Phyllophaga blanchardi* larva collected from a corn field in Tres Marı´as, Morelos, Mexico (Nun˜ez-Valdez *et al.*, 2007).

The hazelnut leaf holer (*Anoplus roboris*, Coleoptera: Curculionidae) is a devastating pest of hazelnut and oak trees. It causes approximately 20-30% economic damage to hazelnut production per year in Turkey. In order to find a more effective and safe biological control agent against *A. roboris*, the bacterial flora of the hazelnut leaf holer were investigated and tested for insecticidal effects. According to morphological, physiological and biochemical tests, bacterial flora were identified as *Bacillus circulans* (Ar1), *Bacillus polymyxa* (Ar2), *Enterobacter* sp. (Ar3) and *Bacillus sphaericus* (Ar4) (Demir *et al.*, 2002).

The alder leaf beetle (*Agelastica alni* L., Coleoptera: Chrysomelidae) is one of the sources of damage to hazelnut and alder trees throughout the world. In order to isolate more effective and safe biological control agent against *A. alni*, bacterial flora of the alder leaf beetles were isolated that were collected from the vicinity of Trabzon, Turkey. Based on morphological, physiological and biochemical tests, bacterial flora were identified as *Enterobacter agglomerans* (Aa1), *Listeria* sp. (Aa2), *Pseudomonas chlororaphis* (Aa3) and *Pseudomonas fluorescens* (Aa4) (Sezen *et al.*, 2004).

Despite a more than 10 fold increase in insecticide use since 1940 (Lysansky and Coombs, 1994), crop losses due to insects have nearly doubled in the same period. The situation demands movement towards better control methods among which microbial control is most efficient. The most promising biological control agent is *Bacillus thuringiensis*, the leading organism being used as commercial microbial pesticides (Lambert and Pferoen, 1992; Meadows, 1993; Lysansky and Coombs, 1994).

The microbial control of insect pests is of crucial importance in developing countries (Dulmage, 1993). Overuse or misuse of chemical pesticides and their negative impacts on soil, and water quality, human health, wild life and ecological balance within agro-ecosystems are increasingly becoming causes of concern, underlining the need for development of alternative pest control methods (Meadows, 1993).

2.c Importance of isolation of new strains of bacteria for developing potential biopesticides

Bacillus thuringiensis is a bacterium known for producing protein crystals with pesticidal properties. These toxins are widely sought after for controlling agricultural pests due to both their specificity and their applicability in transgenic plants. Although any particular toxin has a desirably restricted host range, there is a large number of different toxins, each showing toxicity to one or many diverse pests (Rampersad and Ammons, 2005). For these reasons there is currently great interest in isolating strains of *Bt* with either unique host specificity or elevated toxicity.

Constraints to greater use of *Bt* in developing countries are: 1) scientific and technical: the difficulty in increasing the effectiveness of the products against specific pests and under specific agro-ecological conditions. 2) micro and macro-economics: efforts to reduce costs of production in developing countries, thus making *Bt* useful only in small scale application and limiting its large scale commercialization. 3) farmer acceptability: the longer period necessary to obtain high levels of mortality of pest larvae with *Bt* compared to chemical pesticides often prevent farmers from readily accepting *Bt*-based insecticides.

The main and the foremost problem encountered commercial formulation of *Bt* was the development of resistance in the insect pests against *Bt*. A number of insect populations of several different species with different levels of resistance to *Bt* have been reported (Schnepf *et al.*, 1998). The pest resistance was registered for *Bacillus*

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thuringiensis kurstaki, *Bacillus thuringiensis israelensis* and other *Bt* subspecies. The insects resistance to *Bt* include that of *Plodia interpunctella*, *Cadra cautella*, *Leptinotarsa decemlineata*, *Chrysomela seripta*, *Trichoplusia ni*, *Spodoptera litoralis*, *S. exigua*, *Helicoverpa virescens*, *Ostrinia nubilalis* and *Culex quinquefaciatus*. It is clear that this wide spread appearance of resistance to *Bt* presents a cautionary tale for the way of using *Bt* and *Bt* toxin genes in pest management with a review of the resistance management program of *Bt* (Schnepf *et al.*, 1998).

Due to different constraints of using *Bt* in the field, scientists all over the world are engaged to isolate new strains of *Bt* and develop them for controlling different pest insects. Selection of *Bt* strains for controlling fall armyworm (*Spodoptera frugiperda*) has already been worked out (Polanczyk *et al.*, 2000). Any particular *Bt* toxin has a desirably restricted host range, there is a large number of different toxins each showing toxicity to one of many diverse pests. For this reason currently research is going on to isolate novel strains of *Bt* with either unique host specificity or elevated toxicity. There are numerous reports on isolation of novel *Bt* strains from the environment (Rampersad and Ammos, 2005; Bernard *et al.*, 1997; Vilas-Boas and Lemos, 2004; Mohan *et al.*, 2009).

The great extension of tea belts in North Bengal region especially in the Terai and the Dooars regions, and diversity of insects provide the opportunity of isolating novel entomopathogenic bacteria. The identified bacterial strains analyzed in this research investigation represent a sample of diversity and natural resource.

Under the new plant protection strategy especially organic farming in tea, biological control plays an important role. Knowledge based technologies, free from inputs of harsh chemicals are now needed to produce bio-organic tea. Pest management technologies must be economical, robust, reliable, practical and ecologically benign. Once such integrated pest management modules are developed using appropriate control techniques including biological control in a mutually reinforcing manner, a check on the tea pest populations to a non-damaging level can be easily obtained. Entomopathogenic microbials like bacteria hold a great promise for developing such ecofriendly pest management strategies.