Impact of Science on the Economics of Tea Industry

3rd International Conference on
Global Advances in Tea Science
Held at Kolkata November 20-22, 2003

PART III
IMPROVING REALIZATION

Technical Session 4
Quality Improvement Through Safe Phyto-sanitation

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Technical Session 5
Improving Realization: Non-use of Chemicals

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Technical session 4
Quality improvement through safe phyto-sanitation
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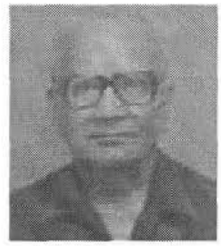
Chapter 16

TEA QUALITY, TEA PESTS, THEIR NATURAL ENEMIES AND PESTICIDES

B. Banerjee*



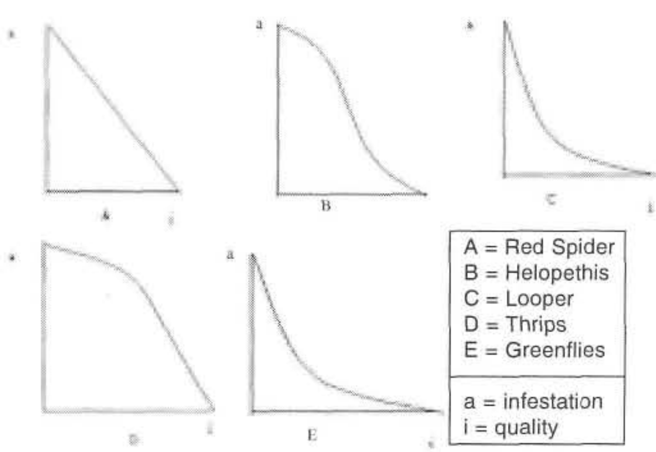
Dr. Barun Banerjee has had a long association with tea research. He was educated at Universities of S. Illinois, London and Oxford; Dr. Banerjee headed Tocklai as Director and later served as its Advisor. He held the World Bank chair in Applied Entomology at the University of Nairobi and at Washington. He is Adviser to National Tea Research Foundation.

Quality is intrinsic to some varieties of tea, and for others it can be developed and sustained by improvements in field and factory practices at different levels. Though no fixed norm exists for measuring, quantifying or even defining quality, insect infestations and disease infections could affect the quality of tea even within the remit of the current understanding of quality in a generic sense. Somehow this aspect did not receive the attention it deserved, despite apprehensions about probable and possible impact of pests on quality. Traditionally pests are viewed in terms of crop loss rather than loss in quality, though the latter could have long-term repercussions on the quality base of tea, even if crop losses are reversed. Some data, albeit their preliminary nature, are presented here on the significance of pest-quality interactions, and if an optimal combination of natural enemies and pesticides could inverse these negative interactions is discussed.

LEVEL OF INFESTATION AND QUALITY

Expect for red spiders, no linearity exists between the degree of infestations by different pest species and tea quality as perceived by tea tasters. The level at which quality starts declining varies with species. Thus while a mild infestation (<5%) of looper caterpillars may drastically affect quality, the flush-worms at that level may not. Even at an infestation level of >10% greenflies in Darjeeling do not seem to affect quality. If anything as a current study shows they might even help enhancing quality by secreting an enzyme that is precursor to the Kawakami molecular pathway to quality (Kwakami et al., 1995). However, the benefit may be short lived because quality starts deteriorating with increase in the levels of infestations. The way the quality degradation could be characteristic of some major pests is presented in Fig. 1.

Fig. 1. Quality degradation characteristics of some major pests in tea



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The five patterns of temporal quality changes suggest they are independent of the nature of damage by the pests, i.e. whether they are chewing or sucking up the leaves. With red spider attack quality declines proportionately as the infestation spreads horizontally, but under a comparable situation, *Helopeltis* presents a sigmoid response. Looper causes drastic reductions in quality even at a relatively low level of abundance, but thrips cause the decline almost logarithmically. Greenflies present a rather intriguing picture as quality is actually enhanced at low level of infestations but this advantage is lost at higher levels of infestations.

PESTICIDE USE AND QUALITY

It is axiomatic that pest related deterioration in quality has to be averted even if only patches of tea fields are the initial casualty. To contain these pests, pesticides are at present evaluated, apart from bio-effectivity, for their tainting and residual toxicities (MRL). Their effects on quality have always been suspected (Wallace *et al.*, 1962), but are not well documented for tea.

The residues that remain on tea leaves after knocking down the pests could create an inadequate quality related enzyme profile by any of the three ways:

1. Such enzymes are not constitutive.
2. Enzymes are produced but accesses to labile sites within the cells are blocked by crossing linkages.
3. Enzymes may be inactivated.

THE IDEAL SITUATION

The ideal situation from the perspective of both quality and quantity would be to have neither pests nor pesticides! In reality this is not possible because most tea insects and mites have virtually coevolved with tea plants (Banerjee, 2000). Though a totally sanitized tea is neither possible nor desirable, applications of pesticides can be regulated to take advantage of the biological factors in the economic management of pests and diseases. Natural enemies are crucial here, but they also have they limitations (Banerjee 1982, 1996; Mukhopadhyay, 2003; Singh, 1998). Their utilization, rather than mere preservation, is central to any worthwhile concept of pest management. More research on this aspect is certainly called for, particularly for predators that are highly effective at the low levels of pest populations (Banerjee, 1983). This would be virtually analogous to prophylaxis by pesticides.

It is nevertheless a tribute to Indian tea industry that over the decades it has progressively gone for a degree of self-regulation in the use of pesticides as Table 1 would indicate :

Table1. Pesticide regulation by Indian tea industry

Decade	Generic pesticides in use	Emphasis
Sixties	15	Broad spectrum
Seventies	10	Selective
Eighties	7	Biodegradability
Nineties	6	Biopesticides & natural enemies

The trend obviously is towards least or minimal use of pesticides as evident from the declining number of generic pesticides and preference for ecofriendly pesticides. This has resulted in a significant reduction in the number of tea samples with residue levels above the threshold. A moot point however. Will the nonselective natural enemies interfere with quality in the absence of their prime targets which are tea mites and insects?

CONCLUSION

I posit that integrated pest management in tea has to be oriented towards sustainability of quality in addition to crop quantity. The functional profiles of individual predators will have to be sorted to find out the pesticides they are compatible with and at what pesticide levels. Finally, the cost benefit aspect of this integrated approach must be evaluated in terms of environment protection and quality of tea.

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Chapter 17

STRATEGIES FOR REDUCING PESTICIDE RESIDUES IN TEA

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Dr. N. Muraleedharan is presently Director of UPASI Tea Research Foundation at Valparai in Tamil Nadu. He joined the Tea Research Foundation in 1980 and has served in different capacities there. He obtained his Ph.D. from University of Madras. He has authored two books, and several chapters in multi-authored books. Over 200 scientific research papers are to his credit. Recently the Indian Society of Plantation Crops honored him with the Fellowship of the Society. His areas of interest include insect ecology, biological control, pesticide residue and integrated pest management.

Pests, pathogens and weeds are important factors limiting the productivity and quality of tea in almost all the tea-growing countries of the world. While mites, thrips, scale insects, *Helopeltis* and caterpillars are the major pests of foliage, shot hole borer and termites infest the stem. White grubs, nematodes and pathogenic fungi attack roots. Blister blight is a major leaf disease responsible for considerable crop loss. Weeds are problematic in new clearings, pruned fields and vacant patches of tea fields. Recently, Muraleedharan and Chen (1997) reviewed the pest and diseases of tea and their management. The loss in crop due to pests, diseases and weeds is estimated to be between 15 and 20 per cent. During the last few decades control of pests, diseases and weeds in tea fields had been achieved predominantly by the use of synthetic chemicals. Though broad spectrum chemicals offer powerful incentives in the form of good control, increased yield and high economic returns, they have serious drawbacks such as resistance to pesticides, pest resurgence, outbreak of secondary pests, harmful effects on human health and environment and presence of undesirable residues. In the last one decade there have been welcome efforts to adopt non-chemical control methods and to evolve an integrated management programme against pests, diseases and weeds infesting tea.

In India the registration of pesticides is done by the Central Insecticides Board (CIB) under the Insecticides Act and the Maximum Residue Limits (MRL) on food materials by the Ministry of Health and Family Welfare under the Prevention of Food Adulteration Act (PFA Act). Compounds such as DDT, BHC, aldrin, heptachlor and tetradifon are banned in India. For use in tea fields the CIB has accorded registration for the following chemicals *viz.* carbofuran, dicofol, deltamethrin, dimethoate, ethion, endosulfan, fenazaquin, oxydemeton - methyl, propargite, profenofs, quinalphos, sulphur (insecticides / acaricides), copper oxychloride, hexaconazole, propiconazole, tridemorph (fungicides), glufosinate ammonium, glyphosate, paraquat-dichloride and 2,4-D. It may be emphasized that tea being an important export commodity, the use of pesticides on tea in India had largely been guided by the tolerance limits prescribed by the EPA of USA and Codex Alimentarius Commission of FAO/WHO. However, the national laws of tea importing countries vary on the MRL of different pesticides on tea. In other words, there is no harmonization of the MRL of different pesticides on tea. The EU / Germany had

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declared MRLs for a few pesticides on tea. They are ethion (3 ppm) dicofol (20 ppm) endosulfan (30 ppm) deltamethrin (5 ppm), propargite (5 ppm) lamda-cyhalothrin (1 ppm) malathion (0.5 ppm) fenitrothion (0.5 ppm) cypermethrin (0.5 ppm) and tridemorph (20 ppm). By a recent regulation (2070/2002) in November 2002, EC had withdrawn authorisation for more than 300 compounds in member countries of EU (Anon, 2002). This regulation has an impact on tea exports since it withdraws the approval for ethion, which is extensively used for mite control in tea, and quinalphos, an insecticide with limited usage. It is understood that the EU has proposed a draft regulation to the European parliament and EU council to replace the earlier directives on MRLs for plant protection products. The new draft regulation allows the possibility to set import tolerances where different agricultural practices outside the EU lead to different residue levels on imported products. Tea is not grown in the member countries of EU and, therefore, India and other countries exporting tea to EU should explore the possibility of this provision of the draft regulation to obtain import tolerances on an acaricide like ethion, by submitting to EU the relevant data on MRL. These data will have to be based on field and laboratory studies carried out on the principles of GAP, GLP, HACCP and as per FAO paper No.170.

Pesticides applied on tea bushes are degraded in several ways such as growth dilution, weather factors and processing. Photolabile compounds undergo degradation due to sunlight. The pesticide present on harvested shoots undergoes thermal degradation during manufacturing, at the high temperature, especially if the compound is thermolabile. In short, the level of residues of any pesticide on made tea depends on its response to temperature. Based on the degradation pattern and MRL, safe harvest intervals have been fixed for the more commonly used pesticides (see infra). Just as temperature influences the residues in made tea, the solubility of the compound decides the level of residues in the tea infusion.

All over the world, regulations on pesticide residues in food and environment are becoming more the more stringent. All food industries, including tea industry, will have to take note of the emerging scenario. The tea industry in India is in an advantageous position, since it had all along been adhering to the tolerance levels or MRLs declared by the EPA of USA or the EU in view of the large export market for Indian tea. The tea garden managements have to look at ways and means to further reduce the usage of pesticides. This, to a very large extent, can be achieved by adopting the following measures.

PEST MONITORING

Agro Ecosystem Analysis

Field officers and supervisors can carry this out to analyse the field conditions with regard to plant health, pest and natural enemy situation, soil condition and weather factors based on past experience. Such a critical analysis will help the management in taking appropriate decision on management practices.

Field Monitoring and Sampling of Pests / Pathogens

The supervisors and field officers should conduct regular surveys and record the incidence of pests or diseases or weed growth. The sampling procedures for different pests such as mites, thrips, *Helopeltis,* caterpillar pests, shot hole borer, termites and blister blight are already available.

INTEGRATED PEST MANAGEMENT STRATEGIES

Certain routine cultural operations such as plucking, pruning, shade regulation and weed control can be manipulated to reduce the incidence of pests, diseases and weeds.

Plucking and Manual Removal

Populations of leaf folding caterpillars such as flush-worms, leaf rollers and tea tortrix can be reduced by their manual removal while harvesting. Areas, which would be severely infested by tea mosquito bug, could be earmarked and black plucked to retard the growth of its population.

Populations of foliage feeding caterpillars such as loopers, bunch caterpillars and faggot worms can be reduced to a great extent by manual removal of their larvae and pupae. Tea mosquito bugs lay eggs on the broken ends of plucked shoots. Intensive manual removal of stalks during plucking will help to reduce the incidence of tea mosquito bug.

Pruning and Skiffing

When an attack by *Helopeltis* becomes unmanageable the affected bushes may be skiffed to reduce the damage. Medium prune (60-70 cm) is best suited for shot-hole borer infested fields (except when other factors demand a different height of pruning). Longer pruning cycles will tend to increase the intensity of borer damage, especially in mid and low elevation areas.

Shade Regulation

Unshaded areas are more prone to the attack of thrips and mites. The recommendations on shade management, if adopted, will help to prevent the excessive build up of thrips, mites and reduce the incidence of blister blight disease.

Field Sanitation

Weeds like *Mikania cordata, Bidens biternata, Emillia sp., Polygonum chinense* and *Lantana camara* offer excellent hiding places and serve as alternate hosts for the tea mosquito. Growth of weeds and wild host plants in and around tea fields should be controlled and this will help to reduce the growth of *Helopeltis* population. In the case of primary root diseases such as *Poria hypolateritia* and *Fomes noxius*, isolation of diseased patches by making 120 cm deep and 45 cm wide trenches surrounding the diseased plants help in preventing the spread of these primary root diseases.

Fertilizer Application

Application of higher levels of potassium fertilizers is known to reduce the incidence of pests and disease in several crops. Application of higher levels of potassium fertiliser ($N:K_2O$ = 1:2) in the pruned year had helped to decrease the incidence of shot-hole borer in the infested fields.

Infilling Vacancies in Field

Weeds are problematic in young tea clearings, pruned fields and vacant patches in mature tea fields. These patches have to be in-filled while pruning the field to avoid weed growth and to increase bush population and yield.

Host Plant Resistance

Use of pest resistant varieties is one of the important components of non chemical control strategies. Even low levels of resistance are important since the need for other control methods can be reduced. Being a perennial crop, research on clonal selection and breeding is primarily aimed at the production of high yielding and superior quality plants with practically no emphasis on resistance of pests or diseases. The emerging information could be successfully utilised in the management of pests and diseases.

It is now realised that Chineric varieties are more susceptible to the attack of red spider and scarlet mites while Assam cultivars are favoured by eriophyid mites. Incidence of tea mosquito is more on China jats and the clone TV-1 is highly susceptible to the pest. Flush-worm incidence is more on the clone UPASI-17, considered "Cambod" in nature. Soft wooded tea plants are known to be easily attacked by termites in Sri Lanka. Clones with high alpha spinasterol content are susceptible to damage by shot hole borer. Certain Sri Lankan selections like TRI 2024 and TRI 2025, which are popular in south India, should not be planted in shot hole borer prone areas. Screening of cultivars for susceptibility or tolerance to major pests must become a prerequisite for the release of new cultivars.

Heat Treatment and Soil Solarisation

Soil used in the nursery may be heated to 60-62^{O}C for killing the infective juveniles of root knot nematodes. Care may be taken that the soil is not over heated since this will lead to phytotoxicity. Soil solarisation during summer is also found quite effective in reducing the *Meloidogyne javanica* populations in the infested soil.

Use of Light Traps

Fluorescent light traps are useful in attracting the moths of caterpillars and other insects. These can be set up during the seasons of moth and beetle emergence, and the attracted moths and beetles can be killed mechanically or by using insecticides. These traps are useful for monitoring the activity of the pests and also as a tool in their suppression. Yellow pan water traps have been found useful to trap thrips and aphids. Use of yellow sticky traps has been found to reduce thrips attack by trapping the adult thrips.

Biocontrol Using Multicellular Organisms

The minor status of several pests such as aphids, scale insects, flushworms, leaf rollers and tea tortrix is due to the action of natural enemies. Often, the work of these beneficial arthropods goes unnoticed, especially when their hosts are minor pests. Efforts towards the conservation and augmentation of natural enemies in the tea ecosystem could offer significant advances biological control programme in tea in future.

Biocontrol Using Entomopathogens

Several microbes are pathogenic to tea pests. Formulations of the bacterial insecticide, *Bacillus thuringiensis* have been effectively used for the control of lepidopterous pests. A local strain of *Beauveria bassiana* has been found effective against the shot hole borer. This strain is now being marketed as a wettable powder formulation for the control of shot hole borer.

Certain commercial formulations of the entomopathogenic fungi, *Verticillium lecanii, Paecilomyces fumosoroseus* and *Hirsutella thompsonii* were evaluated and found effective against pink, purple and red spider mites. These formulations are recommended for the control of mites. *Trichoderma viride, T. harzianum* and *Gliocladium virens* are used to suppress the populations of soil borne pathogenic fungi causing root and stem diseases of tea.

Behavioral Control

Sex pheromones form an important component of IPM. Pheromone traps of *Spodoptera litura* (cutworms) infesting tea are commercially available and can be used for controlling the populations of the moths. The pheromones of tea leaf roller have been field tested and are used in Japan. Similarly, the female sex attractants of the flush-worm *(Cydia leucostoma)* have also been identified recently. Studies on the sex pheromones of tea mosquito are in progress. Kairomones from the partly dried stems of *Montanoa bipinnatifida* are found to attract shot hole borer beetles. At present these stems are used to attract shot hole borer beetles in tea fields.

Use of Plant Products for Pest Control

Certain products derived from plants are used for tea pest control. Azadirachtin, obtained from the seed kernels of the neem tree, *Azadirachta indica*, has been found effective against pink and purple mites and certain caterpillars. Formulations containing azadirachtin and their combination with synthetic insecticides are recommended for pest control in tea. Products from several other plants such as *Acorus calamus, Annona squamosa, Lantana camara, Ocimum basilicum, Bidens pilosa, Calotropis gigantea* and *Vitex negundo* have pesticide properties. Products such as rotenone, nicotine, pyrethrum extract and garlic extract derived from plants can also be used for controlling pests.

Use of Inorganic Compounds and Hydrocarbon Oils

Formulations of sulphur and lime sulphur are effective against mites. Recently, spray oil of paraffinic base has been found effective against eriophyid and red spider mites. Since this oil does not leave any residues in tea, it could be incorporated in to the mite control programme in tea. Copper oxychloride (COC) is used in combination with triazole fungicides for the control of blister blight disease of tea.

Chemical Control

Safety harvest intervals have been established for the commonly used pesticides, based on the field data generated during the last few years. These data will help the industry to decide the harvesting interval after the application of the chemicals such as ethion (7 days), dicofol (6 days), endosulfan (3 days), quinalphos (4 days), chlorpyrifos (12 days) and deltamethrin (6 days) and to keep their residue levels below the MRL prescribed by EU. Need based, judicious and safe application of approved pesticides is the most vital aspect of chemical control measures under IPM strategy. It involves developing IPM skills to play safe with environment by proper crop health monitoring, observing ETL and conserving the natural bio-control potential before deciding in favour of the use of chemical pesticides as a last resort.

ACKNOWLEDGEMENTS

I am thankful to Dr. R. Selvasundaram, Dr. B. Radhakrishnan and Mr. K.N.Manikandan of UPASI Tea Research Foundation for their help in the preparation of this paper.

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Chapter 18

ROLE OF BIO-PESTICIDES IN TEA CULTIVATION

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Dr. S. Ramarethinam obtained a PhD in Botany from the University of Bombay 1963. From 1963 to 1969 he worked in academia in various posts and in CSIR. From 1969 to 1984 he worked in EID Parry as a senior executive where he developed the Seeds Division and established a number of seed processing units. From 1988 to present he has been the Director of projects, T. Stanes & Company and Director of Research and Development of United Nilgiri Estates in Coimbatore where he has been involved in development of a number of products including neem formulations for which he got a national award. He has also developed products based on various entomophagous pathogens including Beveria bessiana as well as the antagonistic fungi of the genus Trichoderma. He is also the author of numerous national and international papers.

ABSTRACT

The international movement for sustainable tea cultivation through IPM and organic farming and the advocation of use of bio-rational molecules as an alternative to synthetic chemicals to achieve the sustenance of ecological balance and a better sustained productivity are the point of interest. All the more tea, the most popular common natural drink next to water with most of the nutrition and therapeutic properties, if chemicalised will only lead to cause ill effects in human health world over. Management for integration of non-chemical methods of controlling insect pests by using bio-pesticides is essential for successful organic farming in general and tea in particular. The biopesticides for organic tea cultivation includes botanical pesticides; microbial pesticides of fungal, bacterial, and viral origin, parasites and predators are discussed elaborately.

INTRODUCTION

Tea, the world's most popular common natural drink shrouded in myth and legend, has always been looked upon as a drink with rejuvenating and energizing properties. Greater understanding of tea has also revealed its remarkable health benefits. When its usage has increased in leaps and bounds from 0.53 kg in 1981-83 to 0.66 kg in 1997-98, greater production of tea has become a necessity. However, the question on the increased tea productivity at an economical cost without involving chemicals to meet the pallet requirement of million and billions of mouth needs to be given due consideration.

CURRENT INDIAN PESTICIDES SCENARIO

True there is no dearth for chemical pesticidal molecules all over the world in general and in India in particular where the usages have been slowly and steadily increased in the past few decades which polluted the atmosphere beyond redemption. In the past 5 decades the use of chemical pesticides steadily increased to about 300 fold i.e. from 2.2 gram per hectare of active ingredient in 1950 to the current level of 650 gram per hector.

IMPORTANCE OF BIOLOGICALS INPUTS IN TEA CULTIVATION

Tea is a crop that is cultivated all over the world as commercial cash crops. The agronomical practices

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of tea involved usage of synthetic chemicals fertilizers for nutrient management, synthetic chemical pesticides for pest and disease management, synthetic chemical weedicides for weed management coupled with appropriate technology for water conservation, harvesting and scientific processing have enabled the tea industry to improve the yield and the profitability as well. However the awareness created among the consumers and the changes in the perception of importers in the maximum residual level (MRL) of chemical pesticides (Table 1) based on the health ground have necessitated the tea producers to look for an alternative to the present day of cultivation practice. IPM involving the biologicals as a major input in tea cultivation has come as a handy tool.

Table 1. MRL value for tea (Muraleedharan and Selvasundaram, 2002)

Pesticide rate	Application tea in ppm	Residue on black	M RL
Dicofol	185	45	20
Endosulfan	350	24	30
Ethion	500	10	2

WHAT ARE BIO-PESTICIDES

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Biopesticides are pesticides, which use insect pathogens as the active ingredient. Although they have been known for hundreds of years, only in the last twenty years have commercial products become available. Despite their favorable properties, they have remained a minority interest, and sales account for only about 2% of the worldwide market in agrochemicals (Ramarethinam, 2002).

WHAT ARE THE ADVANTAGES OF USING BIOPESTICIDES

Biopesticides are inherently less toxic than conventional chemical pesticides. Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional chemical pesticides that may affect organisms as different as birds, insects, and mammals. Biopesticides often are effective in very small quantities and often degrade quickly, avoiding the connected pollution problems, which is associated with conventional pesticides. It is also significant to note that some of the pests resistant to pesticides can be effectively controlled by the biopesticides.

IMPORTANT BIOTIC STRESSES OF TEA

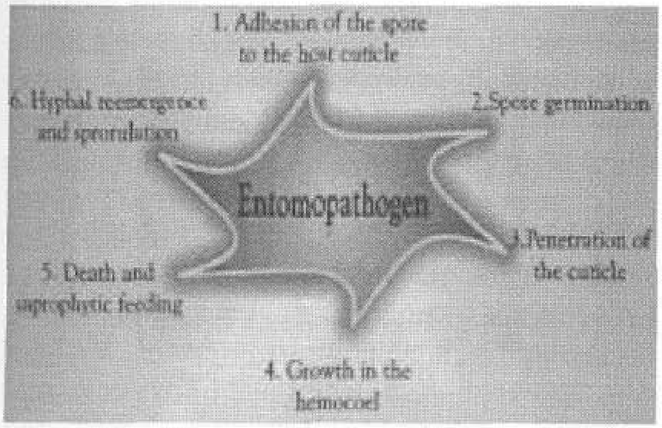
The Biotic stress encountered in tea falls into tow category. They are major and minor pests from among different orders of insects (Table 2).

Table 2. Biotic stresses of tea

Pests		Category	Pest	
			Common name	Scientific name
Insect Pest	Acarinae	Major pests	Pink Mite	*Acaphylla theae*
			Purple mite	*Calacarus - carinatus*
		Minor pests	Scarlet Mite	*Brevipalpus Sp.*
			Yellow Mite	*Polyphagotar-sonemus latus*
	Lepidoptera	Minor pests	Flush worm	*Cydia leucostoma*
		Minor pest	Hairy Caterpillar	*Euptorate sp.*
			Looper Caterpillar	*Buzura Suppressaria*
			Bunch caterpillar	*Andraca bipuncatata*
	Coleoptera	Minor Pests	Shot Hole Borer	*Euwallacea fornicatus*
	Hemiptera	Major pest	Tea Mosquito Bug	*Helopeltis theivora*
	Thysonaptera	Major pest	Thrips	*Scirtothrips Sp*
Diseases	Fungi	Major disease	Blister blight	*Exobesidium vexans*
		Major disease	Brown blight	*Colletotrichum camellia*
		Major disease	dieback	*Alternaria*
		Major disease	brown root disease	*Fomes*

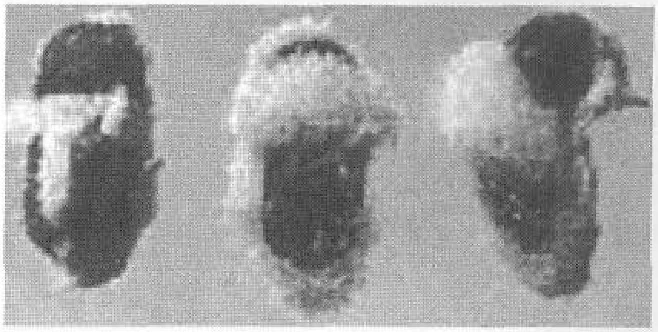
(Source: Muraleedharan and Selvasundaram, 2002).

MODE OF ACTION OF BIOPESTICIDES VS CHEMICAL PESTICIDES



Unlike chemicals, which exhibit a "knock-down" effect, the biopesticides will not give any instantaneous death to insects. Rather its action on pest will be manifested slowly but exerts a long lasting control over the pests, as most of these biocontrol agents are living organisms, which are self-perpetuating in nature (Table 3).

USAGE OF BIOPESTICIDES IN THE CONTROL OF TEA PESTS AND DISEASES

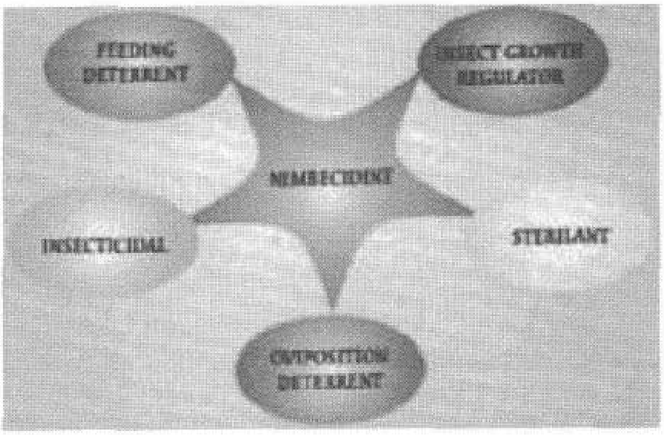


Several microbes are pathogenic to tea pests. Formulations based on fungal, bacterial and viral origin are now used effectively in the control of lepidopterous, acarine, hemipterous and coleopterous pests of tea (Selvasundaram and Muraleedharan, 2000; Ramarethinam et al., 2000). The important pests, the chemicals currently used in their control and the suggested biocontrol agents for the respective pests as supplement if not substitute are as listed in Table 3.

Table 3 : Bio-rational molecules, their active ingredients and the target pest controlled by these molecules

Biopesticides	Species	Secondary Metabolites produced	Tea Pest controlled
Botanicals			
Neem	*Azadirachta indica*	Azadirachtin Salanin, Meliantriol, Nimbin, Nimbidine, Nimboline	Hairy Caterpillar, Flush worm Mites, Cutworm Tea Mosquito Bug, Tea Tortrix
Entomopathogens			
Fungal origin	*Beauveria sp*	Bassianin, Oosporein Tennelin, Beauvericin	Flush worm, Tea Mosquito Bug, Cutworm
	Verticillium sp.	Balanol, Cephalochromin, Dihydroisoustilaginoidin, Dihydroxychaetocin, Iso-ustilaginoidin A	Aphids Thrips
	Paecilomyces sp.	Belfedrin A Byssotoxin, Ergosterol peroxide Variotin	Mites
	Metarhizium sp	Destruxin	Tea Mosquito bug, Aphids
Bacterial origin	*Bacillus thuringiensis (Bt.)*	Protein crystals, Beta endotoxin, Delta endotoxin	Caterpillars Cutworm

NEEM IN TEA PEST MANAGEMENT



Neem has also been accepted as an important ecofriendly tool to manage the pest in tea cultivation (Ramarethinam et al, 2000, 2001). Neem with its multiple biological effects like Antifeedancy, repellency, oviposition deterrency, Insect growth regulation and insecticidal properties has offered a effective control over the pest like flush worm, mites, cutworms, hairy caterpillar, aphids, and *nematodes* (Ramarethinam et al 2004; Muraleedharan and Selvasundaram, 2002).

BIOLOGICALS VS COST EFFECTIVENESS

Though the cost involved in the adoption of biologicals as a major pest control input is high it is more justifiable keeping in view of the high environmental cost in terms of environmental degradation, depletion of natural enemies and ecological imbalance incurred through the usage of chemical pesticides (Table 4).

Table 4. Common tea pest and its present and suggested control measures including economics*

	Chemical Method			Botanical/Biological Method			Cost difference	
Pests	**Product**	**Dosage/ha**	**Cost/ha.**	**Product**	**Dosage/ha**	**Cost (Rs.) / ha**	**Chemical**	**Biological**
Mites (Red spider mite, Pink mite, Purple mite)	Dicofol	1000 m l	Rs. 280	Nimbecidine	1000 ml.	Rs. 208	Rs. 72	-
	Sulphur – 80%	1000 gm	Rs 55	Nimbecidine + Priority	500 ml+ 2.5 kg	Rs.104+ Rs.302	-	Rs. 351
	Quinolphos 25EC	750 ml	Rs. 195	Nimbecidine	1000 ml	Rs. 208	-	Rs. 13
	Dicofol	1000 m l	Rs. 280	Nimbecidine + Priority	500ml+ 2.5kg	Rs.104+ Rs.302	-	Rs. 126
	Cost		**Rs. 810**			**Rs.1228**		**Rs. 418**
SHB	Quinolphos 25EC+ Dichlorovos 76 EC	750 ml+ 250 ml	Rs.195+ Rs. 95	Nimbecidine+ Bio-Power	500 ml+ 1.5 kg	Rs. 104 + Rs. 105	-	-
	Lambbdacy halothrin 5EC	120 ml	Rs.130	Nimbecidine+ Bio-Power	500 ml+ 1.5 kg	Rs. 104 + Rs. 105	-	Rs. 79
	Fenvalerate 20 EC	500 ml	Rs.103	Nimbecidine+ Bio-Power	500 ml+ 1.5 kg	Rs. 104 + Rs. 105	-	Rs. 106
	Quinolphos+ Dichlorovos	750 ml+ 250 ml	Rs.195+ Rs. 95	Nimbecidine+ Bio-Power	500 ml+ 1.5 kg	Rs. 104 + Rs. 105	Rs. 195	-
	Cost		**Rs. 813**			**Rs. 836**	-	**Rs. 23**
TMB	Endosulfan 35EC	1000 ml	Rs. 210	Nimbecidine+ Bio-Power	500 ml+ 2.5 kg	Rs. 104 + Rs. 362	-	Rs. 256
	Quinolphos 25EC	750 ml	Rs.195	Bio-Power	2.5 kg	Rs. 362	-	Rs. 167
	Endosulfan+ Dichlorovos	1000 ml+ 250 ml	Rs.105+ Rs.95	Nimbecidine+ Bio-power	500 ml+ 2.5 kg	Rs. 104 + Rs. 362	-	Rs. 266
	Endosulfan+ Dichlorovos	750 ml+ 250 ml	Rs. 195+ Rs.95	-	-	-	Rs.290	-
	Cost		**Rs. 895**			**Rs.1294**	-	**Rs. 399**
Flush worm	Endosulfan	1000 ml	Rs. 211	Nimbecidine	1000 ml	Rs. 208	Rs. 3	-
	Quinolphos 25EC	750 ml	Rs.195	Nimbecidine+ Bio-Power	500 ml+ 1.5 kg	Rs. 104 + Rs. 105	-	Rs. 14
	Fenvalerate	500 ml	Rs.103	Nimbecidine	1000 ml	Rs. 208	Rs. 103	-
	Cost		**Rs. 509**			**Rs. 625**	-	**116**

** The number of sprays as given in the table for the control of various pests is only approximate and it will vary depending upon the prevailing abiotic and biotic conditions in a given area.*

STATUS OF ORGANIC TEA CULTIVATION IN INDIA AS A MEANS FOR VALUE ADDITION

The concept of organic farming is gaining ground with Indian tea planters too. Unlike the market trend for tea in general, demand for organic tea like other food items has also been growing rapidly since it was introduced to the world market in the late 1980's. The growth in the production of organic tea from 1990 to 2000 has been 20 fold. The production of organic/organic in conversion tea was 1, 50, 000 kg in 1990 and it increased to 21, 50, 000 kg in 2000 (Chaudhuri, 2000). In India, the cultivation of organic tea started in Darjeeling during 1986 and gradually spread to the tea areas of Assam and South India. Hence, organic tea production is more remunerative than conventional tea production even after taking into consideration the lower productivity and higher production costs (Damodaran, 2000; Damodaran, 2002).

CONCLUSION

Use of chemical insecticides is quite indispensable in any agricultural system, for which tea is no exception. However to elevate the vibrant image, it is imperative to grow tea organically without the use of hazardous chemical in tea. In this context biopesticides play an important role in tea cultivation. The most popular and natural, common man drink next to water – The tea, unpolluted and un-poisoned with chemical pesticides will thus be used to deliver its rightful nutritional and therapeutic properties to the needy. Some of the Biopesticides including Neem and Microbial pesticides can be used to contain the pests successfully - major pests of today can become minor pests of tomorrow, besides rejuvenating tea drink itself to be rejuvenated in the marketing front through value addition of tea as pesticide free health beverage.

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Chapter 19

MICROBIAL PESTICIDES AND INSECT SEMIOCHEMICALS FOR CONTROL OF TEA PESTS IN CHINA

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Now he is in a professional position. His research fields are chemical ecology of insects, biological control and tea science and he is going to talk about the control of tea pests in China.

ABSTRACT

More than 800 species of pests in Chinese tea gardens had been recorded, in which the around several decimal pests usually occurred during the past many years. Over 60 species of insect pathogenic viruses from tea plant pests were collected and identified, including Nuclear polyhedrosis viruses (NPV), Granulosis viruses (GV), and a few of Cytoplasmic polyhedrosis viruses (CPV), from the end of seventies to the early eighties in 20st century. Some universities, institutes and large tea farms have produced NPV and GV preparations by the live worm bodies, and utilized them to control *Euproctis pseudoconspersa* Strand, *Ectropis oblique* Prout, *Buzura supressaria* Guenee, *Adoxophyes orana* Fischer von Rosierstamm, and *Andraca bipunctata* Walker, etc. The research results showed that the latency of the pathogens was from three to four days, and control effects range from 80 % to 95 %. Several researchers gleaned and identified more than 40 species of entomogenous fungi, e. g. *Beauveria bassiana* (Bals.) Vuill., *Metarhizjum anisopliae* (Metschn.) Sorokin, *Paecilomyces* spp., *Aschersonia aleyrodis* Webb, *Aegerita webber* Fawcett, etc. from the middle eighties to the middle nineties. A few tea farms and institutes have cultivated those fungi with the method of three-grade enlarging culture, processed them into preparations, and sprayed them to control *A. orana*, *Curculio chinensis* Chevrolat, *Aleurocanthus spiniferus* (Quaintance) , etc. The latency of the pathogens was from six to seven days and the control effect was from 80 % to 85 %. Two species of insect pathogenic bacteria, i. e. *Bacillus thuringiensis* and *Serratia marcescens*, were found out. The latency of *B. thuringiensis* was about one day. *B. thuringiensis* was used to control the pests from 1970s to 1980s, and its control effect to Lepidopterous larvae was more than 95 %. Generally, the control strategy follows as: processing various pathogens into preparations, and then releasing them to control pests during the population peak by inundative method. Several components of sexual pheromones were identified during the middle 1980s. The lures made of the components showed the certain attractive effect to male moths. Tritrophic chemical communications in tea garden were surveyed since 1990s, viz., tea plant-*Toxoptera aurantii* – ladybeetles, lacewings and *Aphidius* sp., tea plant – *E. oblique* – *Apanteles* sp., and tea plant – *Empoasca vitis* – *Evarcha albaria*. It was detected that the volatiles from tea

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shoots attracted *T. aurantii*, *E. oblique* and *E. vitis* intensively. The synomones emitted by pest-damaged tea shoots, the excretion and the residue material from the pests elicited the attracting activity to the natural enemies. We suppose that the insect semiochemicals will be applied to suppress pests in tea gardens to certain scale in the future.

Key words: China; insect pathogenic viruses; entomogeous fungi; *Bacillus thuringiensis*; inundative release; inoculative release; insect semiochemicals; mechanism of chemical communication.

INTRODUCTION

Tea is one of major economic crops in China, being grown in the extensive area to the south of Qinling Mountains and Huaihe River. In 2002, the tea garden area reached 1160 thousand ha, with tea output 720,000 tons and tea export 252,300 tons. More than 800 species of tea plant pests have been reported during the past years in China (Han, 1998; Zhang and Han, 1999), 10 % of which are the important pests, and 5 % of which usually occur and have to be controlled. Such as, *Empoasca vitis* (Göthe), *Ectropis oblique* Prout, *Aleurocanthus spiniferus* (Quaintance), *Euproctis pseudoconspersa* Strand, *Agrotis caneacens* (Butler), *Toxoptera aurantii* Boyer, *Adoxophyes orana* Fischer von Rosierstamm, *Myllocerinus aurokineatus* Voss, *Thosea sinensis* (Walker), *Cryptothelea minuswcula* Butler, *Andraca bipunctata* Walker, *Helopeltis* sp., *Contarinia* sp., *Acaphylla theae* (Watt), and *Xylebors fornicatus* Eichh. In some tea growing areas insecticides are sprayed around ten times per year, which have induced the insecticide residues and insect resistances. New approaches to controlling tea insects have been explored for a long time. A great deal of insect pathogenic viruses and entomogenous fungi were collected, identified and applied since the early 1970s. The semiochemicals of insects in tea gardens have been deeply investigated since the early 1990s to and are expected to control pest in the future.

VARIOUS PATHOGENIC MICROORGANISM RESOURCES IN TEA GARDEN ECOSYSTEMS

Insect Pathogenic Viruses

Euproctis pseudoconspersa Nuclear polyhedrosis virus (*Ep*NPV) was first found in the tea gardens of Tea Research Institute of Hunan Agricultural Academy in 1957. Sixty-nine species of viruses from near to fifty species of pests had been isolated and identified, most of whom were belonged to A sub-group (NPV) and B sub-group (GV) of Genus *Baculovirus* of Baculoviridae, and CPV group of Reoviridae, and a few were little viruses without capsids (Liang and Zheng, 1986; Zhang, 1990; Zheng, *et al.*, 1985; Hong, 1991). They follow as:

Host insect	**Year and province where found first**	
A Sub-group of genus *Baculovirus* of Baculoviridae (NPV)		
1. *Adoxophses reticulama*	?	
2. *Agrosea sinensis*	1964	Hebei
3. *Amata germane*	1985	Guizhou
4. *Arctornis aiba*	1977	Guizhou
5. *Biston marginata*	1980	Hunan
6. *Buzuraai sappressra*	1977	Hunan
7. *Buzura thibetariu*	1977	Guizhou
8. *Cipuna locuples*	1981	Guizhou
9. *Cnidocampa flavescens*	1970	Jiangxi
10. *Cryptothelea minuswcula*	1975	Shanghai
11. *Cryptothelea mvariegata*	1983	Guizhou
12. *Culcula panterinania*	1973	Beijing
13. *Dappala tertia*	1986	Guangdong
14. *Dassyhira glaucinopera*	1981	Guizhou
15. *Ecxtropis grisescens*	1987	Hubei
16. *Ectropis oblique*	1979	Anhui
17. *Euproctis bipunctapex*	1983	Guizhou
18. *Euproctis flavanata*	1977	Liaoning
19. *Euproctis pseudoconspersa*	1957	Hunan
20. *Euproctis varians*	1985	Guizhou
21. *Heliothis zea*	?	
22. *Jankowskia athieta*	1981	Hubei
23. *Lepede nobilis*	1978	Hunan
24. *Lragoides fasciata*	1979	Hubei
25. *Mahasena colona*	1983	Guizhou

26. *Parasa lepida*	1981	Guizhou
27. *Pasychira baibarana*	1977	Guizho
28. *Porthesis aterta*	1981	Guizhou
29. *Pradenia lifura*	1973	Guangdong
30. *Scoplodes venosa*	1985	Guangdong
31. *Scopule subpunctaria*	1977	Guizhou
32. *Setora suberecta*	1983	Guizhou
33. *Spitarctic oblique*	1983	Guizhou
34. *Thosea haibarana*	1974	Guangdong
35. *Thosea sinentis*	1964	Beijing

B Sub-group of genus *Baculovirus* of Baculoviridae (GV)

36. *Adoxophyes orana*	1979	Hubei
37. *Adoxophyes prinatana*	1979	Guizhou
38. *Agrotis segetum*	1974	Beijing
39. *Adoxophyes* sp.	?	
40. *Agrotis tokionis*	?	
41. *Agrotis ypsilon*	?	
42. *Ameacta lactinca*	1979	Guangdong
43. *Andraca bipunctata*	1984	Guizhou
44. *Clania variegata*	?	
45. *Clostora anastomosis*	1983	Guizhou
46. *Cnidocampa flavescens*	?	
47. *Darna trima*	1989	Guizhou
48. *Dasschira baibarana*	1985	Guizhou
49. *Ectropis obliqus*	?	
50. *Eterusia aedea*	1985	Zhejiang
51. *Homena coffearia*	1979	Chongqin
52. *Homena magnanima*	?	
53. *Lragoides fasciata*	1986	Guizhou
54. *Parasa bicolor*	?	
55. *Parasa consocia*	1978	Shandong
56. *Parasa lepide*	1980	Guangdong
57. *Prodenia titura*	1964	Beijing
58. *Setora suberecta*	1984	Guizhou
59. *Thosea sp.*	?	

Cytoplasmic polyhedrosis virus group of Reoviridae (CPV)

60. *Adoxophyes fasciata*	?	
61. *Adoxophyes orana*	?	
62. *Agrotis segetum*	1974	Beijing
63. *Cnidocampa flavescons*	?	
64. *Euproctis pseudoconspersa*	1981	Hubei
65. *Homon magnima*	1989	Jiangshu
66. *Setora suberecta*	1983	Guizhou

Genus *Entomopoxvirus* of Poxviridae (EPV)

67. *Adoxophyes* sp.	?	

Viruses without Capsids

68. *Betora* sp.	1983	Guizhou
69. *Netria viridescens*	1984	Guizhou

ENTOMOGENOUS FUNGI

Entomophthorales

1. *Zoophthora radican*. Hosts: *Ectropis oblique*, *Empoasca vitis*. Places: Guangdong, Yunnan, Anhui.
2. *Neozygites fresenii*. Hosts: *Toxoptera aurantii*. Places: Hainan, Anhui.
3. *Entomophthora* sp. Hosts: *Amata germana*, Places: Guizhou.
4. *Entomophaga grylli*. Hosts: Several species of locusts. Places: Zhejiang, Anhui, Guizhou.

Ascomycotina

5. *Cordycepes militaris* Hosts: *Thosea posotnata*, *Iragoides fasciata*. Places: Guizhou, Anhui.
6. *Cordycepes gunnii*. Hosts: Larvae of a species of swiffmoth. Places: Guizhou.
7. *Cordycepes hawkesii*. Hosts: Larvae of a species of swiffmoth. Places: Guizhou.
8. *Hypocrella palmae*. Hosts: *Ceroplastes ceriferus*. Places: Sichuan
9. *Nectria flammea*. Hosts: *Lepidosaphes ume*, *Aspidiotus dustructor*, *Aspidioutus hederae*, *Pseudacnida paeoniae*. Places: Sichuan.
10. *Cordycepes fruinosa*. Hosts: *Setora postornata*, *Iragoides fasciata*. Places: Guizhou.
11. *Sphaerostilbe* sp. Hosts: *Paralepidosaphes tubulorum*. Places: Guizhou.
12. *Cordycepes* sp. Hosts: *Buzura thibetaria*. Places: Guizhou.

Septobasidiales

13. *Septobasidium pedicellatum*. Hosts: Various scales on tea plants. Places: Anhui, Yunnan, Hainan.

Deutoromycotina

14. *Beauveria bassiana*. Hosts: *Empoasca vitis*, *Adoxophyes orana*, *Homona coffearia*, etc.

Places: Throughout the tea plant growing area.

15. *Metarhizium anisopliae*. Hosts: Larvae of a species of swiffmoth, Adult of a species of Cicada. Places: Guizhou, Anhui.
16. *Metarhizium cylindrosporae*. Hosts: Larvae of a species of swiffmoth. Places: Guizhou.
17. *Mariannaea pruinosa*. Hosts: larvae of *Setora postornata* and *Iragoides fasciata*. Places: Guizhou.
18. *Paecilomyces gunnii*. Hosts: Larvae of a species of swiffmoth. Places: Guizhou.
19. *Paecilomyces cateniannulatus*. Hosts: *Homona coffearia, Adoxophyes orana*, Adults from Coleoptera. Places: Guizhou, Anhui.
20. *Paecilomyces fumosoroseus*. Hosts: Larvae of *Adoxophyes orana*. Places: Anhui, Guizhou.
21. *Paecilomyces cateniobliquus*. Hosts: *Homona coffearia, Adoxophyes orana*. Places: Guizhou.
22. *Paecilomyces farinosus*. Hosts: Larvae and pupae of *Setora postornata*, Pupae of *Iragoides fasciata*. Places: Guizhou.
23. *Paecilomyces tenuipes*. Hosts: Larvae of *Iragoides fasciata*. Places: Guizhou, Anhui.
24. *Paecilomyces cicadae*. Hosts: A species of cicada. Places: Anhui.
25. *Paecilomyces* sp. Hosts: Pupae of *Parasa lepida*. Places: Anhui, Zhejiang.
26. *Nomuraea rileyi*. Hosts: Larvae of Noctuidae. Places: Anhui.
27. *Verticillium lecanii*. Hosts: *Adoxophyes orana, Iragoides fasciata, Ceroplastes ceriferus*, Pupae of a species of Lepidoptera. Places: Yunnan, Guizhou.
28. *Fusarium moniliforme*. Hosts: Larvae of *Ectropis oblique*. Places: Zhejiang.
29. *Fusarium moniliforme* var.*subglutinans*. Hosts: Larvae of *Ectropis oblique*. Places: Zhejiang.
30. *Fusarium semitectum*. Hosts: Larvae of *Ectropis oblique*. Places: Zhejiang.
31. *Aschersonia aleyrodis*. Hosts: *Aleurocanthus spiniferus, Dialeurodes citri*. Places: Anhui, Zhejiang, Fujian, Guangdong, Hainan.
32. *Aschersonia duplex*. Hosts: A species of scales. Places: Zhejiang.
33. *Aschersonia fimbriata*. Hosts: *Aleurocanthus spiniferus*. Places: Zhejiang, Jiangshu, Anhui.
34. *Aegerita weberi*. Hosts: *Aleurocanthus spiniferus, Dialeurodes citri*, Several species of scales. Places: Anhui, Zhejiang, Fujian, Hubei, Hunan, Yunnan, Guangdong, Hainan.
35. *Cladosporium cladosporioides*. Hosts: *Aleurocanthus spiniferus*, etc. Places: Throughout tea plant growing area.
36. *Acremonium* sp. Hosts: *Aleurocanthus spiniferus*, etc. Places: Anhui.
37. *Pleurodesmospora coccorum*. Hosts: *Aleurocanthus spiniferus*, etc. Places: Anhui, Shanxi.
38. *Hirsutella* sp. Hosts: *Aleurocanthus spiniferus*. Places: Anhui.
39. *Paecilomyces* sp. Hosts: *Aleurocanthus spiniferus*. Places: Anhui.
40. *Cephalosporium coccorum*. Hosts: *Pulvinaria horii*. Places: Sichuan
41. *Aspergillus oryzae*. Hosts: *Aleurocanthus spiniferus*. Places: Anhui.

Insect Pathogenic Bacteria

1. *Bacillus thuringiensis*. Hosts: Larvae of Lepidoptera. Places: Throughout tea plant growing area.
2. *Serratia marcescens*. Hosts: Some pests of Orthoptera. Places: Anhui.

Entomogenous Nematodes

1. A species of entomogenous nematode (?) . Hosts: Mantid adults. Places: Henan.

STUDIES AND APPLICATION OF PATHOGENIC MICROORGANISMS ON PESTS IN TEA GARDENS

Insect Pathogenic Viruses

Generally, the ultra-structures of insect viruses have been observed by electro-microscopes and their pathogenicity also has been experimented. The serology and the security to humans and animals on some important insect viruses have also been studied. Some of them have been greatly produced and applied in field with a large scale, e.g. *Ep*NPV (Yao *et al.*, 1987)· *Ao*GV (Du *et al.*, 1984), *Eo*NPV (Wu and Hu, 1987), etc.

*Eo*NPV has been deeply studied. Generally, the dead insect carcasses were collected from tea garden, grinded, filtrated, centrifuged, then the rude viruses extraction were separated. The larvae were infected by viruses from their mouths. After the tea leaves were sprayed or effaced by virus, they were fed to larvae to expand the amount of the virus. The most important factor affecting the yield of *Eo*NPV was the concentration of the virus, and the next was instars of host larvae fed with the virus. The high yield was obtained when the early fourth instar larvae were fed with virus at the concentration of 2.2 × 10 ^{6} PIB / ml for 48 h. The optimum temperature ranges from 18 to 26^{0}C, and the highest yield was got at 25^{0}C. The middle and late fifth instar larvae produced the highest yield (Wu and Hu, 1987). After the larvae were infected by the virus, their survival rate, pupation rate, emergence rate, reproductive rate, net reproductive rate, innate capacity for increase and finite rate of increase were lower or significantly lower than those of CK; however, their average generation duration was distinctly longer than that of CK (Ye *et al.*, 1994). Usually, as a microbial insecticide, it is sprayed to control larvae. When adding a little of FSS and fluorescein-sodium into the purified *Eo*NPV, a new formulation of UV-protecting NPV could be obtained. The UV-protecting efficiency of the formulation was 41.0 %, and the control efficiency under field condition was 12.1 % higher than that of CK. The additive of synergist could be in favour of enhancing the effect (Yin *et al.*, 1996; Ye and Hu, 1991). The formulation is used to control the first and fifth generations larvae. As to control 2 to 4 generations larvae, the formulation is often added into a little insecticide. The optimum control periods were the second and the third instars, and the hatching period may be the optimum period (Tables 1 & 2) (Hu *et al.*, 1997). The research results showed that the latency of the pathogens was from three to four days, and control effects were listed in Table 3 (Zhang, 1990).

Table 1. Experimental results of *Eo*NPV to larvae during hatching period

Concentration (PIB/ml)	Day	Mortality (%) $\bar{X}$ ±S.D.			Accumulative descent of leaf area (%) $\bar{X}$±S.D.			Pupation rate (%) $\bar{X}$±S.D.		
	0		b	B	92.95±0.13	ab	AB	1.93±2.72	cd	BC
1.5×10^{5}	1	83.72±4.17	cd	CDE	81.32±0.28	bcde	AB	8.07±11.41	cd	BC
	2	44.79±5.10	de	DE	61.82±4.82	f	C	21.43±5.06	bc	BC
	3	31.48±4.11	de	CDE	67.14±20.83	ef	BC	18.39±15.90	bc	BC
	4	36.18±14.49	e	E	68.84±4.71	def	BC	33.60±16.77	b	AB
	0	18.40±12.55	a	A	98.63±0.38	a	A	0.00	d	C
1.5×10^{6}	1	100.00±0.00	b	B	87.91±3.43	bcd	ABC	9.84±0.23	bcd	BC
	2	80.27±5.09	b	BC	76.22±4.33	cdef	BC	16.13±9.12	bcd	BC
	3	70.79±4.56	b	B	88.25±8.00	bcd	ABC	9.09±12.86	bcd	BC
	4	78.74±14.93	bc	BCD	85.20±3.40	bcde	ABC	4.26±0.13	cd	BC
CK		62.10±7.88	f	F	0.00	g	D	75.33±8.15	a	A

0.00

Notes: Differences in averages with the same letter or Capital do not reach the level of 5 % and 1 %.

Table 2. Application results of *Eo*NPV to larvae

Date	Generation	Dose	Spray	Area (666.7m²)	No 12 d Mortality (%)	No 12 d Accumulative descent of leaf area (%)	No 14 d Mortality (%)	No 14 d Accumulative descent of leaf area (%)	Notes K
1989.7	3	A	Dongfanghong-18	51.0	88.12	87.69	92.53	86.01	
1989.8	4	B		25.0	76.33	65.01	83.14	61.98	
	4	C		25.0	67.95	53.09	77.75	53.09	
1990.4	1	D		5.0	100.00	74.45	—	—	L
	1	E	Gongnong-16	10.0	78.98	52.45	96.67	52.45	
1990.6	2	F	Dongfanghong-18	5.0	89.33	61.29	100.00	61.29	
1991.4	1	G		5.0	90.32	63.22	100.00	66.13	K
1991.6	2	H		5.0	100.00	72.16	—	—	K
1991.7	3	I	Gongnong-16	0.5	86.97	—	100.00	—	
1991.9	5	J	DongFanghong-18	20.0	85.26	76.28	97.85	76.28	

Notes: A - 1.5×10^{10} PIB+5.5ml Fenvalerate+54.5ml Dimethoate / 666.7m²;
B - 1.5×10^{10} PIB+3ml Fenvalerate /666.7m²
C - 1.5×10^{10} PIB+1.5ml Fenvalerate+3g Red dye /666.7m²
D - 2.3×10^{10} PIB / 666.7m²
E - 1.5×10^{10} PIB+50ml Dichlorphos (DDVP) / 666.75.0m²
F - 1.5×10^{10} PIB+5ml Fenvalerate/666.7m²
G - 1.5×10^{10} PIB / 666.7m²
H - 1.5×10^{10} PIB+5ml Fenvalerate+25g Buprofezin / 666.7m²
I - 3.0×10^{10} PIB+3ml Fenvalerate+25mg Buprofezin / 666.7m²
J - 1.5×10^{10} PIB / 666.7m²
K - Control *Empoasca vitis* at the same time
L - Control *Cryptothelea minuswcula* at the same time

Table 3 Suppression results of insect pathogenic viruses to tea pests

Target pest	Virus type	Dose	Mortality (%)
Euproctis pseudoconspersa	NPV	6×10^7 PIB / ml	94
	NPV	1×10^5 PIB / ml	90
	NPV	$3\text{-}6\times10^7$ PIB / ml	71-97.0
	NPV	$250\text{-}500\times10^8$ / 667 m²	93-97.5
Buzura supressaria	NPV	$5\text{-}20\times10^{12}$ PIB / hr	86.9-94.0
	NPV	0.2×10^8 / ml	93-100
Buzura thibetaria	NPV	$0.5\text{-}1\times10^8$/ ml	98.0
Ectropis oblique	NPV	1×10^{10} PIB / 667 m²	83-96
Adoxophyes orana	GV	7.5-50 mg / 667 m²	76.6-100
	GV	12.5-25 mg / 667 m²	87.5-93.8
Andraca bipunctata	GV	200 mg / 667 m²	88.69-97.67

Entomogenous Fungi

The pest carcasses were fetched from tea gardens, on which hyphae and spores were set aside and transferred onto inclined medium, such as Czapek, PDA, etc. respectively, for culture under 20-25°C. After a week they were transferred into triangle bottles, which contained liquid medium and were laid on orbital shaker, for the second grade culture.

In 5 to 7 days, they were transferred into the tray for the third grade culture. Most of media used for the second and third grade cultures were inexpensive agricultural products, such as, wheat bran, rice chaff, rice, etc. A little additive of sucrose acts as carbon sources, and a little bean powder and corn power as nitrogen sources. After two weeks, the media were reversed, and then the fungi were fostered for a week (Tang *et al.*, 2003; Pu and Li, 1996; Han and Li, 2001). Sifting some medium out of the mix of fungi and medium, adding talcum power or clay into the mix, then the mix was made into power formulation. If other appropriate additives are used, the mix can be made into the soluble power formulation, or other types. Afterwards, detect the formulation quality, such as, spores content, living spore rate, and moisture content, toxicity tested by bioassay, etc. (Pu and Li, 1996; Han and Li, 1992).

While pests occur severely in tea gardens, the soluble power formulations are usually added water, and sprayed in the morning, evening or the rainy days. The application way is the inundutive spraying. The latency of the pathogens was from six to seven days.

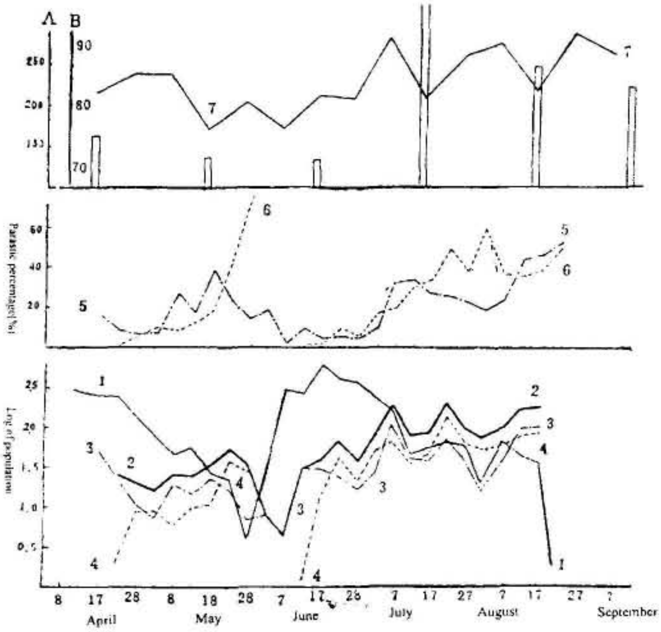
Beauveria bassiana is used to control *Adoxophyes orana* Fischer von Rosierstammand, *Homona coffearia* Nietner and *Myllocerinus aurolineatus* Voss, etc. The control results range from 80 % to 85 %. The control results of it to *A. orana* are better than those to *H. coffearia*. *Paecilomyces aleurocanthus* and *Aegerita weberi* are two species of important entomogenous fungi isolated from whiteflies. The both isolation, identification, culture, toxicity and the field application were surveyed (Tang *et al.*, 2003; Han and Li, 1992; Han, 1997). The both have the stronger pathogenicity and prevailing potential. Both formulations are sprayed in "Mildew water" (May) period and autumn rain period (September - November), and they control results are around 85%. If adding a little insecticide, the control results would surpass 98 %.

NATURAL INFECTIONS PREVAILING

Tea gardens are closing, shady and humid. The average relative humidity per day in middle and lower tea clumps was more than 85 % (Han, 2000). The plenty of entomogenous fungi resources are contained in tea ecosystem. If pests outbreak, entomogenous fungi may be brew epizootic. For example, *Aleurocanthus spiniferus* outbroke all over the tea plant growing area in China, however, three species of entomogenous fungi came into being epizootic to a broad extent (Fig. 1) (Han and Li, 1993). The high moist, high population density and certain fungi amount are responsible for the epizootic.

Entomogenous fungi and parasitic wasps jointly suppress pests. For example the warm and moist weather was favorable to pupation, eclosion and hatching in spring of 1999. The first generation outbroke severely. The suppressing effect of the parasitic wasps on the pest population did not exhibit obviously until the pupal stage of the whitefly. Then, a lot of these adult wasps deposited their eggs into the larvae of the first and second instars of the second generation. Several species of entomogenous fungi resulted in the epizootic in the third instar larvae of this generation. The population trend index of the first, second and third generation was estimated at 0.47, 0.09 and 0.02, respectively. *A. spiniferus* population decreased sharply from generation to generation, and finally collapsed in the late August. Over 10 species of parasitic wasps and 4 species of entomogenous fungi were jointly responsible for dynamics of the pest population (Han and Cui, 2003). And the parasitic wasps' effect was stronger slightly (Tables 4-6) (Han and Li, 1993). Entomogenous fungi and insect pathogenic viruses jointly suppress pests. For instance, *Ectropis oblique* injure tea plants severely in the eastern Chinese tea gardens. However, since the 1970s, within the adjoining area of Zhejinag and Jiangsu and Anhui Provinces, during autumn rain period of September to October, *Zoophthora radican* and *Eo*NPV caused epizootics in the fifth generation population of *E. oblique*, with natural infective rate over 95 %. The next year the population density of the first generation was low. The density did not increase until the third generation. The fungi and wasps became the key suppressing factors to the pest for a long time (Li *et al.*, 1989).

Fig. 1. Changes in pupae of *A. spiniferus* and parasitic wasps and entomogenous fungi (lower), fluctuation of infective rate of fungi and parasitic rate of wasps (middle), and relative rainfall per month and average RH per 10 days (upper)



1 – pupae of *A. spiniferus*,
2 - fungi and wasps joint group,
3 - entomogenous fungi,
4 – parasitized pupae,
5 - infective rate of fungi,
6 - parasitic rate of wasps,
7 - average RH per 10 days

Table 4. Average infective or parasitic percentage of fungi or wasps per month.

Control factors	Infective (Parasitic) percentage						t-test
	Apl	May	Jun	July	Aug	Avg	
Fungi	10.25	20.91	7.00	24.91	32.59	19.13	0.158
Parasitic wasps	2.10	28.15	2.72	31.05	42.24	21.25	$t_{0.05}$

Table 5. Control effect of fungi and wasps in different types of tea gardens

Tea garden types	Control factors	Average infective or parasitic (%)	t	$t_{0.05}$	$t_{0.01}$
Young tea garden	fungi	20.03	8.94**	2.10	2.88
	wasps	44.22			
Mature tea garden	fungi	35.44	1.54		
	wasps	30.96			
Tea garden forward the Sun	fungi	29.97	1.12		
	wasps	34.83			
Tea garden against the sun	fungi	33.50	1.07		
	wasps	37.76			

Table 6. Control effect of fungi and wasps in different tea garden most seriously damaged by insects

Layers of tea plant	Total of pupae	Parasitic wasps		Fungi		Survival pupae	
		No.	Parasitic (%)	No.	Infective (%)	No.	(%)
Up	556	186	33.45	73	13.13	297	53.42
Middle	1541	531	34.46	658	42.70	352	22.84
Down	1565	783	50.03	478	30.45	304	19.43
Total	3662	1500	40.96	1209	33.02	953	26.02

PROGRESS IN RESEARCHES ON SEMIOCHEMCIALS OF TEA INSECTS

Sex Pheromones

Based on the researches on anatomy, bioassay, and EAG studies, the sex pheromone gland of *Ectropis obliqus* was found, locating between the eighth and ninth abdominal segments. The releasing amount of sex pheromones from two-day-old females after 6 to 8 h dark phase was the biggest. Five components had been purified from sex gland extraction and synthesized, in which (Z, Z)-3, 9-6, 7-epoxy-octadedicene had the most intense activity, eliciting the calling of 30 % to 47 % males. Unfortunately, the field application of the components acquired inferior results (Yin *et al.*, 1993). The synthetic sex pheromones lures showed significant attraction to *Homona magnannima* Diakonoff, in which the ratio among Z-11-tetradecenyl acetate, Z-9-dodecenyl acetate and 11-dodecenyl acetate was 80:10:10, and dose was 1 mg (Xiao, 1998). The primary component of *Euproctis pseudoconspersa* is 10,14-

dimethylpentadecyl isobutyrate (Zhao *et al.*, 1998), the lures made from the component were used to trap the males, and the larva population of the next generation decreased by 81.15 % (Ge *et al.*, 2002).

Studies on Chemical Communication in Tea Plant-Pest-Natural Enemy

Three chemical communication systems have been studied since the 1990s: tea plant-tea aphid-seven spot ladybird, lacewing, and *Aphidius* sp. (Han and Chen, 2002 a, 2002 b, 2000 a), tea plant-*Ectropis oblique-Apanteles* sp. (Xu *et al.*, 1999), and tea plant-*Empoasca vitis – Evarcha albaria* (Zhao *et al.*, 2002). Green leaf volatiles emitted from the fresh tea shoots elicited intense attraction and EAG response to pests, while elicited weak ones to the natural enemies. The metabolism in tea shoots would be changed and released the specific synomomes after damaged by the pests. Tea aphid damage induced the liberation of benzaldehyde, *Ectropis oblique* damage induced C_5-C_6 aldehydes emitted, and *Empoasca vitis* damage induced the releases of 2,6-dimethyl-3,7-octadien-2,6-diol, attracting spiders. The rinse from pest body surface (Han, 2001) and the secretion of pests (Han and Chen, 2000) attracted the natural enemies intensely, too. The detected main components from intact tea shoots were *E*-2-hexenal, ocimene, *Z*-3-hexenyl acetate, *Z*-3-hexen-1-ol, butanoic acid-3-hexenyl ester, linalool, 1-octanol, geraniol and indole. The detected main components from tea shoot-tea aphid complex were Z-3-hexen-1-ol, benzaldehyde, E-2-hexenal, Z-3-hexenyl acetate, ocimene, linalool, geraniol, indole, and E-2-hexenoic acid. The main components in a hexane rinse from tea aphid cuticle were benzaldehyde, undecane, 2,5-hexanedione, 2,5-dihydrothiophene, linalool, 4-methyl-octane and eicosane, whereas the main components in a ether rinse from tea aphid cuticle were E-2-hexenoic acid, heptadecane, pentadecane, eicosane, tetratetracontane and nonadecane.

DISCUSSION AND PROSPECTS

1. A large number of tea gardens have been contracted by smallholders since the 1990's. It is difficult for the individual smallholders to produce the microbial pesticides. After completing the field practices in tea gardens, the farmers still hope to seek other job for making more money. Therefore, the farmers like to apply insecticides, because insecticides are simple, convenient and more effective. The yield of the microbial pesticides reduced sharply. During the past several years, Chinese government advocates non-polluted teas and organic teas, and the microbial insecticides are emphasized again.

2. First of all, the strong toxicity microbial species or strains should be screened, and can selected out, because a plenty of microbial resources exist in tea gardens. They may be acquired through protoplasm induction, radiation induction and so on, and by means of the modern molecular biological techniques, gene engineering, etc.

   Secondly, strengthen the applying techniques. Except the inundative way, the inoculative way should be adopted before the pest peak to efface "time lag effect" between pests and natural enemies. The insect community are complicated, and *Aegerita weberi* and other fungi can infect many whiteflies and scales, and should be introduced and set in host populations (Han, 2000). As an element of IBM, combined with other agents. For example, adding Buprofezin into *Aegerita weberi* formulation can promote the effect, and control tea leafhoppers. It can be combined with parasitic wasps effectively (Han and Li, 1993). We suggest that it should integrate with tea plant cultivation. China possesses a great deal of intercrop tea gardens (Han *et al.*, 2001), within whose clumps RH usually exceed 85 %. So do the closeplanting tea gardens in double row, which distribute on the middle and low reaches of Changjiang River. The entomogenous gungi easily live in such tea garden habitats.

3. Up to now, few semiochemicals has been applied to the suppress tea pests. We suppose to combine them with microbial insecticides. For example, lure pests to be infected by pathogenic microorganisms, let them spread pathogens in the healthy pest individuals, then result in epizootics. The chemical ecology of tea insects needs to combine with other disciplines as so to enhance it. For instance, 2,6-dimethyl-3,7-octadien-2,6-diol can be released due to *Empoasca onukii* damage. 2,6-dimethyl-3,7-octadien-2,6-diol was an important aroma component from Darjeeling black teas in India as well as Pomfon Oolong teas injured by *Empoasca flavescens* F.(川上美智子 *et al.*, 1997) . By means of biochemical and molecular biological techniques, we maybe probe into the mechanism for synomones to form.

ACKNOWLEDGMENTS

This work is supported by the subject from the science and research foundation for outstanding talents of Chinese Academy of Agricultural Sciences (2002-382 of Plan and Finance of CAAS), and the national item of tackling key problem of the science and technology (2001BA502B02).

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Chapter 20

SOME OBSERVATIONS ON THE MYCOFLORA OF TEA ENVIRNOMENT WITH SPECIAL REFERENCE TO THEIR MYCOTOXIN PRODUCING POTENTIAL IN BLACK TEA

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ABSTRACT

A total of 34 fungal species were observed in the tea factory atmosphere (ambient air), phyllosphere and soil, out of which a few are mycotoxin producers. The toxin producing mycoflora inside the tea factory include *Aspergillus niger, A. flavus, A. fumigatus, Fusarium lactus.* The dominant and frequently occurring mycoflora were *Cladosporium herbarum, Curvularia interseminata, Rhizopus nigricans and Penicillium spinulosum.* Ten isolates, which were isolated from air, phylloshpere and soil, were found to be toxin producers. Eight of them were strains of *Aspergillus flavus* and two were *A. parasiticus.* The various parameters of aflatoxin investigation taken up in this work include the potential of aflatoxin production in black tea under different moisture regimes, temperature regimes, time span and strain variation. The importance of aflatoxin producing organisms, toxin production in food / beverage and its impact on human health are discussed.

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Keywords : India; aflatoxin; mycoflora; tea environment

INTRODUCTION

Tea of commerce (*Camellia sinensis* (L.) O. Kuntze) is extensively cultivated in northeast India since the beginning of the 19th century and it has become the most popular beverage world over. India is the leading country for the production, consumption and export of quality tea all over the world . India had 4,32,297 ha area under tea cultivation with a production of 8,10,613 billion kg in1997, of which northeast India contributed 4,998.9 billion kg. Cachar's contribution has been 48 million kg during 1997 from 35,000 ha area under tea (Tea Statistics, Tea Board, Calcutta, 2002). It has been reported that the common moulds observed on made tea in the tea factory premises were *Aspergillus flavus* Link and other Aspergilli and Penicillia (George *et al.*, 1994). The possibility that tea may serve as vehicle for pathogen has also been reported (Ekanayaka et al.,1987).

The information available on the fungal air spora of tea plantations of Assam is fragmentory. Also very little information on the factory atmosphere is available so far. The only available data on tea air spora is from the Tocklai Experimental Station of

TRA, as reported in their annual reports published since 1967 onwards. Further Devi (1984) reported that basidiospores and ascospores constitute major components in the atmosphere of the tea fields and it was possible to forecast the disease development to issue suitable and fairly accurate warning to the need for spraying for controlling the black rot and red rust diseases effectively. Dutta and Debnath(1990) also reported on "Blister blight disease" of tea in relation to forecasting and management of the disease in Darjeeling, where it still is a serious problem.

Tea is one of the most important beverages produced by India and is known to carry various mycoflora which may secrete toxins. No systematic study has been made on this aspect under the specific agroclimatic conditions of Cachar District in Assam. The present work is aimed at studying the possibility of mycotoxin production on the processed tea by the mycoflora with the objective to work out preventive measures.

MATERIALS AND METHODS

Isolation of Mycoflora

Petri plates containing Czapek (Dox) agar medium (Rangaswami, 1988) were exposed for 5 minutes in fermentation, drying and sorting rooms. A 2-stage Anderson sampler was also used. Exposures/ Observations were performed at a monthly interval starting from the month of January to December, 2002. Mycoflora were also isolated from the tea phyllosphere and soils from tea fields following Deb *et al.* (1999).

Identification and Maintenance of Culture

Fungal species were identified based on characteristics as well as microscopic observations on the size and shape of conidia, conidiophores, sporangia etc. and related parameters etc. (Thom & Raper, 1945 ; Raper & Thom,1949; Skinner *et al.*, 1961; Smith, 1969). Pure cultures of various isolates were maintained by subculturing. The identifications were confirmed through Indian Type Culture Collection Centre, Division of Plant Pathology, IARI, New Delhi.

Aflatoxin Production Potential

The aflatoxin producing potential was studied by individually inoculating the pure cultures of mycoflora on 'YES' (Yeast Extract Sugar) medium. The medium was incubated for 7 days at a temp. of 28±2^{0}C. Afterwards the cultures were mixed with a blender, and the detection of aflatoxin was carried out by the method of Nabney and Nesbitt (1965) and dip-strip method of Sashidhar (1993). The inoculated processed tea sample (50 g) was taken into a 500 ml methanol : water (55:45). The sample was blended with the hand-held blender for 3 minutes at 12,000 rpm. The sample was filtered and 50 ml of the filtrate was taken into separating funnel, along with 100 ml hexane followed by 50 ml of petroleum ether to defat the sample. The aqueous phase was separated and aflatoxin was extracted into 20 ml of chloroform. The chloroform layer collected was passed through a column containing neutral alumina (500 mg) and anhydrous sodium sulfate (2 g). The chloroform layer was evaporated to dryness, and the extracted toxin was redissolved in benzene : acetonitrile (98:2, 50-250 ml). Twenty microliters of aflatoxin extract was spotted onto a dip-strip and developed in the chloroform : acetone (95:5) solvent system for 12-15 min. This was done in a minideveloping chamber. Silica gel coated TLC plates of 0.5 mm thickness were used in the experiment. A mixture of standard aflatoxin sample (Sigma) containing aflatoxin B_1, B_2, g_1 and g_2 was also spotted on the TLC plate along with the experimental aflatoxin extract. The TLC plates were subsequently observed under the UV light in order to locate the fluorescent spots.

After the detection of aflatoxin on the TLC, the toxin was quantified by the spectrophotometer (Nabney and Nesbitt 1965).

Calculation of Aflatoxin Concentration

The aflatoxin concentration in the samples was calculated by taking optical density (OD) difference at 363 nm and 420 nm, and setting values according to the following formula :

$$\frac{D \times M \times 10^6}{E \times 1 \times 2000}$$

Where

D = Optical density value at 363 nm minus 420 nm

M = Molecular weight of aflatoxin

E = Molar extinction co-efficient

The values of M and E are shown in Table 1.

Table 1. Values of M and E for different aflatoxins (Nasney and Nesbitt, 1965).

Toxins	Wavelength (nm)	Mol. wt.	Molar extinction co-efficient
B_1	360	312	22,000
B_2	362	314	23,400
G_1	360	328	18,700
G_2	362	330	21,000

Study of Aflatoxin Producing Ability of Toxin Producers in Connection with Various Factors

After screening, the aflatoxin producers were used in the experiments. A study was conducted on the presence/absence of aflatoxin in processed tea/ medium under laboratory conditions. The media used are "YES medium + processed tea" , "tea extract" and "YES medium" (Bilgrami *et al.*, 1977-80). Tea samples were inoculated separately with the 10 strains of *Aspergillus* spp. isolated from the tea factory atmosphere (ambient air), tea phyllosphere and tea soil. They were studied for their growth and aflatoxin production potential under varying moisture regimes, temperature regimes, time span (in days) and strain variation. The temperature regimes used were 0°C, 10°C, 20°C, 30°C & 40°C. The moisture regimes include control, 5%, 7%, 10%, 15%, 20%, 25% & 30%. The time span taken was for 3, 5, 7, 9 and 11 days respectively.

Data Analysis

The occurrence of mycoflora in the fermentation, drying and sorting rooms was recorded. In the present investigation seasonal variation in the total population of mycoflora was also recorded. Data on the seasonal variation were analysed statistically and represented graphically. Correlation coefficient (r) was calculated for the data obtained from fermentation, drying and sorting rooms.

Statistical Analysis

Some statistical analyses were carried out using two way analysis of variance (ANOVA), critical difference (C.D.) and coefficient of variation (C.V.).

RESULTS AND DISCUSSION

A total of 34 fungi were found to be present in the tea atmosphere (ambient air), i.e. air, phyllosphere and soil. The mycoflora observed inside the tea factory were *Aspergillus clavatus, A. niger, A. ustus, A. flavus, A. parasiticus, A. ochraceous, A. nidulans, A. amstelodami, A. fumigatus, Penicillum sp., P. turbatam, P. frequentens, P. spinulosm, P. admetzi, P. Chrysogenum, P. brevicompactum, Fusarium lactus, F. moniliforformae, Helminthosporium sp., Cladosporium herbarum, Curvularia interseminata, Rhizopus nigricans, Rhodotorula sp., Saccharomyces cerevisiae, Mucor mucedo, Alternaria fasciculata, Humicola brevis, Gilmeniella humicola.* The dominating organisms included *Cladosporium herbarum, Curvularia interseminata, Rhizopus nigricans* and *Penicillium spinulosum.* Some of the organisms are known mycotoxin producers viz., *Aspergillus niger, A. flavus, A. fumigatus, Fusarium lactus* etc. (Monoharachari, 1986) However, the percentage occurrence of these toxin producers was low in comparison to that of the other species.

A uniformity in the pattern of occurrence in the site of observation i.e. fermentation, drying and sorting rooms exists. However, in case of fermentation room, there is a sudden fall of the total population in the month of December. Such decline in the occurrence may be due to the low temperature during winter. Moreover, drying and sorting rooms did not show any increase in population, rather a slight increase in the trend of total population was observed in the month of December. This may be due to the prevailing higher temperature in the drying and sorting room.

The seasonal variation of the toxin-producing mycoflora in terms of percentage of total occurrence is shown in Fig.1. *Aspergillus niger* had the highest occurrence during June, July and September in the sorting rooms, while *A. flavus, A. fumigatus and Fusarium lactus* were at their highest levels in drying room followed by sorting and fermentation rooms. From the above observations it is clear that the occurrence of the probable toxin-producing mycoflora is comparatively low in fermentation room than in the drying and sorting rooms; such low occurrence of the mentioned mycoflora did not exist in the fermenting room because of the good maintenance of hygienic conditions in the fermenting room. However, there is a probability that such organisms may occur in the processed tea as evident from their presence in the sorting room. *Aspergillus fumigatus* occurred during March and November-December. Due attention must be given to eliminate the probable toxin-producing mycoflora from the tea factory because of their implications in human health.

Fig. 1. Percentage occurrence of mycotoxin-producing organism inside tea factory showing seasonal variation

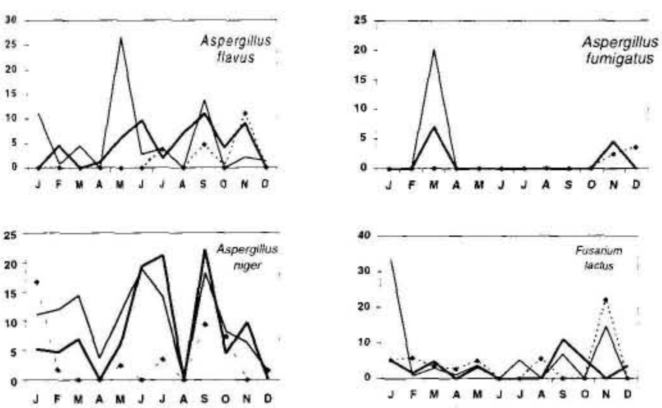
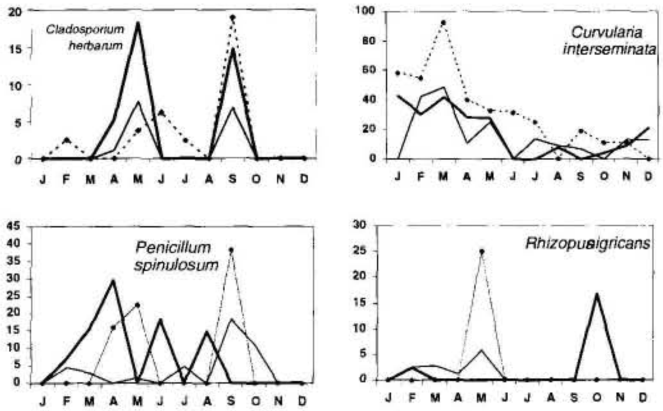


Fig. 2. Percentage occurrence of dominant mycoflora inside tea factory showing seasonal variation



Dominant mycoflora inside tea factory can be seen in the Fig.2. In all the cases there is a similarity in the pattern of occurrence with respect to seasonal variation. *Cladosporium herbarum* and *Penicillium spinulosum* had high density occurrence though fluctuating, during April to October. *Rhizopus nigricans* had high occurrence in May and October. *Curvularia interseminata* was present in all the three processing segments of tea factory in an uniform pattern, the highest being in the month of March.

Toxins are produced by the Aspergilli (i.e. aflatoxin, ochratoxin etc.), Fusaria (trichothecenes, zerarlenone etc.) and other fungi. Therefore, appropriate measures should be taken to keep the food stuff free from air borne mycotxin producing micro-organisms (Bilgrami *et al.* 1977-1980). Pallavi *et al.* (1997) extracted aflatoxin M1 from *Aspergillus parasiticus* (AP 456) in a semi–synthetic medium by solvent extraction procedure. One dimensional TLC in a solvent system (Tolune:ethyl acetate:formic acid; 6:3:1 v/v) showed the presence of a bluish green spot which corresponded to aflatoxin G-2 standard.

Sometime back reports appeared saying that two consignments of tea were allegedly returned from some foreign countries alleging that the said consignment of tea was infested with aflatoxin/toxin producing micro-organisms (source : Tea Board, personal communication).

Keeping the above in view, the information regarding the possible mycotoxin producing air mycoflora in relation to tea field and factory, and their mycotoxin producing ability in tea should be given due importance. Mycotoxins are secondary metabolites produced by the activity of some specialized fungi; poisonous and elicit a toxic response known as mycotoxicoses when food or feed containing them is eaten by human or animals (Monoharachary, 1986).There is evidence that aflatoxin can become air-brone and circumstantial evidence suggests that aflatoxin may be produced in the air. Their inhalation can be up to 40 times higher than by ingestion (Craesia et al., 1987).

Ingested toxins may cause liver cancer; while other mycotoxins can affect other organs and interfere with the body defences against infection through immune suppresion or cytotoxic effects on alveolar macrophages (Gerberick et al., 1984; Pier and Mc-Loughlin, 1985; Sharma, 1991). Mycotoxins can also have a role in illness, including leukemia, associated with the fungi infested building (Samson, 1985).

No satisfactory measure is known to prevent the mould growth and detoxification of the contaminated commodity. It is, therefore, urgently needed to investigate mycotoxin problems in edible commodities in diverse geographical regions and to develop effective and economical method for controlling the fungal mould growth and subsequent production of mycotoxins.

Screening of aflatoxin producers from the isolates of mycoflora from the atmosphere (i.e. air, tea phyllosphere and tea soil) shows that ten isolates were possible toxin producers. However eight of them belong to *Aspergillus flavus* and the remaining two are the strains of *A. parasiticus* (Table 2).

Table 2. Screening of mycotoxin producers from the isolates of mycoflora.

Fungal strain	Mycotoxin production
Aspergillus candidus	–
A. clavatus 30/S	–
A. flavus 61/S	+
A. flavus 102/S	+
A. flavus 102/A	+
A. flavus 102/P	+
A. flavus 30/P	+
A. flavus 30/A	+
A. flavus 61/A	+
A. fumigatus	–
A. niger	–
A. ochraceous	–
A. parasiticus 61/S	+
A. parasiticus 102/P	+
Alternaria fasciculata	–
Cladosporium herbarum	–
Curvularia interseminata	–
Fusarium oxysporum	–
F. lactus	–
Fusarium sp.	–
F. moniliformis	–
Mucor mucedo	–
Helminthosporium sp.	–
Penicillium citrinum	–
P. brevicompactum	–
P. sclerotium	–
P. rubrum	–
P. chrysogenum	–
Taloromyces flavus	–
Rhizopus nigricans	–

\+ : *Toxin production*

\- : *No toxin production*

Table 3. Aflatoxin production in "YES medium + processed tea"

Organism	Rf Value	Aflatoxin type	Quantity (MeanValue) µg/ mL
Aspergillus flavus	0.320	B_1	0.071
Strain 1	0.300	B_2	0.134
Aspergillus flavus	0.322	B_1	0.134
Strain 2.	0.305	B_2	0.134
Aspergillus	0.325	B_1	0.425
parasiticus	0.302	B_2	0.370
	0.285	G_1	0.422

S.D = 0.1447

C.V.= 59.29%

Table 4. Aflatoxin producation in tea extract

Organism	Rf Value	Aflatoxin type	Quantity (MeanValue) µg/ mL
Aspergillus flavus	0.282	G_1	0.135
Strain 1			
Aspergillus flavus	0.320	B_1	0.025
Strain 2.	0.304	B_2	0.015
Aspergillus	0.323	B_1	0.284
parasiticus	0.301	B_2	0.134
	0.280	G_1	0.023

S.D = 0.0957
C.V.= 93.23%

Table 5. Aflatoxin production in YES medium

Organism	Rf Value	Aflatoxin type	Quantity (MeanValue) µg/ mL
Aspergillus flavus	0.323	B_1	0.275
Strain 1	0.300	B_2	0.342
Aspergillus flavus	0.320	B_1	0.322
Strain 2.	0.302	B_2	0.335
Aspergillus	0.320	B_1	0.450
parasiticus	0.304	B_2	0.389
	0.283	G_1	0.460

S.D = 0.0634
C.V.= 17.25%

In "YES medium supplemented with processed tea extract (10:100 v/v), three of the toxins were produced by *A. parasiticus* ; however, the other two strains viz. *Aspergillus flavus* strain 1 and *A. flavus* strain 2 could produce aflatoxins B_1 and B_2 only. Similar results were obtained in the case of YES medium. Such similarities must be due to metabolic activities of the toxin producing strains. Again in case of tea extract all the three toxins were produced by *A. parasiticus*; however, *A. flavus* strain 1 could produce only aflatoxin G_1 under the same laboratory conditions (Tables 3-5).

Fig. 3. Quantity of various Aflatoxins (mg/mg) produced by *A. flavus* 61 on processed tea (after inoculation) with respect to various moisture regimes.

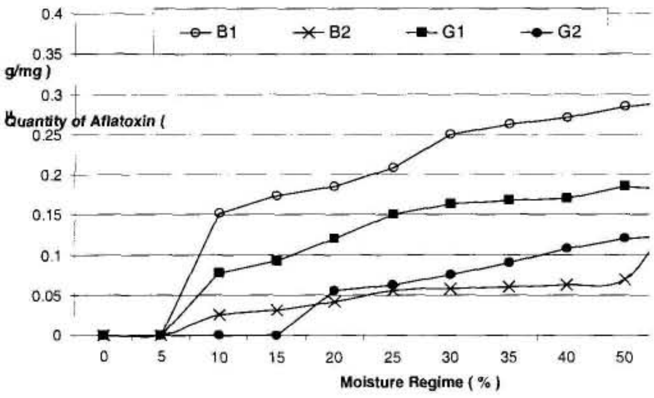


Fig. 4. Concentrations of various Aflatoxins (µg/mg) produced by *A. flavus* 61 on processed tea in different time after inoculation (in days)

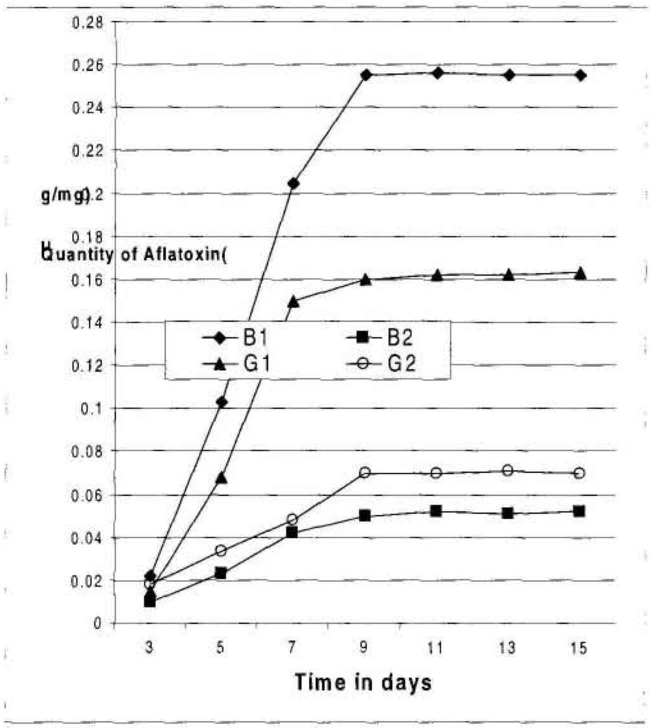
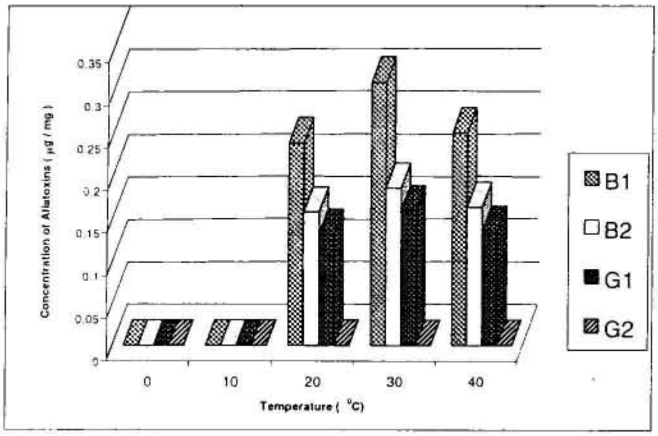


Fig. 5. Concentration of various Aflatoxins (µg/mg) produced by *A. flavus* 102 on processed tea (after inoculation) with respect to various temperature regime



In the processed tea under laboratory condition with respect to various moisture levels, the maximum quantity of aflatoxin production was observed in case of *A.flavus* 61 (aflatoxin B_1) at 90% moisture level, the value being 0.763 mg/mg. Control, 5% & 7% moisture levels did not result in any toxin production. But the moisture levels at 10% and above caused gradual increase in the amount of aflatoxin production (Fig. 3). Regarding the time duration, a time span of 3 days showed no aflatoxin production. Very little amount of toxin was detected after 5 days of incubation. Greater amount of toxin was observed almost uniformly after 7, 9 & 11 days of incubation (Fig. 4). The higher quantity of aflatoxin production was shown by *A. flavus* 102 in the temperature range of 20° to 40°C. Though, the highest production of aflatoxin was observed at 30°C (Fig. 5). So 30°C could be the crucial factor in aflatoxin production.

The aflatoxin producing potential in terms of the percentage variation in mean level was maximum in the tea extract (C.V. = 93.23% with S.D.=0.0957). Such observation implies that all the three Aspergillus strains are not equally potent for Aflatoxin production on the tea extract. In case of YES medium the percentage variation in the mean level was minimum (C.D. = 17.25%). It implies that all the strains can grow in the YES medium with equal potency. When YES medium is supplemented with processed tea the percentage variation in the mean level increased to some extent (C.D. = 59.29%). Such increase in variation may be due to the interference of processed tea on YES medium.

From the statistical analysis (ANOVA) it is observed that variation regarding quantity of aflatoxin production was highly significant with respect to strain variation. Regarding the quantity of toxin production variation due to different strains isolated from different sources (i.e., air, phyllosphere and soil), the fungal strains isolated from phyllosphere showed the highest variation (C.V. = 68.36%) followed by air (66.33%) and soil (63.75%). Both the quantity of toxin variations as well as the temperature variation are significant. However, the variation regarding the quantity of aflatoxin production due to temperature difference is highly significant.

Variation in the quantity of toxin production as well as variation in time is highly significant. ANOVA shows that variation in the quantity of toxic metabolite is highly significant with respect to various moisture regime. In the ANOVA for moisture vs. quantity of aflatoxin, the statistical analysis has been carried out by considering the quantities of aflatoxins produced from the treatments of 10% moisture level onwards. Since there was no production of aflatoxin in case of 0% and 5% moisture level, the values were discarded in the statistical analysis.

Aflatoxins are naturally occurring toxic chemicals produced by fungi infecting agricultural crops during crop growth, in processing, in storage and in transportation. The possibility of aflatoxin production in made tea as such is very low but the possibility of its occurrence in storage and transportation can not be ruled out. The aflatoxin producing micro-organisms (i.e. *Aspergillus flavus, A. parasiticus*) are common in the tea growing/ manufacturing atmosphere and they will definitely infest on the manufactured black tea and if they get appropriate moisture level (10% and above), they will definitely produce aflatoxin under the appropriate temperature condition (10°C and above). It has been observed in the present work that they can use black tea as substrate for their growth and produce aflatoxin in the process. Therefore large scale survey in different tea growing areas will possibly be helpful to determine the extent of aflatoxin contamination in black tea and other tea at large so that appropriate preventive measures can be taken up for the same.

Some general conclusions on the above can be made from this :

- *Aspergillus flavus, A. parasiticus* are present in the tea growing and manufacturing atmosphere (i.e. air, leaf surface and soil).
- Standard dry black tea (having less than 3% moisture) is not prone to Aspergilli development and contamination of aflatoxin.
- A high frequency of Aspergilli in the atmosphere, however, does not necessarily indicate a high level of aflatoxin contamination/ accumulation.
- Prevention of the growth of aflatoxin producing microorganisms during storage and transport including shipment (for export) by preventing them to gain moisture should be the best procedure to prevent aflatoxin contamination, if any, in black tea at large.
- Initial small scale survey/screening of black tea samples from the tea factory/market did not show any aflatoxin contamination in black tea.
- Large scale survey in the tea growing area can be helpful to determine the extent of aflatoxin contamination, if any.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. R. B. Sashidhar, Deptt. Of Biochemistry, Osmania University, Hyderabad for his help and cooperation. The authors are also thankful to Dr. S.K.Bhattacharjee, Director I.G.M.R.I., Jorhat for providing valuable suggestions and laboratory facilities. Special thanks are also due to Dr Barundev Banerjee, Advisor NTRF, Tea Board, Calcutta, for critically going through the manuscript.

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Closing Remarks by Dr. Peter Baker, Session Chairman

I would like to thank all the speakers for their concise presentations. Let me recapitulate the salient points made in these presentations:

Intrinsic quality of tealeaf determines the price realized from the manufactured teas. Leaf quality is influenced, inter alia, by the degree of pest infestation and the after effects of chemical/s used for pest control, i.e. pesticide residues left in the made tea. The phyto-sanitary measures should allow retaining the health giving properties of tea, which is going to be USP or the most important selling point in future. Bad publicity from a single, fairly minor poisoning problem could be splashed around the world through electronic media and seriously jeopardize the sale price of tea. This is going to become increasingly important as the number of approved pesticides shrinks, and the European Union and other tea importers impose greater restrictions.

Bio-control of pests has a great potential of eliminating pesticide residues. However, the experience of my Organization CABI shows that the farmers fail to adopt many bio-control measures due to lack of work in product development and field application of these products. Information transfer, which we shall discuss tomorrow, also has an important role to play in advancing their useful application. Lack of investment by large pesticide manufacturers has resulted in manufacture of biocides by small entrepreneurs. Analysis by CABI has shown that their product quality is variable due to poor quality control. The industry needs an independent regulatory body to test the viability of biocides and monitor quality control.

A lot more work is needed for effective implementation of Integrated Pest Management. The problem with many IPM schedules is their complexity, which the farmers find difficult to adopt, due to lack of knowledge, information and training. Implementation of these seemingly complex ways of controlling pests is a great area to focus on.

Aflatoxins are very much in the news, especially in coffee, where consignments from several countries have been returned, causing extreme hardship in some of the poorest countries. I do not think you have much of a problem in tea. However, monitoring aflatoxin build up in the blood of the people in tea processing factories is a really useful, cost effective way. If blood stream of workers is free from appreciable level of aflatoxin, you can be reasonably certain that the problem of aflatoxin does not exist. A routine monitoring of blood samples will also protect the health of your workers in the industry.