Microbial Bioremediation of Pesticide Residues in Tea Soil

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Abstract

Tea (*Camellia sinensis*) belonging to family *Theaceae*, is one of the largest consumed beverages in the world. Tea contains several naturally occurring dietary polyphenols such as catechins possessing anticarcinogenic activity that act as effective chemopreventive agents against the initiation, promotion and progression stages of multistage carcinogenesis. To meet the needs of consumers, tea industry largely rely on use of chemicals in the form of fertilizers and pesticides for better production. Increased use of agrochemicals for better production has resulted in pollution of garden tea soil, besides rendering native microbiota resistant to these chemicals. Identification of soil microbes thriving in such polluted soil would be helpful in screening for tolerant bacteria that can be exploited for pesticide degradation.

Introduction:

Tea is cultivated in different type of terrains in the North eastern parts of India. It is planted on the mountain slopes of the eastern Himalayas upto a height of 2000 m in Darjeeling and in undulating flat lands ranging from 20-250 m in the Dooars and Assam regions. Tea requires a moderately hot and humid climate. Climate influences yield, crop distribution and quality. Tea grows best on well-drained fertile acid soil on high lands (Bezbaruah, 1994).

In South Assam, tea is an important perennial cash crop. Tea is unique in that, besides being an agricultural crop, it has also provided an industrial base. The cultivation, maintenance, harvesting and processing of tea are labour-intensive and provide a regular employment to millions of people. Number of Tea Estates in Cachar district is 116 and the area under tea (2004) is 32124 Ha. (Ubhadia, 2009). Tea has special importance in the Indian Economy in view of the fact that India is one of the major producers of this crop, which again is one of its major foreign exchange earners. The tea industry employs more than one million workers. It is a highly input based crop and requires heavy chemicals for good production over and above extensive care. Tea garden soils are therefore highly polluted with chemical pesticides. The shade trees of tea gardens like *Albizia, Dalbergia*, etc provide two-in-one purpose. One, they give shade to the tea plants and secondly, being leguminous, their root nodules form an ideal niche for nitrogen fixing organisms i.e., *Rhizobium.*

Tea plantation is managed by use of a large amount of chemical fertilizers and pesticidal compounds. It has led to a global concern for environmental pollution as well as harmful side effects created by their excessive use in tea plantation.

The tea-growing environment in the North East India is also conducive to a large number of pests and diseases. Studies have been carried out at Tocklai Tea Research Association, Jorhat on the biology and control of tea pests during the last decades. Pesticides invariably leave residues and their indiscriminate use may render the tea unsuitable for consumption and trade. With ever growing concern over pesticide residues

and the rising costs of the pesticides, the concept of pest control has undergone radical changes. Therefore, monitoring of pests for their early detection, integrated management of pests (IPM) and discretion on the choice of pesticides to be used on tea is of utmost importance (Tea Research Association, Tocklai).

The common pesticides used in the tea cultivation are endosulfan, dicofol, fenazaquin, glyphosate, 2,4-D, paraquat dichloride, etc. Endosulfan(6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) is a neurotoxin organochlorine insecticide of the cyclodiene family of pesticides. It is an endocrine disruptor, and is highly toxic. It is a mixture of sterioisomers, designated "α" and "β," in a ratio of 7:3. It may also contain small amounts of endosulfan sulfate and related chemicals. Endosulfan controls a wide range of sucking and chewing insect pests. Its residues have been detected in the atmosphere, soils, sediments, surface water and foods. The recommended dose is 1:400 (HV).

Microorganisms play a key role in the mineral cycles on earth. They are involved in the biodegradation of many compounds; these processes occur not only in the soil environment, but also in symbiosis with other organisms (eg. lichens, intestinal and rumen bacteria).

This review examines the potential uses of bacteria that may degrade pesticide residues in the soil and help in establishing a clean and green environment.

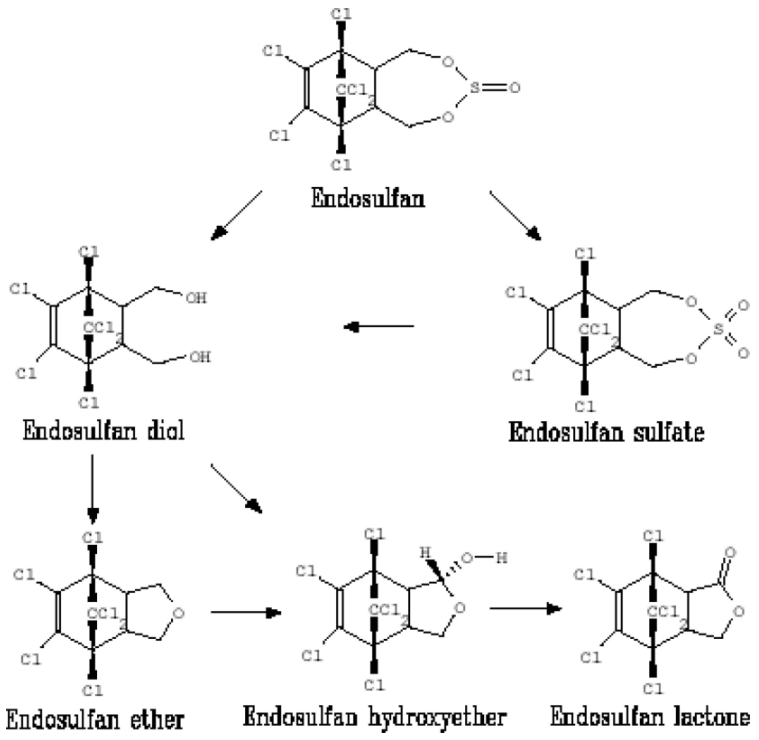

Figure 1: Schematic pathway of endosulfan degradation (Shivaramaiah & Kennedy 2006)

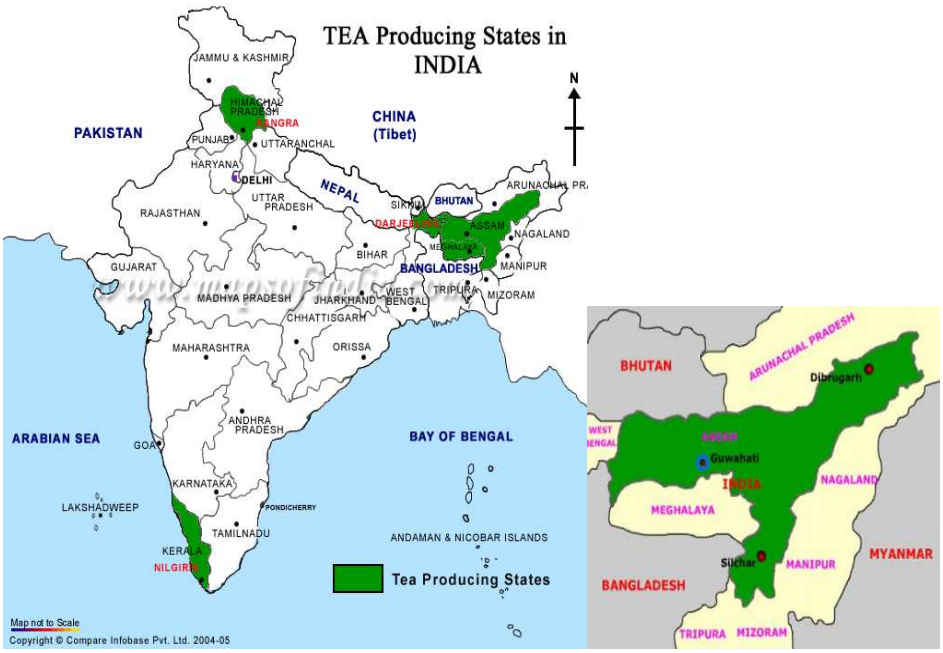

Figure 2: India map showing tea producing states and a highlighted Assam map.

Table 1: Area under Tea, Plucked Area, Production & yield of tea in total India

Year	**Area under Tea** (Hectare)	**Area under Plucking** (Hectare)	**Production** (Th Kgs.)	**Yield** (Kg/plucked Hect)
1991	420470	407576	754192	1850
1992	420289	407539	732322	1796
1993	418363	405658	760826	1875
1994	425966	412232	752895	1826
1995	427065	414367	756016	1824
1996	431245	414733	780140	1881
1997	434294	418007	810031	1939
1998	474030	419873	874168	2081
1999	490200	426045	825935	1939
2000	507196	429114	846483	1972
Average	444912	415514	789295	1898
% Increase	20.63%	5.28%	12.24%	6.59%

Source: The Journal of the Tea Board (India), Tea Digest-2001, P.2

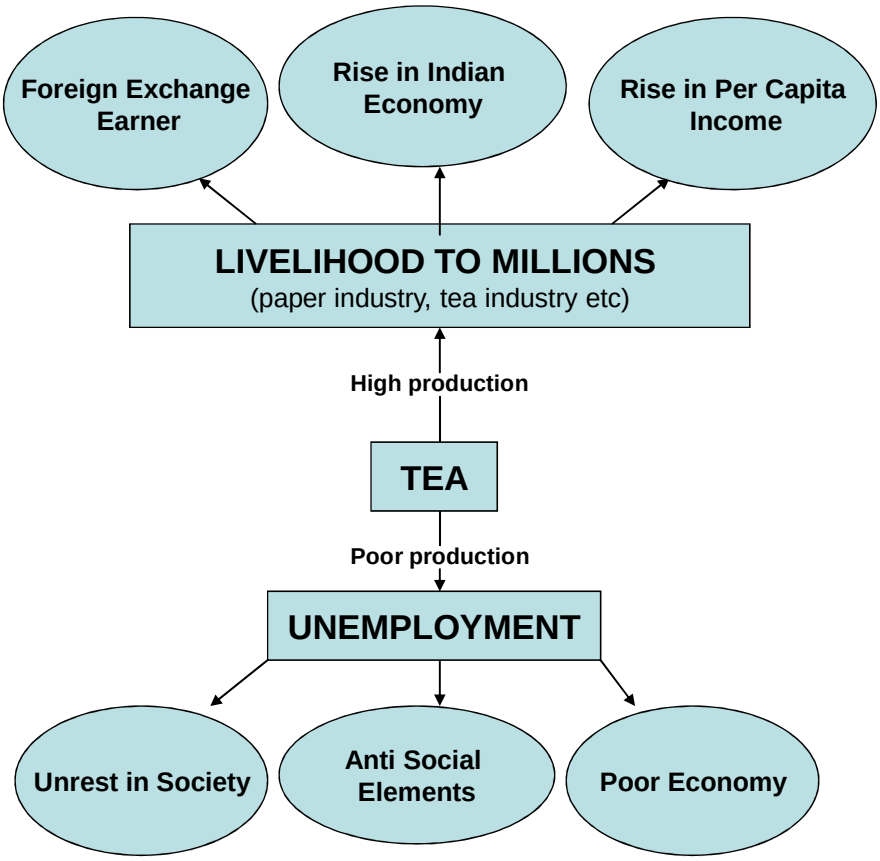

Figure3: A hypothetical representation of the importance of tea

Microbial diversity in tea ecosystem

The composition and population of microbes in the rhizosphere microflora at the tea gardens located in Red-Yellow earth region of south-Anhui showed that there were various microbial groups in rhizosphere habitat of tea plant, and some of then which increased the soil fertility significantly, for example, *Azotobacter*, ammonifying bacteria, cellulose decomposing bacterium etc. (Zhenrui et al. 1985).

Genus *Bacillus* was well adapted to the rhizoplane and rhizosphere of tea bushes. *B. subtilis* and *B. mycoides* appeared to be closely associated with tea roots. The two species comprised a major part of the bacterial population, even during unfavourable periods (Pandey and Palni, 1997).

A large number of bacteria and fungi were isolated from the rhizosphere of established tea bushes and were tested for their antifungal activity (Pandey et al., 1997).

A pot experiment was carried out to study the effects of two species of VAM fungi (*Glomus* sp.) on the vegetative growth and physiology of tea trees (*Camellia* sp.) and the quality of tea. The results showed that infection of host plants with mycorrhizal fungi markedly raised the activity of phosphorylase in the soil and the dehydrogenase of

their roots, promoted the absorption of P, Cu and Mn, and increased their growth volumes and rates. In addition, the contents of chlorophyll and caffeine increased, but the activity of peroxidase and the contents of Fe and catechins reduced (Shousheng et al. 1997).

Zoysa et al. (1998) conducted a study to test the effect of N form (NH +4, NO-3 , or both) on the transformation of soil P in the rhizosphere and its availability to tea (*Camellia sinensis* L.) plants fertilised with sparingly soluble Eppawala phosphate rock (EPR).

Neurospora sp., an aluminum resistant fungus, was isolated from the rhizosphere soil of tea plant. It could grow normally in the medium containing 20mmol/L of aluminum and decreased the medium aluminum concentration and faded the haematoxylin color of the medium (Yuerong et al. 1999).

Effect of ALF 1 fungus (*Neurospora* sp.) on the pH value of acidic tea soil was studied. The results showed that the growth of ALF 1 fungus was closely related to the organic nutrients in the soil and its growth rate increased with soil organic nutrients. Soil pH was significantly increased and activated aluminum content in the uncultivated acidic soil significantly decreased when the soil was added organic matters and inoculated with the ALF 1fungus (Yuerong et al. 1999).

Acid and aluminum (Al) tolerant microorganisms were isolated from tea fields, from which six strains were selected and identified as *Cryptococcus humicola, Rhodotorula glutinis, Aspergillus flavus Link, Penicillium sp., Penicillium janthinellum Biourge and Trichoderma asperellum* (Kawai et al., 2000).

Tian Yong-hui (2000) investigated the composition and diversity of N-fixing bacteria in the rhizosphere of tea plants with various plant ages. The results showed that the N-fixing bacteria could be divided into three types, among which, ten species of the bacteria were found in the rhizosphere of tea plants at young and prime life, only five species of the bacteria in that of tea plants at decrepit life.

Studies of microbial population densities and species distributions from various types of tea plantations indicated that rich tea plantation soils had higher rhizosphere microbe population densities than poor tea plantation soils. In the tea plantations of similar fertility, the population density of rhizosphere bacteria varied with cultivation age of the plantations, following a pattern of 10 year>4 year>20 year. The population density of rhizosphere fungi in rich tea plantations was more than that in normal and poor tea plantations. The population density of actinomyces in 20-year tea plantations was significantly higher than that in 4-or 10-year tea plantations; and moreover, poor tea plantation soils had the greatest population density of actinomyces (Sun and Liu, 2004).

The rhizosphere of cultivated tea bushes was dominated by *Glomus* morhpotypes (88.89%) along with three morphotypes of *Acaulospora;* occurrence of 35 morphotypes belonging to four genera viz. *Acaulospora* (11.43%), *Gigaspora* (11.43%), *Glomus* (68.57%) and *Scutellospora* (8.57%) was recorded in the rhizosphere of tea plants from the natural ecosite in the Kumaun region of Uttaranchal Himalaya, India (Singh et al., 2007).

Species of *Trichoderma, Penicillium, Aspergillus* and *Mucor* were found to dominate the rhizosphere of tea bushes in different regions of the Indian Himalayas. In addition, the dominant bacteria in the tea rhizosphere, i.e., *Bacillus subtilis* and *B. mycoides*, showed antagonistic activity against fungal isolates by inhibiting the growth

and causing structural abnormalities in mycelium (Singh et al., 2007). Tea rhizospheres have some specific characteristics viz lowering of soil pH, antagonistic activity among microbial communities and dominance of certain species (Lynch, 1987, Sood et al 2007). Tea rhizosphere favors the growth of microbes, which are known to produce strong antibiotics with potential biocontrol agents. Sood et al (2007) carried out a research to investigate the role of *Bacillus* and *Pseudomonas* species producing bacteriocin like compounds.

Nine florescent Pseudomonas isolates obtained on King's B agar from rhizosphere of tea plant were studied for their biochemical and functional characteristics. They were also tested for their ability to promote growth of tea seedlings. The isolates produced IAA like substances, siderophores and soluble phosphate in the range of 8.7-32.1, 13.6-196.3 and 1.4-15.7 µg/ml culture filtrate, respectively. Four isolates were able to utilized cellulose as carbon source and another four were capable of inhibiting growth of the saprophytic Rhizoctonia solani in laboratory bioassay. The growth parameters of tea seedlings in fertilizer P added pot was statistically at par with those of superior strain inoculated seedlings (Mazumder et al. 2007).

Xue et al (2007) extracted total microbial DNA from the soils in 8, 50 and 90 years old tea orchards, adjacent wasteland, and 90 years old forestland in Meijiawu tea area of Hangzhou. The 16S rDNA V3 fragment was amplified by PCR, and the polymorphism of this fragment was analyzed by DGGE. The results indicated that both the tea orchard age and the land use type had significant effects on soil microbial genetic diversity.

Eupenicillium parvum was recorded for first time during isolation of phosphate solubilizing microorganisms from the tea rhizosphere. The fungus developed a phosphate solubilization zone on modified Pikovskaya agar, supplemented with tricalcium phosphate (Vyas et al., 2007).

Representatives of *Bacillus* and *Pseudomonas* genera were found to dominate the rhizosphere of established and abandoned tea bushes, respectively in some parts of the Indian Himalayan Region. *B. subtilis* and *B. mycoides* appeared to be closely associated with roots of established tea bushes while the rhizosphere of abandoned tea bushes was dominated by *P. putida* (Sood et al., 2008).

Sarkar et al., (2009) studied on the degradation potential of *Pseudomonas* sp. isolated from tea rhizosphere towards the pesticide dicofol.

Biodegradation of triazole propiconazole fungicide by selected *Pseudomonas* strains isolated from tea rhizosphere was reported by Sarkar et al. (2009).

Effects of Aluminium and Fluorine toxicity on soil microbes of tea rhizosphere were studied. It indicated that low concentration of aluminum and fluorine exerted a positive effects on increasing the population quantity of rhizosphere microbes significantly, such as bacteria and actinomycetes. It suggested that appropriate concentration of Al and F could promote the growth and propagation of these microbes while high concentration of Al and F had a negative effects on them, except fungi (Shaoguang et al. 2009).

Ochrobactrum anthropi, isolated from the rhizosphere of healthy tea plants growing at the foothills of Darjeeling and Dooars, was found to be antagonistic to several root rot pathogens of tea plant. The cell free culture filtrates of the bacterium was assayed for antagonistic effects. Further, a series of in vivo experiments were conducted on tea

plants. Treating the different varieties of tea plants with *O. anthropi* significantly increased the growth and development of the plant. The bacterium was able to colonize and maintain population in the rhizosphere and was able to control the root rot disease incidence in tea plants (Anonymous, 2010).

Molecular identification and phylogenetic analysis of 16S rRNA gene sequences revealed that tea garden soils of South Assam harbored bacterial species belonging to *Rhizobium, Burkholderia* and *Enterobacter* (Huidrom et at, 2011).

Biodegradation of insecticide fenpropathrin was carried out in vitro by selected *Pseudomonas* strains isolated from tea rhizosphere (Soumik Sarkar et al, 2011).

Balamurugan et. al. (2011) reported on cellulose degrading bacteria of tea garden soil. Their cellulase activity was studied in vitro.

A bacterium, *Bacillus megaterium* (TRS-4) isolated from the rhizosphere of tea bushes of Darjeeling and Dooars regions of West Bengal was tested on 5 varieties of tea for its plant growth promoting ability (Chakraborty et al, 2012).

Sharma et. al. (2012) isolated *Kurthia* sp., a new novel member as phosphate solubilisers from the rhizospheric soil of tea bushes of Darjeeling.

Pesticide Degrading Microbes

Rainer Martens in 1976 isolated 16 fungi, 15 bacteria and 3 actinomycetes capable of metabolizing more than 30% of endosulfan. The major metabolites detected were endosulfate, formed by oxidation of the sulfite group, and endodiol, formed by hydrolysis of the ester bond.

It is noted that nearly 43 pesticidal compounds were degraded by a wide variety of microorganisms (Francis, 1992). Mustafa et al. (1972) reported that *Rhizobium leguminosarum* and *R. trifolii* isolated from Egyption soil can hydrolyse melathion by producing carboxyesterase. Extended studies by Francis (1992) indicated that 22 rhizobial isolates tolerated endosulfan, carbofuran, carboryl and melathion at the range of 25 to 125 ug /ml. Isolates from the nodules of *Indigofera echinata* and *I. duthei* tolerated melathion upto 125ug/ml. (Gangawane et al, 2007; Reddy et al., 1997).

Kothari et al. (1998) reported on the biodegradation of 2,4-D by *Penicillium citrinum* and *P. oxalicum* isolated from paint coated teak wood.

Phorate [O,O-diethyl-S-(ethylthio)methyl phosphoradiothioate] degrading bacteria were isolated from agricultural soil and characterized based on their morphological and biochemical characteristics. The selected isolates PS-1, PS-2 and PS-3 were presumptively identified as *Rhizobium, Pseudomonas* and *Proteous* species, respectively. The HPLC analysis of phorate in bioaugmented soil revealed its complete disappearance within 40 days. (Bano and Musarrat, 2003).

The potential of rhizosphere microbes isolated from common reed [*Phragmites australis* (Cav.) Trin. ex Steud] plants grown in a subsurface-flow constructed wetland to biomethylate selenate or selenite was studied in liquid cultures under controlled conditions. Total mean percentages of volatilized Se from half-strength Hoagland culture solutions (low C content) supplemented with selenate or selenite and inoculated with cultured rhizosphere microbes after 15 d of incubation were 7.9 and 49.1%, respectively. There was a relative best fit (r = 0.87) between total number of rhizosphere and cultured microbes and the percentage of volatilized Se in Hoagland solution after 15 d of incubation. However, when the same microbes were cultured in tryptic soybean broth

(TSB) medium (high C content), the percentages of volatilized Se from selenate and selenite were 1.3 and 1.9%, respectively. The volatilization percentages of Se from selenate or selenite in culture solutions inoculated with rhizosphere suspension instead of cultured rhizosphere microbes were very low (1.2–3.0%) in both cultivation media. In all experiments, selenite was volatilized significantly ($p < 0.05$) in higher amounts by cultured rhizosphere microbes after 15 d of incubation compared with selenate. Dissolved biomethylated dimethylselenide (DMSe) in water samples taken from the sub- surface-flow bed was determined by purging with helium. The DMSe in water samples was indirectly detected up to 2.4 µg Se L-1, which indicates that part of the produced DMSe was dissolved in the matrix before being released into the atmosphere (Azaizeh et al. 2003).

Pravakaran and Allenpeterson (2005) investigated on the degradation of endosulfan by a *Bacillus* species.

Biodegradation of endosulfan into endosulfan sulfate by a soil bacterium, *Bacillus* sp. was reported by Shivaramaiah & Kennedy (2006). The bacterium degraded 50% of the compound within 3 days of incubation.

A mixed bacterial culture of *Staphylococcus sp*., *Bacillus circulans-I* and *-II* was studied for degradation of endosulfan in aerobic and facultative anaerobic conditions via batch experiments with an initial endosulfan concentration of 50 mg/L. After 3 weeks of incubation, mixed bacterial culture was able to degrade 71.58±0.2% and 75.88±0.2% of endosulfan in aerobic and facultative anaerobic conditions, respectively (Mathava and Phylip, 2006).

Mathava and Phylip, (2006) studied endosulfan mineralization by bacterial isolates and identified their possible degradation pathway. It was postulated that endosulfan was mineralized via hydrolysis pathway with the formation of carbenium ions and/or ethylcarboxylates, which later converted into simple hydrocarbons.

Inoculation of *Pseudomonas fluorescence* and *P. aeruginosa* degraded 78 and 85% of chlorpyrifos in plots without cotton plants whereas 99% degradation of chlorpyrifos was observed in soil, where cotton plants were inoculated with either *P. fluorescence* or *P. aeruginosa* as compared to un-inoculated control soil (Vidya Lakshmi et al., 2009).

Multiclass pesticide residues viz. endosulphan, cypermethrin, monocrotophos and chlorpyriphos have been estimated qualitatively and quantitatively in two vegetables, tomato (*Lycopersicon esculentum*) and radish (*Raphanus sativus*) by adopting gas liquid chromatographic and high performance liquid chromatographic methods (Kumar et. al., 2011).

Sunitha et al 2012 reported about degradation of endosulfan upto 70% and endosulfan sulphate upto 100% by organisms isolated from endosulfan contaminated soils of South Indian States (Kerala and Karnataka) by the process of enrichment.

Table 2: Some results of work on microbial degradation of pesticides

Pesticide	Organism	Result	Reference
Endosulfan	16 fungi, 15 bacteria and 3 actinomycetes	Metabolized more than 30% of endosulfan	Rainer Martens 1976
Melathion	*Rhizobium leguminosarum* and *R. trifolii*	Hydrolysed melathion by producing carboxyesterase	Mustafa et al 1972
Endosulfan, Carbofuran, Carboryl and Melathion	Rhizobial isolates	22 rhizobial isolates tolerated these pesticides at the range of 25 to 125 ug /ml.	Gangawane et al 2007; Reddy et al 1997
2,4-D	*Penicillium citrinum* and *P. oxalicum*	Degradation of 2,4-D	Kothari et al 1998
Phorate	*Rhizobium, Pseudomonas* and *Proteous* species	The HPLC analysis of phorate revealed its complete disappearance within 40 days.	Bano and Musarrat 2003
Endosulfan	*Staphylococcus sp., Bacillus circulans-I* and *-II*	After 3 weeks of incubation, mixed bacterial culture was able to degrade 71.58±0.2% and 75.88±0.2% of endosulfan	Mathava and Phylip 2006
Endosulfan	*Bacillus* sp.	The bacterium degraded 50% of the compound within 3 days of incubation.	Shivaramaiah and Kennedy 2006
Dicofol and Triazole propiconazole	*Pseudomonas* sp.	Degradation was observed	Sarkar et al 2009
Chlorpyrifos	*Pseudomonas fluorescence* and *P. aeruginosa*	99% degradation of chlorpyrifos was observed in soil, where cotton plants were inoculated with either *P. fluorescence* or *P. aeruginosa*	Vidya Lakshmi et al 2009
Fenpropathrin	*Pseudomonas* strains	Biodegradation of fenpropathrin.	Soumik Sarkar et al 2011
Endosulfan	Organisms from endosulfan contaminated soil.	Degradation of endosulfan upto 70% and endosulfan sulphate upto 100%	Sunitha et al 2012

Conclusion:

Producing nutritious foods sufficiently and sustainably is the goal of modern agriculture. Increasing crop yields by enhancing the concentrations of agrochemicals represents a challenging problem that requires concerted efforts from researchers amid their distribution along with potential deleterious effects on human health. Plants enhance degradation of soil contaminants by releasing exudates that nourish microbes in the rhizosphere, besides inducing biochemical pathways within bacteria. Keeping in view that neither every microbe possesses the ability to thrive in the sites where the contamination is present nor have the ability to degrade toxic compound, it would be of great advantageous to search for the one that possesses the ability to survive in a given environment, besides possessing the capability to degrade the contaminant present.

Keeping in view of the fact that pesticides are unavoidable in the present scenario, it would be of immense value to apply microbial inoculum technology having the potential of pesticide degradation. This would most definitely help to solve more than 50% of the problem of pesticide contamination in soil, water and food and help in establishing a safe tea beverage and a clean soil environment.

Future Prospects:

The above reported works on pesticide degradation are quite heartening, however, diversity study and exploitation of beneficial microbes is very less known in the northeastern part of India in spite of its rich biodiversity. Therefore studies on microbial diversity and development of consortium technology for their application in tea agro-ecosystem should be developed. This will help the tea growers to enhance the tea productivity and reduce the soil and water pollution caused by the pesticides.

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