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Impact of agrochemicals on endophytic bacterial population in tea (*Camellia sinensis* L.) shrubs

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ABSTRACT
The present study deals with the isolation of endophytic bacterial isolates and the study of their population in the tea leaf, stem and root samples collected from different tea gardens of Assam, India. Out the six tea gardens studied three were commercial agrochemical based gardens and the rests were maintained organically without direct application of agrochemicals. The endophytic bacterial population in the root samples collected from the agrochemical based gardens ranged between 9.2 x10^3 to 8.7 x 10^3 followed by stem (8.7 x 10^2 - 5.8 x 10^2) and leaves (3.4 x 10^2 - 3.1 x 10^2). Similarly, the endophytic bacterial population in the root samples collected from the organically maintained agrochemical free gardens ranged between 10.2 x 10^4 - 8.8 x 10^4 followed by stem (9.3x 10^3 - 7.8 x 10^3) and leaves (8.6x 10^2 - 7.3 x 10^2). Hence, the endophytic bacterial population in the samples collected from organically maintained agrochemical free gardens was significantly higher in comparison to agrochemical based gardens.

***Key words**: Agrochemicals, endophytes, Camellia sinensis*

INTRODUCTION

Microorganisms present within plant tissues for all or a part of their life cycle without showing any visible symptom of their presence are defined as endophytes [1]**.** It is an endosymbiont, often a bacterium or fungus that lives within a plant for at least a part of their life without causing any apparent disease. Endophytes are ubiquitous and are found in all plant species studies to date; however most plant endophyte relationships are not well understood. Endophytes inhabit in majority of healthy and symptomless plants, in various tissues, seeds, roots, stems and leaves [2]**.** A large number of experimental evidences demonstrate that bacterial endophytes support the plant growth, development and yielding by synthesizing different plant hormones,[3, 4, 5, 6] solubilizing the insoluble mineral salts and confer enhanced resistance to various pathogens [7, 8] by producing antimicrobial agents [9, 10]**.** It has been suggested that endophytes act as a biological trigger to activate the stress response system more rapidly and strongly than non mutualistic plants [11]**.** As these microbes are symptomless with plant growth promoting activities they may definitely have remarkable contribution in plant growth and development and hence may be a good source for isolation of new biofertilizers and biocontrol agents to maintain sustainable organic agriculture.

Tea is one of the most popular non alcoholic beverages of the world and one of the major crops in India, especially in Assam. At present, our tea cultivation is based on high input of agrochemicals. To obtain maximum yield, planters often use agrochemicals beyond the prescribed level. That is why, foreign markets often reject tea from Assam because of its high chemical residues in finished tea.

Moreover, agrochemicals like pesticides and herbicides are detrimental to non target organisms too. The excess part of agrochemicals is one of the major causes of air, water and soil pollution causing a serious problem up to the sites away from their application.

Lots of studies have been carried out to know the adverse affects of agrochemicals on plants and animals. However affect of agrochemicals on microorganisms, especially on endophytes are very less studied till now. The present study deals with the comparison of endophytic bacterial population of agrochemical based commercial gardens and organically maintained chemical free gardens to depict the adverse affect of agrochemicals on endophytic microbial population.

MATERIALS AND METHODS

Sample collection

Tea (*Camellia sinensis*) leaf, stem and root samples were collected from healthy and mature tea plants. Samples were collected from six different tea gardens located at different districts of upper Assam within May to September, the sprouting season of tea in the year 2013. Healthy young shoot tips with upper most four leaves and disease free young stems were collected in sterilized polythene bags and immediately kept into 4º C ice box. For root samples, young roots along with some rhizospheric soil were collected in to sterilized polythene bags and kept immediately at 4º C in ice box. All samples were brought to the laboratory and further processing was done in the next day.

For random collection of samples, five plots (1600 sq. metre) of the garden with healthy tea shrubs were selected and samples were collected from seven different tea shrubs of each plot and mixed. Hence, a total of five subsamples were collected from each gardens.

Isolation of endophytes

To remove the debris, root, stem and leaf samples were washed separately with tap water for seven times followed by distilled water for five times. After proper washing, about ten grams of root samples were dipped in 70% ethanol for five minutes followed by 0.1% $HgCl_2$ for 1minute. However, equal amount of stem and leaf samples were treated with 70% alcohol for five minutes followed by 0.05% $HgCl_2$ for 1minute. To remove the effect of surface sterilizing agents, samples were washed with sterilized distilled water for ten times [12]**.** After proper surface sterilization as well as removal of surface sterilizing agents, samples were homogenized in 100 ml of sterilized distilled water to prepare the stock tissue homogenate. From this tissue homogenate, proper dilution was done according to the standard process of serial dilution [12]**.** Serially diluted sample tissue homogenates were spread on different types of culture media like tryptic soya agar (TSA), nutrient agar (NA), *Pseudomonas* agar (PA) medium to isolate endophytic bacteria [13]**.** After inoculation and spreading of serially diluted samples, plates were incubated at 30ºC for 48 for proper growth of the microbial colonies. Number of colony forming units (cfu) was calculated with the help of a colony counter and the cfu population in per ml of tissue homogenate was calculated. To avoid the unexpected contamination, whole process was done in front of laminar air hood.

Efficacy of the process of surface sterilization was confirmed by rolling a piece of leaf, stem or root on nutrient agar plate and incubated for 24 hours. The plate without any microbial colony after incubation period confirmed the proper sterilization of the samples.

RESULTS

The endophytic bacterial population in the root samples collected from the agrochemical based gardens (Table 1) ranged between 9.2×10^3 to 8.7×10^3 followed by stem (8.7×10^2 - 5.8×10^2) and leaves (3.4×10^2 - 3.1×10^2).

Similarly, the endophytic bacterial population in the root samples collected from the organically maintained agrochemical free gardens ranged between 10.2×10^4 - 8.8×10^4 followed by stem (9.3×10^3 - 7.8×10^3) and leaves (8.6×10^2 - 7.3×10^2).

Table 1. Population of endophytic bacteria in agrochemical based tea gardens

Samples	Bacterial population		
	Root	**Stem**	**Leaf**
Agrocgemical based garden A (ABG-A)	$9.2x\ 10^3$	$8.7\ x\ 10^2$	$3.4x\ 10^2$
Agrocgemical based garden B (ABG-B)	$7.4\ x\ 10^3$	$5.8\ x\ 10^2$	$3.3\ x\ 10^2$
Agrocgemical based garden C (ABG-C)	$8.7\ x\ 10^3$	$7.7\ x\ 10^2$	$3.1\ x\ 10^2$

Values are average of five replicates

Table 2. Population of endophytic bacteria in organically maintained tea gardens

Samples	Bacterial population		
	Root	**Stem**	**Leaf**
Organic Garden A (ORG-A)	$10.2x\ 10^4$	$9.3x\ 10^3$	$8.6x\ 10^2$
Organic Garden B (ORG-B)	$9.4\ x\ 10^4$	$7.8\ x\ 10^3$	$7.3\ x\ 10^2$
Organic Garden C (ORG-C)	$8.8\ x\ 10^4$	$8.7\ x\ 10^3$	$7.6\ x\ 10^2$

Values are average of five replicates

Fig. 1: Comparison of endophyte population in root samples of organic and agrochemical based gardens

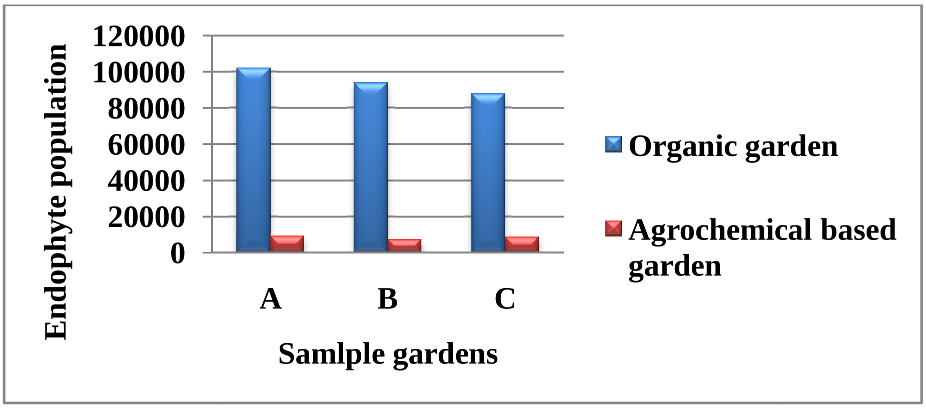


Fig. 2: Comparison of endophyte population in stem samples of organic and agrochemical based gardens

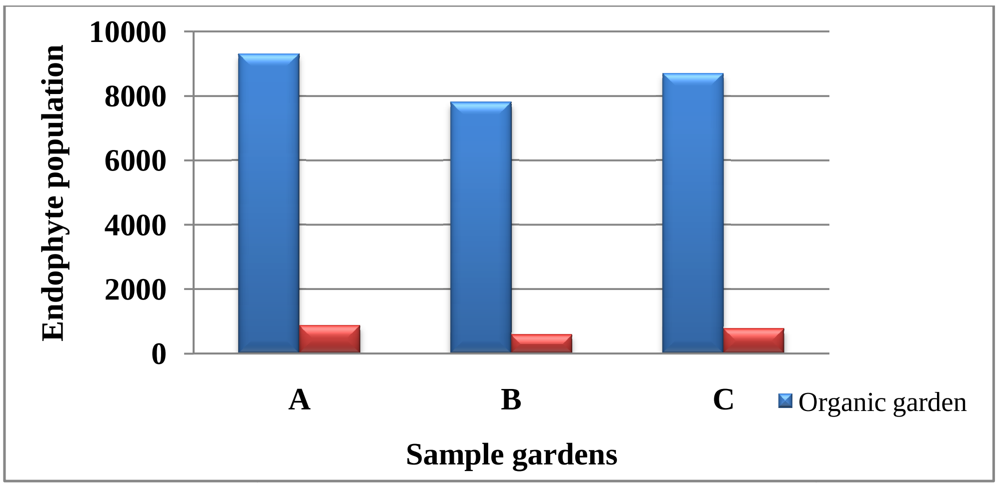
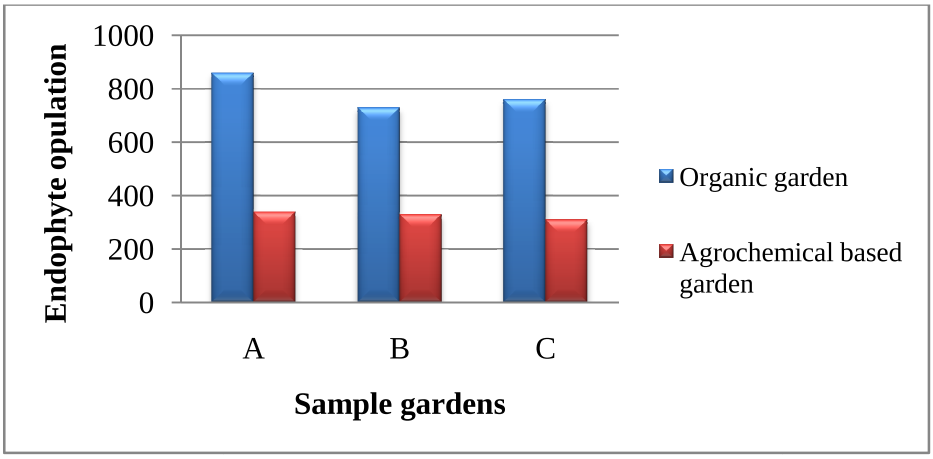


Fig. 3: Comparison of endophyte population in leaf samples of organic and agrochemical based gardens



DISCUSSION

In the present study it was observed that the endophytic bacterial population was highest in the roots followed by the stem and leaves. This is because the main source of endophytes of a plant is its rhizospheric microbes. Some of the rhizospheric microbes which are best compatible to the plant internal environment may enter into the plant roots mainly through the cracks at the site of emergence of lateral roots [14] and hence the endophytic microbial community of a plant resembles its rhizospheric partners [15]**.** After crossing the root epidermis, endophytes colonize in the cortex and some of them may invade the casperian strip and xylem vessels to migrate towards the aerial parts [10, 16]. Though, major parts of endophytic microbes enter into plant tissue through root, many of them may enter into the aerial part of the plant body though natural openings like stomata, hydathodes, lenticels and natural wounds [17]**.** As endophytic microbes can reside inside the fruits and seeds, vertical transfers of them from seeds to the aerial parts is also a common mode of endophytic entry.

In the present study the endophytic bacterial population in root, stem and leaf samples collected from the tea gardens maintained organically was significantly higher in comparison to the samples collected from the agrochemical based tea gardens. That is why it may be assumed that the agrochemicals used in the commercial tea gardens are detrimental to some of the endophytic bacteria present in the tea plants. Lots of earlier works are also in support of the findings of the present study. Ampofo et al. [18] reported that regular application of different kinds of pesticides and herbicides resulted in lower microbial population in rhizosphere of different crop plants in comparison to agrochemical free rhizospheric soil. As agrochemicals are detrimental to soil microbes, it may be assumed that they are detrimental to endophytes also. Wei-Dong et al. [19] reported that long term application of NPK fertilizers caused reduction of microbial load in soil in comparison to soil without NPK application. As soil is the main source of endophytes, lower microbial population in soil may be another cause of lower microbial population in the samples collected from commercial agrochemical based gardens. Hence, the present study comes to a conclusion that the main cause of lower microbial loads in the samples collected from agrochemical based tae gardens is the negative impact of the agrochemicals.

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