RESEARCH

Study of tolerance ability in phosphate solubilising microorganisms isolated from tea plantations soil of lower Darjeeling hills

Sumedha Saha, Sukanya Acharyya, Sahadeb Sarkar and Malay Bhattacharya[*]

Abstract

Background: Tea is an important plantation crop in India and world. Introduction of fertilisers and pesticides for better production polluted tea garden soils. Phosphates in agricultural soils are converted into soluble forms by phosphate solubilising microorganisms (PSMs). Consortium of PSM having high tolerance level is an attractive option for bioremediation of degraded tea plantation soils. This research initiative was to isolate PSM from tea plantation soil and detect their tolerance against pesticide, antibiotic and antifungal.

Results: Isolated consortia from organic and inorganic tea plantation soils of Darjeeling showing halo was considered for tolerance study. Phorate was most and Deltamethrin was the least tolerant pesticide for the isolated PSM consortia. So, Phorate may be considered as most used or most accumulated pesticide. Erythromycin was most and Ofloxacin was the least tolerant antibiotic while Fluconazole was most and Itraconazole was the least tolerant antifungal agent for the isolated PSM consortia. It was noted that there was no or partial inhibition of PSM growth by some pesticide, antibiotic and antifungal agents. In all the three tolerance studies it was observed that there is no relation between collection sites but in all the assays average inhibition zones were more in organic plantations than inorganic plantations.

Conclusions: Thus, it may be said that inorganic practice induces tolerance to microbes. So, other than cultural practice use of chemicals, micro-ecosystem and antibiosis exhibited by microbes do play a role in conferring tolerance.

Keywords: Darjeeling, Tea plantation, Soil, PSMC, Tolerance

Background

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most common and widely consumed beverages not only in India but also worldwide. It is one of the most important plantation crops in the world. The tea industry is the oldest organised industries in India and Indian tea is appreciated world over as health drink for its unique flavour, aroma and medicinal properties. India produces three speciality teas—Darjeeling, Assam and Nilgiris. Thus from Indian Economy point of view, tea is a major foreign exchange earner (https://www.ibef.org/exports/indian-tea-industry.aspx).

Organised tea plantations in India were initiated by the British. Thereafter, there has been a steady increase in the production over the years since its day of first cultivation, which is mainly due to extensive planting, improved technology, nutrition and fertility management (Saraswathy et al. 2007), introduction of high yielding clones and longer pruning cycle. These factors, on the other hand, have encouraged biotic stresses like insect pests and diseases that limit the productivity of this crop (Gurusubramanian 2005). More than one thousand species of arthropod pests are known to attack tea all over the world, though only about 300 species of insects are recorded from India, in that 167 species from North-east

*Correspondence: malaytsnbu@gmail.com
Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, Darjeeling 734013, India

Saha *et al. Bull Natl Res Cent*   (2021) 45:109

Page 2 of 11

India (Das 1965) resulting to 55% loss in yield. Therefore, to meet the needs of consumers, tea industry largely relies on use of chemical fertilisers and pesticides for better production. And thus, tea garden soils are highly polluted with chemical pesticides. It has led to a global concern for environmental pollution as well as harmful side effects created by their excessive use in tea plantation (Igbedioh 1991; Brauer et al. 2019).

The pesticides applied to tea bushes not only affect the pests, but also hinder the essential microflora of the soil that helps in nutrient recycling, solubilisation and uptake of nutrients by the plant (Aktar et al. 2009; Saha et al. 2020). Phosphorus, one of the major macronutrients for plants, remains unavailable for their uptake due to its fixation with other metallic elements such as calcium, aluminium and iron to form their respective salts in soil. Plant cell might take up several forms of phosphorus, but the greatest part is absorbed in the forms of phosphate anions mainly or depending upon soil pH (Rodríguez and Fraga 1999; Mahidi et al. 2011; Walpola and Yoon 2012; Satyaprakash et al. 2017; Kumar et al. 2018).

The main input of inorganic phosphorus in agricultural soil is by applying fertilisers. Nearly, 70 to 90% of phosphorus fertilisers applied to soils is fixed by cations and converted to inorganic form and these accumulated phosphates in agricultural soils are adequate to maintain maximum crop yields worldwide for about 100 years (Walpola and Yoon 2012) if it could be mobilised, and converted into soluble forms of phosphorus salts with the aid of phosphate solubilising microorganisms (PSMs). A greater concern has been made to get an alternative system yet low-priced technology that could supply adequate phosphorus to plants (Sharma et al. 2013). Thus, there is a need to explore microbial diversity which can either transform toxic pesticides to non-toxic forms or could survive in presence of high concentrations. In this regard, the consortium based on stable association of microbes, belonging to phosphate solubilising microorganisms, having resistance to high concentrations of toxic pesticides is an attractive option for further studies and application in biodegraded tea plantation soils. A previous study on tolerance capability of PSMC isolated from non-tea growing region of Darjeeling hills was reported by Saha et al. (2020). However, there are no reports on isolated phosphate solubilising microbes from tea garden soil. So, this research initiative was undertaken to isolate PSM from tea plantation soil and detect their tolerance against pesticide, antibiotic and antifungal.

Methods
Collection of soil samples
Collection of soil samples were conducted following the protocol of Saha et al. (2020). Top soil from tea plantations of lower Darjeeling was collected during the month of April after the first shower. Each sample consists of a mixture of five pre-samples collected from four different corners and diagonal bisector of an imaginary square of 10 m side. The collected samples were transferred to sterile zipper bags and after proper labelling were placed in styrofoam box filled with ice packs. Altitude, soil temperature, latitude and longitude of the collection sites were recorded. The collected soil samples were brought back to laboratory for downstream experiments.

Soil analysis
pH, organic carbon (OC), organic matter (OM) content, total nitrogen and available phosphorus contents of the collected soil samples were analysed. The collected soil samples were completely air-dried. Soil samples were passed through 2-mm sieve and crushed with mortar and pestle. For organic carbon determination, the samples were further passed through a finer mesh sieve (0.5 mm). Estimation of soil pH was conducted following Mukherjee et al. (2020) and calculations were made following Baruah and Barthakur (1997). Quantification of soil organic carbon and organic matter was conducted by the chromic acid method proposed by Walkley and Black (1934) with minor modification following Mukherjee et al. (2020) and Saha et al. (2020). Total soil nitrogen was determined by the Kjeldhal method (Jackson 1973). Estimation of phosphorus as phosphate was conducted following protocol of Bray and Kurtz (1945), Baruah and Barthakur (1997), Mukherjee et al. (2020) and Saha et al. (2020).

Isolation and authentication of PSMC
Selective media (Pikovskaya's agar) was used for isolation of phosphate solubilising microorganisms (Schoebitz et al. 2013; Saha et al. 2020). 500 mg of collected soil sample was weighed being careful to exclude any stone chips and was mixed with 1 ml of sterile distilled water in an autoclaved eppendorf. The solution was then vortexed for a minute ensuring proper mixing of the soil. From this sample solution 500 µl was pipetted into one-fourth strength of autoclaved Pikovskaya's agar media. The culture was then incubated at 30 °C for 48 h. The isolated culture was then placed on Pikovskaya's agar plates for further screening and authentication of phosphate solubilising activity by appearance of halo around the colonies (Dias et al. 2009; Saha et al. 2020). The colonies showing prominent halo were picked by sterile loops and further cultured in Pikovskaya's media to preserve at 4 °C for further downstream experiments.

Saha *et al. Bull Natl Res Cent*   (2021) 45:109

Page 3 of 11

Pesticide tolerance assay

Pesticides are widely used in tea plantations to control pests (Roy et al. 2008; Huidrom and Sharma 2014; Saha et al. 2020). Commonly used pesticides in the tea gardens of the study area like Fipronil (FP), Phorate (P), Emamectin benzoate (EB), Quinalphos (Q), Thiomethoxame (T), Fenazaquin (FN), Spiromesifen (S), Deltamethrin (D) and Flubendiamide (FB) were chosen to detect the tolerance ability of the isolated PSMCs. Protocol of Iqbal and Bartakke (2014) was followed with slight modifications. 100 µl of the isolated culture was pour plated in a Petri dish with Pikovskaya's media. Four different concentrations of pesticides—25, 50, 100 and 200 mg/10 ml, were added in the bored wells and their inhibition was studied using well diffusion method. The plates were incubated at 30 °C for 48 h.

Antibiotic tolerance assay

The presence of antibiotic tolerance was tested for the isolated culture (Hameeda et al. 2008; Saha et al. 2020) with few modifications by using antibiotic and antifungal discs from HiMedia (Catalogue No. HX032-1PK and HX038-1PK). Twelve common antibiotics—Bacitracin (B10), Chloramphenicol (C30), Penicillin-G (P10), Polymixin B (PB300), Gentamicin (Gen10), Neomycin (N30), Cefotaxime (CTX30), Augmentin (AMC30), Erythromycin (E10), Chloramphenicol (C30), Ofloxacin (OF5) and Co-Trimoxazole (COT25), were used in 100 µl of isolated culture that was pour plated in Pikovskaya's media.
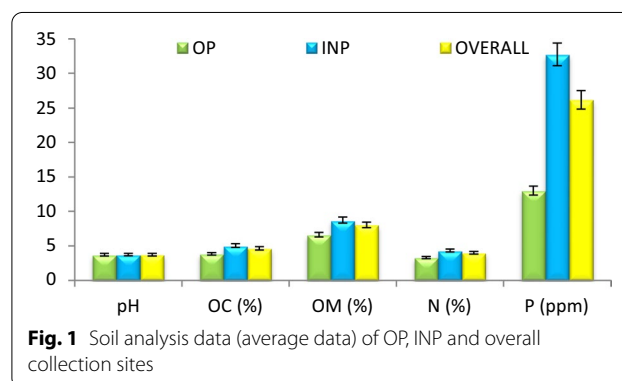
Antifungal tolerance assay

The presence of antifungal tolerance was tested for the isolated culture using antibiotic and antifungal discs from HiMedia (Catalogue No HX104-1PK) following Saha et al. (2020). Six antifungals—Amphotericin-B (AP100), Clotrimazole (CC10), Fluconazole (FLC25), Itraconazole (IT10), Ketoconazole (KT10) and Nystatin (NS100), were used in 100 µl of isolated culture that was pour plated in Pikovskaya's media.

Correlation studies

Correlation between pesticide, antibiotic and antifungal resistance was worked out by using Microsoft Office Excel Worksheet. Relations of soil collection site and tolerance ability of PSMC were also studied. More is the magnitude of inhibition zone less is the tolerance and vice versa was accepted as the relation between tolerance and inhibition zone. This logical point was used to conclude relationship.

Table 1 Information of collection sites of soil samples

Sample ID	Elevation (ft)	Latitude	Longitude	Soil temperature (°C)
4535/7	4535	26.52070	88.11430	14
4648/8	4648	26.52200	88.11380	14
3871/15	3871	26.51330	88.15460	15
847/1	847	26.80056	88.27106	22
847/2	847	26.80056	88.27106	23
3210/4	3210	26.84353	88.22483	19
4117/5	4117	26.50420	88.11360	16
3261/13	3261	26.84361	88.21536	19
515/14	515	26.79053	88.38985	24
4556/17	4556	26.87170	88.26710	14
3308/18	3308	26.85788	88.28739	15

Fig. 1 Soil analysis data (average data) of OP, INP and overall collection sites

Results

Collection of soil samples

Samples from nineteen different locations were considered for the present study of which eleven samples showing the reproducible results were considered for analysis in Table 1. Sample ID 4535/7, 4648/8 and 3781/15 were collected from gardens practicing organic cultivation while rest of the samples were from gardens with inorganic practice of cultivation.

Soil analysis

The average results of soil analysis are represented in Fig. 1. pH of both OP (3.735 ± 0.1909188) and INP (3.752 ± 0.423113) were below the recommended level (4.5–5.5). Organic carbon (1–2%) considered as indicator of organic richness of soil were above the recommended level in both OP ($3.585 \pm 0.4200214\%$) and INP ($5.0635 \pm 1.887816\%$). The status of organic matter was also found to be high. Percentage of total nitrogen in OP ($3.3178 \pm 0.3609073\%$) and INP ($4.3546 \pm 1.623653\%$) was higher than the recommended level (0.1–0.2%). Phosphorus as P_2O_5 was higher than recommended level

Saha *et al. Bull Natl Res Cent* (2021) 45:109

Page 4 of 11

(10–20 ppm). In OP (13.0 ± 4.2426407 ppm) P_2O_5 was within the range but in INP (32.75 ± 11.29528 ppm) it was very high.

Isolation and authentication of PSM

The isolated culture in one fourth strength of Pikovskaya's media was plated in Petri plates and incubated to check the formation of transparent halo (Fig. 2A, B) around the colonies for qualitative evaluation of phosphate solubilising potential (Vazquez et al. 2000; Zhang et al. 2017). PSM consortium isolates showing positive responses were selected. Out of nineteen soil samples eleven showed halo and were considered for downstream experiments.

Pesticide tolerance assay

Our isolated PSM exhibited notable tolerance to such high concentrations of pesticides (Fig. 2C, D). Table 2 shows a representation of the overall data depicting the variable tolerance level of the PSM consortia. Out of the nine pesticides tested, Phorate (0.404 ± 001206 cm) showed average minimum and Deltamethrin (1.413 ± 0.070251 cm) showed maximum inhibition zone. So, it can be considered that Phorate was most and Deltamethrin was the least tolerant pesticide for the isolated PSM consortia (Fig. 3).

Antibiotic tolerance assay

Table 2 is a total representation of the data for Antibiotic tolerance assay in a nutshell. Representative plates showing antibiotic resistance is depicted in Fig. 2E, H. Out of the twelve antibiotics tested E10 (0.090 ± 0.017121 cm) showed average minimum and OF5 (0.681 ± 0.213652 cm) showed maximum inhibition zone. So, it can be considered that E 10 was most and OF 5 was the least tolerant antibiotic for the isolated PSM consortia. The decreasing order of tolerance observed by us in our experiment is E 10, AMC 30, B 10, P10, C30, PB300, CTX30, Gen10, N30, C30, COT25 and OF5 (Fig. 4).

Antifungal tolerance assay

The data for the antifungal assay are represented in Table 2 with respect to their tolerance ability. The data clearly substantiates the commendable tolerance that is induced in the isolates. Representative plates showing antifungal resistance are depicted in Fig. 2F, G. Out of the six antifungals tested FLC 25 (0.020 ± 0.093421 cm) showed average minimum and IT10 (0.559 ± 0.081221 cm) showed maximum inhibition zone. So, it can be considered that FLC25 was most and IT10 was the least tolerant antifungal for the isolated PSM consortia. The decreasing order of tolerance observed by us in our experiment is FLC25, KT10, AP100, NS100, CC10 and IT10 (Fig. 5).
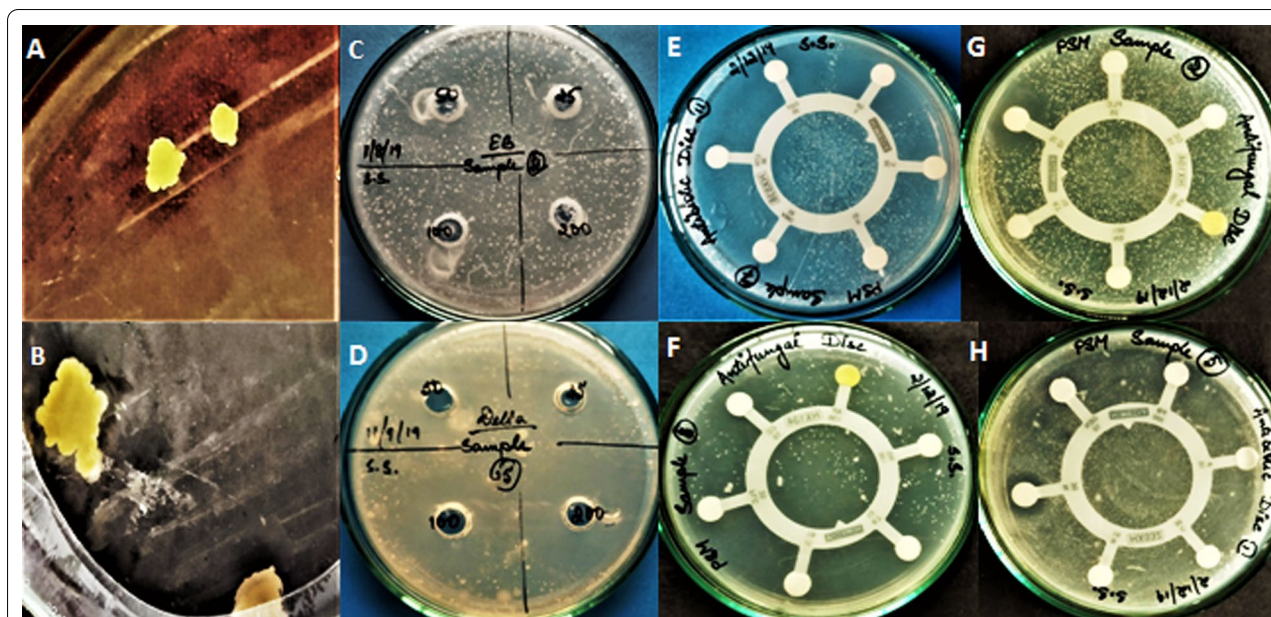

Fig. 2 **A**, **B** Transparent halos around the colonies; plates **C**, **D** pesticide, **E**, **H** antibiotic and **F**, **G** antifungal agents exhibiting different level tolerance by PSMC against antibiotic, antifungal and pesticide

Saha *et al. Bull Natl Res Cent* (2021) 45:109

Page 5 of 11

Table 2 Overall data of inhibition zone of our isolated PSMC

Pesticide/antibiotic/antifungal	4535/7	4648/8	3871/15	847/1	847/2	3210/4	4117/5	3261/13	515/14	4556/17	3308/18
Pesticide											
Fipronil											
Phorate											
Emamectin benzoate											
Quinalphos											
Thiomethoxame											
Fenazaquin											
Spiromesifen											
Deltamethrin											
Flubendiamide											
Antibiotic											
N 30											
Gen 10											
PB 300											
P 10											
C 30											
B 10											
AMC 30											
E 10											
C 30											
OF 5											
COT 25											
CTX 30											
Antifungal											
CC 10											
IT 10											
FLC 25											
AP 100											
NS 100											
KT 10											

Colour code

0 | 0.1–1 | 1.1–2 | 2.1–3 | 3.1–4 | 4.1–5 | 5.1–6

Tolerance level of PSM

The overall pesticide, antibiotic and antifungal tolerance capability of our isolated PSMC in a nutshell is depicted in Fig. 6. In OP two-third of Deltamethrin added plates showed no inhibition. One-third of Phorate, Quinalphos, Thiomethoxame, Fenazaquin and Spiromesifen and none of Fipronil, Emamectin benzoate and Flubendiamine added plated lacked inhibition zone. In INP Phorate, Emamectin benzoate, Quinalphos, Thiamethoxame, Spiromesifen and Flubendiamide exhibited lack of
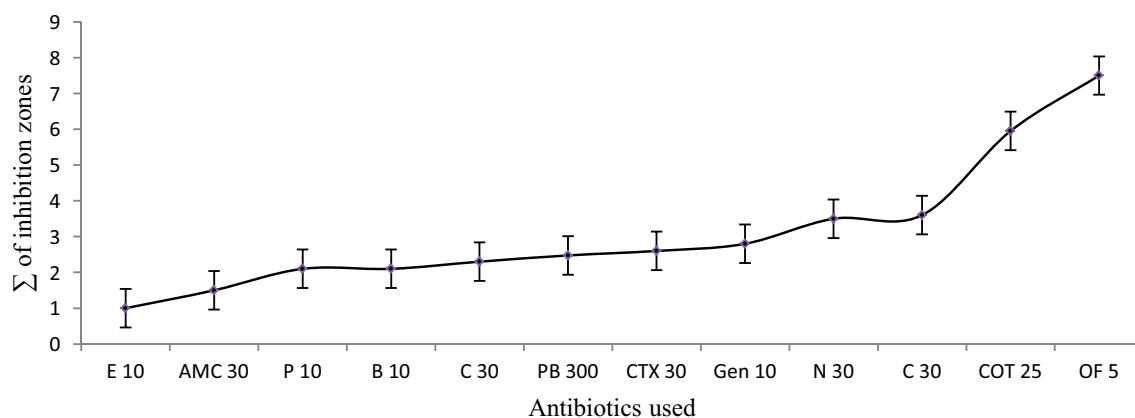
Fig. 3 Tolerance pattern (mean of replications) of PSMC isolates against some common pesticides used in tea gardens

Fig. 4 Tolerance pattern (mean of replications) of PSMC isolates against some common antibiotics
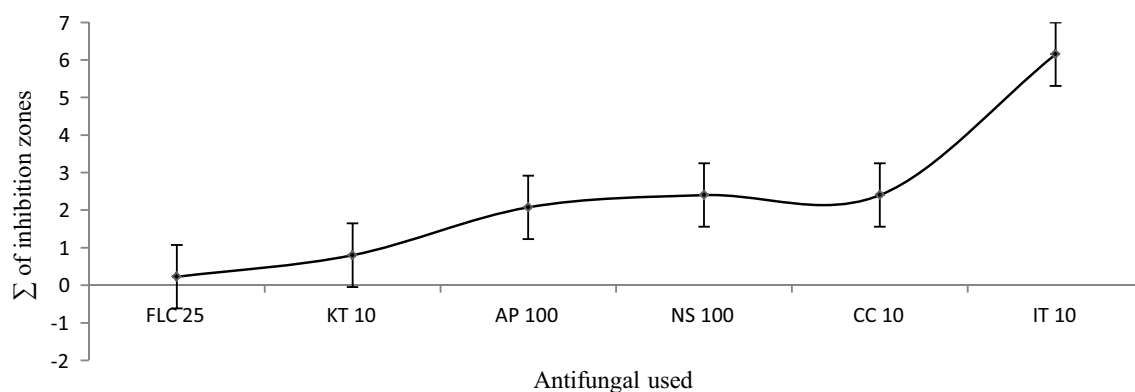

Fig. 5 Tolerance pattern (mean of replications) of PSMC isolates against some common antifungal
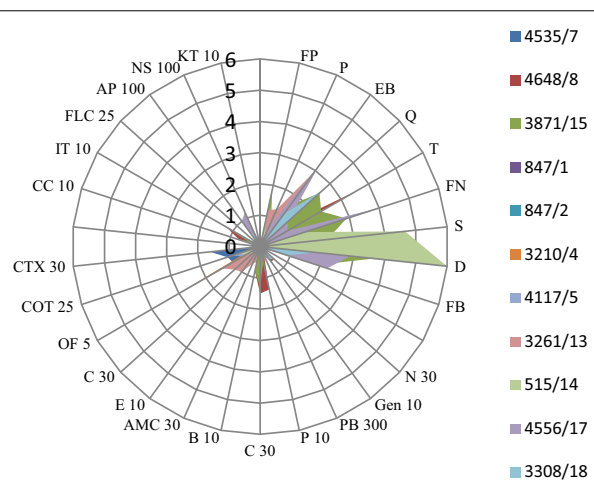

Fig. 6 Overall pesticide, antibiotic and antifungal tolerance capability of our isolated PSMC

inhibition zones in more than half of the plates. In antibiotic tolerance assay 49.69% of the data showed complete tolerance of which 25% and 56.81% were from OP and INP, respectively. Almost twenty-seven per cent of the data showed an inhibition ranging from 0.01 to 1 cm. In OP two-third of E10 added plates showed no inhibition. One-third of P10, B10, AMC30, C30, OF5, COT25 and CTX30 and none of N30, Gen10 and PB300 added plates lacked inhibition zone. Fifty-nine per cent of the data exhibits complete tolerance against the antifungal of which 50% and 62.5% are from OP and INP, respectively. About fourteen per cent of the data though exhibited moderate inhibition ranging from 0.01 to 1 cm, but only around 4.55% showed susceptibility against the antifungals. In OP all the FLC25 and NS100 added plates showed no inhibition. One-third of CC10, AP100, KT10 and none of IT10 added plates lacked inhibition zone.

Correlation studies

In our correlation studies between pesticide, antibiotic and antifungal, a positive correlation was observed among pesticide-antibiotic (0.3802765), pesticide-antifungal (0.4622746) and antibiotic-antifungal (0.0543082) tolerance. This indicates that there seems to be a dominant activity of pesticide in imposing selection or tolerance to other stress factors.

Relation of soil collection site and tolerance was also studied during our research. Soil collections were made from plantations categorised as OP and INP on the basis of their mode of cultivation practices. Average of inhibition zones produced in soil samples of each sample are represented in Fig. 7.

Discussion

Tea plantations of lower Darjeeling were considered for the present study as in this area both inorganic and organic additives are used for pest control. First shower prior to pre-monsoon triggers microbial activities so, this particular time was selected for soil collection. Presample mixing, sterile zipper bags and ice-packing were undertaken to avoid error during sampling, contamination and degradation respectively.

Analyses of collected soil samples were as prerequisite to detect the nutritional status of collected soil samples, as microorganisms also require nutrients from the environment for their growth. pH and other nutritional parameters recommended by Tea Board of India were regarded as standard. The status of organic matter in soil samples were also found to be high. Richness of organic matter present in soil favours growth of microorganisms. So, our collected soil samples were not deficient of nutritional factors that may hinder their presence in the study area. Soil of Darjeeling hills were previously reported by Bhattacharya (2014) in which Darjeeling soil was described as brown, loamy in nature, acidic in reaction and rich in organic matter and nutrients. In another work on non-tea growing region of Darjeeling hills by Saha et al. (2020). pH (3.87), organic carbon (1.187%), total nitrogen (1.02%) and phosphorus in P_2O_5 form (10 ppm) were reported. The low and high level of P_2O_5 in OP and INP, respectively, is obviously due to lack and abundance of inorganic inputs provided to the plantation soil. Our main focus is to find out the tolerance capacity of microorganisms present in soil that converts this P_2O_5 to the form that can easily be absorbed by plants so we mainly concentrated on in vitro tolerance assay and the results.

Pesticides are the most toxic chemical additives in tea plantations. According to the Tea Board of India, the average use pattern of chemical pesticides was estimated to be 7.35 kg per hectare in Darjeeling (Barbora and Biswas 1996). They not only are toxic to the operators spraying them but are deleterious to the ecosystem as they finally accumulate in the soil. Moreover, some amount of toxic chemical additives though claimed to be in very small amount reach our cup of tea that we sip throughout the day for regaining energy and health. Based on
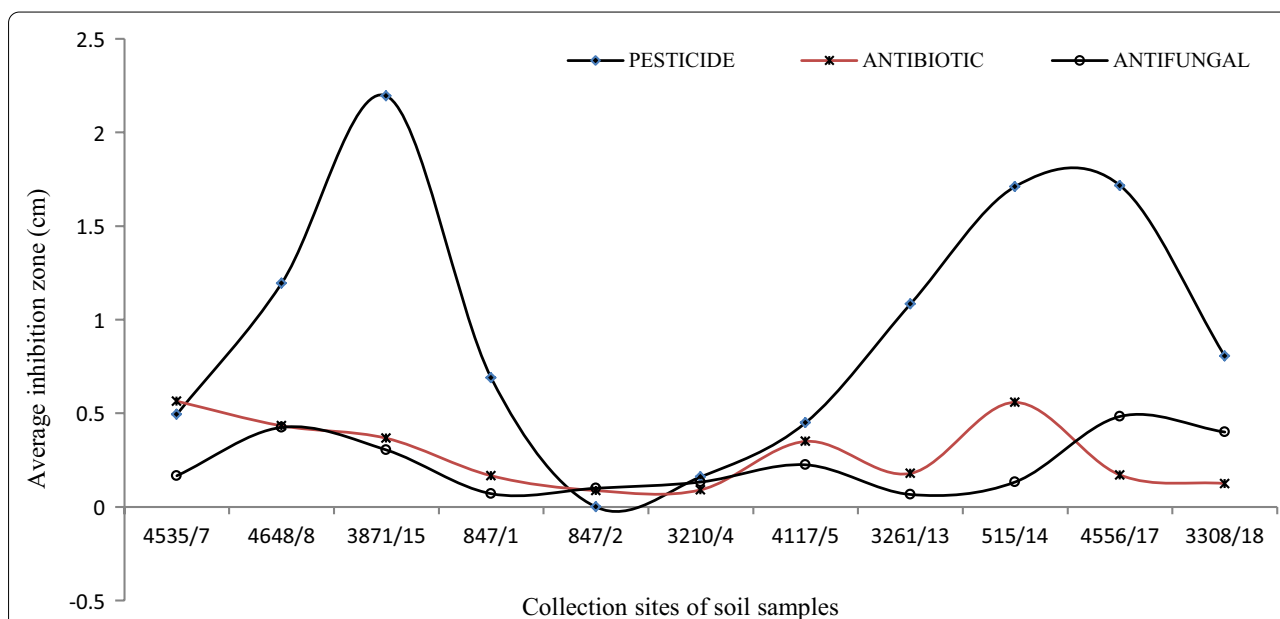

Fig. 7 Comparative illustration of pesticide, heavy metal salt, antibiotic and antifungal tolerance of PSMs isolated from organic practising (OP) and inorganic practising (INP) tea gardens

Saha *et al. Bull Natl Res Cent*   (2021) 45:109

Page 8 of 11

our survey we selected nine most abundantly used pesticides for our present study. The names of the pesticides, chemical nature and mode of action are represented in Table 3. All these pesticides though applied on tea bushes finally reach soil. Over passage of time pesticides degrade or accumulate in soil to modify microbes inhabiting in it. Modifications of soil microflora can be due to death and decline or may incite tolerance for resisting pesticides (Saha et al. 2020).

The eleven PSMC isolates when in vitro assessed with all the nine different pesticides in concentrations ranging from the recommended dosage by the Tea Board of India to the concentration which is four times the recommended levels. Based on the magnitude of inhibition zones produced by pesticides; Phorate was most and Deltamethrin was the least tolerant pesticide for the isolated PSMC. Phorate which is used against tea pests like red spider mites is widely used in the tea gardens to protect the bushes from the pest invasions (Bhattacharyya and Kanrar 2013). The decreasing order of tolerance observed by us in our experiment is Phorate, Thiomethoxame, Flubendiamide, Quinalphos, Fipronil, Emamectin benzoate, Fenazaquin, Spiromesifen and Deltamethrin (Fig. 3). From Fig. 3, it can be concluded that though all the nine pesticides are used in tea gardens and as a response has made the PSMs tolerable to such high concentrations.

The plantations were categorised on the basis of their mode of cultivation practices. Tolerance levels exhibited by the isolated PSMC on pesticides were not similar for both OP and INP. The decreasing order of tolerance observed PSMC isolates of OP are Phorate, Emamectin benzoate, Flubendiamide, Quinalphos, Deltamethrin, Fenazaquin, Spiromesifen, Fipronil and Thiamethoxame while that of INP are Thiamethoxame, Phorate, Flubendiamide, Fipronil, Quinalphos, Spiromesifen, Fenazaquin, Emamectin benzoate and Deltamethrin. Phorate and Thiomethoxame are the pesticides showing maximum level of tolerance. So, they may be considered as most

used or most accumulated pesticide in tea plantation soil that induced tolerance to PSMC. In tolerance study of soil from non-tea growing region pesticides like Fipronil, Thiomethoxame, Emamectin benzoate, Deltamethrin, Flubendiamide, Spiromesifen, Fenazaquin and Phorate showed complete to high degree of tolerance while Quinalphos showed least tolerance for the isolated PSMC (Saha et al. 2020).

Antibiotics have been a pre-eminent discovery of the early twentieth century (Lobanovska and Pilla 2017). After which the widespread use of antibiotics has been inevitable. Not only has it gained its popularity in terms of usefulness as an antibacterial agent in medicines, but its uses have also reached to animal rearing such as maintaining livestock, and so as well in agricultural fields (Kumar et al. 2005). Often planters use antibiotics in their lands with an intent to get rid of the harmful bacteria that might harm the plants (McManus et al. 2002; Shea 2003). But while doing so they even strip their land off the useful microbes that in turn helps in increasing the fertility of the soil. This effect is also known as the 'antibiotic winter' (Blaser 2014). This also aggravates the chances of developing antibiotic resistant microbes. Taking this into consideration, we went forward to study the antibiotic resistance gained by our isolates if any.

Tolerance levels exhibited by the isolated PSM on antibiotics were different for OP and INP. The decreasing order of tolerance observed PSM isolates of OP are E10, C30, PB300, AMC30, OF5, Gen10, N30, CTX30, B10, COT25 and P10 while that of INP are C30, P10, B10, AMC30, E10, CTX30, Gen10, PB300, C30, N30, COT25 and OF5. In OP antibiotics like E10, C30 and PB300 while in INP C30, P10 and B10 are most tolerance antibiotics. Saha et al. (2020) in their experiment on soil of non-tea growing region reported that antibiotics like AMC30, E10, C30, OF5, COT25 and CTX30 are fully tolerant to our isolated PSMC. Other antibiotics like PB300, N30, GEN10 and B10 are high while P10 is mild tolerant. As

Table 3 Chemical nature and mode of action of the pesticides experimented

Pesticide	Chemical nature	Mode of action
Fipronil	Phenylpyrazole, neonicotinoid	Act and disrupts central nervous system
Phorate	Organothiophosphate	Inhibits acetylcholinesterase and butyrylcholinesterase
Emamectin benzoate	Emamectins	Inhibits muscle contraction
Quinalphos	Organothiophosphate	Acetylcholinesterase inhibitor
Thiomethoxame	Oxadiazane	Interferes with nicotinic acetylcholine receptors in central nervous system
Fenazaquin	Quinazoline	Mitochondrial NADH:ubiquinone reductase inhibitor
Spiromesifen	Butenolide	Inhibitor of lipid synthesis
Deltamethrin	Cyclopropanecarboxylate ester (Pyrethroid)	Neurotoxin
Flubendiamide	Organofluorine	Ryanodine receptor modulator

Saha *et al. Bull Natl Res Cent*   (2021) 45:109

Page 9 of 11

antibiotic resistance can be induced by antibiotic producing microbes or due to its abuse, so we cannot be conclusive for the reason of gaining antibiotic resistance.

Various reports have also suggested that antifungal resistance has emerged just like antibiotic resistance due to improper usage of antifungal (Damalas and Eleftherohorinos 2011; Silva and Costa 2012; Lucas et al. 2015). Hence, an experiment for antifungal tolerance assay was conducted to examine if the isolates have any tolerance against the antifungal as well. According to a study, azole fungicides constitute the most widely used class of antifungal agents for control of fungal plant pathogens (Jensen and Jørgensen 2013). Out of the six commonly used antifungals that were screened for this assay, four of them (CC10, FLC25, IT10 and KT10)) were of azole group.

Out of the most commonly used antifungals, other than IT10, the PSMC isolates exhibited high/considerable tolerance towards antifungals like CC10, FLC25, AP100, NS100 and KT10. FLC25 exhibited highest tolerance among all the antifungals that were used for the experiment. Also we can see that out of the four azoles used for the experiment, the PSMC isolates were tolerant to only three azoles, whereas IT10 showed highest inhibition in most of the isolates. Therefore, we could infer that probably the use of IT10 is minimum or nil in these plantations, thus proving to be harmful or growth inhibiting for the PSMC isolates.

Tolerance levels exhibited by the isolated PSM on antifungal were not similar for both OP and INP. The decreasing order of tolerance observed PSM isolates of OP are FLC25, NS100, AP100, KT10, CC10 and IT10 while that of INP are FLC25, KT10, AP100, NS100, CC10 and IT10. In OP antifungals like FLC25 and NS100 have no inhibition zones, so they are totally tolerant to these antifungal. In INP, FLC25 and KT10 are most tolerant antifungals. Saha et al. (2020) reported that IT10 and FLC25 are completely tolerant. KT10, AP100 and CC10 are high while NS100 is mild tolerant. As antifungal tolerance can be induced by antifungal producing microbes or due to its abuse (Cowen 2008; Srinivasan et al. 2014), so we cannot be conclusive for the reason of gaining antifungal resistance.

During our experiment we have also noted that there was no inhibition or partial of PSM growth by some pesticide, antibiotic and antifungal in some soil samples. Compared to antibiotic and antifungal agents, tolerance towards pesticides are most prominent. Antibiotic and antifungal tolerance may arise from interaction of microbial population. But for pesticide, indiscriminate and overuse can be the possible reasons for imparting tolerance to PSMs. Partial inhibition against the antibiotics, i.e. growth of only a certain group of the microorganisms, was noticed in some plates. Therefore from the overall data, we can clearly posit that most of the isolates have high tolerance against the antibiotics that they were tested for. Some plates exhibited partial tolerance, i.e. the antifungal could inhibit only a certain group of fungi.

Correlation studies are important to find out whether tolerance is individually active or coactive. In our correlation studies between pesticide, antibiotic and antifungal a positive correlation existed between pesticide-antibiotic, pesticide-antifungal and antibiotic-antifungal tolerance indicating a dominant activity of pesticide to impose selection or tolerance to other stress factors. More is the magnitude of inhibition zone less is the tolerance and vice versa was accepted as the relation between tolerance and inhibition zone. In pesticide tolerance assay maximum inhibition zone was observed sample 3871/15 while minimum inhibition zone was observed in sample 847/2. The plantation from which sample 3871/15 and sample 847/2 was collected practice organic and inorganic mode of cultivation, respectively. The average inhibition zone observed in samples collected from OP (1.294 ± 0.264354 cm) was much higher than samples collected from INP (0.827 ± 0.123083 cm). In case of antibiotic tolerance assay maximum inhibition zone was observed sample 4535/7 while minimum inhibition zone was observed in sample 847/2. The plantation from which sample 4535/7 and sample 847/2 was collected practice organic and inorganic mode of cultivation, respectively. The average inhibition zone observed in samples collected from OP (0.454 ± 0.173246 cm) was more than twice the samples collected from INP (0.219 ± 0.112413 cm). Maximum antifungal inhibition zone was observed in sample 4556/17 while minimum inhibition zone was observed in sample 847/1. The plantation from which both the samples were collected were from INP, though the average inhibition zone observed in samples collected from OP (0.298 ± 0.201452 cm) was more than samples collected from INP (0.201 ± 0.268921 cm). In all the three tolerance studies using pesticide, antibiotic and antifungal it was observed that there is no relation between altitudes of collection site. But in all the three assays average inhibition zones were more in OP than INP. Thus, it may be said that INP induces tolerance to microbes. However, the variable results were observed in antibiotic and antifungal assays in sample level. So, other than being in OP or INP, judicious use of chemicals, micro-ecosystem and antibiosis exhibited by microbes do play a role in conferring tolerance.

Saha *et al. Bull Natl Res Cent*   (2021) 45:109

Page 10 of 11

Conclusion

Chemical additives in agricultural field finally accumulate in top soil where microbial diversity and their activity are at the highest level. We tried to assess the degree of tolerance that our PSM isolates gained over past years. Our results are quite alarming as the isolates not only from inorganic plantations but from organic tea plantations showed much tolerance towards our tested pesticides, antibiotics and antifungal agents. It has also been observed that there are no relations to increased tolerance by the PSM isolates with change in location, but related to cultural practises of the tea gardens. The well-being of soil, soil microbes and soil nutrition status are interrelated. Imbalance of this equilibrium may result in serious consequences in years to come. These results can be considered as an eye-opener and proper corrective measures must be initiated to maintain harmony in soil ecosystem.

Abbreviations

PSM: Phosphate solubilising microorganisms; PSMC: Phosphate solubilising microorganism consortium(s); OC: Organic carbon; OM: Organic matter; FP: Fipronil; P: Phorate; EB: Emamectin benzoate; Q: Quinalphos; T: Thiomethoxame; FN: Fenazaquin; S: Spiromesifen; D: Deltamethrin; FB: Flubendiamide; B10: Bacitracin; C30: Chloramphenicol; P10: Penicillin-G; PB300: Polymixin B; Gen10: Gentamicin; N30: Neomycin; CTX30: Cefotaxime; AMC30: Augmentin; E10: Erythromycin; C30: Chloramphenicol; OF5: Ofloxacin; COT25: Co-trimoxazole; AP100: Amphotericin-B; CC10: Clotrimazole; FLC25: Fluconazole; IT10: Itraconazole; KI10: Ketoconazole; NS100: Nystatin; OP: Organically practising; INP: Inorganically practising.

Authors' contributions

MB and SS contributed to the study conception and design. Material preparation, data collection and analysis were performed by SS, SA and SDS. The draft manuscript was written by SS and MB. All authors read and approved the final manuscript.

Availability of data and materials

All data analysed during this study are included in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1):1

Barbora BC, Biswas AK (1996) Use pattern of pesticides in tea estates of North East India. Two Bud 47:19–21

Baruah TC, Barthakur HP (1997) A textook of soil chemical analysis. Vikash, New Delhi

Bhattacharya M (2014) Floral components of Darjeeling with special reference to its forest areas. A comprehensive district profile of Darjeeling. N.L. Publishers, Darjeeling

Bhattacharyya A, Kanrar B (2013) Diversity of pesticides in tea. In: Preedy VR (ed) Tea in health and disease prevention. Academic Press, London, pp 1491–1501

Blaser MJ (2014) Missing microbes: how the overuse of antibiotics is fueling our modern plagues. Emerg Infect Dis 20(11):1961. https://doi.org/10.3201/eid2011.141052

Brauer VS, Rezende CP, Pessoni AM, De Paula RG, Rangappa KS, Nayaka SC, Gupta VK, Almeida F (2019) Antifungal agents in agriculture: friends and foes of public health. Biomolecules 9(10):521

Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 59(1):39–46

Cowen LE (2008) The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. Nat Rev Microbiol 6(3):187–198

Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 8(5):1402–1419

Das GM (1965) Pests of tea in North East India and their control. Memoir Tea Res Assoc Tocklai 27:115

Dias AC, Costa FE, Andreote FD, Lacava PT, Teixeira MA, Assumpçao LC, Araújo WL, Azevedo JL, Melo IS (2009) Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. World J Microbiol Biotechnol 25(2):189–195

Gurusubramanian G (2005) Safe and economic pest management in tea, shade trees and green crops. In: Dutta AK, Ahmed N, Kotoky B, Burugohain D (eds) Manual on tea culture for condensed course. Tocklai Experimental Station, TRA, Jorhat Assam Printing Works Private Limited, Jorhat, pp 113–127

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2008) Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol Res 163(2):234–242

https://www.ibef.org/exports/indian-tea-industry.aspx

Huidrom P, Sharma GD (2014) Microbial bioremediation of pesticide residues in tea soil. Int Interdiscip Res J 4:261–275

Igbedioh SO (1991) Effects of agricultural pesticides on humans, animals, and higher plants in developing countries. Arch Environ Health Int J 46(4):218–224

Iqbal MA, Bartakke KV (2014) Isolation of pesticide degrading microorganisms from soil. Adv Biores 4:164–168

Jackson ML (1973) Soil chemical analysis. Prentice-Hall of India Private Limited M-97, New Delhi

Jensen HE, Jørgensen LN (2013) Use of antifungals in animals and agriculture-any impact on human infections? Mycoses Suppl 56(Suppl. 3):32–32

Kumar K, Gupta SC, Chander Y, Singh AK (2005) Antibiotic use in agriculture and its impact on the terrestrial environment. Adv Agron 1(87):1–54

Kumar A, Kumar A, Patel H (2018) Role of microbes in phosphorus availability and acquisition by plants. Int J Curr Microbiol Appl Sci 7(5):1344–1347

Lobanovska M, Pilla G (2017) Focus: drug development: Penicillin's discovery and antibiotic resistance: lessons for the future? Yale J Biol Med 90(1):135

Lucas JA, Hawkins NJ, Fraaije BA (2015) The evolution of fungicide resistance. Adv Appl Microbiol 1(90):29–92

Mahidi SS, Hassan GI, Hussain A, Faisul-Ur-Rasool (2011) Phosphorus availability issue-its fixation and role of phosphate solubilizing bacteria in phosphorus solubilization-case study. Res J Agric Sci 2:174–179

McManus PS, Stockwell VO, Sundin GW, Jones AL (2002) Antibiotic use in plant agriculture. Annu Rev Phytopathol 40(1):443–465

Mukherjee M, Chakraborty S, Sarkar S, Saha S, Majumder S, Ghosh A, Bhattacharya M (2020) Soil nutritional status of tea plantations in plains of Sub Himalayan West Bengal. India Curr Agric Res 8(3):239–246

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17(4–5):319–339

Roy S, Mukhopadhyay A, Gurusubramanian G (2008) Use pattern of insecticides in tea estates of the Dooars in North Bengal, India. North Bengal Univ J Anim Sci 2(1):35–40

Saha S, Chakraborty S, Sarkar S, Acharyya S, Majumder S, Ghosh A, Bhattacharya M (2020) Phosphate solubilizing microorganism consortium of virgin Tiger hill forest soil shows high level tolerance to pesticide, antibiotic, antifungal and heavy metals. Ecobiotech 3(4):578–588

Saraswathy R, Suganya S, Singaram P (2007) Environmental impact of nitrogen fertilization in tea eco-system. J Environ Biol 28:779

Satyaprakash M, Nikitha T, Reddi EUB, Sadhana B, Vani SS (2017) Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. Int J Curr Microbiol Appl Sci 6(4):2133–2144

Schoebitz M, Ceballos C, Ciamp L (2013) Effect of immobilized phosphate solubilizing bacteria on wheat growth and phosphate uptake. J Soil Sci Plant Nutr 13(1):1–10

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2(1):1–4

Shea KM (2003) Antibiotic resistance: what is the impact of agricultural uses of antibiotics on children's health? Pediatrics 112(Supplement 1):253–258

Silva MF, Costa LM (2012) The pesticide industry. BNDES Sectorial, No. 35, sea. 2012, pp 233–276

Srinivasan A, Lopez-Ribot JL, Ramasubramanian AK (2014) Overcoming antifungal resistance. Drug Discov Today Technol 1(11):65–71

Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol Fertil Soils 30(5–6):460–468

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37(1):29–38

Walpola BC, Yoon MH (2012) Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: a review. Afr J Microbiol Res 6(37):6600–6605

Zhang J, Wang P, Fang L, Zhang QA, Yan C, Chen J (2017) Isolation and characterization of phosphate-solubilizing bacteria from mushroom residues and their effect on tomato plant growth promotion. Pol J Microbiol 66(1):57–65

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.