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PREVALENCE OF PESTICIDE TOLERANCE IN SOIL BACTERIA FROM DARJEELING HIMALAYAS: A GROWING CONCERN OF GRASSHOPPER EFFECT

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Abstract

Agricultural development involves the use of pesticides that have ended up in the soil or water bodies. These chemicals can accumulate and control microbial population by imposing tolerance to them. Pesticides can accumulate in soil or water by a multi-hopping phenomenon, termed the 'Grasshopper effect' that requires one or more volatilization-migration-deposition cycles. This study is an attempt at assessing the possible occurrence of this phenomenon in the Darjeeling Himalayas. A total of 39 sites of varied biotopes were sampled and their soil bacterial populations assessed based on the acquired degree of tolerance against pesticides. Soil microbial consortia isolated from all the regions showed total tolerance against the volatile pesticides emamectin benzoate, thiamethoxam, quinalphos, deltamethrin, spiromesifen and flubendiamide. Out of 47 isolates obtained, 29 exhibited full tolerance against them. Based on altitudinal zones, all the isolates from below <2000 ft depicted no inhibition zones against the tested pesticides while some inhibition was observed for the isolates from 2000-4000 ft (57.14%), 4000-6000 ft (28.57%), 8000-10000 ft (14.29%) and >10000 ft (75%). This work provides evidence for the occurrence of the 'Grasshopper effect' in the Darjeeling Himalayas facilitating the long-range transport, deposition and accumulation of harmful volatile pesticides in the region.

Keywords: Pesticides, soil, bacteria, Darjeeling Himalayas, Grasshopper effect

Introduction

Agricultural development remains the prime objective for every country's planning to cope with an increasing population. Although pesticides have become one of the most important tools as a plant protection agent in increasing crop yield, they may have also altered the soil biological activity. Such an effect, when present, may be direct or indirect and stimulatory or depressive with regard to the growth of microorganisms, when applied at normal rates (Chi-Chu Lo, 2010). Pesticides may arise from various sources and eventually reach soil or water bodies where they accumulate over time, forming reservoirs of toxic chemicals. These conditions control microbial population by imposing a 'die or tolerate situation', in which either they embrace death or adjust to the environmental toxicity gaining tolerance against the chemical.

The 'Grasshopper effect' is a phenomenon in which persistent organic pollutants and other volatile or semi-volatile chemicals are transported from one region to another through wind cycles and/or air pressure differences. Slowly degradable and semi-volatile organic compounds may undergo more than one volatilization-migration-deposition cycle (multi-hopping) through the atmosphere (Semeena and Gerhard, 2005). It has already been established that such volatilized chemicals accumulate in cold environments (cold condensation) and their large-scale fractionation is controlled by the vapour pressure of the substance (Wania and Mackay, 1993). Persistent organic pollutants (POPs) like pesticides are no exception to this phenomenon. They are constantly volatilized and transported from warm areas and condense to be deposited in colder areas through a series of "volatilization-migration-deposition" processes (Chai *et al.*, 2022). Pesticides, in fact, have been known to undergo efficient

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atmospheric transport across considerable distances, getting deposited at remote high-altitude locations (Loewen *et al*., 2005). This phenomenon is exacerbated due to climate change, population increase and industrialization.

Geologically, the young Himalayas covering 6 x 10^5 km^2 consist of parallel and converging ranges (Loewen *et al*., 2005). Darjeeling and Sikkim hills, a part of the eastern Himalayas, are globally important by their biodiversity, manifested by topographic complexity, altitudinal and climatic variations (Bhattacharya, 2014). Credited with plantations producing the best quality tea, Darjeeling-Sikkim share political boundaries with countries like Nepal, Tibet, Bhutan and Bangladesh. The Darjeeling Himalayas have been extensively studied on several aspects by researchers from all around the globe. Soil microbes of Darjeeling hills exhibiting pesticide (Saha *et al*., 2020 and Saha *et al*., 2021) and mercury tolerance (Acharyya *et al*., 2021; Acharyya *et al*., 2024) has been reported but without discussion on the origin of pesticides and mercury in the region other than anthropogenic inputs. Some historical mercury may be naturally present due to volcanic eruption during the formation of Himalayas from Tethys (Sial *et al*., 2016).

This study was an attempt to assess the possible 'Grasshopper effect' and 'cold trapping' phenomenon by sampling soil from varied biotopes of Darjeeling and Sikkim and ascertaining the degree of tolerance acquired by soil bacteria against pesticides. This will probably be the first report to understand the effect of pesticides' volatilization-migration-deposition cycle in the Darjeeling hills.

Materials and Method

Study area

The study area covers the Darjeeling Himalayas within the altitude of 453 ft to 11929 ft. The soil sampling sites were spread over thirty-nine locations of diverse biotopes viz. inorganic tea plantations (ITP), organic tea plantations (OTP), paddy field (PF), orange orchard (OO), vegetable field (VF) and natural forest (NF).

Collection of soil samples

The soil samples were collected in early mornings of April-May 2021, following the protocol of Mukherjee *et al*., 2020. Five pre-samples were collected from the corners of an imaginary square with sides measuring ten metres and also from the diagonal bisector of this said square. These pre-samples samples were mixed in equal proportions to make the final sample. The collected samples were filled in airtight zipper bags, kept in ice boxes and transported to laboratory for downstream analysis.

Isolation of soil microbial consortia

Untargeted soil microbial consortia were grown in nutrient broth (HiMedia M001). A quantity of 500 mg collected soil sample was mixed with 1 ml of sterile distilled water and vortexed for a min to ensure proper mixing of the soil. From this sample solution, 500 µl was pipetted into autoclaved media and incubated at 30°C for 48 h. The isolate was further cultured in the same media and preserved at 4°C for further downstream experiments.

Isolation of pure culture

The isolated consortia were inoculated onto nutrient agar plates containing phorate (O,O-diethyl S-ethylthiomethyl phosphorodithioate) to select for colonies tolerant to the added pesticides at a concentration of 5 mg/ml. The plates were incubated at 30°C for 48 h. Pure cultures were obtained by repeated streaking of the colonies on nutrient agar plates. All the isolated pure cultures were preserved at 4°C for further downstream experiments.

Pesticide tolerance assay

Consortia and pure culture isolates (100 µl) were separately plated on semisolid nutrient media containing pesticides (emamectin benzoate, thiamethoxam, quinalphos, deltamethrin, spiromesifen and flubendiamide) at a concentration of 10 mg/ml (solid form of pesticide) and 10 µl/ml (liquid form of pesticide) in the bored wells of the Petri plates and incubated at 30°C for 48 h. Inhibition zones, if any, were recorded following incubation.

Results and discussion

Soil samples were collected from 39 sites representing diverse biotopes and altitudes (Figure 1). Isolation of microbial consortium was carried out from soil samples of tea plantations following inorganic mode of cultivation (ITP), organic mode of cultivation (OTP), paddy fields (PF), orange orchards (OO), vegetable fields (VF) and natural forests (NF) (Table 1). Complete growth of isolated microbial consortia was observed in the presence of pesticides, even at four times (2.5x4mg/ml), the concentration used by tea gardens practicing inorganic mode of cultivation (Plant Protection Act, 2010). ITPs (16 collection sites) use a substantial amount of pesticides to keep away insects and other pests. Thus, the high tolerance to pesticides observed in their microbial populations is expected. However, biotopes like OTP (9 collection sites) use plant derived formulations or biopesticides for managing pests. Similarly, the consortia from PF (1 collection site), OO (1 collection site), VF (2 collection sites) practicing organic mode of cultivation were supposed to show some degree of susceptibility towards the tested pesticides. Moreover, consortia from NF (10 collection sites) which were entirely virgin with no chances of human intervention, also exhibited tolerance against pesticides, contrary to expectations.

Table 1. Soil sampling sites

Index	Collection site	Latitude	Longitude	Altitude (ft)	Land use
1	North Bengal University	26.71316	88.34845	453	ITP
2	Gulma Tea Estate	26.78097	88.38805	515	ITP
3	Simulbari 1	26.80050	88.27095	847	ITP
4	Simulbari 2	26.80052	88.27091	847	ITP
5	Gyabaree	26.83739	88.22350	2946	ITP
6	Singbulli 1	26.84353	88.22490	3210	ITP
7	Singbulli 2	26.84367	88.21564	3261	ITP
8	Rohini Gidda Pahar	26.83876	88.29038	3308	ITP
9	Kalimpong (Deorali 2)	27.02790	88.47364	3396	OO
10	Makaibari	26.85921	88.26282	3871	OTP
11	Castleton	26.85873	88.26876	3871	ITP
12	Kalimpong (Deorali 1)	27.02793	88.47356	3996	PF
13	Kalimpong (Comesi Forest)	27.02602	88.48021	4101	NF
14	Tingling	26.84498	88.19339	4117	ITP
15	Sourenee 1	26.86719	88.19530	4535	OTP
16	Sourenee 2	26.86854	88.19539	4535	OTP
17	Spring Sidi	26.87174	88.26736	4556	ITP
18	Sourenee 3	26.87221	88.19399	4648	OTP
19	Kalimpong (Delo)	27.09474	88.50617	5590	NF
20	Phoobshering 1	27.06095	88.27708	5671	ITP
21	Okayti 2	26.90801	88.15425	5702	OTP
22	Okayti 1	26.90802	88.15388	5735	OTP
23	Phoobshering 2	27.06093	88.27710	5742	ITP
24	Margaret's Hope	26.92941	88.29914	5763	ITP
25	Phoobshering 3	27.06099	88.27700	5851	ITP
26	Sikkim (Singtam)	27.33674	88.49352	5868	VF
27	Lebong	27.05203	88.27127	6150	ITP
28	Happy Valley	27.05047	88.26015	6531	OTP
29	Happy Valley	27.05031	88.26019	6544	OTP
30	Happy Valley	27.05040	88.26054	6621	OTP
31	Sikkim (Raley Khesey)	27.33525	88.49545	8238	VF
32	Dhotrey	27.04794	88.10725	8340	NF
33	Tiger Hill	88.28588	26.99465	8484	NF
34	Gairibas	27.04963	88.03166	8600	NF
35	Tonglu	27.03449	88.08795	10070	NF
36	Kalipokhri	27.07727	88.01966	10500	NF
37	Sandakphu 1	27.10670	88.00507	11464	NF
38	Sandakphu 2	27.11058	88.01089	11464	NF
39	Sandakphu Top	27.10524	88.00183	11929	NF

ITP=inorganic tea plantation, OTP=organic tea plantations, PF=paddy field, OO=orange orchard, VF=vegetable field, NF=natural forest

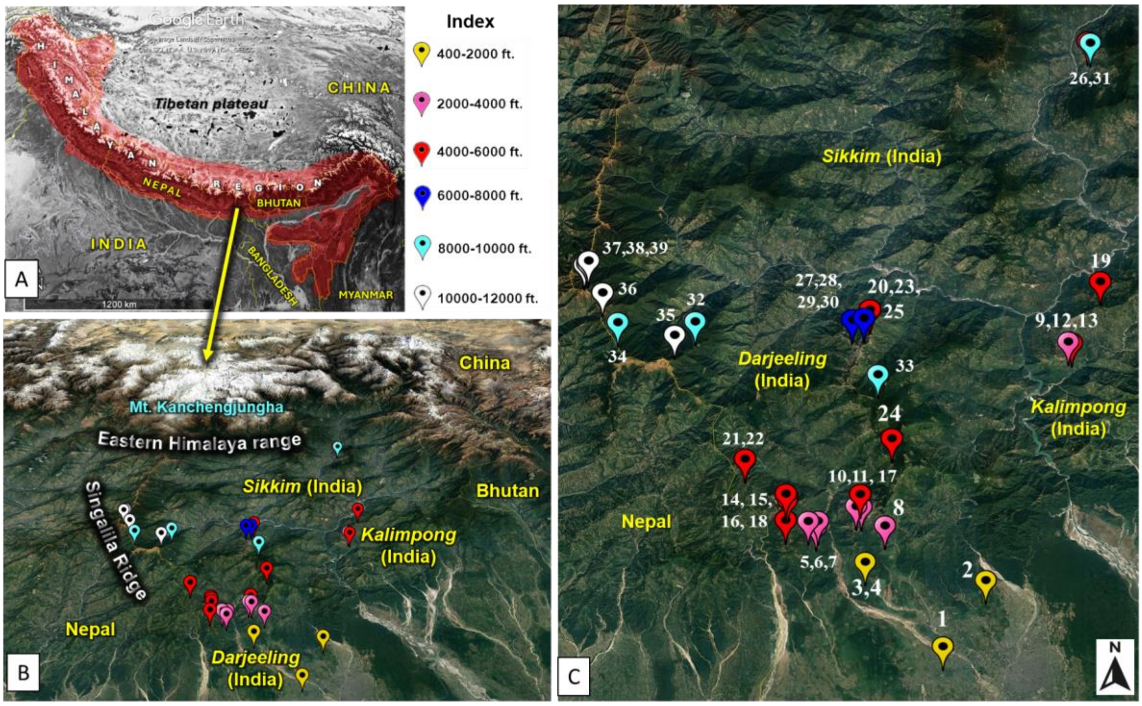

Figure 1. Map of study area; A: Location of the study area in the Himalayan Mountain system, B and C: Satellite view of the study area/soil collection sites (Source: Google Earth Pro v.7.3.6.9345) [1-39 = Sample collection sites in accordance with the index given Table 1]

Pure cultures (47 isolates) were obtained from the consortia of the biotopes, viz., ITP (7 isolates), OTP (6 isolates), PF (2 isolates), OO (6 isolates), VF (3 isolates) and NF (24 isolates). Out of these, 29 isolates exhibited complete tolerance against all the pesticides tested for without any inhibition zones (ITP-100%, OTP- 86.11%, PF- 83.33%, OO- 80%, VF-100% and NF- 78.47%) (Figure 2). Isolates from <2000 ft showed no inhibition zones in any of the tests while inhibition zones were noted in 57.14% of the tests from 2000-4000 ft, 28.57% of the tests from 4000-6000 ft, 14.29% of the tests from 8000-10000 ft and 75% of the tests from above <10000 ft (Figure 3). The isolates showed varied inhibition zones towards the pesticides, displaying the highest tolerance to thiamethoxam (97.87%), followed by quinalphos (91.48%), spiromesifen (91.48%), flubendiamide (89.36%), deltamethrin (72.34%) and emamectin benzoate (63.82%).

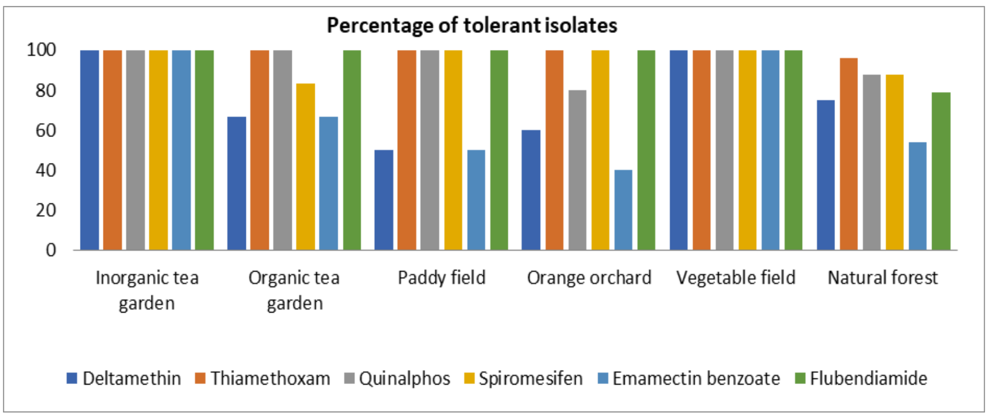

Figure 2. Percentage distribution of tolerance to pesticides among different biotopes

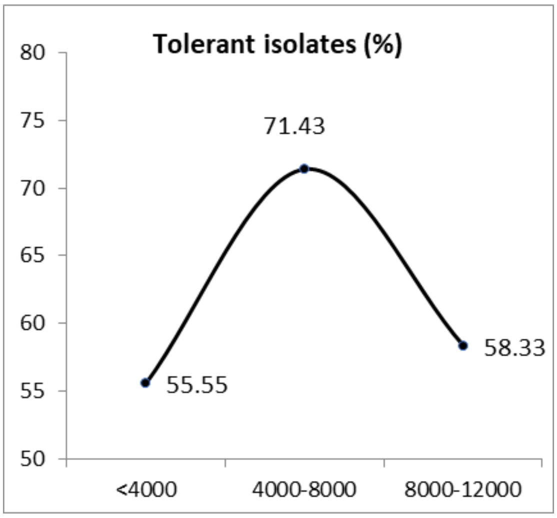

Figure 3. Distribution of pesticide tolerance against altitude

The soil microbial consortia isolated from varied biotopes showed complete tolerance against the tested pesticides. India, with 234 pesticides registered for use, is one of the largest producers as well as consumers (http://indiaforsafefood.in/farminginindia/). Pesticides in large quantities are often used in agricultural practices in the lower Himalayan and sub-Himalayan plains to control pest infestations resulting from a highly humid climate (Ghosh *et al.*, 2015). Moreover, tea plantations of the Himalayas and sub-Himalayan region use excessive quantities of pesticides (https://www.teaboard.gov.in/TEABOARDCSM/NDQ=). Studies have investigated the tolerance in phosphate solubilizing bacterial consortia from Tiger hills (8500ft), a virgin natural forest (Saha *et al.*, 2020), and from tea plantations of lower altitudes (847-4648 ft) of Darjeeling hills (Saha *et al.*, 2021) against pesticides like fipronil, phorate, emamectin benzoate, quinalphos, thiamethoxam, fenazaquin, spiromesifen, deltamethrin and flubendiamide. Complete tolerance was reported against fipronil and thiamethoxam and a high level of tolerance was observed against emamectin benzoate, deltamethrin, flubendiamide, spiromesifen, fenazaquin and phorate, implicating pesticide abuse in tea plantations as the probable cause for conferring tolerance to resident microbes. It may be argued that the exhibition of high levels of pesticide tolerance by the isolated bacteria from ITP of lower altitudes may be due to substantial use of pesticide in the region and further accumulation of pesticides brought down by water from the ITPs of higher altitudes. However, the same observation in consortia from NF, OTP and other organically cultivated fields is unprecedented.

Isolation of pure culture was conducted using phorate as a standard. This pesticide has low volatility and the least chance of distant migration. Phorate is not extensively used in tea plantations of the area except during planting of bushes to prevent attack from nematodes and termites. The isolates from varied biotopes and elevations showed variation in results. The isolates obtained from the ITPs showed 100% tolerance against all the pesticides used, which may be due to the same reasons as discussed in the previous paragraph for ITP consortia. The organic tea gardens do not use chemical pesticides but instead use plant-derived formulations, biopesticides and/or cultural methods to control pests. The isolates from the OTPs in this study, showed complete tolerance against thiamethoxam, quinalphos, flubendiamide but some level of growth inhibition on application of deltamethrin, spiromesifen and emamectin benzoate (Table 2). The tolerance may possibly be due to pesticides that have migrated through rivers and waterfalls, and accumulated from surrounding inorganic tea gardens or other crop fields in the lower altitudes. The paddy fields of Kalimpong also employ organic cultivation methods. The isolates from the collected PF soil showed some inhibition zones with deltamethrin and emamectin benzoate but were 100% tolerant to thiamethoxam, quinalphos, spiromesifen and flubendiamide. The isolates from OO of Kalimpong, which also follows organic form of practice, showed complete tolerance against thiamethoxam, spiromesifen and flubendiamide. Minor inhibition zones were observed against quinalphos but larger inhibition zones were observed against deltamethrin and emamectin benzoate. In hilly terrains, migration of pesticides is possible along with flow of water from the higher to lower elevations. Thus, the collections that were made from the paddy field, orange orchard and vegetable fields may have gathered some pesticides with the flow of water from higher elevations.

The isolates from VF of Sikkim showed complete tolerance against the pesticides. The Indian state Sikkim prohibits use of inorganic pesticides by state law; as such, samples collected from two altitudinal zones (Singtam and Raley Khesey) were expected to be free from pesticides. There is also no other region above this collection site that practices inorganic mode of cultivation wherefrom migration of pesticides with water would be possible. Comesi forest of Kalimpong district is a NF with very less human intervention, yet showed complete tolerance against thiamethoxam and quinalphos. Although small, but some level of growth inhibition was observed upon the application of the other four pesticides (deltamethrin, spiromesifen, emamectin benzoate and flubendiamide). The nine isolates obtained from NF of Tiger hill showed complete tolerance. Tiger hill, situated at an elevation of 8500 ft, is the highest point close to Darjeeling municipality and is a virgin land. The question of pesticide accumulation from nearby agricultural fields along flow of rain water does not arise. Sandakphu, the highest peak in Darjeeling hills, is uninhabited except by very few tribals of the region. The area is completely organic and is mostly covered by natural forests. As there is no other region from which migration of pesticides is possible along with flow of water, the only possibility left is the process of volatilization-migration-condensation or so called 'Grasshopper effect'.

The Himalayas are a high-altitude barrier surrounded by lowlands (Indian subcontinent and Tibetan plateau) of two of the most populous countries of the world. The two countries have adopted extensive use of inorganic inputs in agriculture to feed their growing population in the last century. In our study region, an altitudinal difference of around 11500 ft is covered within an aerial distance of around 50 km. Widespread tolerance of soil bacteria against commonly used pesticides suggests volatilization, long and short distance transportation (from distant places to mountains and from valley to mountain of close proximity) followed by cold condensation. A tropical monsoon system operates in this region. Warm summers in the Indian subcontinent creates a low-pressure region causing the cool humid air from southern hemisphere to cross the equator and push the airmass over India where it hits the Himalayas resulting in precipitation. Moreover, precipitation in the form of heavy rain in the lower altitudes and snow in the higher elevations of the Himalayas function as a strong pump in removing pesticides from the atmosphere to the surface. There are several reports on snow being an efficient scavenger of persistent organic pollutants (Franz and Eisenreich, 1998; Wania *et al.*, 1998). The tropical monsoon has previously been found to be responsible for the transportation process of pollutants from the Indian subcontinent to the Himalayas (Arndt *et al.*, 1998; Hindman and Upadhayay, 2002; Loewen *et al.*, 2005).

Mountain regions are the reservoirs of pollutants, accumulating higher than expected concentrations in several regions around the world (Wania, 1999). The phenomenon has been observed in vegetable fields (Calamari *et al.*, 1991), Canadian Rocky Mountains (Davidson *et al.*, 2004), European mountain regions (Weiss *et al.*, 2000; Carrera *et al.*, 2001), and the American Sierra Nevada (Dutta *et al.*, 1999). Weiss *et al.*, 1998, in his work on Austrian Alps reported higher concentrations of POPs in comparison to lower altitudes. Studies indicate that higher altitudes of mountain regions function as a convergence zone for POPs (Daly and Wania, 2005).

Deltamethrin ([cyano-(3-phenoxyphenyl)methyl] 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate) is a pyrethroid insecticide that kills by disrupting normal nervous system function. Exposure is via direct contact or ingestion. It has a tendency to bind tightly to soil particles and has a half-life ranging from 5.7-209 days in soil and a half-life of 5.9-17 days on plant surfaces. It does not break down as quickly in soil with a high clay or organic matter content. Himalayan soil with its diverse and dense vegetation accumulates huge quantities of organic matter. It is not likely to evaporate into the air, thus making it almost non-volatile, or dissolve easily into water. It is broken down by microbes, light and water, the latter releasing two of its major breakdown products into the soil (Deltamethrin General Fact Sheet, 2010). Thiamethoxam (4H-1,3,5-oxadiazin-2-imine, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methylN-nitro), a nicotinoid compound is used to systematically control a broad spectrum of chewing pests and sucking pests (Schwartz *et al.*, 2000). This low volatile pesticide has a half-life of 140 to 180 days (Todey *et al.*, 2018). The studied soil bacteria exhibited the maximum tolerance against it. Quinalphos (O,O-diethyl O-quinoxalin-2-yl phosphorothioate), a widely used organophosphate pesticide, is used for controlling caterpillar and scale insects (Reddy and Ghewande, 1986). It inhibits acetylcholinesterase (AChE) (Vig *et al.*, 2006) and also has activity as a neurotoxicant as well as a respiratory tract, skin, and eye irritant (Dhanjal *et al.*, 2014). Quinalphos persists for 125 days with a half-life of 20 days in the soil and is susceptible to hydrolysis. It is also highly volatile and its degradation is heavily influenced by the nature of the soil (Schwartz *et al.*, 2000).

Table 2. Inhibition zone diameter (mm) displayed by the isolates

Biotopes	Isolates	Delta	Thia	Quin	Spiro	EB	Flub
Inorganic tea garden	NBU1	0	0	0	0	0	0
	TIN1	0	0	0	0	0	0
	TIN2	0	0	0	0	0	0
	TIN3	0	0	0	0	0	0
	TIN4	0	0	0	0	0	0
	TIN5	0	0	0	0	0	0
	SIM1	0	0	0	0	0	0
Organic tea garden	SOU1	0	0	0	0	0	0
	SOU2	0	0	0	0	0	0
	MTD10A	2	0	0	2.1	1.6	0
	MTD10B	0	0	0	0	0	0
	MTD10C	1.2	0	0	0	1.1	0
	MTD10D	0	0	0	0	0	0
Paddy field	MTK3	0	0	0	0	0	0
	MTK4	1.4	0	0	0	1.4	0
Orange orchard	MTK5	0	0	0	0	0	0
	MTK6	0	0	0	0	0	0
	MTK7	1.5	0	0	0	1.6	0
	MTK8	0	0	0	0	1.4	0
	MTK9	1.7	0	1.2	0	1.3	0
Vegetable field	MTSK1	0	0	0	0	0	0
	MTSK2	0	0	0	0	0	0
	MTSK3	0	0	0	0	0	0
Natural forest	MTK1	2.1	0	0	1.8	1.6	1.9
	MTK2	1.8	0	0	1.3	1.8	1.5
	MTS1A	0	0	0	0	0	0
	MTS1B	0	0	0	0	0	0
	MTS1C	1.3	0	0	0	0	0
	MTD11A	0	0	0	0	0	0
	MTD11B	0	0	0	0	0	0
	MTD11C	0	0	0	0	0	0
	MTD11D	0	0	0	0	0	0
	MTD11E	0	0	0	0	1.1	0
	MTD11F	0	0	0	0	1.1	0
	MTD11G	0	0	0	0	0	0
	MTD11H	0	0	0	0	0	0
	MTD11I	0	0	0	0	0	0
	MTS3A	0	0	0	0	0	0
	MTS3B	1.8	0	0	0	1.9	1.1
	MTS2A	0	0	0	0	0	0
	MTS2B	0	0	0	0	1.2	0
	MTS2C	0	0	0	0	1.2	0
	MTS2D	1.7	0	1.9	0	2.2	1.8
	MTS4A	2.7	1.4	2.5	1.4	2.2	1.8
	MTS4B	0	0	0	0	0	0
	MTS5	2.3	0	1.3	0	1.9	0
	MTS6	2.4	0	0	0	2.1	0

Del=deltamethrin, Thia=thiamethoxam, Quin=quinalphos, Spiro=spiromesifen, EB=emamectin benzoate, Flu=flubendiamadine

Thus, accumulation and persistence of this pesticide is variable. Spiromesifen (3-mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate), a low volatile insecticidal cyclic ketoenole, is an acetyl coenzyme A carboxylase inhibitor. The biological activity of cyclic ketoenoles correlates with the inhibition of lipogenesis, resulting in decreased lipid content, especially of triglycerides and free fatty acids, in treated insects. Its half-life depends on the moisture content in the soil ranging from 14.3-16.7 days in submerged soil, 18.7-20 days in field capacity soil and 21.9-22.9 days in dry soil (Mate *et al*., 2015.). *Emamectin benzoate* (4'-deoxy-4'-epi-methyl-amino benzoate) is a salt of avermectin B1 (abamectin) with low volatility. Its half-life in soil is only 3.65-5.78 days as it undergoes rapid photolytic degradation with a dissipation rate of 90% over 7 days (Zhu *et al*., 2011; Li *et al*., 2011). Lastly, flubendiamide (1-N-[4-(1,1,1,2,3,3,3-heptafluoropropan-2-yl)-2-methylphenyl]-3-iodo-2-N-(2-methyl-1-methylsulfonylpropan-2-yl) benzene-1,2-dicarboxamide) is an organofluorine insecticide with low volatility. It has a role as a ryanodine receptor modulator and is functionally related to a phthalamide. It degrades slowly in field conditions. In terrestrial field experiments, its half-life ranged from 210 to 770.2 days in loamy sand to silty loam and 322 days in the case of sandy loam soil.

The general transportation and accumulation factors of POPs such as rivers, run-down water, etc. can only aid in the development of tolerance by the bacteria isolated from the OTPs, VF, OO and PF up to a certain extent. Yet such huge tolerance by most of the isolates from VF of Sikkim and NFs with no known nearby sources of contaminating agent raises a question on its origin. The tolerance pattern was also seen to differ among the pesticides included in this study. Of all the 47 isolates studied in this work, only 2.13% of them exhibited a slight susceptibility against thiamethoxam, 8.51% against spiromesifen and quinalphos, 10.64% against flubendiamide, 27.66% against deltamethrin and 36.17% against emamectin benzoate. Deltamethrin is a non-volatile stable compound, and hence cannot migrate through wind currents. Yet the bacteria displayed a higher susceptibility percentage towards it. Spiromesifen and flubendiamide with their low volatility can be migrated to higher altitudes, and effectively increase the tolerance of the bacteria in regions that are not subjected to direct exposure to such pesticides. Emamectin benzoate, though has low volatility, degrades easily through photolysis and bacterial interference. Subsequently, the shortened half-life of emamectin benzoate (approx. 5 days) leaves fewer residues in the soil for the bacteria to develop an enhanced tolerance mechanism for survival, thus explaining the susceptibility of the bacterial isolates against the pesticide. Thiamethoxam, also a low volatile pesticide, was seen to impact the extent of tolerance of the bacteria to maximum degree may be particularly owing to their prolonged half-life in the soil. Quinalphos, on the other hand, is highly volatile and can be re-emitted and relocated from one region to another giving it the ‘hopping’ characteristic. Thus, with the data presented, the volatility and half-life of these pesticides, it can be concluded that the exposure of the resident microflora of these areas to pesticides is occurring under the influence of the “Grasshopper effect” or on a smaller scale the “mountain trapping effect”.

Besides physical and chemical characteristics, soil fertility depends on microbial diversity in which the bacterial populations may reach around 10^6 in one gram of soil (Thompson *et al*., 2017). The microbial richness depends on pH, nutritional status, climate, vegetation and the quantity of toxicants present in the soil. Sarkar *et al*., 2022 in a research work by metagenomic approach compared the soil samples from two tea plantations (ITP and OTP) of Darjeeling and found that the overall microbial diversity was higher in OTP compared to ITP along with reduction of pathogenic bacteria in OTP soil. Four pesticides thiamethoxam, quinalphos, spiromesifen and flubendiamide have conferred more tolerance to the soil microbes over deltamethrin and emamectin benzoate in this study. It may be deduced that these pesticides are present in all the biotopes in higher quantity which has led to the tolerance observed within the soil bacterial microflora.

Conclusion

Presently, pesticides use and crop production go hand in hand and reliance on such chemicals is only growing day by day. Although concerns over the environmental consequences of unchecked pesticide use and its impact on public health have been raised, the transport mechanisms of its volatile counterparts are yet to be properly addressed. It is important to note that pesticide pollution does not remain limited to the confines of the region where they are used. Volatile pesticides can undergo long range transport to settle in remote locations with no history of prior exposure and subsequently pollute its vulnerable ecosystem. The Himalayas have emerged as such a convergence zone, facilitated by wind currents and altitudinal temperature gradients. Moreover, several rivières originate in the Himalayan region and flow to the plains which becomes the water supply for many. Thus, the pesticides that accumulate in this area as a result of the so called ‘Grasshopper effect’ is of great concern to pollution control and must be recognized as an important contributor to environmental pollution in the Darjeeling Himalayas and monitored regularly.

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Conflict of interest
The authors do not have any conflict of interest.

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