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# A Study on the Diversity of Pesticide-Resistant Bacterial Population from Different Agricultural Fields of Manjoor

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#### ABSTRACT

The regular usage of pesticides in agricultural fields results in the development of a pesticide-resistant microbial population. Vegetable cultivation is a common practice in the agricultural growing areas of Manjoor, Kerala. The present study was envisaged to understand the resistance of microorganisms to different types and doses of pesticides. The study revealed that heterotrophic bacteria are capable of resisting lower concentrations (0.01 and 0.001%) of the pesticides lindane and methyl parathion while a higher concentration of carbaryl (0.1%) could also be tolerated. In the soil sample where there was no prior addition of pesticides, the heterotrophic bacteria could only tolerate very low concentrations of pesticides The results of mean pesticide-resistant bacterial load when compared to normal Total Heterotrophic bacteria of soils collected from different agricultural fields and the pesticide-resistant bacterial load was lower than normal THB.

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#### INTRODUCTION

Pesticides encompass a variety of different types of chemicals including herbicides, insecticides, fungicides, and rodenticides. These synthetic organic compounds intended to regulate the pests constitute an important aspect of modern agriculture, as they are necessary for economical pest management. Pesticides are broadly divided into many classes based on their chemical structure as the organochlorines, organophosphates, and carbamates of which the most important are organochlorine pesticides (OCPs).

Organochlorine pesticides are broad spectrum chlorinated hydrocarbons and representative compounds in this group include the chlorinated derivatives of diphenyl ethane (dichlorodiphenyltrichloroethane-DDT, its metabolites dichlorodiphenyldichloroethylene-DDE, dichlorodiphenyldichloroethane-DDD, and methoxychlor), hexachlorobenzene (HCB), the group of hexachlorocyclohexane ( $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH and  $\delta$ -HCH,), the group of cyclodiene (aldrin, dieldrin, endrin, chlordane, nonachlor, heptachlor, and heptachlor-epoxide), and chlorinated hydrocarbons (dodecachlorine, toxaphene, and chlordecone). Organochlorine pesticides are very stable compounds and it has been cited that the degradation of dichlorodiphenyltrichloroethane (DDT) in soil ranges from 4 to 30 years. They are liposoluble compounds and are capable of bioaccumulating in the fatty parts of biological beings. Organophosphates are phosphoric acid esters or thiophosphoric acid esters which include pesticides like Malathion, dibrom, chlorpyrifos, temephos, diazinon, and terbufos. The organophosphate insecticides bind with the cholinesterase (ChE) enzyme at the neuromuscular junction and deactivate or inhibit the activity of the enzyme by irreversible phosphorylation. This results in elevated levels of acetylcholine (ACh), which acts on the muscarinic receptors situated at cholinergic junctions in skeletal nerve-muscular junctions, at nicotinic receptors in autonomic ganglia, and receptors in the central nervous system (CNS) (Kwong 2002). Carbamate pesticides are esters of carbamates and organic compounds derived from carbamic acid. This group of pesticides can be divided into benzimidazole-, N-methyl-, N-phenyl-, and thiocarbamates. Aldicarb, carbaryl, propoxur, oxamyl and terbucarb are carbamates. The carbamates are relatively unstable compounds that break down in the environment within weeks or months. These pesticides affect the nervous system by disrupting an enzyme that regulates acetylcholine, a neurotransmitter. The enzyme effects are usually reversible.

Many soil microorganisms have the ability to act upon pesticides and convert them into simpler non-toxic compounds. Most microbial degradation of pesticides occurs in the soil, which is the storehouse of multitudes of microbes both in quantity and quality, receives the chemicals in various forms, and acts as a scavenger of harmful substances. The rate of biodegradation in soil depends on the availability of pesticide or metabolite to the microorganisms, physiological status of the microorganisms, survival and/or proliferation of pesticide degrading microorganisms at the contaminated site, and sustainable population of these microorganisms. Even though the physical and chemical forces also act in degrading the pesticides to some extent, microorganisms play a major role in the degradation of pesticides (Kumar et al. 2021).

The halogenated aliphatic compound, position, and the number of halogens are important in determining both rate and mechanism of biodegradation (Mulligan 2005) and since organochlorine pesticides possess halogen electron-withdrawing groups that generate electron deficiency in the molecule, they resist aerobic degradation (Rieger et al. 2002). However, these compounds can be attacked more readily under reductive conditions, which could be enhanced by the addition of auxiliary electron donors. For most pesticides, aerobic decomposition proceeds much faster than anaerobic decomposition; however, an exception to this is DDT, whose decomposition proceeds ten times faster under anaerobic conditions (Scott 2000).

Organophosphorus pesticides consist of ester or thiol derivatives of phosphoric, phosphonic, or phosphoramides acids. The main degradation pathway starts with the hydrolysis of the P-O alkyl or P-O aryl bonds (Ang et al. 2005) which diminishes as much as 100 times the toxicity of these compounds. Bacterial enzymes have been found to achieve such detoxifying reactions (Yañez- Ocampo et al. 2009). This reaction is performed by esterases or phosphotriesterases that have been described for a number of different genera of bacteria and fungi.

A number of bacterial genera have been identified as carbamate degraders (Parekh et al. 1995, Satish et al. 2017) and microorganisms in soil are known to degrade carbamate pesticides via hydrolysis or oxidation, of which microbial hydrolysis of carbofuran is well established (Chaudhry et al. 2002). The enzymatic hydrolysis of carboxyl esters by carboxyl esterases (CbEs) is based on the reversible acylation of a serine residue within the active center of the protein (Gupta 2006).

In vegetable growing areas, where immense pesticides of different pesticide groups are applied, a natural microbial population efficient to resist these pesticides might have evolved over time. But the effects of these pesticides on these pesticide degrading strains and the emergence of pesticide-resistant strains are not studied so far. In field or laboratory studies, pesticide impact on microbial biota is most often assessed by measuring the number of microorganisms (Cycon et al. 2009). Hence the present study was conducted to understand the existence of a pesticide-resistant population and to compare the pesticide-resistant bacterial load associated with soils from six different agricultural fields of Manjoor, which is a predominant agricultural field in Kerala.

#### MATERIALS AND METHODS

Soil samples are collected from six agricultural areas of Manjoor (S1 to S6). Six sites that practiced crop rotation after two or three life cycles of a crop were selected. The six sites were representing regions that produce different vegetables like Pea plant (station 1), Bitter gourd (station 2), Snake gourd (station 3), little gourd (station 4), and Cucumber (station 5). Station 6 is a pea plant growing area where pesticides are not added. A garden soil sample (station 7), from the nearby area, was also collected during the period which was used as a control.

For the estimation of the pesticide-resistant population, the pesticides used were lindane (an organochlorine pesticide manufactured by Jayakrishna Pesticides (P) Ltd, Salem), methyl parathion (an organo phosphorous pesticide manufactured by Agro Chemicals of India, Nashik), and carbaryl (a carbamate pesticide manufactured by S.S. Cropcare Ltd, Bhopal and marketed by Rhone-Poulenc Agrochemicals India Ltd).

#### **Quantification of Pesticide-Resistant Population**

To estimate the pesticide-resistant population, 10 g of soil samples were homogenized with 90 mL sterile distilled water using a rotary shaker and serially diluted up to  $10^{-2}$ . Between each dilution, the samples were mixed thoroughly on a vortex mixer to release bacterial cells from soil particles. One hundred microliters of various dilutions of each sample were pipetted onto a surface dried mineral medium plate supplemented with various concentrations (0.1%, 0.01%, and 0.001%) of different pesticides. The sample was spread aseptically using a sterile L- rod and incubated in an inverted position at 37°C for 6-7 days. All the plating was done in duplicates. After incubation, the colony was enumerated using the conventional plate count method. The pesticide-resistant population was recorded as colony-forming units per gram of soil.

#### **Statistical Analysis**

Results of the study were compiled and subjected to statistical analysis for drawing inferences. The techniques employed for the analysis of the data were Analysis of Variance (ANO-VA) for comparison of treatment means and the  $\chi 2$  test for goodness of fit of proportions. For framing the ANOVA table for comparison of data on bacterial load, the figures

were converted to their corresponding logarithm values after adding 1 to all the observations wherever necessary.

#### RESULTS

The present study was carried out to determine the diversity of pesticide-resistant bacterial populations from different agricultural fields. The bacterial population's resistance to organochlorine pesticide (lindane), organophosphorus pesticide (methyl parathion), and carbamate pesticide (carbaryl) was estimated.

#### **Quantification of Pesticide-Resistant Bacteria**

**Pesticide-resistant bacterial load in soils of pea plant:** The pesticide-resistant bacterial load of station 1 is represented in Table 1. The lindane resistant bacterial load in station1 ranged from  $4.8 \times 10^5$  to  $3.2 \times 10^6$  cfu.g<sup>-1</sup> and the mean lindane resistant bacterial load was  $1.21 \times 10^6$  cfu.g<sup>-1</sup>. The pesticide-resistant bacterial load was observed only when lindane impregnation was provided at a concentration of 0.01% and 0.001%. When the dosage was increased to a concentration of 0.1%, the bacterial growth was arrested. The methyl parathion-resistant bacterial load ranged from  $2.3 \times 10^6$  cfu.g<sup>-1</sup>. At a concentration of 0.1%, the bacterial load varied from  $3.8 \times 10^6$  to  $9.5 \times 10^7$  cfu.g<sup>-1</sup> and the mean carbaryl resistant bacterial load was  $1.8 \times 10^7$  cfu.g<sup>-1</sup>. A higher concentration of 0.1% of

carbaryl did not suppress the bacterial load in the samples collected from station 1.

Pesticide-resistant bacterial load in bittergourd growing fields: The results of the pesticide-resistant bacterial load of station 2 (Bittergourd growing fields of Manjoor) are represented in Table 2. In station 2 the bacterial load resistance to lindane varied from  $1.2 \times 10^5$  to  $9.6 \times 10^6$  cfu.g<sup>-1</sup> and the mean lindane resistant bacterial load was 3.64×10<sup>6</sup>cfu.g<sup>-1</sup>. In station 2 the growth of heterotrophic bacteria was arrested at 0.1% of lindane concentration. The methyl parathion-resistant bacterial load in station 2 varied from  $2.6 \times 10^6$  to  $2.5 \times 10^7$  cfu.g<sup>-1</sup> and the mean bacterial load was 1.11×10<sup>7</sup>cfu.g<sup>-1</sup>. The heterotrophic bacteria of station 2 could not tolerate a higher percentage of methyl parathion and hence no growth was noticed at a concentration of 0.1% pesticide. The carbaryl resistant bacterial load in station 2 ranged from  $0.4 \times 10^5$  to  $3.0 \times 10^7$  cfu.g<sup>-1</sup>. The mean pesticide-resistant load was  $8.45 \times 10^6$  cfu.g<sup>-1</sup>. The bacteria at station 2 could exhibit tolerance to carbaryl at all the three concentrations (0.001%, 0.01%, and 0.1%) studied.

**Pesticide-resistant bacterial load in snakegourd growing fields:** The results of the pesticide-resistant bacterial load of station 3 are represented in Table 3. The result indicates that in station 3 the heterotrophic bacteria could tolerate a pesticide concentration of 0.001% and 0.01% for lindane and methyl parathion, and for the pesticide carbaryl, it could tolerate all three concentrations (0.001%, 0.01%, and

Table 1: Load of bacteria from pea plant growing field capable of resisting various concentrations of Lindane, methyl parathion, and carbaryl pesticides.

Sampling months	Load of THB from station1 (Pea plant soil) tolerant to the pesticides										
		Lindane		М	ethyl parathion		Carbaryl				
	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%		
January	3.2×10 <sup>6</sup>	5.4×10 <sup>5</sup>	-	6.0×10 <sup>6</sup>	2.3×10 <sup>6</sup>	-	3.4×10 <sup>7</sup>	1.2×10 <sup>7</sup>	5.5×10 <sup>6</sup>		
February	4.8×10 <sup>5</sup>	6.2×10 <sup>5</sup>	-	9.3×10 <sup>6</sup>	$5.4 \times 10^{6}$	-	9.3×10 <sup>6</sup>	$8.7 \times 10^{6}$	6.3×10 <sup>6</sup>		
March	5.9×10 <sup>5</sup>	$2.4 \times 10^{6}$	-	$4.2 \times 10^{6}$	6.7×10 <sup>6</sup>	-	$1.7 \times 10^{7}$	$9.5 \times 10^{6}$	3.8×10 <sup>6</sup>		
April	1.0×10 <sup>6</sup>	8.6×10 <sup>5</sup>	-	9.8×10 <sup>6</sup>	2.9×10 <sup>6</sup>	-	2.0×10 <sup>7</sup>	9.2×10 <sup>7</sup>	$4.0 \times 10^{6}$		

Table 2: Load of bacteria from the Bittergourd growing field capable of resisting various concentrations of Lindane, methyl parathion, and carbaryl pesticides.

Sampling months	Load of THB from station 2 (Bittergourd soil) tolerant to the pesticides									
	Lindane			Methyl parathion			Carbaryl			
	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	
January	5.2×10 <sup>5</sup>	4.5×10 <sup>6</sup>	-	2.5×10 <sup>7</sup>	8.4×10 <sup>6</sup>	-	2.7×10 <sup>7</sup>	7.4×10 <sup>6</sup>	3.6×10 <sup>5</sup>	
February	9.6×10 <sup>6</sup>	6.0×10 <sup>6</sup>	-	9.4×10 <sup>6</sup>	$2.6 \times 10^{6}$	-	$1.2 \times 10^{7}$	$5.6 \times 10^{6}$	$2.8 \times 10^{5}$	
March	6.7×10 <sup>6</sup>	$1.2 \times 10^{5}$	-	$7.1 \times 10^{6}$	$1.5 \times 10^{7}$	-	3.0×10 <sup>7</sup>	$4.7 \times 10^{6}$	1.3×10 <sup>5</sup>	
April	8.5×10 <sup>5</sup>	8.4×10 <sup>5</sup>	-	$1.2 \times 10^{7}$	9.4×10 <sup>6</sup>	-	9.7×10 <sup>6</sup>	$6.2 \times 10^{6}$	$0.4 \times 10^{5}$	

0.1%) studied. In station 3 the lindane resistant bacterial load exhibited a variation from  $1.0 \times 10^6$  to  $6.0 \times 10^6$  cfu.g<sup>-1</sup>. The mean lindane-resistant bacterial load was  $3.5 \times 10^6$  cfu.g<sup>-1</sup>. The methyl parathion-resistant bacterial load fluctuated from  $5.3 \times 10^6$  to  $3.4 \times 10^7$  cfu.g<sup>-1</sup>. The mean pesticide-resistant bacterial load was  $1.19 \times 10^7$  cfu.g<sup>-1</sup>. In station 3 the carbaryl resistant bacterial load encountered varied from  $8.4 \times 10^5$  to  $1.2 \times 10^7$  cfu.g<sup>-1</sup>.  $5.22 \times 10^6$  cfu.g<sup>-1</sup> was the mean carbaryl-resistant bacterial load.

**Pesticide-resistant bacterial load in littlegourd growing fields:** The results of the pesticide-resistant bacterial load are represented in Table 4. In station 4 (Littlegourd soil), the heterotrophic bacteria could not tolerate a higher concentration (0.1%) of all the three pesticides, and the tolerance to lindane and methyl parathion at a concentration (0.01%) was expressed intermittently. In station 4, the lindane resistant bacterial load exhibited a variation from  $4.2 \times 10^5$  to  $2.8 \times 10^6$  cfu.g<sup>-1</sup>. The mean lindane-resistant bacterial load was  $1.01 \times 10^6$  cfu.g<sup>-1</sup>. The methyl parathion-resistant bacterial load showed a variation from  $8.7 \times 10^5$  to  $5.5 \times 10^6$  cfu.g<sup>-1</sup>. The mean methyl parathion-resistant bacterial load was  $3.04 \times 10^6$  cfu.g<sup>-1</sup>. The carbaryl-resistant bacterial load recorded a variation from  $7.4 \times 10^5$  to  $5.8 \times 10^6$  cfu.g<sup>-1</sup>. The mean carbaryl-resistant bacterial load was  $2.24 \times 10^7$  cfu.g<sup>-1</sup>.

**Pesticide-resistant bacterial load in cucumber growing fields:** The results of the pesticide-resistant bacterial load are represented in Table 5. The result indicates that in station 5

Table 3: Load of bacteria from the Snakegourd growing field capable of resisting various concentrations of Lindane, methyl parathion, and carbaryl pesticides.

Sampling months	Load of THB from station 3 (Snakegourd soil) tolerant to the pesticides										
		Lindane		M	lethyl parathion		Carbaryl				
	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%		
January	6.0×10 <sup>6</sup>	5.4×10 <sup>6</sup>	-	3.4×10 <sup>7</sup>	7.5×10 <sup>6</sup>	-	9.2×10 <sup>6</sup>	6.3×10 <sup>6</sup>	8.4×10 <sup>5</sup>		
February	2.3×10 <sup>6</sup>	$1.0 \times 10^{6}$	-	1.6×10 <sup>7</sup>	9.2×10 <sup>6</sup>	-	$1.2 \times 10^{7}$	$5.7 \times 10^{6}$	$2.0 \times 10^{6}$		
March	$3.6 \times 10^{6}$	$3.2 \times 10^{6}$	-	$7.5 \times 10^{6}$	5.3×10 <sup>6</sup>	-	$8.7 \times 10^{6}$	$2.9 \times 10^{6}$	9.7×10 <sup>5</sup>		
April	$2.9 \times 10^{6}$	3.8×10 <sup>6</sup>	-	8.9×10 <sup>6</sup>	6.8×10 <sup>6</sup>	-	9.3×10 <sup>6</sup>	$3.5 \times 10^{6}$	1.3×10 <sup>6</sup>		

Table 4: Load of bacteria from the Littlegourd growing field capable of resisting various concentrations of Lindane, methyl parathion, and carbaryl pesticides.

	Load of THB from station 4 (Littlegourd soil) tolerant to the pesticides										
Sampling months		Lindane		Ν	lethyl parathion		Carbaryl				
	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%		
January	4.2×10 <sup>5</sup>	-	-	$1.0 \times 10^{6}$	8.7×10 <sup>5</sup>	-	$5.8 \times 10^{6}$	7.4×10 <sup>5</sup>	2.5×10 <sup>5</sup>		
February	$7.5 \times 10^{5}$	6.0×10 <sup>5</sup>	-	$4.8 \times 10^{6}$	-	-	$3.2 \times 10^{6}$	9.6×10 <sup>5</sup>	4.0×10 <sup>5</sup>		
March	$2.8 \times 10^{6}$	-	-	$2.5 \times 10^{6}$	-	-	$2.5 \times 10^{6}$	2.3×10 <sup>6</sup>	1.7×10 <sup>5</sup>		
April	9.0×10 <sup>5</sup>	6.2×10 <sup>5</sup>	-	3.6×10 <sup>6</sup>	5.5×10 <sup>6</sup>	-	3.9×10 <sup>6</sup>	3.0×10 <sup>6</sup>	0.2×10 <sup>5</sup>		

Table 5: Load of bacteria from the Cucumber growing field capable of resisting various concentrations of Lindane, methyl parathion, and carbaryl pesticides.

Sampling months	Load of THB from station 5 (Cucumber growing soils) tolerant to the pesticides									
		Lindane		М	lethyl parathion		Carbaryl			
	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	
January	8.9×10 <sup>5</sup>	1.8×10 <sup>6</sup>	-	3.5×10 <sup>6</sup>	$1.4 \times 10^{6}$	-	6.5×10 <sup>6</sup>	2.0×10 <sup>6</sup>	4.7×10 <sup>5</sup>	
February	$1.4 \times 10^{6}$	7.5×10 <sup>5</sup>	-	2.8×10 <sup>6</sup>	9.6×10 <sup>5</sup>	-	$4.0 \times 10^{6}$	$1.8 \times 10^{6}$	2.9×10 <sup>5</sup>	
March	9.2×10 <sup>6</sup>	6.7×10 <sup>5</sup>	-	9.3×10 <sup>5</sup>	7.8×10 <sup>5</sup>	-	$1.4 \times 10^{6}$	3.2×10 <sup>6</sup>	2.2×10 <sup>5</sup>	
April	$0.5 \times 10^{6}$	9.8×10 <sup>5</sup>	-	$2.4 \times 10^{6}$	$1.0 \times 10^{6}$	-	3.1×10 <sup>6</sup>	$1.5 \times 10^{6}$	$0.5 \times 10^{5}$	

(Cucumber growing soils) the heterotrophic bacteria could tolerate a pesticide concentration of 0.001% and 0.01% for lindane and methyl parathion, and for the pesticide carbaryl it could tolerate all the three concentrations (0.001%, 0.01%, and 0.1%) studied. In station 5, the lindane resistant bacterial load varied from  $6.7 \times 10^5$  to  $6.7 \times 10^5$  cfu.g<sup>-1</sup> and the mean lindane resistant bacterial load was  $2.02 \times 10^6$  cfu.g<sup>-1</sup>. Methyl parathion bacterial load varied from  $7.8 \times 10^5$  to  $3.5 \times 10^6$  cfu.g<sup>-1</sup> with a mean population of  $1.72 \times 10^6$  cfu.g<sup>-1</sup>. The pesticide-resistant bacterial load exhibited a variation from  $1.5 \times 10^6$  to  $6.5 \times 10^6$  cfu.g<sup>-1</sup> with a mean population of  $3.19 \times 10^6$  cfu.g<sup>-1</sup>.

#### Pesticide-Resistant Bacterial Load in Agricultural Field Soils of Pea Plant (Without Pesticide Addition)

The results of the pesticide-resistant bacterial load are represented in Table 6. The result indicates that in station 6 (soils of pea plant without pesticide addition), the heterotrophic bacteria could not tolerate a pesticide concentration of 0.01% and 0.1% for all the three pesticides, lindane methyl parathion, and carbaryl. The lindane resistant bacterial load varied from  $3.7 \times 10^5$  to  $1.4 \times 10^6$  cfu.g<sup>-1</sup> and the mean lindane resistant bacterial load was  $8.7 \times 10^5$  cfu.g<sup>-1</sup>. Methyl parathion bacterial load fluctuated from  $2.0 \times 10^6$  to  $5.8 \times 10^6$  cfu.g<sup>-1</sup> with a mean population of  $3.32 \times 10^6$  cfu.g<sup>-1</sup>. The pesticide-resistant bacterial load exhibited a variation from  $2.9 \times 10^6$  to  $5.3 \times 10^6$  cfu.g<sup>-1</sup>.

## Spatial Variation of Pesticide-Resistant Bacterial Load in Different Crops

Spatial variation of pesticide-resistant bacterial load in different crops is represented in Fig 1, 2, 3. and 4. There is a significant difference in bacterial resistance to lindane and methyl parathion pesticides in different crops (P<0.05) and between concentrations (P<.01). Stations 2 (bitter gourd soils) and 3 (snake gourd soils) are having significantly higher lindane and methyl parathion-resistant bacterial loads compared to stations 1, 4, 5, and 6. Between stations 2 and 3, the difference in Total Heterotrophic Bacteria (THB) is not significant. 1% concentration is having significantly lower lindane and methyl parathion-resistant bacterial load compared to 0.01% and 0.1%. There is a significant difference in THB between crops (P>0.05) and no significant difference in concentrations (P>0.05) when the pesticide carbaryl was applied. Station 1 (pea plant soil) has a significantly higher carbaryl-resistant bacterial load compared to other stations.

#### Comparison of Pesticide-Resistant Bacterial Loads With Culturable Heterotrophic Bacterial Loads of Each Sampling Station

The results of the comparison of pesticide-resistant bacterial loads with culturable heterotrophic bacterial loads are represented in Fig. 5. The pesticide tolerant bacterial load was comparatively lower than the culturable bacterial loads of each sampling station.

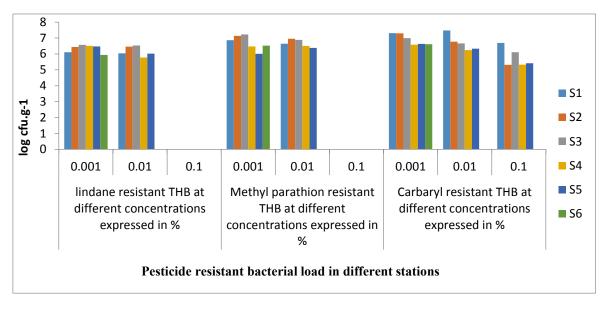


Fig 1: Spatial variation in a load of pesticide-resistant bacteria from different agricultural field soils of Manjoor.

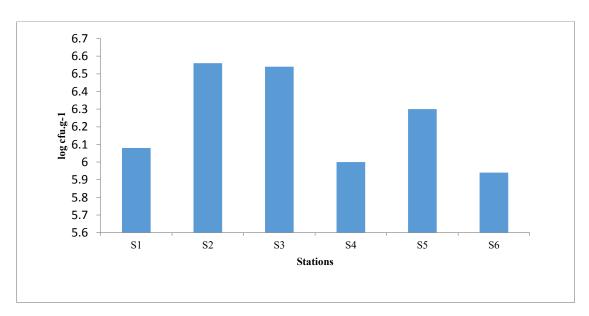


Fig. 2: Spatial variation of lindane resistant bacteria at different agricultural field soils of Manjoor.

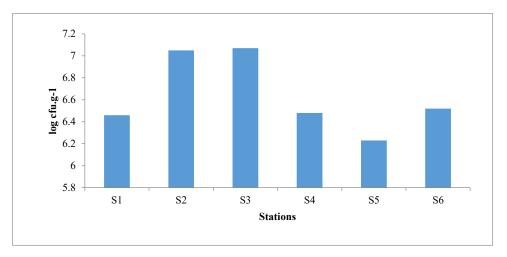


Fig 3: Spatial variation of methyl parathion-resistant bacteria at different agricultural field soils of Manjoor.

Table 6: Load of bacteria from the Pea plant growing field (without pesticides), capable of resisting various concentrations of Lindane, methyl parathion, and carbaryl pesticides.

Sampling months	Load of THB from station 6 (pea plant without pesticide addition) tolerant to the pesticides								
		Lindane		М	ethyl parathior	1	Carbaryl		
	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%
January	3.7×10 <sup>5</sup>	-	-	5.8×10 <sup>6</sup>	-	-	$2.9 \times 10^{6}$	-	-
February	9.2×10 <sup>5</sup>	-	-	$2.4 \times 10^{6}$	-	-	3.6×10 <sup>6</sup>	-	-
March	$1.4 \times 10^{6}$	-	-	$2.0 \times 10^{6}$	-	-	$4.7 \times 10^{6}$	-	-
April	7.8×10 <sup>5</sup>	-	-	3.1×10 <sup>6</sup>	-	-	5.3×10 <sup>6</sup>	-	-

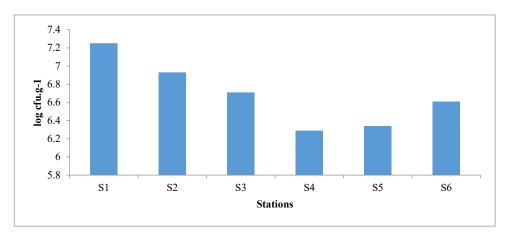


Fig 4: Spatial variation of carbaryl resistant bacteria at different agricultural field soils of Manjoor.

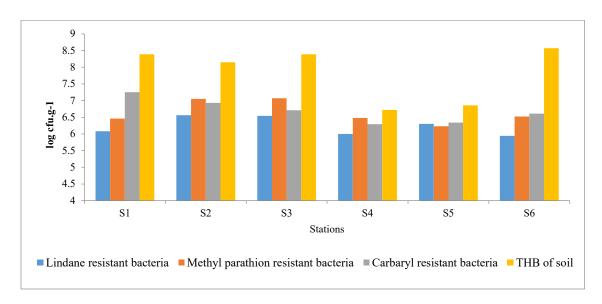


Fig. 5. Relative load of pesticide-resistant bacterial loads with that of total culturable heterotrophic bacterial loads of various agricultural field soils of Manjoor.

### DISCUSSION

The result of the heterotrophic bacteria capable of resisting pesticides indicated that pesticides used in Manjoor affected the total numbers of heterotrophic bacteria and the observed effects are strongly related to pesticide type and its dosage. The results indicate that at a small dosage the pesticides did not show any characteristic effect on the growth of the organisms while the higher dosage of 0.1% suppressed the growth of the organisms when lindane and methyl parathion were given as the sole carbon source. Similar results were observed when Singh (2001) conducted an in vitro study with endosulfan and nivar. When the dosage of pesticide was increased to 0.1% and 1% the growth of bacteria was arrested.

The results when compared to normal soils showed that pesticides exhibited an inhibitory effect on the heterotrophic bacteria of soils collected from different agricultural fields at various doses. Busse et al. (2001) also observed a decrease in the total number of heterotrophic bacteria after glyphosate application. In contrast, an increase in the total number of soil microorganisms in the field, as a response to pesticide application was observed in some studies (Monkiedje & Spiteller 2002, Amal et al. 2005). Hence the results obtained showed that pesticides depending on type and dosages might decrease or increase the microbial counts.

An organic substance of any kind cannot escape the onslaught of microbial degradation, insecticides and pesticides are no exception. Pesticide residues are generally degraded and degradation products are assimilated by soil microorganisms (Rache & Coats 1988). This phenomenon of decrease or increase in the microbial counts could be expected as a result of the utilization of applied pesticides as a source of carbon, energy, and other nutrient elements by some soil microorganisms (Bhuyan et al. 1993) resulting in either proliferation or decrease in the number of organisms.

The load of the pesticide-resistant population in the soil samples incorporated with lindane was very low compared to pesticide-resistant populations of methyl parathion and carbaryl. The results indicate that the load of the pesticide-resistant population is dependent on the nature of pesticides and also is field-dependent. The result also indicated that they are able to utilize all the three pesticides at lower concentrations, although at varying degrees, and in our observations, 0.1% concentration of the pesticide suppressed the growth of heterotrophic bacteria in the soils of most of the cultivating fields.

When pesticides (lindane, methyl parathion, and carbaryl) were added to sample 6 (pea plant without pesticides), the heterotrophic bacteria could only tolerate very low concentrations of pesticides. The explanation for this could be that in soils where there have been no previous applications of the same pesticide or another pesticide with a similar chemical structure, heterotrophic bacteria will not be able to degrade or utilize the compound at an accelerated or enhanced rate (Arbeli & Fuentes 2007).

#### CONCLUSION

The pesticide residues in the agricultural field soil can give the required selective pressure for the emergence of pesticide-resistant microorganisms. The load of the pesticide-resistant population in the soil samples incorporated with lindane was very low compared to pesticide-resistant populations of methyl parathion and carbaryl. The lindane and methyl parathion-resistant bacterial load were high in snake gourd and bitter gourd areas while the carbaryl resistant bacterial load was high in pea plant growing area.

Results of this study indicated that heterotrophic bacteria can withstand lower concentrations of the pesticides lindane and methylparathion (0.01% and 0.001%), while a higher concentration of carbaryl (0.1%) could only be tolerated. When the pesticides (lindane, methyl parathion, and carbaryl) were added to sample 6 (pea plant soil where there was no addition of pesticides), the heterotrophic bacteria could only tolerate very low concentrations of pesticides. The results of the mean pesticide-resistant bacterial load, when compared to normal THB of soils, indicate that pesticides exhibited an inhibitory effect on the heterotrophic bacteria of soils collected from different agricultural fields and the

pesticide-resistant bacterial load was lower than normal THB.

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