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Residual effect of chlorpyriphos and monocrotophos on soil bacteria and growth of earthworm

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Abstract
Incresed demand for food to sustain the ever increasing world population has led to massive increase in industrial and agricultural activities. Modern agricultural practices also increased the usage of pesticides and fertilizers. The indiscriminate usage of pesticides may lead to soil contamination. About 99% of applied pesticides are not reached to the target organism and persist in the environment for a long time. Soil samples were collected from five intensive vegetable growing area of Ottanchathram Block. Conduted pot culture study to monitor survival percentage, weight loss of earthworm, soil bacterial population and microbial activity for six weeks. The concentration of chlorpyriphos ranged from 0.04 mgkg^{-1} to 0.1 mgkg^{-1} and maximum (0.1mgkg^{-1}) was recorded in the Cauliflower field of Thasarapatti and Arasappapillaipatti village. The result revealed that.th survival and weight loss was maximum in soil samples collected from Cauliflower field when compared to Bhendi and Chilli field. The observation taken on first week showed 63% reduction in weight and then weight loss is very slow from second week. At the end of sixth week 40% reduction was observed. Village of Veeralapatti soils showed 60% reduction in Cauliflower, Brinjal, Bhendi and Chilli field samples. The CO_2 production during first and 6th week of incubation by 17% and 38% respectively. The most adverse effect was seen with soil contaminated with chlorpyriphos when compared to monocrotophos.

Keywords: vegetable field, soils, chlorpyriphos, monocrotophos, effect, earthworm, soil bacteria, microbial activity

Introduction

Protection of the soil habitat is the first step towards sustainable management of its biological properties that determine long-term quality and productivity. The Soil macrofauna, such as EWs, modify the soil and litter environment indirectly by the accumulation of their biogenic structures (casts, pellets, galleries, etc.). The cycling of nutrients is a critical ecosystem function that is essential to life on earth. At present, there is increasing evidence that soil macro invertebrates play a key role in Soil Organic Matter (SOM) transformations and nutrient dynamics at different spatial and temporal scales through perturbation and the production of biogenic structures for the improvement of soil fertility and land productivity (Beeby, 2001) [2]. EWs are a major component of soil fauna communities in most natural ecosystems of the humid tropics and comprise a large proportion of macrofauna biomass. In cultivated tropical soils, where organic matter is frequently related to fertility and productivity, the communities of invertebrates—especially EWs—could play an important role in (SOM) dynamics by the regulation of the mineralization and humification processes (Sandal *et al*., 2001) [26].

A greater proportion (>80%) of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality (Sorour and Larink, 2001) [28]. The suitability of earthworms as bioindicators in soil toxicity is largely due to the fact that they ingest large quantity of the decomposed litter, manure, and other organic matter deposited on soil, helping to convert it into rich topsoil (Sandal *et al*., 2001) [26]. Moreover, studies have shown that earthworm skin is a significant route of contaminant uptake (Lord *et al*. 1980) [13] and thus investigation of earthworm biomarkers in the ecological risk assessment (ERA) can be helpful (Hernandez, 2006) [25]. *Eisenia fetida* is the standard test organism used in terrestrial ecotoxicology, because it can be easily bred on a variety of organic wastes with short generation times [OECD 1984). Its susceptibility to chemicals resembles that of true soil organisms.

Soil microbes metabolize recalcitrant forms of soil-borne nutrients to liberate these elements for plant nutrition. In natural ecosystems, most nutrients such as N, P, and S are bound in organic molecules and are therefore minimally bioavailable for plants. To access these nutrients, plants are dependent on the growth of soil microbes such as bacteria and fungi, which possess the metabolic machinery to depolymerize and mineralize organic forms of N, P, and S. It has been long recognized that some of these microbes, such as mycorrhizal fungi or nitrogen fixing symbiotic bacteria, play important roles in plant performance by improving mineral nutrition. (Jacoby *et al*., 2017).

Sustanable management of soil, environmental stability, bioaccumulation, and toxicity to non-target species have brought about the restricted use of some organochlorine insecticides; these properties have also led to many studies being carried out on the microbiological breakdown of these pesticides, as reviewed by Lal and Saxena 1982 [14] and Motosugi and Soda 1988 [15]. Catabolism and detoxification metabolism occur when a soil microorganism uses the pesticide as a carbon and energy source. The latter process is facilitated by resistant microorganisms. The reduced persistence of organophosphate insecticides attributed to soil microorganisms has been described by Chapman and Harris 1982 [5], Gorder *et al*. 1982 [10] and Sharmila *et al*., 1989 [27]. The degradation of xenobiotic compounds by members of the soil microflora is an important means by which these compounds are removed from the environment, thus preventing them from becoming pollution problems. Much work has been directed towards understanding the complexity of pesticide-microbial interactions in soil. Microbial communities composed of several species are more likely to cause pesticide biodegradation in soil and rhizosphere environments than are single species. Pesticides applied to soil at planting should persist during the development of plant roots. Therefore, a portion of the pesticide probably interacts with microorganisms in the soil and rhizosphere The biodegradation of organophosphorus insecticides by microorganisms in soil has been widely reported, however, the effects of organophosphorus pesticides on soil microorganisms has received less attention. Hence, the present study aimed to assess the effect of chlorpyriphos and monocrotophos on soil bactera and growth of earthworm in soil samples collected from intensive vegetable growing area of ottanchathiram block

Materials and Methods

Study Area

The study was taken in the Oddanchatram village; It is a town in Dindigul district in the Indian state of Tamil Nadu. Oddanchatram is a region in the southwest of Tamil Nadu. Oddanchatram is also famous for vegetable and cattle market. It is located at the base of the Western Ghats in South India. It is known as vegetable city of Tamil Nadu. Oddanchatram vegetable market is the largest supplying of vegetables in Tamil Nadu and Kerala. Agriculture is the major economic support for the town. Region.

Five intensive vegetable growing villages, Thasaraipatti, Veeralapatti, Ambilikai, Arasappapillaipatti and Vadakadu was selected to test the residual effect of pesticides. Soil samples were collected immediately after pesticide treatment from Cauliflower, Brinjai, Bhendi and Chilli growing field randomly at depth 0–20 cm with a soil auger and put together to form a composite sample. The composite soil samples were well mixed and three soil replicates were collected from each vegetable growing village. All soil samples were transported to the pots in the laboratory to test the residual effect of chlorpyriphos and monocrotophos on earthworm and soil bacteria. The portion of soil samples were air dried and sieved using 2 mm nylon mesh and sieved soils was taken for pesticide residue analysis.

Extraction of pesticide residues from soil samples

Ten gram (10 g) of the representative soil samples were weighed and quantitatively transferred into 250 mL separating flasks. A 10 mL of acetonitrile was added to each of the soil samples in the flasks and ultra-sonicated for 5 min. An additional 10 mL of acetonitrile was added, and the flasks closed tightly. The samples were placed on a horizontal mechanical shaker and set to shake continuous for 30 min at 300 rpm. The contents were then allowed to stand for 10 min to sufficiently separate the phases or layers. A 10 mL of the supernatants were carefully taken by pipette and dried over 2 g anhydrous magnesium sulphate through filter paper into 50 mL round bottom flasks. The concentrates were then adjusted to about 2 mL using the rotary film evaporator at 35 °C, and made ready for silica clean up step.

Clean up procedure for soil samples

Extracts clean up were done, using polypropylene. cartridge columns, packed with one-gram silica gel previously activated for 10 h in an oven at 130 °C, which have 1 cm thickness layer of anhydrous magnesium sulphate on top and conditioned with 6 mL acetonitrile. The concentrated extracts were then loaded onto the columns/ cartridges, and 50 mL pear shape flasks placed under the columns to collect the eluates. A 10 mL acetonitrile was used to elute the columns/cartridges afterwards. The total filtrates (eluents) collected were concentrated to dryness using the rotary evaporator set at 40 °C. The residues were re-dissolved in 1 mL ethyl acetate by pipetting and transferred into 2 mL standard opening vials prior to quantitation by gas chromatography (GC).

GC-MS Analysis

The Gas Chromatography - Mass Spectrometer from Thermo fisher, Trace-1300 series, were engaged for analysis. The instrument was set as follows, Injector port temperature set to 220 ºC, Interface temperature set as 250 ºC, source kept at 220 ºC. The oven temperature programmed as available, 75 ºC for 2 mins, 150 ºC at 10 ºC min^{-1}, up to 250 ºC at 10 ºC min^{-1}. Split ratio set as1:12 and the injector used was splitless mode. The DB-5 MS capillary standard non - polar column was used whose dimensions were 0.25mm OD x 0.25µm ID x 30 meters length procured from Agilent Co., USA.Helium was used as the carrier gas at 1.5 mL min^{-1}. The MS was set to scan from 50 to 550 of ion source. The source was maintained at 220 ºC and $4.5e^{-6}$ mtorr vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre- filter which reduced the neutral particles. The data system has inbuilt libraries for searching and matching the spectrum.

Effect of chlorpyriphos and monocrotophos residues on earth worm

Eisenia fetida is a favorite worm species for composting and is frequently used as a testing organism for biological monitoring of contaminants on soil biota. Earth worm:

Eisenia fetida was collected from S.S vermicompost unit Vadipatti, Madurai and grown in partially degraded kitchen waste collected from AC & RI, Madurai in a bucket. Earth worms are allowed to grow for a week time to attain considerable size. Ten numbers of earthworms are introduced into the soil and survival percentage of Earth worm population, weight loss and coiling behaviour was monitored at weekly intervals (upto six weeks).

Effect of chlorpyriphos and monocrotophos residues on soil bacteria

Soil sample preparation and Experimental design

Soil samples were collected from Cauliflower, Brinjal, Bhendi and Chilli field and 200 gm of sieved soil was placed in 250 ml flask replicated three times. The moisture content of soil was got to 60% water holding capacity. Distilled water was added to maintain them 60% of WHC. Ten ml of 2M sodium hydroxide solutions was placed in a glass tube and put the tube gently on soil surface in each flask. The flasks were closed well with rubber stoppers to avoid any gaseous exchange between the flasks and outside atmosphere. A blank, in three replicate, was also done to account for the quantity of CO_2 already present in the flask's atmosphere. The flasks were incubated at a temperature of 30ºC for 6 weeks. CO_2 production was measured at weekly intervals,

Measurement of microbial activity

Activities of microorganisms were determinded in the form of carbon dioxide production according to Anderson *et al*., 1982. The glass tube was gently got out of flask weekly and the sodium hydroxide solution was transferred to clean flask. For following incubation fresh sodium hydroxide solution was put in clean glass tube and placed in the same flask and it is gave back to the incubator. The process was repeated at the end of an each previous incubation period. After addition 10ml of 1M barium chloride solution and drops of phenolphthalein, to the transferred sodium hydroxide solution and titrated against 1 M hydrochloric acid solution. During the reaction one mole of carbon dioxide equalize two moles of sodium hydroxide. The quantity of released CO_2 was adjusted as mg CO_2/100g soil $2NaOH + CO_2$ Na_2CO_3 $Na_2CO_3 + BaCl_2$ $2NaCl + BaCO_3$

Microbial analysis -Assessing microbial population

Sstandard plate count methods were used to prepare nutrient agar (NA) for assessment of the bacteria population, One gram each of the soil samples were measured into the test tube containing 9 ml sterile distilled water and serially diluted to dilution factor 10^5 and 1 ml of the proper dilutions was pipette into sterile plate with appropriate medium which were incubated at 30 ˚C. All plates were incubated inverted wise. Microbial counted were done at 48 hours for NA.

Result and Discussion

Effect of chlorpyriphos and Monocrotophos residues on survival percentage and weight of *Eisenia foetida*

The five villages, Thasaraipatti, Veeralapatti, Ambilikai, Arasappapillaipatti, Vadakadu, are selected to study the residual effect of chlorpyriphos and monocrotophos on soil bacteria and growth of earthworm. The concentration of chlorpyriphos ranged from 0.04 $mgkg^{-1}$ to 0.1 $mgkg^{-1}$ and maximum (0.1 $mgkg^{-1}$) was recorded in the Cauliflower field of Thasarapatti and Arasappapillaipatti village. Twenty numbers of earthworms are introduced into the pot and observed the survival rate and weight loss at weekly intervals. The effect of chlorpyriphos on survival percentage of earth worm and weight loss is presented in the Table 1 and 2. In the first week, the numbers reduced from 20 to 15 and then there is a slight reduction in numbers from 15 to 12 on second week and from third week to the end, the reduction was 50%. In Cauliflower field. The soil sample collected from Chilli (14) and Bhendi (12) field showed maximum number of population and 40% of reduction was observed in Chilli field, when compared to Cauliflower field samples in all the villages at the end of sixth week. (Table 1). There wont be any any significant changes in survival percentage was not observed among the different concentrations. This may be due to continuous application of pesticides and it may persist in the soil for longer period to affect the population of earthworm.

Table 2 showed the weight loss of introduced earthworm. The observation taken on first week showed weight loss of introduced earthworm. The observation taken on first week showed 63% reduction in weight and then weight loss is very slow from second week. At the end of sixth week 40% reduction was observed. Village of Veeralapatti soils showed 60% reduction in Cauliflower, Brinjal, Bhendi and Chilli field samples.

Table 1: Average Concentration of Chlorpyriphos in soils of Ottanchathiram Block and effect on survival perccentage of Earthworm

Villages	Vegetables	Chlorpyriphos $mgkg^{-1}$	Survival (Numbers)						
			Weeks						
			0	1	2	3	4	5	6
Thasaraipatt	Cauliflower	0.10	20	15	12	10	10	10	10
	Brinjal	0.08	20	16	14	12	12	12	12
	Bhendi	0.08	20	18	16	16	14	14	14
	Chilli	0.06	20	18	16	16	14	14	14
Veeralapatti	Cauliflower	0.09	20	14	13	12	11	11	11
	Brinjal	0.07	20	15	12	12	11	11	11
	Bhendi	0.06	20	16	13	13	13	11	11
	Chilli	0.05	20	16	14	13	13	13	12
Ambilikai	Cauliflower	0.09	20	15	13	12	12	11	11
	Brinjal	0.08	20	16	13	12	12	11	11
	Bhendi	0.08	20	18	15	13	13	13	13
	Chilli	0.04	20	18	16	15	14	14	14
Arasappapillaipatti	Cauliflower	0.10	20	14	12	11	10	10	10
	Brinjal	0.09	20	16	13	12	11	11	11
	Bhendi	0.07	20	16	14	13	13	13	13
	Chilli	0.05	20	18	16	14	14	14	13

Vadakadu	Cauliflower	0.09	20	14	12	12	12	12	10
	Brinjal	0.07	20	16	14	14	12	12	11
	Bhendi	0.09	20	16	14	14	14	13	13
	Chilli	0.05	20	18	17	16	16	15	15

Table 2: Average Concentration of Chlorpyriphos in soils of Ottanchathiram Block and weight loss of Earthworm

Villages	**Vegetables**	**Chlorpyriphos mgkg^{-1}**	**Weight loss (g)**						
			Weeks						
			0	**1**	**2**	**3**	**4**	**5**	**6**
Thasaraipatt	Cauliflower	0.10	4.3	2.8	2.3	2.0	1.8	1.8	1.8
	Brinjal	0.08	4.5	3.0	2.2	2.0	2.0	2.0	2.0
	Bhendi	0.08	4.8	2.8	2.3	2.0	2.0	2.0	2.0
	Chilli	0.06	4.5	2.5	2.1	2.0	2.0	2.0	2.0
Veeralapatti	Cauliflower	0.09	4.2	2.2	2.1	1.8	1.8	1.8	1.8
	Brinjal	0.07	4.1	1.9	1.9	1.8	1.8	1.8	1.8
	Bhendi	0.06	3.8	1.9	1.9	1.9	1.9	1.8	1.8
	Chilli	0.05	4.3	2.5	2.1.	1.9	1.9	1.8	1.8
Ambilikai	Cauliflower	0.09	4.1	2.0	1.8	1.7	1.7	1.7	1.7
	Brinjal	0.08	4.3	2.2	2.0	1.9	1.9	1.9	1.9
	Bhendi	0.08	4.5	2.8	2.0	2.0	2.0	2.0	2.0
	Chilli	0.04	4.6	2.3	2.0	2.0	1.9	1.9	1.9
Arasappapillaipatti	Cauliflower	0.10	4.5	2.2	2.0	1.9	1.9	1.8	1.8
	Brinjal	0.09	4.3	2.2	2.0	2.0	2.0	1.8	1.8
	Bhendi	0.07	4.3	2.6	2.2	2.0	2.0	1.9	1.9
	Chilli	0.05	4.3	2.5	2.0	2.0	2.0	2.0	2.0
Vadakadu	Cauliflower	0.09	4.5	2.5	2.0	1.8	1.7	1.7	1.7
	Brinjal	0.07	4.5	2.3	2.0	2.0	1.9	1.9	1.9
	Bhendi	0.09	4.5	2.5	2.0	2.0	1.9	1.9	1.9
	Chilli	0.05	4.5	2.8	2.5	2.2	2.0	2.0	2.0

Table 3 showed monocrotophos residues in five intensive vegetable growing villages. The concentrations of monocrotophos ranged from 0.02 to 0.06 mgkg^{-1}. The concentrations of monocrotophos are very less, when compared Chlorpyriphos. First week observation showed 25% reduction and at the end of sixth week the reduction was 40%. In Cauliflower field. The population in Bhendi, Brinjal and Chilli field soils of Vadakadu and Arasappapillaipatti villages showed only 30-35%reduction in population.

Table 3: Average Concentration of Monocrotophos in soils of Ottanchathiram Block and effedct on survival of Earthworm

Villages	**Vegetables**	**Monocrotophos mgkg^{-1}**	**Survival (numbers)**						
			Weeks						
			0	**1**	**2**	**3**	**4**	**5**	**6**
Thasaraipatt	Cauliflower	0.05	20	16	14	14	14	14	14
	Brinjal	0.04	20	17	17	17	16	16	16
	Bhendi	0/04	20	18	18	17	17	16	16
	Chilli	0.02	20	19	18	17	17	17	17
Veeralapatti	Cauliflower	0.06	20	16	15	13	13	13	13
	Brinjal	0.04	20	17	16	16	15	15	15
	Bhendi	0.03	20	17	15	15	15	14	14
	Chilli	0.03	20	18	17	16	16	16	16
Ambilikai	Cauliflower	0.06	20	15	13	12	12	12	12
	Brinjal	0.04	20	16	15	15	14	14	14
	Bhendi	0.03	20	16	16	16	15	15	15
	Chilli	0.02	20	17	16	16	16	15	15
Arasappapillaipatti	Cauliflower	0.05	20	14	13	12	12	12	12
	Brinjal	0.04	20	16	15	15	14	14	14
	Bhendi	0.03	20	17	15	14	14	13	13
	Chilli	0.02	20	18	17	16	15	15	15
Vadakadu	Cauliflower	0.05	20	13	12	11	10	10	10
	Brinjal	0.03	20	17	16	15	15	15	15
	Bhendi	0.03	20	17	16	16	16	15	15
	Chilli	0.03	20	18	17	17	16	16	16

Table 4 showed weight loss of introduced earthworm in soils samples collected from intensive vegetable growing area. During the first week of observation the weight loss is 34% and further it weight loss was 47% at the end of sixth week in Cauliflower field. Weight loss was very less in Chilli field (33%) at sixth week of observation, when compared to other crops.

Table 4: Average Concentration of Monocrotophos in soils of Ottanchathiram Block and weight loss of Earthworm

Villages	Vegetables	Monocrotophos $mgkg^{-1}$	Weight loss (g) Weeks						
			0	1	2	3	4	5	6
Thasaraipatt	Cauliflower	0.05	4.8	3.2	3.0	2.8	2.5	2.5	2.3
	Brinjal	0.04	4.6	3.5	3.2	3.0	2.8	2.5	2.0
	Bhendi	0/04	5.1	4.6	4.3	4.1	4.0	3.8	3.5
	Chilli	0.02	4.7	4.0	3.7	3.5	3.2	3.2	3.2
Veeralapatti	Cauliflower	0.06	4.6	4.0	3.8	3.5	3.0	2.8	2.8
	Brinjal	0.04	4.5	4.3	4.1	4.0	3.8	3.7	3.5
	Bhendi	0.03	4.5	4.2	4.0	3.8	3.5	3.4	3.4
	Chilli	0.03	4.2	4.0	3.5	3.3	3.0	2.9	2.9
Ambilikai	Cauliflower	0.06	4.6	4.3	4.0	3.8	3.5	3.3	3.2
	Brinjal	0.04	4.2	3.4	3.2	3.2	2.9	2.8	2.8
	Bhendi	0.03	4.8	4.3	4.0	3.8	3.3	2.8	2.3
	Chilli	0.02	5.0	4.2	3.7	3.2	3.0	2.8	2.8
Arasappapillaipatti	Cauliflower	0.05	4.3	3.0	2.5	2.0	2.0	1.8	1.8
	Brinjal	0.04	4.6	4.2	3.8	3.2	3.0	2.8	2.8
	Bhendi	0.03	4.6	4.2	3.8	3.4	3.0	3.0	3.0
	Chilli	0.02	3.6	3.1	2.9	2.7	2.7	2.7	2.7
Vadakadu	Cauliflower	0.05	4.2	3.8	3.3	3.0	2.5	2.0	2.0
	Brinjal	0.03	4.1	3.8	3.3	2.9	2.9	2.7	2.7
	Bhendi	0.03	4.5	4.2	4.0	3.9	3.7	3.3	3.0
	Chilli	0.03	4.8	4.3	4.0	3.8	3.4	3.2	3.2

Negative impact of pesticides on earthworm growth has been reported by various researchers. Xiao *et al*. 2006 [32] suggested that growth can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms. Helling *et al*., 2000 [11] tested in laboratory the effect of copper oxychloride, while Yasmin and D'Souza 2007 investigated the impact of carbendazim, glyphosate and dimethoate on *Eisenia fetida* and found a significant reduction in the earthworm growth in a dose-dependent manner. According to Van Gestel *et al*., 1992 [29] parathion affects the growth of *Eisenia andrei*. Booth *et al*. 2000 [3] studied the effect of two organophosphates, chlorpyrifos and diazinon, while Mosleh *et al*., 2003 [17] investigated the toxicity of aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine, and metalaxyl in the earthworm *Aporrectodea caliginosa* and observed a reduction in growth rate in all pesticide-treated worms. Mosleh *et al*. 2003 [17] studied the effects of endosulfan and aldicarb on *Lumbricus terrestris* and have suggested growth rate as important biomarkers for contamination by endosulfan and aldicarb. Zhou *et al*., 2007 [34] assessed and found chlorpyrifos had adverse effect on growth in earthworm exposed to 5 mg/kg chlorpyrifos after eight weeks. Some studies have shown that growth of earthworms appeared to be more severely affected at juvenile stage than at adult stage

Effect of chlorpyriphos and monocrotophos residues on Soil bacteria and microbial activity

Soil samples collected from five intensive vegetable growing (Cauliflower, Brinjal, Bhendi and Chilli) villages and analysed for soil bacterial population at weekly intervals. Table 5 shows the decreased the count of bacteria in all the villages and at all incubation periods. In the first week of incubation period, the population was reduced from 50.6 CFU $x10^5$ to 43.28 CFU $x10^5$and at the end of sixth week the population was 30.12 CFU $x10^5$. In Cauliflower field of Thasarapatti villlage. Almost similar trend was observed in all the five villages Cauliflower field. Soil bacterial population in Bhendi and Chilli field was high when compared to Brinjal and Cauliflower.

Table 6 shows the effect of monocrotophos residues on soil bacterial population in five vegetable growing villges. The initial population was high (60.5 CFU $x10^5$) when compared to chlorpyriphos field samples. As observed in chlorpyriphos contaminated samples, the maximum reduction in soil bacterial population was observed in Cauliflowers field samples. Tbacterial population in Bhendi and Chilli field samples are recorede high population.

This results are consistent with (Newman *et al.*, 2016; Aralujo *et al.*, 2003) [21, 1] who concluded that the presence of glyphosate decreased the number of bacteria, microbial biomass and acidobacteria population. They believed the reduction in the bacteria population for a long time could weaken some biogeochemical reactions accomplished by these microorganisms. (Grossbard and Atkinson, 1985) reported that the toxic effects of pesticides as result of inhibition of amino acid synthesis via the shikimic acid pathway. Similar results were observed by (Goswami *et al.*, 2013; Wesley *et al.*, 2017) [9, 30] who reported that the decrease in the soil microbial count and biomass can be associated with the toxic effect of Cypermethrin on soil microorganisms. The presence of Cypermethrin and thiamethoxam inhibited the metabolic process and significantly decreased ammonifying, nitrifying and denitrifying bacteria compared to the untreated sample (Nicoleta *et al.*, 2015) [22]. These results agree with (Haleem *et al.*, 2013) [12] who concluded the presence of malathion significantly decreased the population of bacteria.

Table 5: Average Concentration of Chlorpyriphos in soils of Ottanchathiram Block and effect on soil bacterial population

Villages	Vegetables	Chlorpyriphos mgkg^{-1}	Soil bacterial population (CFUx10^5/g of soil)						
			Weeks						
			0	1	2	3	4	5	6
Thasaraipatt	Cauliflower	0.10	50.5	43.2	40.5	39.3	35.4	33.0	30.1
	Brinjal	0.08	55.3	47.2	42.1	40.1	27.2	35.2	32.1
	Bhendi	0.08	59.4	52.0	49.3	47.5	45.2	42.2	39.3
	Chilli	0.06	60.7	55.2	50.1	46.2	43.1	40.1	35.6
Veeralapatti	Cauliflower	0.09	56.1	45.1	40.1	36.1	32.3	30.5	29.1
	Brinjal	0.07	60.1	55.2	50.6	48.1	43.2	40.2	39.4
	Bhendi	0.06	62.3	57.1	53.2	50.1	47.3	43.6	40.1
	Chilli	0.05	73.2	68.1	63.2	59.1	53.2	49.2	47.2
Ambilikai	Cauliflower	0.09	50.5	45.3	42.5	40.3	39.4	38.1	38.1
	Brinjal	0.08	53.2	49.2	45.3	42.1	40.1	39.3	39.0
	Bhendi	0.08	55.1	50.1	48.3	45.1	44.1	40.1	39.1
	Chilli	0.04	65.2	58.1	54.1	48.1	43.1	40.1	38.1
Arasappapillaipatti	Cauliflower	0.10	59.2	55.1	50.1	47.1	45.1	42.1	40.1
	Brinjal	0.09	73.2	68.1	63.1	58.1	52.4	48.1	43.1
	Bhendi	0.07	75.1	63.2	60.1	58.1	50.1	47.1	43.1
	Chilli	0.05	79.2.	71.2	65.1	60.1	58.1	52.1	49.1
Vadakadu	Cauliflower	0.09	55.2	50.1	49.1	47.2	45.2	40.1	40.1
	Brinjal	0.07	59.12	53.2	50.2	46.1	43.1	40.1	39.1
	Bhendi	0.09	60.1	57.1	50.2	47.2	43.2	39.1	39.1
	Chilli	0.05	65.3	54.1	43.2	49.5	43.2	40.2	39.1

Table 6: Average Concentration of Monocrotophos in soils of Ottanchathiram Block and effedct on soil bacterial population

Villages	Vegetables	Monocrotophos mgkg^{-1}	Soil bacterial population (CFUx10^5/g of soil)						
			Weeks						
			0	1	2	3	4	5	6
Thasaraipatt	Cauliflower	0.05	60.5	50.2	45.5	40.3	38.4	35.0	32.1
	Brinjal	0.04	62.3	52.2	48.1	45.1	40.4	38.2	35.1
	Bhendi	0/04	69.2	62.0	59.3	57.0	53.2	48.0	43.3
	Chilli	0.02	70.7	65.2	60.1	56.2	50.1	45.2	40.6
Veeralapatti	Cauliflower	0.06	63.1	55.5	50.2	46.8	43.2	38.5	30.8
	Brinjal	0.04	65.2	60.8	55.0	50.8	48.2	45.0	40.5
	Bhendi	0.03	67.6	63.8	60.0	57.8	52.0	49.0	45.2
	Chilli	0.03	75.5	73.2	68.4	63.2	60.0	55.1	50.3
Ambilikai	Cauliflower	0.06	55.3	50.2	48.6	45.3	42.5	39.2	35.2
	Brinjal	0.04	58.0	53.0	50.3	47.5	43.8	40.0	33.0
	Bhendi	0.03	60.2	55.2	53.2	50.2	47.0	45.5	40.2
	Chilli	0.02	67.8	62.2	58.2	53.4	48.0	45.2	42.2
Arasappapillaipatti	Cauliflower	0.05	69.4	63.2	58.5	53.2	50.8	47.5	43.2
	Brinjal	0.04	75.2	70.2	65.2	60.4	56.0	50.2	47.4
	Bhendi	0.03	78.2	73.0	70.2	64.2	60.4	57.3	50.2
	Chilli	0.02	80.0.	72.0	66.2	63.3	58.3	54.4	49.3
Vadakadu	Cauliflower	0.05	58.2	55.4	50.3	47.2	45.8	42.2	40.4
	Brinjal	0.03	60.2	57.3	52.8	50.3	47.2	45.4	42.0
	Bhendi	0.03	62.4	58.3	55.0	50.5	47.0	42.5	37.0
	Chilli	0.03	66.0	57.2	52.2	49.6	45.0	41.0	36.2

Microbial activity

The soil samples collected from intensive vegetable growing villages, showed adverse impact on the microbial activity in the form of CO_2 production (Table 7 and 8). A significant decreases in CO_2 production and these decreases significant in all four vegetable fields of five villages. The CO_2 production during first and 6th week of incubation by 17% and 38% respectively. The most adverse effect was seen with soil contaminated with chlorpyriphos when compared to monocrotophos. The same results were shown by Goswami *et al.*, 2013 [9] who reported that the application of cypermethrin insecticide on soil at high concentration leads to poisonous impact on soil biomass, respiration and FDHA activity. Yousaf *et al.*, 2013 [31] concluded that the pesticides were very poisonous to soil microbes, as showed by the decrease of CO_2 produced.

Table 7: Average Concentration of Chlorpyriphos in soils of Ottanchathiram Block and microbial activity (CO_2 evolution)

Villages	Vegetables	Chlorpyriphos mgkg^{-1}	CO_2 evolution (mg CO_2 per 100g soil) Weeks						
			0	1	2	3	4	5	6
Thasaraipatt	Cauliflower	0.10	80	66	60	58	55	53	50
	Brinjal	0.08	95	90	83	65	60	58	58
	Bhendi	0.08	105	100	98	93	90	75	63
	Chilli	0.06	120	110	95	95	90	90	110
Veeralapatti	Cauliflower	0.09	90	83	75	83	80	75	55
	Brinjal	0.07	105	93	100	95	75	62	60
	Bhendi	0.06	98	93	79	90	75	65	65
	Chilli	0.05	133	120	114	100	95	93	90
Ambilikai	Cauliflower	0.09	88	73	64	60	50	55	58
	Brinjal	0.08	98	93	90	80	73	68	60
	Bhendi	0.08	104	100	98	88	76	72	65
	Chilli	0.04	126	120	105	100	98	88	90
Arasappapillaipatti	Cauliflower	0.10	100	98	93	88	75	76	70
	Brinjal	0.09	98	93	95	86	73	65	60
	Bhendi	0.07	76	77	74	66	60	60	55
	Chilli	0.05	121	118	108	105	103	95	90
Vadakadu	Cauliflower	0.09	102	95	88	85	78	73	65
	Brinjal	0.07	113	100	75	72	68	63	64
	Bhendi	0.09	105	90	85	80	75	70	65
	Chilli	0.05	133	127	120	114	106	100	95

Table 8: Average Concentration of Monocrotophos in soils of Ottanchathiram Block and microbial activity (CO_2 evolution)

Villages	Vegetables	Monocrotophos mgkg^{-1}	CO_2 evolution (mg CO_2 per 100g soil) Weeks						
			0	1	2	3	4	5	6
Thasaraipatt	Cauliflower	0.05	90	76	70	68	65	60	60
	Brinjal	0.04	105	99	93	85	80	78	68
	Bhendi	0/04	115	100	104	95	93	85	73
	Chilli	0.02	130	120	105	100	97	90	85
Veeralapatti	Cauliflower	0.06	92	85	80	83	78	75	70
	Brinjal	0.04	102	98	94	90	88	72	70
	Bhendi	0.03	100	102	99	95	85	83	75
	Chilli	0.03	135	125	118	100	95	93	90
Ambilikai	Cauliflower	0.06	90	83	74	65	61	57	53
	Brinjal	0.04	100	95	92	90	83	78	70
	Bhendi	0.03	110	105	100	98	94	89	75
	Chilli	0.02	135	126	115	108	100	98	90
Arasappapillaipatti	Cauliflower	0.05	95	93	90	89	84	90	85
	Brinjal	0.04	98	95	93	88	83	75	70
	Bhendi	0.03	106	100	94	86	76	70	65
	Chilli	0.02	132	128	115	110	107	100	96
Vadakadu	Cauliflower	0.05	98	95	83	80	75	63	60
	Brinjal	0.03	105	100	95	92	88	73	66
	Bhendi	0.03	109	101	95	93	85	80	85
	Chilli	0.03	123	120	116	110	100	90	85

Conclusion

This study suggests that the pesticide usage was maximum in intensive vegetable growing area of Ottanchathiram Block. Due to long term usage, the chlorpyriphos and monocrotophos are persisting in the soil and affect the growth of earthworm. Fifty percent of weight loss was observed in the Cauliflower field, when compared to Chilli field samples, Soil bacterial population and microbial activity also reduced in soil samples collected from intensive vegetable growing area, which confirms and reinforces previously reported environmental concerns.

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