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Comparison of the Population of Soil-Inhabiting Nematoda in The Context of Pesticide Application with an Observation on Their Trophic Groups

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Abstract

Soil-inhabiting Nematodes are agriculturally important for significant loss of crop production as well as for their beneficial role in soil ecosystem. During a visit to Bali Island of Indian Sundarbans, West Bengal, India, few soil samples were collected from agricultural fields. As reported by the villagers, some fields were applied with pesticides like Furadon (carbofuran 3% g) and Phorate (Phorate 10% g organophosphate) and some were without any pesticide. Both of these pesticides have nematicidal effects besides being contact and systemic insecticide. The collected soil samples were processed by Cobb's Sieving and Decantation Method followed by modified Baermann Funnel Technique to extract the nematodes. The populations of nematodes were counted with the help of a counting dish and a hand tally counting machine under a stereo zoom microscope following standard method. The soils applied with pesticides, collected from cauliflower and tomato fields, showed a lesser population count of 26 and 98 nematodes respectively in 250 gm. of soil for each of the samples, whereas the population count for the soils without pesticides from vegetable and paddy fields were 293 and 625 nematodes respectively in the same quantity of soils. The nematodes were identified up to the generic level and presence of twelve genera was observed which indicates an idea about their trophic groups in the soil micro-habitat of agro-ecosystem being associated with the mangrove ecosystem ('ecotone' of terrestrial and marine ecosystem) of Sundarbans. So this observation can be considered as various trophic levels of nematodes in an 'ecotone'. Soils with the pesticides showed only two trophic groups (omnivorous and predatory-omnivore), whereas soil without pesticides revealed four trophic groups (plant and hyphal feeder, omnivorous, bacterial feeder and predatory) of nematodes. The difference in population in different soils was statistically analyzed to show the relation between the nematode population in the soil applied with pesticides and without pesticides.

Keywords: Soil-inhabiting Nematoda, Sundarbans, Population, Pesticide, Trophic groups

Introduction

A study was initiated on the population of soil-inhabiting nematodes in the Agricultural fields of Bali Number 9 village, P. O. Bali Hathkhola, Bali island of Indian Sundarban, South 24-Parganas districts, West Bengal, India to get an idea about the effects of pesticide usage on the relative population difference of the nematodes in the fields of various agricultural practices such as vegetables and rice cultivation under normal circumstances of weather at any instance. In this study, samples were collected from four different fields *viz*. Cauliflower bed (latitude 22º088000N and longitude 88º754607E), Vegetable bed (latitude 22º087528N and longitude 88º754754E), Tomato field (latitude 22º091680N and longitude 88º761145E) and Paddy field (latitude 22º091715N and longitude 88º761040E). 250 gm of soil samples were collected from each of the fields. Two soil samples were collected from the fields applied with pesticide and two samples were from the fields without any pesticide. The nematodes, extracted from the collected soil samples, first counted for the population comparison and then were identified up to the generic level to observe their

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trophic group and to have an idea about their feeding habits in the area of study. Their total population was counted separately for each of the sample to observe whether there is any comparative difference of population count in two different types of soils. This population data was statistically analyzed.

As reported by the villagers, cauliflower (*Brassica oleracea*) and tomato (*Solanum lycopersicum*) fields were applied with pesticides like Furadon (carbofuran 3% g) and Phorate (Phorate 10% g organophosphate). Vegetable and paddy (*Oryza sativa*) fields were without any pesticide. Fields those are applied with pesticides (Phorate/Furadon) are known to have nematicidal effects and widely used as contact and systemic insecticides for its efficacy (Webb and Corbett, 1973, Oyedunmade *et al.,* 2009, Meher *et al.,* 2010, Chelinho *et al.,* 2011). The soils associated with these crops were chosen to compare the population of nematodes in these two types of agricultural practices in four fields just on the basis of pesticide usage only. Total twelve genera of soil nematodes were recovered of which four genera from cauliflower, two genera from tomato, four genera from vegetable and five genera from paddy field and Some of the same genera have been found in soil samples of different crop fields under study (Table 1). To be more precise, *Aporcelaimellus* was found in the soils associated with cauliflower and tomato, *Laimydorus* was present in those of cauliflower, tomato and paddy. An attempt was made to find out to compare the population of nematodes in two types of agricultural practices.

Soil nematodes are very small in size (0.3 to 10mm), population abundance is very high (generally million/m2) and diverse (>30 taxa/m2) in all kind of soils (Yeates, 1979). They are most abundant in the upper strata of the rhizosphere, they may also be found up to the depth of over 20 feet (Jenkins and Taylor, 1976). Most of the soils contain 90% of the nematodes at top six inches of soil surface (Crofton, 1966). Nematodes are important for their beneficial and harmful role in soil micro-habitat. An average annual yield loss of the world's major crops due to the damage by the soil and phytophagous nematodes has been estimated (Sasser and Freckman, 1987; Dasgupta, 1998; Luc *et al.*, 2005, Perry and Moens, 2006). On the other hand, as nematodes are heterotrophic in soil food webs, they play an important role in the transformation of organic matter into mineral and organic nutrients which are beneficial for the growth of plants resulting in better crop productivity (Ingham *et al.*, 1985; Ferris *et al.*, 1998, 2004) and thus Nematode feeding activity contributes to the stability of soil food web and they play important roles in many ecosystem services and processes (Yeates *et al.*, 2009). The feeding types and feeding groups in plant and soil nematodes was studied (Yeats, 1971) and their trophic groups has been designated as omnivorous, phyto-phagous or plant feeders, fungal feeders, bacterial feeders, predators, predatory-omnivore etc. (Yeates *et al.*, 1993). The feeding types of the genera were also observed in the agro-ecosystem in two types of agricultural practices.

Materials and Methods

For the present study, soil samples were collected from agro-ecosystem closely associated with mangrove ecosystem of Sundarbans. Soil samples of about 250 gm. each were taken from soil of four different agricultural fields as mentioned earlier, from a depth of 2 – 10 centimeters from the surface with the help of a hand-shovel. The geographical co-ordinates were noted with the help of a GPS to get the specific locations. The collected soil samples were processed following standard methods of 'Cobb's sieving and decantation technique' (Cobb, 1918) followed by 'modified Bearmann's funnel technique' (Christie and Perry, 1951) to extract the nematodes present in the samples. The extracted nematodes were then taken for population counting in live condition. After the population count was done and the numbers were noted for further study the nematodes were killed and fixed instantly by Seinhorst's method in hot Formaldehyde-acetic acid solution (FA) solution. These were preserved in the same solution with appropriate labels. The specimens were transferred in cavity blocks containing glycerine-alcohol and were kept in a desiccator for 2 to 3 weeks. After complete dehydration of the specimens, permanent slides were prepared by using anhydrous glycerine as a mountant medium. Paraffin was used to seal the cover glass with the slide containing specimens in anhydrous glycerine. Finally, the nematodes were observed and studied under a Nikon eclipse Ni DIC microscope to identify the genera.

Method counting of nematode population: The live specimens of nematodes for each individual sample were taken for population counting. In this process, the extracted nematodes with 3 – 4 ml of water for a specific sample were taken in a 100 ml measuring cylinder and addition of clean water up to 100 ml was done. Then with the help of a pipette or a glass dropper the sample was made homogenous carefully by bubbling the water repeatedly. 10 ml of this water, containing homogeneously suspended nematodes, was taken in a counting dish by a graduated pipette. Counting of nematodes in the counting dish was done under a stereo-zoom microscope with the help of a 4-digit hand tally counter. The same process of counting was repeated thrice for each of the four samples. The mean of nematode population from three counts of each sample was recorded. The process of calculation for each of the samples was as follows:

Suppose, 10 ml of homogeneous suspension contains x number of nematode.

1ml of homogeneous suspension contains x/10 of nematode.

100 ml of homogeneous suspension contains 100x /10 of nematode.

In this way, the individual mean of nematode population for each of the four samples was calculated. After that the mean of total population was finally calculated and were recorded for statistical analysis.

Results

Twelve genera of soil-inhabiting nematodes were observed to be associated with the soils of four types of crops. These are *Laimydorus* Siddiqi, 1969, *Mesodorylaimus* Andrassy, 1959, *Thornenema* Andrassy, 1959, *Aporcelaimellus* Heyns, 1965, *Discolaimium* Thorne, 1939, *Mylodiscus* Thorne, 1939, *Dorylaimoides* Thorne and Swanger, 1936 under the order Dorylaimida; *Tylenchus* Bastian, 1865 and *Rotylenchus* Filipjev, 1936 under the order Tylenchida; *Rhabditis* Dujardin, 1845 and *Mesorhabditis* (Osche, 1952) Dougherty, 1953 under the order Rhabditida and *Clarkus* Jairajpuri, 1970 under the order Mononchida. Population count of nematodes extracted from the soils applied with pesticides collected from the cauliflower and tomato fields are 26 and 98 respectively whereas, the population count from the soils without pesticides, collected from the vegetables and paddy fields are 293 and 625 respectively. The number of nematode population in four crop fields in two different types of soils and the trend of population increase in both types of soils have been shown (Figure 1 and 2). From the above data, it is evident that the nematode population in the fields of cauliflower & tomato, applied with pesticides with chemical compound Carbofuran 3% and Phorate 10%, having nematicidal effect, clearly indicates a sharp decline in comparison with the population in the fields of vegetables and paddy, which were devoid of any pesticides. As the values indicate a clear comparison with difference in population number, the relation drawn by the above observation can be established statistically.

The samples were tested on the basis of Chi square analysis to justify and to infer the relationship of the population count against the pesticides affecting the nematodes in the agricultural fields. For the study and the calculations of the Chi square variables, the small sample grid was used as the sample size was small (table 2). The values were obtained from the 2x2 contingency table for a special case of a table with only two rows variables to examine and to draw the inference for the test following the proper method & guidelines. The calculation of the chi square variables was performed readily with Brandt & Snedecor's formula (Bailey, 1995). The chi square value is now being discussed below with the Yates' correction. Though the correction is overcautious in its desire to avoid a type-1 error, the corrected value and the original value are both discussed below.

As calculated from the table, the level of significance to consider under the sample size is 0.05 and the degree of freedom *(df)* is 1, as for the two variable parameters were considered under the observation, for one sample is applied with pesticides and the other is devoid of any pesticides. After calculating the chi square analysis, the outcome is 6.165 with the p value of 0.013, which after Yates' correction standardized to 5.661 as for the chi square analysis and 0.017 for the value of p, demonstrating a significant relation between use of pesticide and decline in population (table 2, Figure 5).

The nematodes present in both types of the soils, applied and not applied with pesticides found in the agricultural fields of cauliflower, tomato, vegetable, and paddy are categorized under different trophic groups to observe the feeding habits for each of the genus (table 1). The total number of the trophic groups present in the soil without pesticides is four, in which plant and hyphal feeder, omnivorous, bacterial feeder and predatory feeding groups are present in contrast to the number of the trophic groups present in the soil applied with pesticides is only two in which predatory-omnivorous, and omnivorous nematodes were observed. The percentage of various trophic groups was calculated. The trophic groups are comprised of plant and hyphal feeder (17%), omnivorous (33%), predatory-omnivorous (8%), bacterial feeder (17%) and predatory (25%) in the ecosystem under the study (Figure 3). The percentage of trophic groups present in the soils applied with pesticide (33%) and that of without pesticide (67%) were also observed (Figure 4).

Discussion

An attempt was made to assess the relation between the variation of nematode population and application of pesticide with the help of Chi square analysis. According to the Chi square 'test of independence', two following hypotheses were drawn:

Null Hypothesis - The outcome of the population variation and the effect of the pesticides having nematicidal effect tested at random at any instance are not particularly related. They are of independent relationships.

Alternative Hypothesis – The decline in the population variation and the effect of the pesticides having nematicidal effect tested at random at any instance are related. They are not of independent relationships.

Now to draw the inference based on the result of Chi square test of independence is to analyse the *p* values (level of significance) to determine whether the null hypothesis is accepted or rejected. As it occurs the *p* values in both the cases of original and after Yates' correction is significantly less than the chi square value generated in the considered level of significance ($p < 0.05$), which is at $df = 1$ and at $p = 0.05$ is 3.841, which in turn infer the values are statistically significant and thus the 'Null Hypothesis' is finally rejected and the 'Alternative Hypothesis' [$X^2(1, N=4) = 6.165$, $p=0.013$] is accepted. This fact establishes that the effect of the pesticides having nematicidal effect clearly played a role on the population reduction of nematodes in the fields. The chi square distribution curve which plots χ^2 value against the *p* for the degree of freedom or $df = 1$, shows the cumulative probability associated with the chi square value (Figure 5). It is worthy to mention for the present study that although the individual number of sample count is 4 as they are collected from four different agricultural fields but in the present case they are grouped under 2 categories (soil with pesticide and soil without pesticide), which is considered the special case of a table with only 2 rows, having 2 individual columns, hence according to Brandt and Snedecor's formula (Bailey, 1995) the degree of freedom (*df*) is less than the number of column, $df = c - 1 = 1$. The present study is fully consistent with the studies done on the effect of carbofuran, phorate, other pesticides and organic materials to reduce the population of soil-inhabiting nematodes associated with different types of agricultural crops (Webb and Corbett, 1973, Oyedunmade *et al.,* 2009, Meher *et al.,* 2010).

The area under the study is ecologically significant because the agro-ecosystem here is associated with the mangrove ecosystem ('ecotone' of terrestrial and marine ecosystem) of Sundarbans. So the present observation can be considered as the population of soil-inhabiting Nematoda and their various trophic levels in an 'ecotone'. It was observed that only the nematode with omnivorous and predatory-omnivorus feeding habits were present in the soil applied with pesticides whereas, a variety of feeding habits like plant and hyphal feeder, omnivorous, bacterial feeder and predator, in the soil without pesticide were noted signifying a more stable soil micro-habitat and indicating that more trophic groups with diverse feeding habits are present in the soil without pesticide. A relevant work on the effect of carbofuran on the reduction of population abundance and trophic groups of soil nematodes was done by Chelinho *et.al.*, 2011 which is in conformity with the present investigation. In that study, effects on total nematode abundance, number of families and abundance of nematode feeding groups as well as potential shifts in both trophic and family structure were assessed in soil nematode communities showing a decline of population without significant change in community structure and trophic groups (Chelinho *et.al.*, 2011).

Table 1: Nematode genera observed in the respective agricultural fields with their trophic groups

Associated Plant/field	Name of the genera	Use of pesticides	Trophic Group
Cauliflower	1. *Aporcelaimellus* Heyns, 1965	Soil applied with Phorate/ Furadon	Predatory-omnivore
	2. *Mesodorylaimus* Andrassy, 1959		Omnivorous
	3. *Dorylaimoides* Thorne & Swanger, 1936		Omnivorous
	4. *Laimydorus* Siddiqii, 1969		Omnivorous
Tomato	1. *Laimydorus* Siddiqii, 1969	Soil applied with Phorate/ Furadon	Omnivorous
	2. *Aporcelaimellus* Heyns, 1965		Predatory-omnivore
Vegetable	1. *Thornenema* Andrassy, 1959	Soil without application of pesticide	Omnivorous
	2. *Discolaimium* Thorne, 1939		Predatory
	3. *Mylodiscus* Thorne, 1939		Predatory
	4.*Clarkus* Jairajpuri, 1970		Predatory
Paddy	1. *Laimydorus* Siddiqii, 1969	Soil without application of pesticide	Omnivorous
	2. *Rotylenchus* Filipjev, 1936		Plant feeding
	3. *Tylenchus* Bastian, 1865		Plant and Hyphal feeder
	4. *Mesorhabditis* Osche, 1952		Bacterial feeder
	5. *Rhabditis* Dujardin, 1845		Bacterial feeder

Table 2: (2x2) grid for chi square (χ^2) analysis for special case with only 2 rows

Use of pesticides	Number of nematodes present in reference soil samples		Total
Pesticide applied	26	98	124
Pesticide not applied	293	625	918
Total	319	723	1042

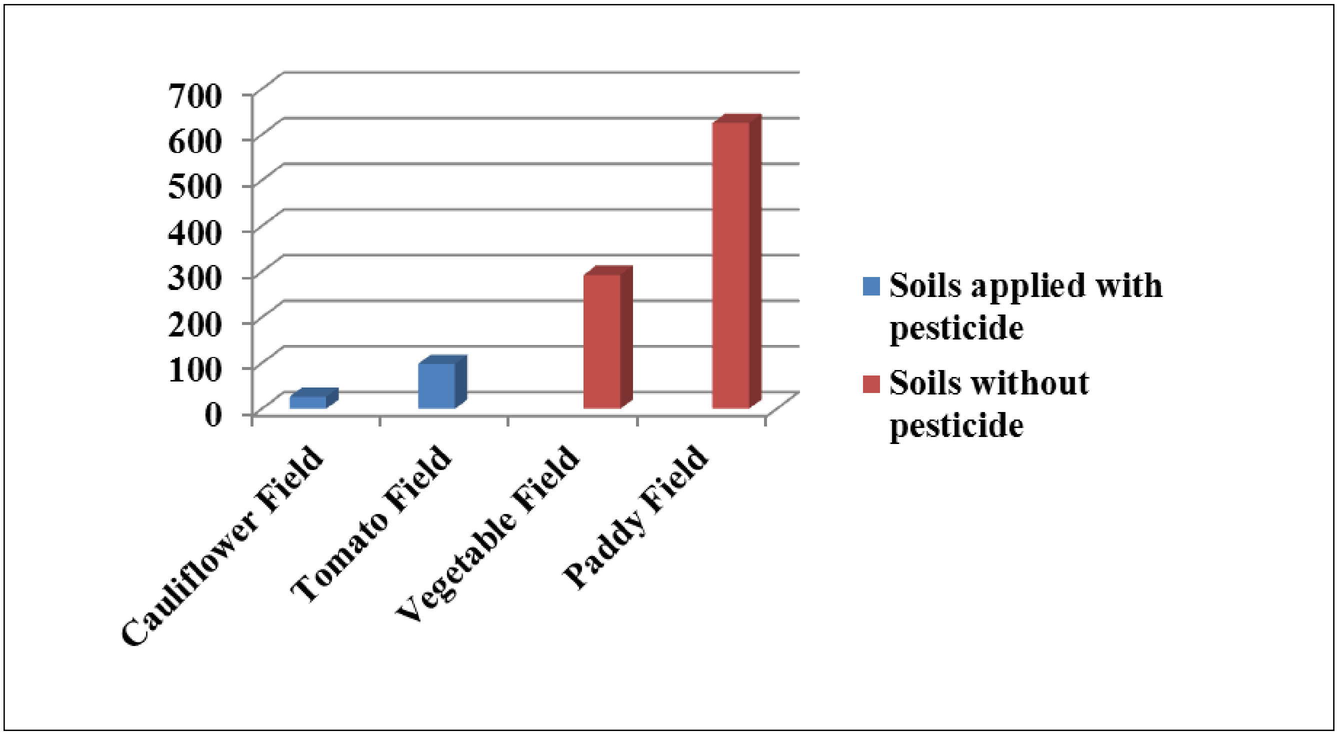

Figure 1: Number of nematode population in four crop fields in two different types of soils

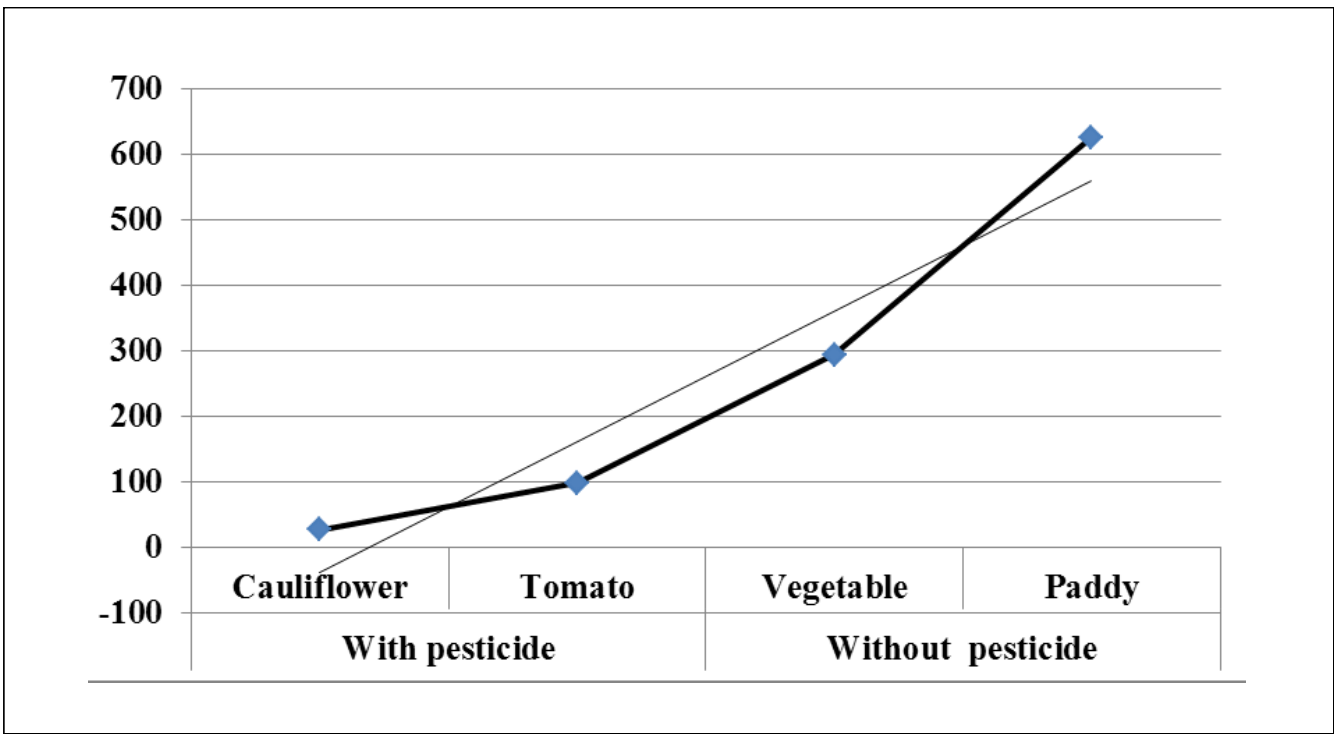

Figure 2: Trend of population increase in the soils applied with pesticide and without pesticide

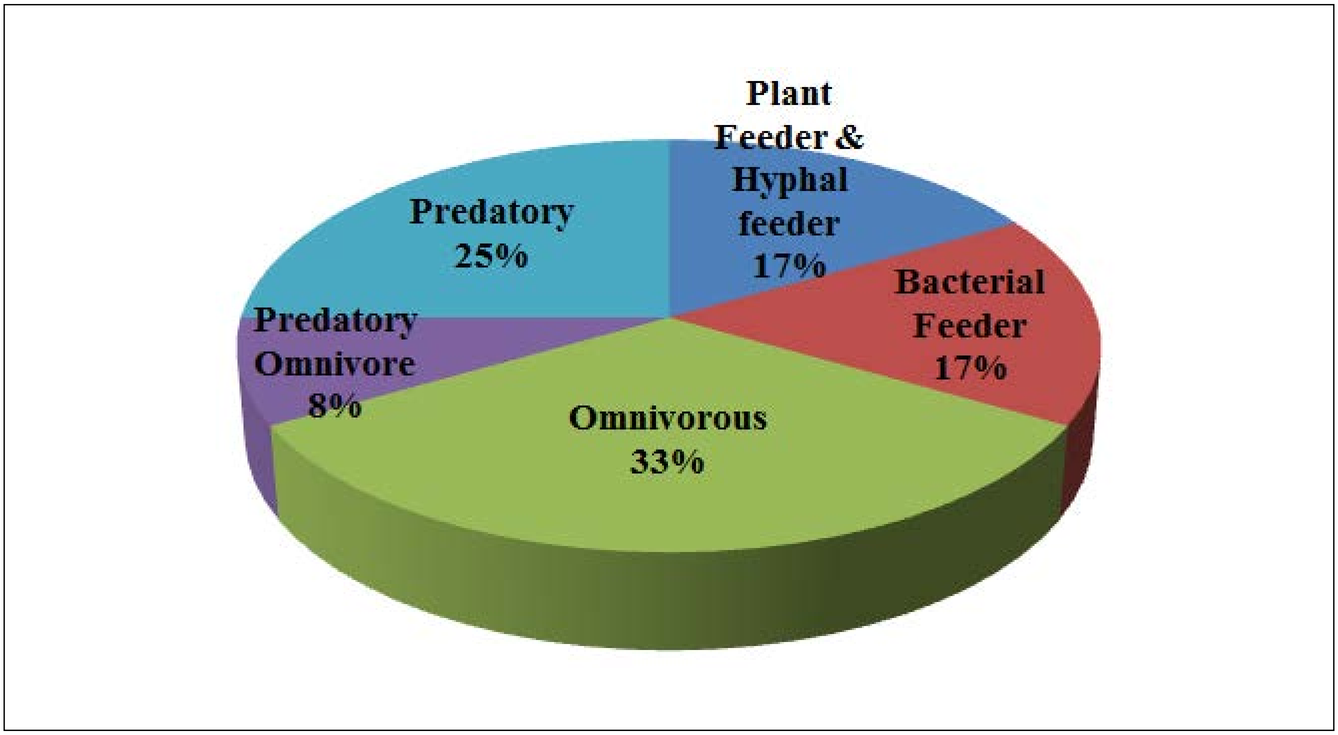

Figure 3: Percentage of different trophic groups

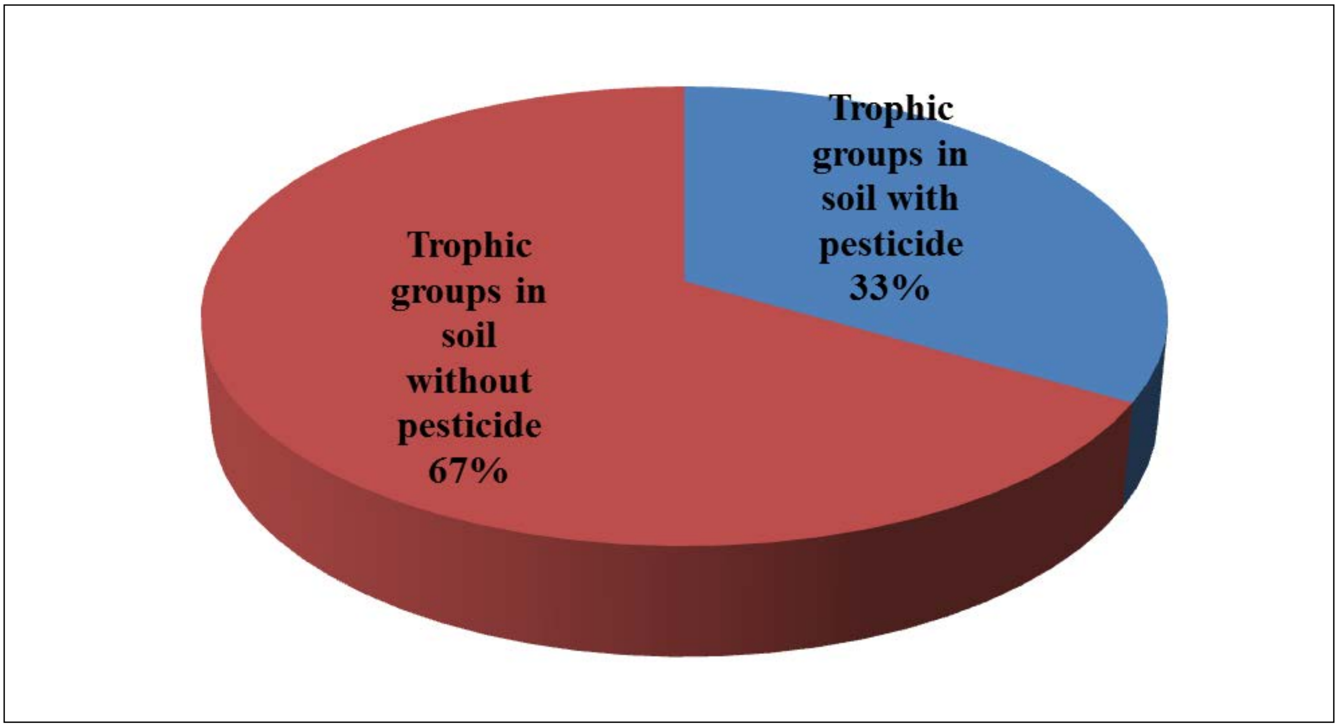

Figure 4: Percentage of trophic groups present in the soils applied with pesticide and without pesticide

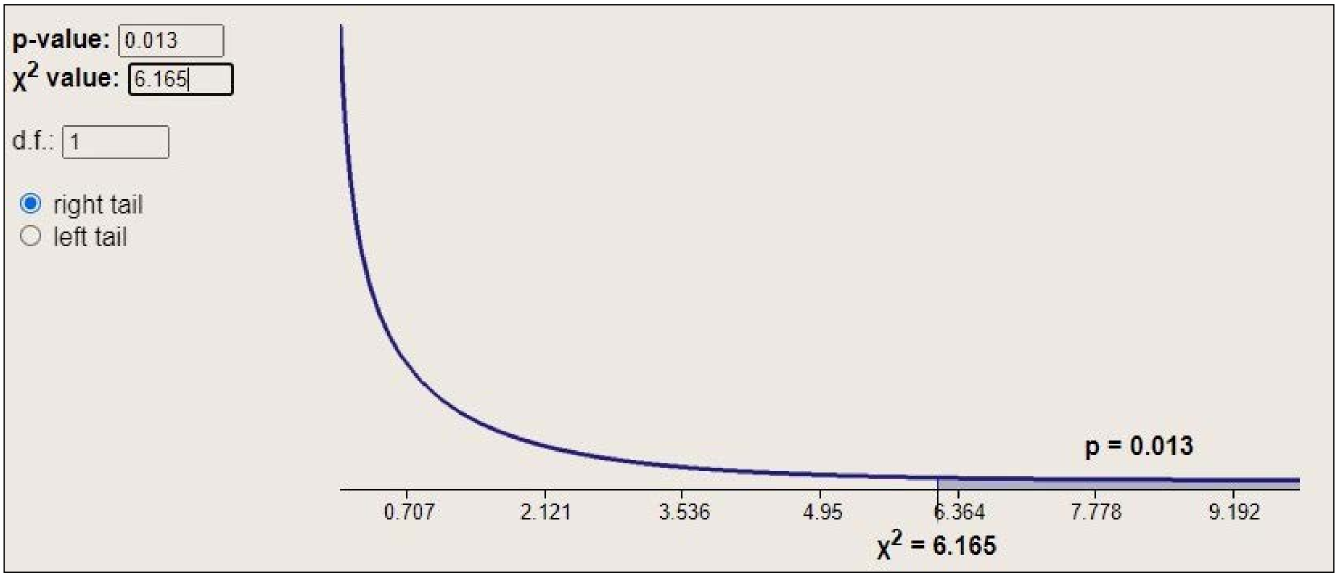

Figure 5: Normal distribution curve of the chi square statistics ($\chi^2 = 6.165$) & *p*-value (0.013)

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