Journal homepage www.jzs.univsul.edu.iq
Journal of ZankoySulaimani
Part-A- (Pure and Applied Sciences)

Impact of Some Pesticides on Total Counts of Soil Non-Symbiotic Nitrogen-Fixing Bacteria,Particularly *Azotobacter* sp.

Nashmeel Saeed Khudhur[1] and Abdul-ghany Omer Ismaeel Sarmamy[2]

1 Department of Environmental Science, College of Science, University of Salahaddin, Kurdistan Region, Iraq

2 Department of Biology, College of Science, University of Salahaddin, Kurdistan Region, Iraq

Email: nashmeel@gmail.com

Article info

Original: 1 June. 2015
Revised:28 June. 2015
Accepted:30 August. 2015
Published online: 20 March. 2016

Key Words:
Soil pollution
Pesticides
Non-symbiotic nitrogen-fixing bacteria
Azotobacter sp.

Abstract

A pot experiment was conducted to evaluate the effect of glyphosate herbicide (n-phosphonomethyl glycine), mancozeb fungicide ([[1,2-ethanediylbis-[carbamodithioato]](2-)] manganese mixture with [[1,2-ethanediylbis-[carbamodithioato]]-(2-)] zinc) and diazinon insecticide (O, O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidynyl]), three soil orders and two soil moisture contents (50% and 100% of field capacity) and their combinations on soil total non-symbiotic nitrogen-fixing bacteria particularly *Azotobacter*sp. counts. This experiment last for two months from the 6th of Jun until the 8th of August 2011 with five sampling periods at biweekly intervals. Factorial experiment (4×3×2) with Completely Randomized Design (CRD) and three replications was used and the means were compared using Revised Least Significant Differences (R.LSD) at the level of significant of 0.05. Significantresults were obtained at the last two scheduled period's sampling of 4th and 5th,and the results showed that: Mancozebfungicide decreased total *Azotobacter*sp.;Diazinoninsecticide decreased both total non-symbiotic nitrogen-fixing bacteria and total *Azotobacter*sp.;Agholan soil (order Entisols) showed the greatest increase in soil total non-symbiotic nitrogen-fixing bacteria. 50% soil moisture content revealed the greatest reduction total *Azotobacter*sp. while, 100% soil moisture content showed the greatest increase in soil total *Azotobacter*sp. count. The combinations among: glyphosate, Agholan soil and 100% moisture combination showed the greatest reduction in total non-symbiotic nitrogen-fixing bacteria and total *Azotobacter*sp.

Introduction

A variety of synthetic chemicals are used throughout the world, and environmental pollution by pesticides is a worldwide problem [1]. Soil pollution is one of the main environmental pollution types which cause ecosystem destruction and disturbance and can be defined as the decline in soil quality caused through its misuse by human activity [2]. Pesticide is a substance or mixture of substances intended for controlling pests and they get into the environment through different routes; consequently they produce many adverse effects on non-target organisms, decrease biodiversity, development of resistance in insects and undesirable health effects [3]. Although, it recognized that pesticides are harmful to human, but it should also be remembered that they are essential to maintain a healthy life and their application can improve crop yields [4]. Soil microorganisms are among the non-target organisms that expose to the undesirable effect of residual pesticides which cause a variety of acute and chronic toxicity such as reducing their numbers, biochemical activity, diversity and changing their community structures [5]. Many authors have studied the effect of pesticides on bacteria playing vital role of nitrogen cycle processes, for example the author [6] had showed that higher doses of 3,3-diaminobenzidine enhanced growth of aerobic nitrogen fixers, but negatively affected denitrifying and nitrifying bacteria; [7] observed that azadirachtin exerted a stimulatory effect on growth of *Azotobacter* sp. in the initial 15 day period; [8] found that *Azotobacterchroococcum* indicated less growth and biomass after addition of lannate and diazinon pesticides at their recommended dose and doubled dose to the media and [9] reported that treflan at lower concentrations appeared to stimulate the growth of pure cultures of *A. Chroococcum*. The excessive use, abuse or repeated application of some pesticides like glyphosate or diazinon in Kurdistan Region may alter the diversity and population of nitrogen fixing bacteria in soil environment. The present investigation was carriedto give some information about the pesticide effects on soil non-symbiotic nitrogen fixing bacteria particularly *Azotobacter*sp. in the study area.

Materials and methods

A.Soil sample collection

Soils of different orders were collected in May 2011 from three different agricultural fields in Erbil province including Agholan, Debaga and Girdarasha(*Figure: 1*).

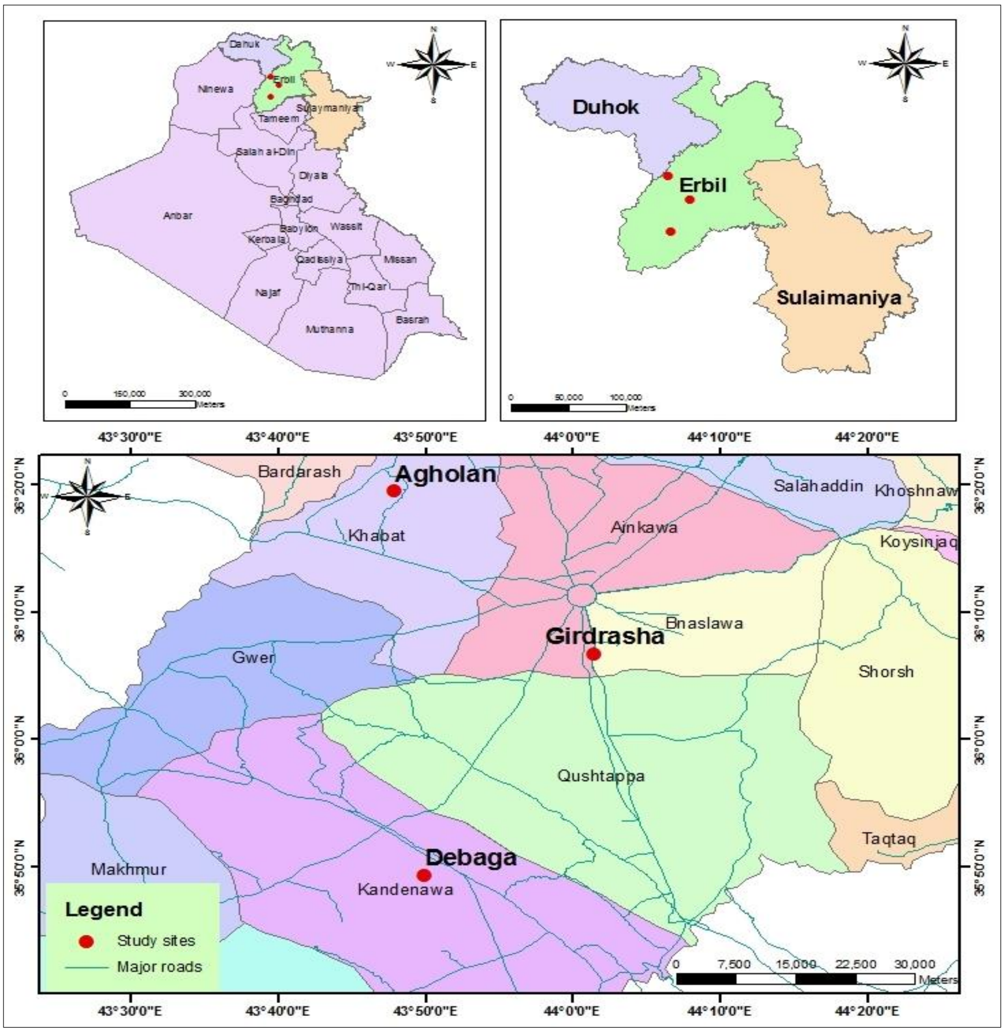

Figure-1: Map showing: Iraq, Erbil and the studied aria.

The soils of these three locations were not used for agricultural purposes for a period of time and had no previous history of pesticide use. More details about the sampling areas are given in (*Table: 1*). Samples were collected from the upper layer of the soils (0-30 cm depth) at a random pattern around each field according to [10]. The soil samples were brought to the greenhouse of the College of Science. The soil samples,free of plant roots, were air-dried, gently crushed and sieved at 2-mm with stainless-steel sieves to avoid any contamination and then stored for subsequent analyses. Four portions from each soil order was separately weighted and placed on 57 cm^2 of nylon-covered floor in dry and cool condition inside the greenhouse, and kept out from sunlight exposure. A sub-sample from each soil order was analysed for physical, chemical and microbiological properties (*Table: 2*). Particle size distribution and soil texture was determined by hydrometer method according to [11]; soil moisture content was determined by gravimetric method in which the samples were dried to constant weight as described by [12];the pH and EC of the soils were determined using a calibrated pH-meter (JENWAY 3505) and an electrical conductivity meter (JENWAY 4510) in 1:1 (soil: water suspension) in line with the method proposed by [11];cation exchange capacity (CEC) was determined by saturating the soils with 1M NaOAc at pH 8.2 and washed with absolute ethanol (exchangeable Na was replaced with 1M NH_4OAc pH 7) then Na concentration was analyzed by flame photometer as described by [13];Walkly-Black procedure (1934) was followed for determination of soil organic matter [14];the micro-Kjeldahl procedure was used as mentioned by [14] for estimation of total nitrogen;ascorbic acid combined with potassium antimonyl tartrate of Murphy and Riley (1962) which described by [12] was used for determination of total phosphorus;and total sulfur was determined by turbidimetric method as described by [12].

Table-1: Description of the studied area.

Location		***Agholan***	***Debaga***	***Girdarasha***
Soil order		Entisols	Vertisols	Inceptisols
Area (donum)		100	30	30
Coordination of locations*	***North***	36˚ 19' 30"	35˚ 49' 24"	36˚ 06' 45"
	East	43˚ 47' 54"	43˚ 49' 59"	44˚ 01' 29"
Elevation (meter)		302	324	419
Short notes		This field was planted with watermelon two years ago, and there was a lot of rainfall during the spring and there is no history of pesticide use in the area.	There were no agriculture activities in this field for a long time because of desiccation. There is no history of pesticide use in the area.	An experimental field for practical studies of agriculture college. There is no history of pesticide use in the area.

** Coordination was read by GPS Accuracy ± 4m.*

Table-2: Physical, chemical and microbiological properties of the studied soils.

	Soil properties		***Agholan***	***Debaga***	***Girdarasha***	***Measuring units***
Physical	Particle size distribution	Sand	23.79	20.51	19.23	$g.kg^{-1}$
		Silt	52.56	58.97	64.10	$g.kg^{-1}$
		Clay	25.64	20.51	16.67	$g.kg^{-1}$
	Texture		Silt loam	Silt loam	Silt loam	-
	Moisture content		16.23	8	4.5	%
	Moisture content		32.50	25.00	24.00	$ml.100g^{-1}$
Chemical	pH		7.22	7.20	7.16	-
	Electrical conductivity (EC)		4.22	4.16	3.16	$dS.m^{-1}$
	Cation exchange capacity (CEC)		33.168	26.959	28.895	$c.mol.kg^{-1}$
	Organic Matter (OM)		15.524	8.624	12.074	$g.kg^{-1}$
	Total nitrogen (N)		2.519	1.079	2.412	$g.kg^{-1}$
	Total phosphorus (P)		0.143	0.127	0.452	$g.kg^{-1}$
	Total sulfur (S)		0.007	0.004	0.008	$g.kg^{-1}$

Microbiological	Total bacterial count	3.80×10^5	1.40×10^5	1.77×10^6	Bacterial cells.g^{-1}dry soil
	Total fungal count	3.70×10^5	6.70×10^4	6.68×10^5	Fungal propagul.g^{-1}dry soil

B.Pesticide preparation

The most abundant and usable pesticides in Kurdistan Region including glyphosate herbicide (48%), mancozebfungicide (80%) and diazinoninsecticide (10%) were selected according to the yearly report of plant protection in the Department of Plant Protection in Erbil/General Directorate of Agriculture during 2010-2011 and used for the present study.These pesticides were prepared at their commercial recommended doses according to their active ingredients (a.i.) as described by [15]. Each pesticide was placed in a sprayer (the total volume of spraying water for each pesticide was 60 litres/donum) and ready for application.

C.Soil treatment by pesticides

The first portion from each soil order was sprayed by glyphosate; the second portion by mancozeb; the third portion by diazinon; and the last portion was control treatment. The soil portions were mixed to obtain a homogenous distribution. Then the pesticide-treated soils were sealed and left for an overnight at room temperature.

D.Pot experiment layout

A factorial experiment (4×3×2) was conducted in the greenhouse of the College of Science/University of Salahaddin using Completely Randomized Design (CRD) with three replications under twenty-four combined treatments (*Table: 3*).For this purpose 72 pre-labelled and similar plastic pots (average diameter 15 cm and height 17 cm) were used and each pot was filled with 4 kg pesticide-treated soil and a sample was taken from each pot. The pots were provided by a below-container to collect the irrigation water and return-back to the pots. Then the pots were covered with filter papers. The pots were irrigated daily by tap water at 50% and 100% moisture content daily and the loss of water were compensated by weighting each pot daily. This experiment last for two months with five sampling periods at biweekly intervals at variable climatic condition (*Table: 4*).

Table-3: Pot experimental design.

No. of treatments		***Pesticide (P)***	***Soil order (S)***	***Moisture content (W)***	***Combined treatments***	***Replications***
1	P1S1W1	Glyphosate	Agholan	50%	Glyphosate.Agholan.50%	3
2	P1S1W2			100%	Glyphosate.Agholan.100%	3
3	P1S2W1		Debaga	50%	Glyphosate.Debaga.50%	3

4	P1S2W2			100%	Glyphosate.Debaga.100%	3
5	P1S3W1		Girdarasha	50%	Glyphosate.Girdarsha.50%	3
6	P1S3W2			100%	Glyphosate.Girdarasha.100%	3
7	P2S1W1	Mancozeb	Agholan	50%	Mancozeb.Agholan.50%	3
8	P2S1W2			100%	Mancozeb.Agholan.100%	3
9	P2S2W1		Debaga	50%	Mancozeb.Debaga.50%	3
10	P2S2W2			100%	Mancozeb.Debaga.100%	3
11	P2S3W1		Girdarasha	50%	Mancozeb.Girdarsha.50%	3
12	P2S3W2			100%	Mancozeb.Girdarsha.100%	3
13	P3S1W1	Diazinon	Agholan	50%	Diazinon.Agholan.50%	3
14	P3S1W2			100%	Diazinon.Agholan.100%	3
15	P3S2W1		Debaga	50%	Diazinon.Debaga.50%	3
16	P3S2W2			100%	Diazinon.Debaga.100%	3
17	P3S3W1		Girdarasha	50%	Diazinon.Girdarsha.50%	3
18	P3S3W2			100%	Diazinon.Girdarsha.100%	3

19	CS1W1	Control	Agholan	50%	Control.Agholan.50%	3
20	CS1W2	(C)		100%	Control.Agholan.100%	3
21	CS2W1		Debaga	50%	Control.Debaga.50%	3
22	CS2W2			100%	Control.Debaga.100%	3
23	CS3W1		Girdarasha	50%	Control.Girdarasha.50%	3
24	CS3W2			100%	Control.Girdarasha.100%	3

Table-4: Climatic condition during the study.

Parameters	***1st sampling***	***2nd sampling***	***3rd sampling***	***4th sampling***	***5th sampling***	***Mean***
	9/6/2011	***24/6/2011***	***9/7/2011***	***24/7/2011***	***8/8/2011***	
Maximum temperature (ºC)	37.0	36.7	37.0	44.6	41.2	39.3
Minimum temperature (ºC)	24.4	26.4	28.6	33.3	30.0	28.54
Dry temperature (ºC)	31.1	31.5	33.3	38.9	35.1	33.98
Humidity (%)	29	31	23	20	29	26.4
Wind velocity ($m.sec^{-1}$)	1.0	1.4	2.5	3.9	1.5	2.06

Wind direction	320	240	220	40	320	228
Maximum wind velocity (m.sec^{-1})	3	4	7	14	6	6.8

From: Directorate of Meteorology and Seismology in Erbil.

E.Preparation of culture media

Nitrogen-free Jensen's brothwas used for counting of total non-symbiotic nitrogen-fixing bacteria. It was prepared according to the instruction of Difco Company by adding and thorough mixing the following components of two solutions as given in [16]. Solution A: [sucrose 20 g; $MgSO_4.7H_2O$ 0.4 g; $CaCl_2$ 0.1 g; $FeSO_4.7H_2O$ 0.012 g and $NaMoO_4.2H_2O$ 0.01 g] and solution B: [K_2HPO_4 2 g and NaCl 0.4 g]. The two solutions were autoclaved separately, and combined in equal volumes after cooling to bring into 1liter. The pH of complete medium was adjusted to 7.0±0.2.Ashbey's Mannitol Agar was used for counting of total *Azotobacter* sp. That can use mannitol and atmospheric nitrogen as a source of carbon and nitrogen respectively. The composition of this medium per a litre of distilled water consists of the following: [mannitol 15 g; $CaCl_2.2H_2O$ 0.2 g; K_2HPO_4 0.2 g; $MgSO_4.7H_2O$ 0.2 g; MoO_3 (11% solution) 0.1 ml and $FeCl_3$ (11% solution) 0.05 ml]. The composition was then mixed, boiled and autoclaved [17].

F.Microbiological analysis

Most probable number (MPN) technique was used for enumeration of total non-symbiotic nitrogen-fixing bacteria as described by [18] and [19]. The incubation period was two weeks at 28±0.5°C. However, total *Azotobacter*sp. was counted by MPN technique as described by [18], using Ashbey's liquid medium by which small numbers can be detected according to the indications of Al-Zubi et al., 2007 described by [17], for a period of two weeks and incubation at 28±0.5°C.

G. Statistical analysis

Data was analysed statistically using SPSS version 11.5 and Microsoft Office Excel 2010 and the means were compared using Revised Least Significant Differences (R.LSD) at the level of significant of 0.05.

Results

Diazinon(P3) caused the highest count of soil non-symbiotic nitrogen-fixing bacteria during the 1stand 5thsamplingperiods(*Figure: 1*), while glyphosate (P1) showed the lowest total non-symbiotic nitrogen-fixing bacterial population during this period; during the 2ndsampling mancozeb(P2) showed the lowest total non-symbiotic nitrogen-fixing bacterial population; during the 3rdand 4thsampling periods glyphosate showed the highest and diazinon showed the lowest population of total non-symbiotic nitrogen-fixing bacteria. According to (*Figure: 2*), Debaga soil showed the highest number of soil total non-symbiotic nitrogen-fixing bacteria during the 1stand 4thsampling periods and Agholan soil showed the highest number during the 3rdsampling, whereas Girdarasha soil caused the greatest population count of non-symbiotic nitrogen-fixing bacteria during the 2ndand 5thsampling periods.At the last sampling periods (4thand 5thsampling periods), 100% soil moisture content was significantly reduced soil total non-symbiotic nitrogen-fixing bacteria(*Figure: 3*). Untreated Agholan soil (CS1) showed the highest non-symbiotic nitrogen-fixing bacterial population during the 1stand 2ndsampling periods(*Figure: 4*). Glyphosate in Agholan soil (P1S1) and Debaga soil (P1S2) showed the highest population of non-symbiotic nitrogen-fixing bacteria during the 3rdand 4thsampling periods(*Figure: 4* and *5*). Mancozeb in Girdarasha soil (P2S3) showed the highest total non-symbiotic nitrogen-fixing bacteria during 5thsampling period(*Figure: 5*).The combined treatment CW2 showed the highest total non-symbiotic nitrogen-fixing bacterial count during the 1stand 2ndsampling periods;

during the 3rdand 4thsampling periods, the combined treatment P1W2 caused the greatest count of total non-symbiotic nitrogen-fixing bacteria; while the combinations P3W1 caused the highest population of non-symbiotic nitrogen-fixing bacteria during the 5thsampling period(*Figure: 6*). Significant differences between all of the combined treatments of pesticides and soil moisture content have been observed(*Table: 5*).The combination S3W2 showed the highest non-symbiotic nitrogen-fixing bacterial population during the 1stand 2ndsamplings, and the combinations of S2W2, S2W1 and S3W1 caused the highest count of total non-symbiotic nitrogen-fixing bacteria during the 3rd, 4thand 5thsampling periods respectively(*Figure: 7*). The combined treatments P3S1W2, CS1W2, P1S1W2, P2S1W2 and P2S3W2 showed the highest total non-symbiotic nitrogen-fixing bacterial population during the five sampling periods respectively(*Figures: 8, 9, 10, 11* and *12*).Mancozeb showed the highest population of total *Azotobacter* sp. during the 1stand 3rdsampling periods. Glyphosate showed the highest total *Azotobacter* sp. count during the 2ndand 4thsampling periods. Diazinon and mancozeb decreased total *Azotobacter* sp. significantly during the last sampling periods (*Figure: 13*). According to (*Figure: 14*), Debaga soil showed the highest number of total *Azotobacter*sp. during the first 3 sampling periods, Agholan soil showed the lowest number of total *Azotobacter*sp. during the experiment starting. At the last sampling periods Girdarasha soil showed the greatest number and Agholan showed the lowest number of total *Azotobacter*sp. As shown in (*Figure: 15*), 100% soil moisture content showed the highest population of total *Azotobacter*sp. at the beginning and the end of the study.Mancozeb in Debaga soil (P2S2) revealed the highest population of total *Azotobacter*sp. during the 1stand 3rdsampling periods(*Figure: 16*). While the combined treatments P1S2, P1S3 and CS2 caused the highest *Azotobacter*sp. count during the 2nd, 4thand 5thsampling periods respectively(*Figure: 16* and *17*).The combination of P2W2 during the 1stand 3rdsampling periods and the combination of P1W1 during the 2ndand 4thsampling periods showed the highest *Azotobacter*sp. count(*Figure: 18*). The combination of P3W2 caused significant increasing in total *Azotobacter*sp. at the end of the study. Significant differences between all of the combined treatments of pesticides and soil moisture content have been observed(*Table: 6*). As shown in (*Figure: 19*), the combined treatment S2W1 during the first two sampling periods showed the highest total *Azotobacter*sp. count and the combination of S2W2 during the 3rdsampling periods showed the highest count of total *Azotobacter*sp., both of the combinations S3W2 and S3W1 during the 4thand 5thsampling periods respectively showed the highest total *Azotobacter*sp. population. The combined treatment S1W1 decreased total *Azotobacter*sp. at the end of the study. The combined treatments P2S3W2, P1S2W1, P2S2W2, P1S3W1 and P3S3W2 showed the highest populations of total *Azotobacter*sp. during the five sampling periods respectively(*Figures: 20, 21, 22, 23* and *24*).

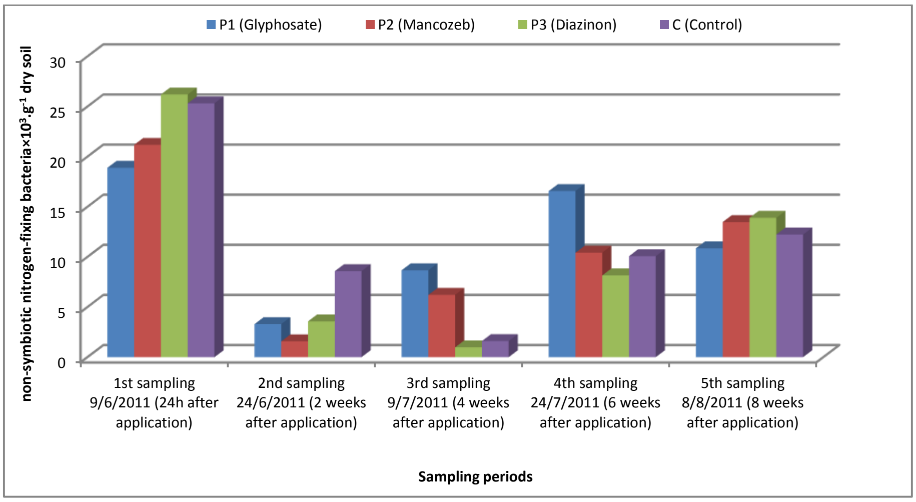

Figure-2: Effects of pesticides on soil total non-symbiotic nitrogen-fixing bacterial population×$10^3.g^{-1}$ dry soil during five sampling periods.

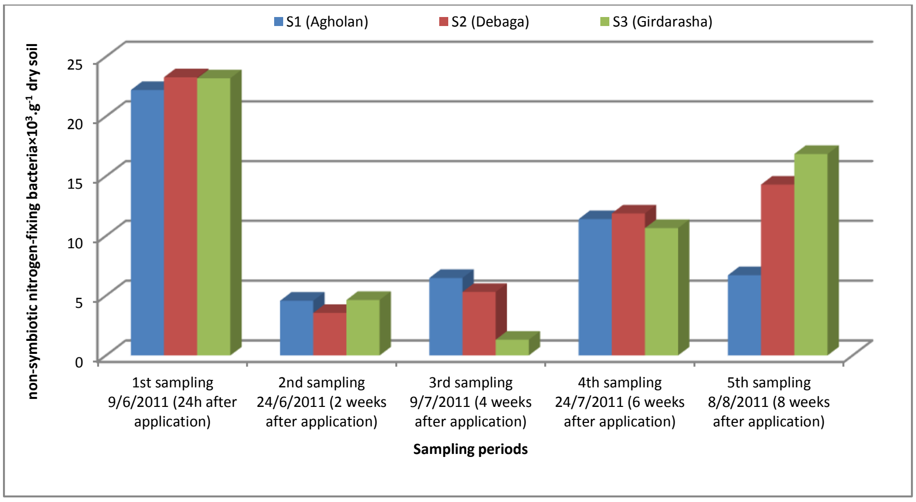

Figure-3: Effects of soil orders on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during five sampling periods.

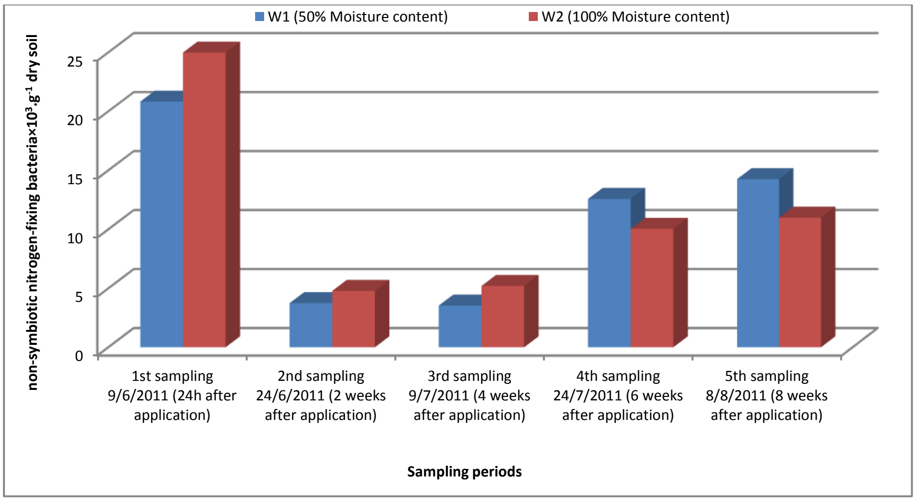

Figure-4: Effects of soil moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during five sampling periods.

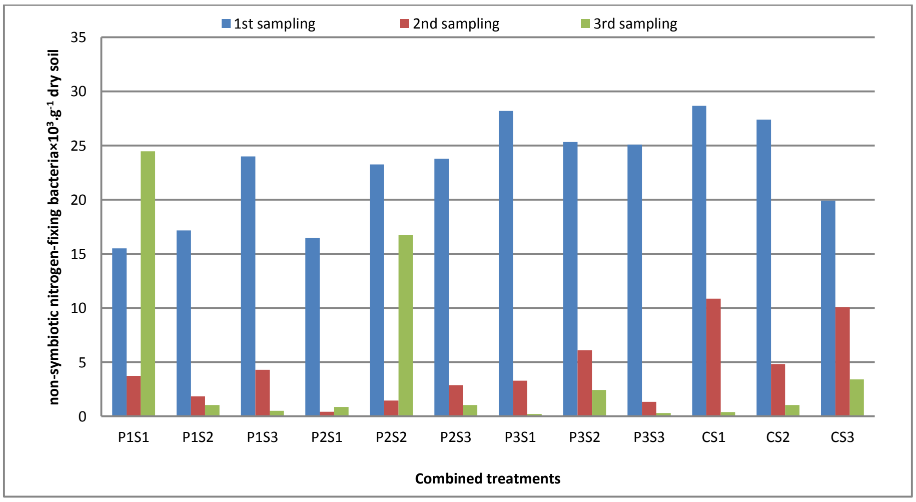

Figure-5: Effects of the combined treatments between pesticides and soil orders on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during the 1^{st}, 2^{nd} and 3^{rd}sampling periods.

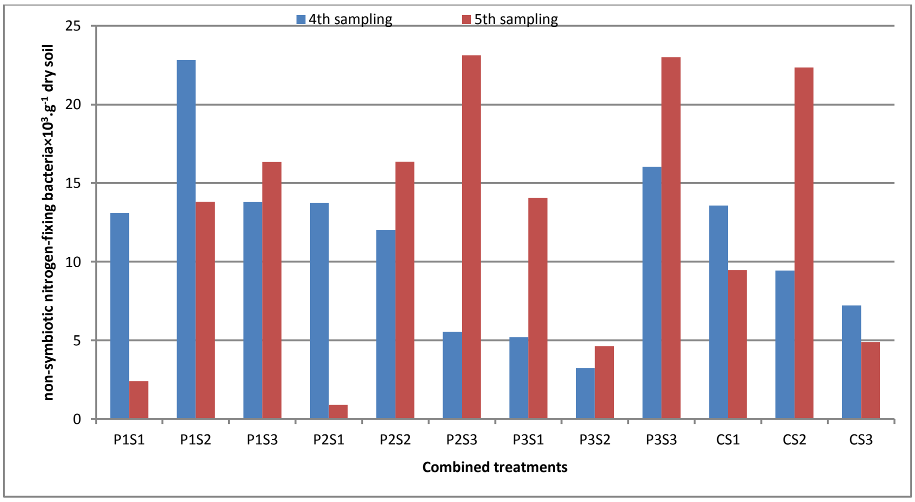

Figure-6: Effects of the combined treatments between pesticides and soil orders on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during the 4^{th} and 5^{th}sampling periods.

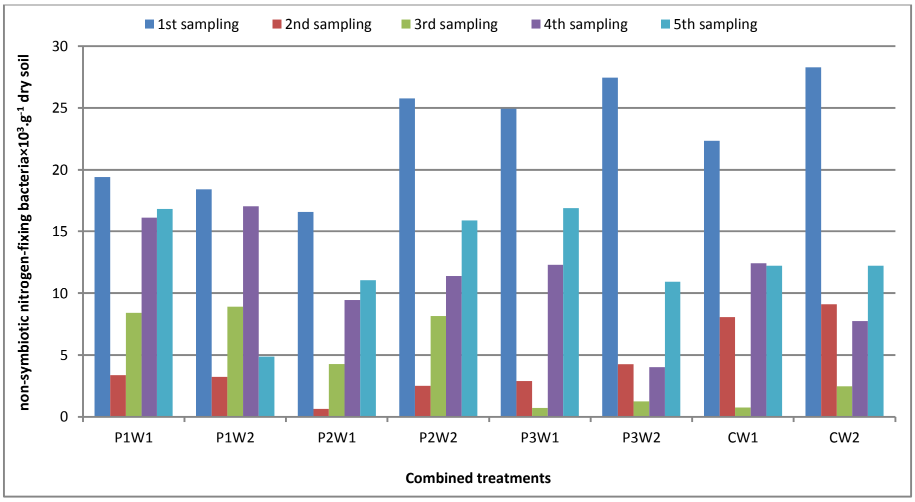

Figure-7: Effects of the combined treatments between pesticides and moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during five sampling periods.

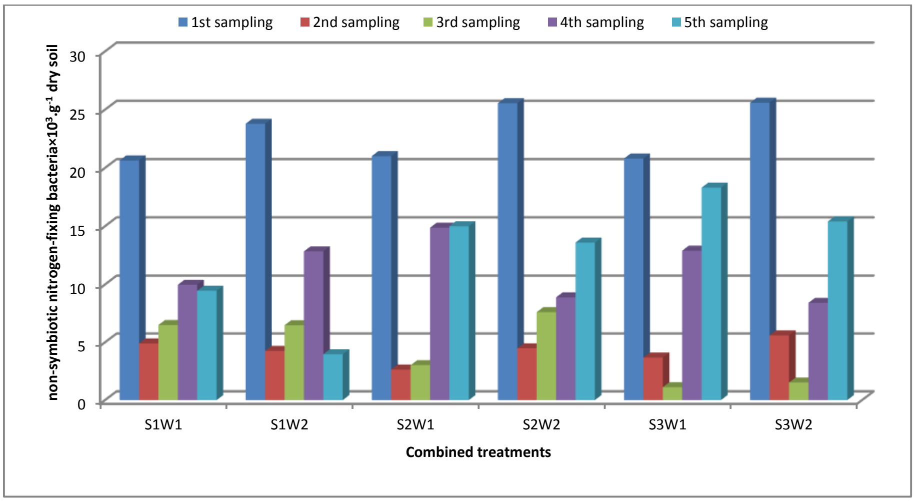

Figure-8: Effects of the combined treatments between soil orders and moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during five sampling periods.

Table-5: Effects of different treatments on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during five sampling periods (Mean±S.E.).

Treatments	***1st sampling*** ***9/6/2011 (24h after application)***	***2nd sampling*** ***24/6/2011 (2 weeks after application)***	***3rd sampling*** ***9/7/2011 (4 weeks after application)***	***4th sampling*** ***24/7/2011 (6 weeks after application)***	***5th sampling*** ***8/8/2011 (8 weeks after application)***
P1 (Glyphosate)	18.891±3.396 d	3.300±0.647 c	8.669±4.998 a	16.572±3.961 a	10.859±4.044 d
P2 (Mancozeb)	21.179±3.714 c	1.583±0.718 d	6.215±3.806 b	10.427±4.082 b	13.476±4.524 b
P3 (Diazinon)	26.204±0.887 a	3.570±1.427 b	0.985±0.513 d	8.161±3.250 d	13.904±4.085 a
C (Control)	25.320±2.787 b	8.581±1.224 a	1.607±0.764 c	10.075±2.734 c	12.236±3.305 c
S1 (Agholan)	22.215±3.840 c	4.577±1.517 b	6.481±3.927 a	11.401±3.498 b	6.710±2.664 c
S2 (Debaga)	23.282±2.004 a	3.560±1.075 c	5.310±2.849 b	11.875±3.343 a	14.293±3.260 b
S3 (Girdarasha)	23.198±1.767 b	4.638±1.323 a	1.316±0.593 c	10.651±2.784 c	16.854±3.073 a
W1 (50% Moisture content)	20.818±2.302 b	3.742±0.945 b	3.540±1.997 b	12.576±2.638 a	14.252±2.664 a
W2 (100% Moisture content)	24.978±1.801 a	4.775±1.146 a	5.198±2.629 a	10.041±2.453 b	10.986±2.705 b
P1S1	15.517±8.944 l	3.751±0.124 f	24.456±0.525 a	13.089±9.966 f	2.414±1.971 k
P1S2	17.164±7.252 j	1.858±0.137 i	1.041±0.564 e	22.831±0.174 a	13.811±9.371 g
P1S3	23.993±0.230 f	4.290±1.057 e	0.510±0.357 h	13.796±8.771 c	16.352±6.589 e

P2S1	16.475±1.317 k	0.412±0.075 l	0.876±0.721 g	13.729±9.344 d	0.908±0.006 l
P2S2	23.266±0.303 h	1.471±0.895 j	16.730±7.114 b	11.998±1.046 g	16.377±6.852 d
P2S3	23.796±0.272 g	2.866±1.971 h	1.039±0.536 e	5.555±0.401 j	23.143±0.420 a
P3S1	28.198±2.069 b	3.287±0.215 g	0.204±0.010 k	5.205±0.430 k	14.061±9.004 f
P3S2	25.316±1.065 d	6.094±3.780 c	2.437±0.875 d	3.239±1.685 l	4.633±0.280 j
P3S3	25.098±0.629 e	1.330±0.418 k	0.312±0.110 j	16.040±6.486 b	23.019±0.355 b
CS1	28.671±0.004 a	10.857±0.811 a	0.389±0.146i	13.582±0.928 e	9.456±0.010 h
CS2	27.384±0.457 c	4.818±0.356 d	1.032±0.612 f	9.429±0.002 h	22.350±0.008 c
CS3	19.904±8.455i	10.067±0.380 b	3.400±1.827 c	7.212±2.275i	4.901±0.006i
P1W1	19.379±4.738 f	3.362±0.133 d	8.425±0.775 b	16.116±6.497 b	16.836±6.226 b
P1W2	18.404±5.916 g	3.238±1.438 e	8.913±0.805 a	17.028±6.002 a	4.882±2.699 h
P2W1	16.598±6.647 h	0.653±0.124 h	4.263±2.677 d	9.463±0.655 f	11.050±6.345 f
P2W2	25.760±1.946 c	2.513±1.301 g	8.167±0.784 c	11.392±6.284 e	15.902±7.495 c
P3W1	24.950±0.593 d	2.887±1.337 f	0.726±0.423 h	12.319±5.272 d	16.881±5.985 a
P3W2	27.458±1.417 b	4.253±2.816 c	1.243±1.035 f	4.004±2.782 h	10.928±6.226 g

CW1	22.348±5.472 [e]	8.065±1.805 [b]	0.745±0.417 [g]	12.407±5.383 [c]	12.240±5.229 [d]
CW2	28.291±0.243 [a]	9.096±1.993 [a]	2.469±1.416 [e]	7.742±1.718 [g]	12.232±5.222 [e]
S1W1	20.641±5.842 [f]	4.899±1.997 [b]	6.491±0.582 [b]	9.968±4.513 [d]	9.455±4.866 [e]
S1W2	23.789±5.748 [c]	4.254±2.585 [d]	6.471±0.617 [c]	12.834±5.947 [c]	3.965±0.210 [f]
S2W1	21.013±3.791 [d]	2.644±0.818 [f]	3.019±0.222 [d]	14.867±4.535 [a]	14.995±4.590 [c]
S2W2	25.552±0.964 [b]	4.476±2.041 [c]	7.601±0.543 [a]	8.882±5.061 [e]	13.591±5.311 [d]
S3W1	20.801±3.124 [e]	3.682±1.076 [e]	1.109±0.283 [f]	12.893±5.616 [b]	18.306±4.471 [a]
S3W2	25.594±0.995 [a]	5.595±1.802 [a]	1.522±0.124 [e]	8.408±1.128 [f]	15.402±4.762 [b]
P1S1W1	24.461±0.006 [j]	3.627±0.001 [l]	23.932±0.011 [b]	3.123±0.001 [s]	4.385±0.001 [s]
P1S1W2	6.574±0.002 [u]	3.876±0.002 [k]	24.981±0.005 [a]	23.054±0.003 [b]	0.444±0.001 [w]
P1S2W1	9.912±0.001 [t]	3.226±0.002 [m]	0.477±0.000 [o]	22.658±0.007 [e]	23.182±0.007 [d]
P1S2W2	24.417±0.008 [k]	0.489±0.000 [u]	1.605±0.001 [h]	23.005±0.003 [c]	4.440±0.006 [r]
P1S3W1	23.763±0.004 [o]	3.233±0.001 [m]	0.867±0.001 [l]	22.567±0.002 [f]	22.941±0.007 [f]
P1S3W2	24.223±0.010 [m]	5.348±0.001 [g]	0.153±0.002 [u]	5.025±0.001 [n]	9.763±0.001 [k]
P2S1W1	3.307±0.001 [v]	0.487±0.001 [u]	1.597±0.000[i]	4.385±0.001 [q]	0.902±0.001 [v]

P2S1W2	29.642±0.005 ^{b}	0.337±0.001 ^{v}	0.155±0.001 ^{u}	23.072±0.007 ^{a}	0.913±0.000 ^{u}
P2S2W1	22.962±0.004 ^{r}	0.576±0.001 ^{t}	9.617±0.001 ^{d}	22.459±0.006 ^{h}	9.525±0.001 ^{l}
P2S2W2	23.569±0.007 ^{p}	2.366±0.001 ^{n}	23.844±0.002 ^{c}	1.537±0.000 ^{u}	23.228±0.012 ^{c}
P2S3W1	23.524±0.009 ^{q}	0.895±0.000 ^{s}	1.576±0.001 ^{j}	1.543±0.001 ^{u}	22.723±0.004 ^{g}
P2S3W2	24.068±0.013 ^{n}	4.837±0.001^{i}	0.503±0.001 ^{n}	9.566±0.001^{i}	23.564±0.002 ^{a}
P3S1W1	26.129±0.012 ^{h}	5.437±0.001 ^{f}	0.195±0.000 ^{t}	9.507±0.001 ^{k}	23.065±0.003 ^{e}
P3S1W2	30.266±0.009 ^{a}	1.137±0.000 ^{q}	0.214±0.001 ^{r}	0.903±0.001 ^{v}	5.057±0.001 ^{o}
P3S2W1	24.251±0.012 ^{l}	2.314±0.001 ^{o}	1.562±0.001 ^{k}	4.925±0.000 ^{p}	4.913±0.000 ^{p}
P3S2W2	26.381±0.005 ^{g}	9.875±0.001 ^{d}	3.312±0.001 ^{f}	1.554±0.001 ^{t}	4.353±0.000 ^{t}
P3S3W1	24.469±0.012 ^{j}	0.912±0.001 ^{r}	0.422±0.001 ^{p}	22.525±0.009 ^{g}	22.664±0.004 ^{h}
P3S3W2	25.727±0.013^{i}	1.748±0.001 ^{p}	0.203±0.001 ^{s}	9.554±0.000 ^{j}	23.373±0.004 ^{b}
CS1W1	28.667±0.010 ^{c}	10.046±0.008 ^{c}	0.243±0.001 ^{q}	22.858±0.007 ^{d}	9.466±0.001 ^{m}
CS1W2	28.675±0.009 ^{c}	11.668±0.004 ^{a}	0.535±0.001 ^{m}	4.306±0.001 ^{r}	9.445±0.000 ^{n}
CS2W1	26.927±0.010 ^{f}	4.462±0.001 ^{j}	0.420±0.000 ^{p}	9.427±0.001 ^{m}	22.358±0.007^{i}
CS2W2	27.840±0.006 ^{e}	5.173±0.000 ^{h}	1.644±0.001 ^{g}	9.432±0.000 ^{m}	22.343±0.004 ^{j}

CS3W1	11.449±0.012 s	9.687±0.001 e	1.573±0.000 j	4.938±0.000 o	4.895±0.000 q
CS3W2	28.359±0.016 d	10.447±0.013 b	5.227±0.001 e	9.487±0.001 l	4.907±0.001 p

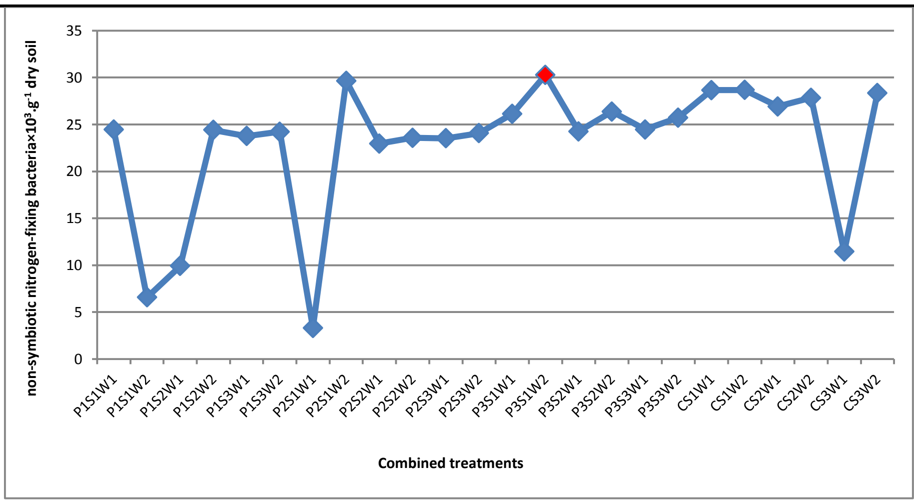

Figure-9: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during the 1^{st} sampling period.

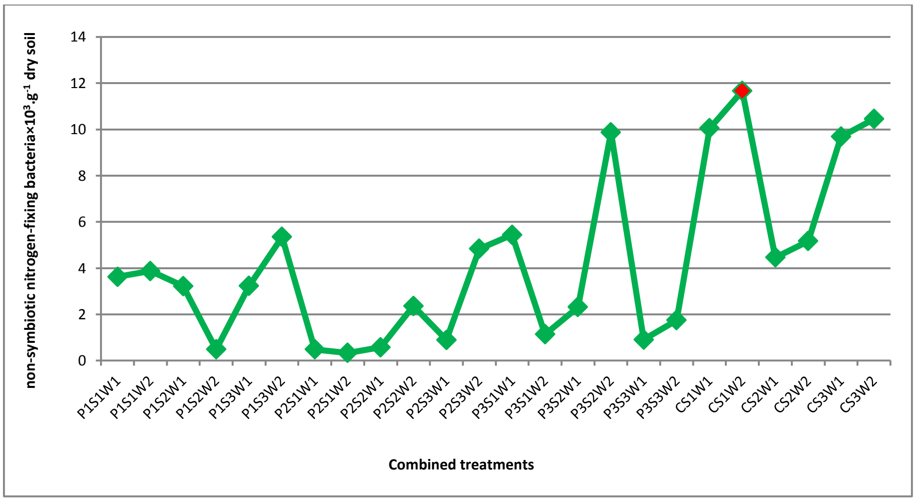

Figure-10: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during the 2^{nd} sampling period.

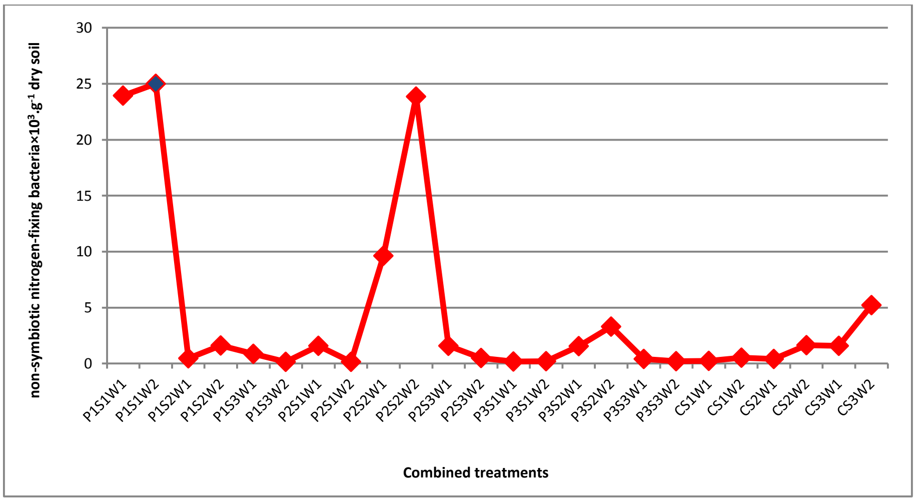

Figure-11: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during the 3rd sampling period.

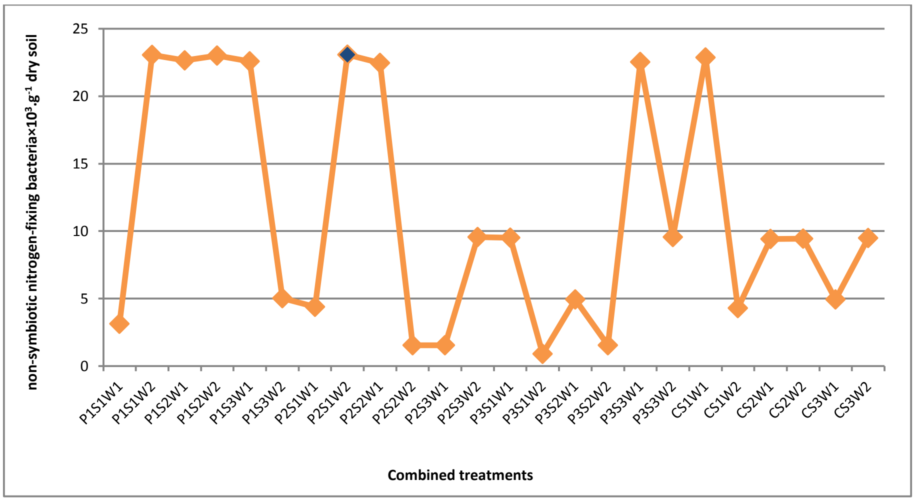

Figure-12: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during the 4th sampling period.

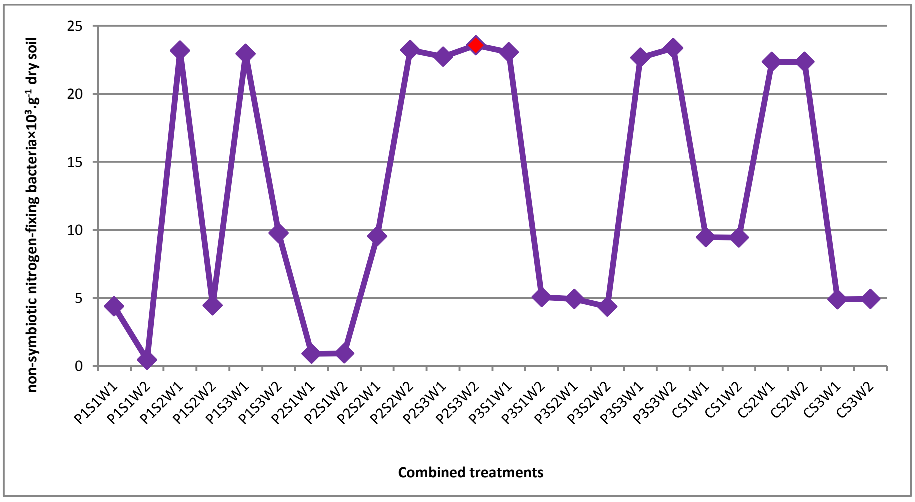

Figure-13: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total non-symbiotic nitrogen-fixing bacterial population×10^3.g^{-1} dry soil during the 5th sampling period.

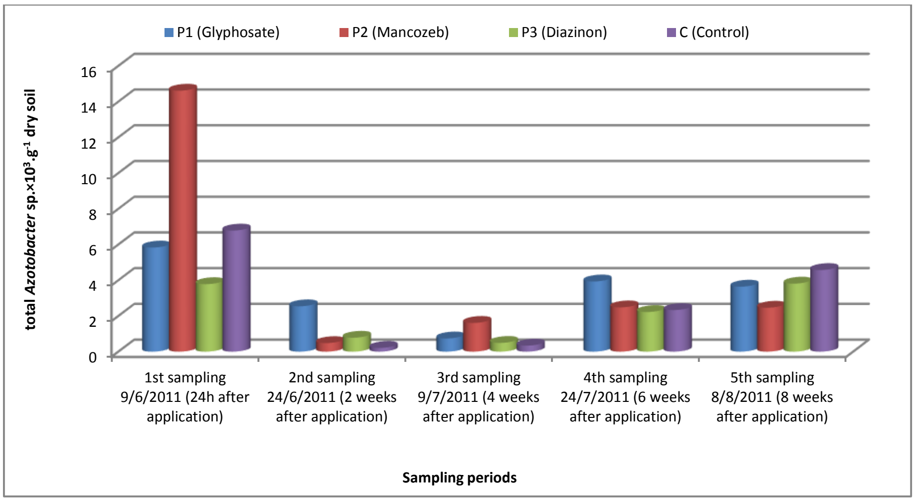

Figure-14: Effects of pesticides on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during five sampling periods.

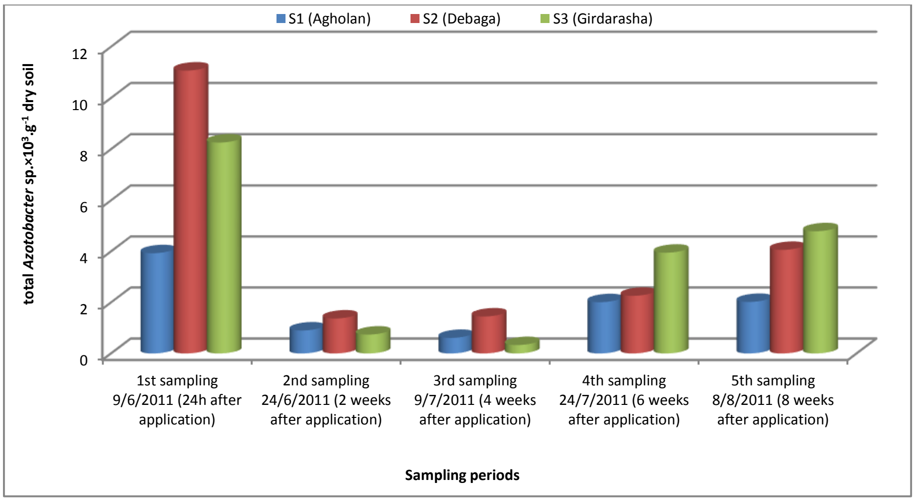

Figure-15: Effects of soil orders on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during five sampling periods.

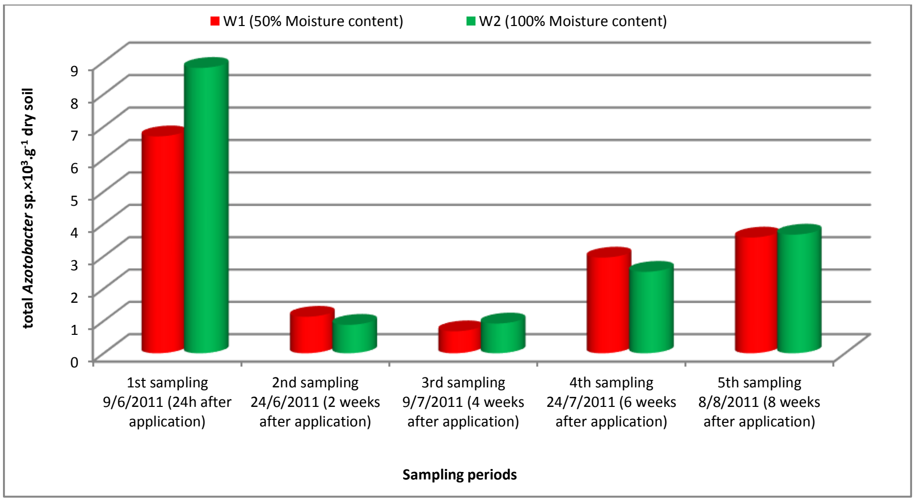

Figure-16: Effects of soil moisture contents on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during five sampling periods.

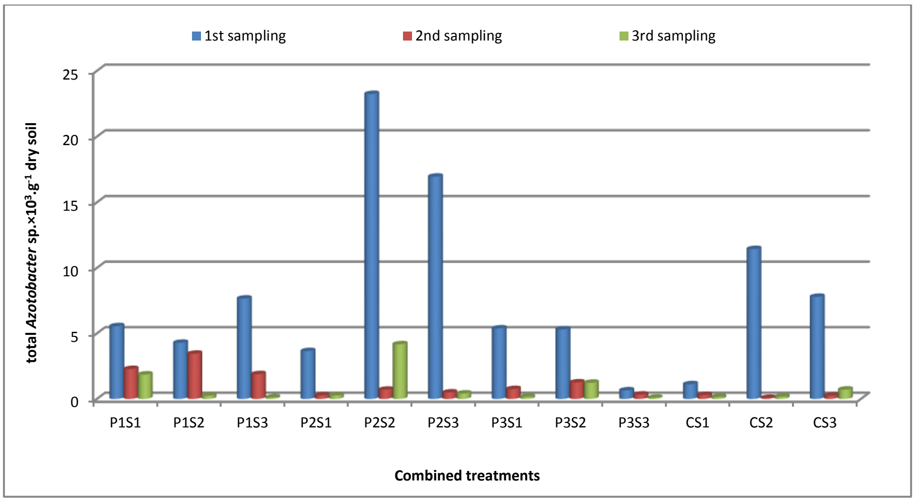

Figure-17: Effects of combined treatments between pesticides and soil orders on soil total *Azotobacter*sp.$\times 10^3.g^{-1}$ dry soil during the 1st, 2nd and 3rdsampling periods.

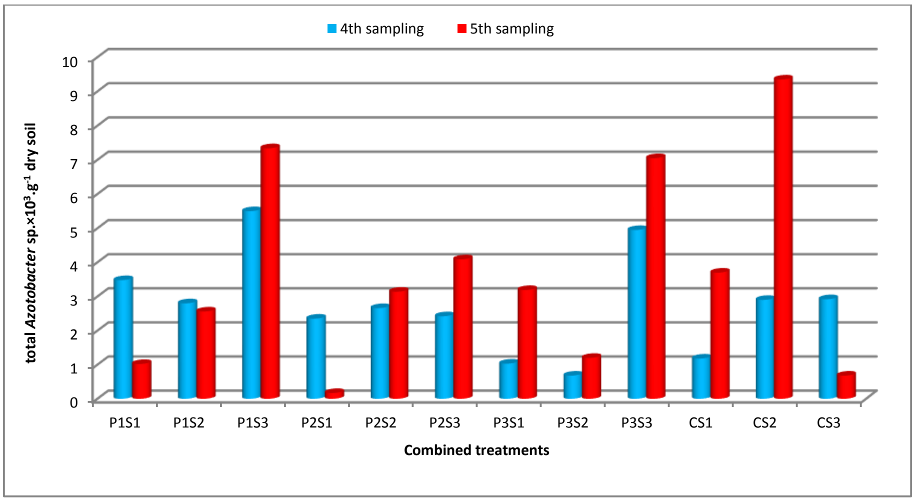

Figure-18: Effects of combined treatments between pesticides and soil orders on soil total *Azotobacter*sp.$\times 10^3.g^{-1}$ dry soil during the 4th and 5thsampling periods.

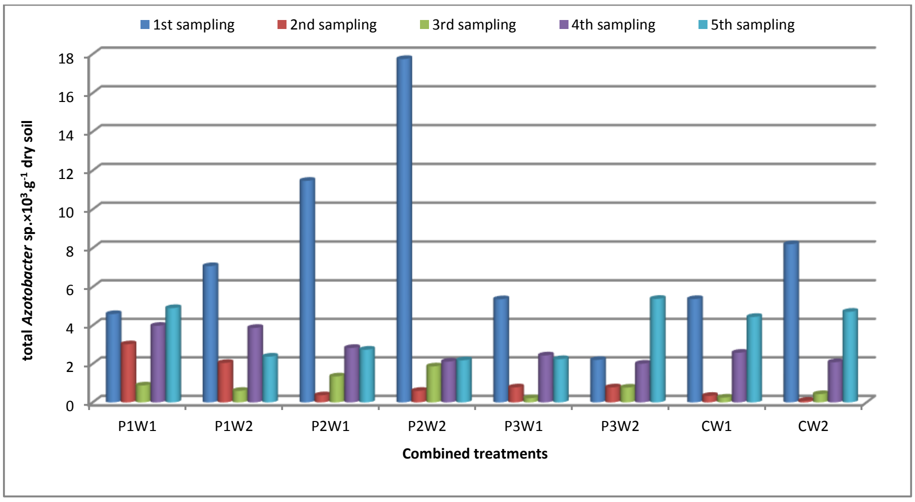

Figure-19: Effects of combined treatments between pesticides and soil moisture contents on soil total *Azotobacter*sp.$\times 10^3.g^{-1}$ dry soil during five sampling periods.

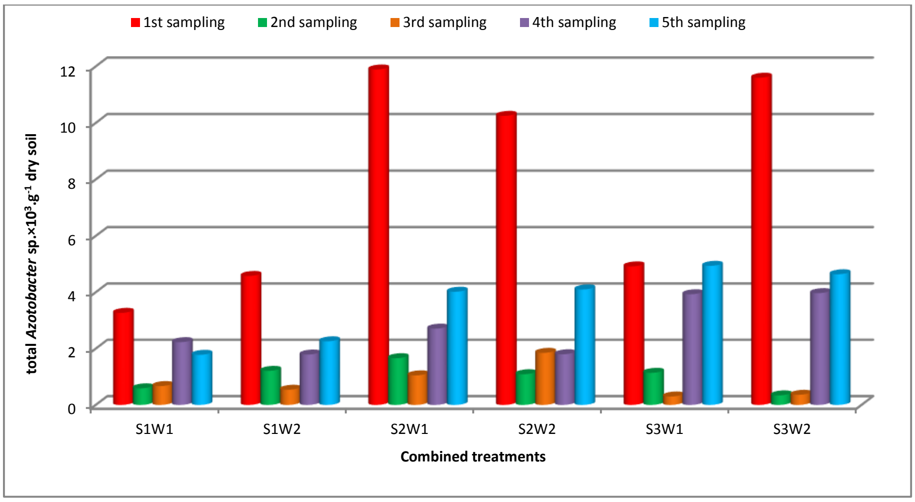

Figure-20: Effects of combined treatments between soil orders and soil moisture contents on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during five sampling periods.

Table-6: Effects of different treatments on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during five sampling period (Mean±S.E.).

Treatments	*1st sampling*	*2nd sampling*	*3rd sampling*	*4th sampling*	*5th sampling*
	9/6/2011 (24h after application)	***24/6/2011 (2 weeks after application)***	***9/7/2011 (4 weeks after application)***	***24/7/2011 (6 weeks after application)***	***8/8/2011 (8 weeks after application)***
P1 (Glyphosate)	5.83±0.934 ^{c}	2.54±0.758 ^{a}	0.74±0.364 ^{b}	3.93±1.335 ^{a}	3.64±1.368 ^{c}
P2 (Mancozeb)	14.61±4.120 ^{a}	0.48±0.101 ^{c}	1.61±0.854 ^{a}	2.48±0.794 ^{b}	2.46±0.779 ^{d}
P3 (Diazinon)	3.78±1.598 ^{d}	0.78±0.204 ^{b}	0.49±0.316 ^{c}	2.22±0.897 ^{d}	3.81±1.346 ^{b}
C (Control)	6.78±2.180 ^{b}	0.21±0.081 ^{d}	0.34±0.131 ^{d}	2.33±0.847 ^{c}	4.57±1.616 ^{a}
S1 (Agholan)	3.93±0.777 ^{c}	0.90±0.437 ^{b}	0.61±0.278 ^{b}	2.01±0.543 ^{c}	2.02±0.638 ^{c}
S2 (Debaga)	11.07±3.000 ^{a}	1.37±0.583 ^{a}	1.45±0.664 ^{a}	2.26±0.788 ^{b}	4.06±1.197 ^{b}

S3 (Girdarasha)	8.26±2.720 b	0.74±0.361 c	0.33±0.106 c	3.95±1.039 a	4.79±1.230 a
W1 (50% Moisture content)	6.70±1.791 b	1.13±0.437 a	0.68±0.270 b	2.96±0.773 a	3.58±0.899 b
W2 (100% Moisture content)	8.81±2.318 a	0.88±0.317 b	0.92±0.437 a	2.52±0.612 b	3.67±0.930 a
P1S1	5.55±0.209 f	2.28±1.602 b	1.86±0.160 b	3.48±1.544 c	1.02±0.927 j
P1S2	4.28±1.046i	3.45±1.707 a	0.24±0.239 g	2.80±0.222 f	2.56±0.600 h
P1S3	7.66±2.476 e	1.89±1.349 c	0.11±0.029 k	5.50±3.931 a	7.34±2.251 b
P2S1	3.65±2.001 j	0.27±0.072 j	0.26±0.169 f	2.35±0.780i	0.17±0.022 l
P2S2	23.24±0.308 a	0.70±0.223 f	4.17±1.034 a	2.66±2.238 g	3.14±0.029 g
P2S3	16.95±7.113 b	0.49±0.048 g	0.41±0.079 e	2.42±1.947 h	4.09±0.870 d
P3S1	5.38±0.399 g	0.75±0.225 e	0.16±0.006i	1.03±0.888 k	3.19±1.238 f
P3S2	5.31±4.830 h	1.26±0.354 d	1.23±0.819 c	0.68±0.210 l	1.20±0.725i
P3S3	0.65±0.300 l	0.33±0.116 h	0.09±0.005 l	4.95±0.034 b	7.05±2.723 c
CS1	1.12±0.001 k	0.30±0.199i	0.15±0.060 j	1.18±0.750 j	3.70±0.614 e
CS2	11.44±0.190 c	0.08±0.000 l	0.17±0.015 h	2.90±0.202 e	9.35±0.001 a
CS3	7.79±4.068 d	0.25±0.178 k	0.71±0.228 d	2.92±2.034 d	0.68±0.200 k

P1W1	4.59±0.677 ^{g}	3.02±1.298 ^{a}	0.88±0.579 ^{c}	3.98±2.755 ^{a}	4.90±2.374 ^{b}
P1W2	7.07±1.537 ^{d}	2.05±0.977 ^{b}	0.60±0.554 ^{e}	3.87±1.149 ^{b}	2.38±1.460 ^{f}
P2W1	11.47±6.197 ^{b}	0.37±0.089 ^{f}	1.35±0.894 ^{b}	2.83±1.285 ^{c}	2.74±1.401 ^{e}
P2W2	17.75±6.051 ^{a}	0.60±0.172 ^{e}	1.87±1.667 ^{a}	2.12±1.172 ^{f}	2.19±1.000 ^{h}
P3W1	5.36±2.660 ^{f}	0.78±0.166 ^{d}	0.22±0.101 ^{h}	2.44±1.308 ^{e}	2.25±1.122 ^{g}
P3W2	2.21±1.788 ^{h}	0.78±0.424 ^{c}	0.77±0.642 ^{d}	2.01±1.504 ^{h}	5.38±2.315 ^{a}
CW1	5.37±3.036 ^{e}	0.34±0.129 ^{g}	0.25±0.118 ^{g}	2.58±1.208 ^{d}	4.44±2.537 ^{d}
CW2	8.20±3.541 ^{c}	0.09±0.010 ^{h}	0.43±0.252 ^{f}	2.09±1.437 ^{g}	4.71±2.570 ^{c}
S1W1	3.27±1.097 ^{f}	0.59±0.164 ^{e}	0.67±0.456 ^{c}	2.23±0.301 ^{d}	1.78±0.607 ^{f}
S1W2	4.58±1.152 ^{e}	1.21±0.893 ^{b}	0.54±0.388 ^{d}	1.79±1.120 ^{f}	2.26±1.222 ^{e}
S2W1	11.89±4.086 ^{a}	1.66±1.179 ^{a}	1.05±0.697 ^{b}	2.71±1.265 ^{c}	4.02±1.882 ^{d}
S2W2	10.25±4.986 ^{c}	1.09±0.381 ^{d}	1.85±1.210 ^{a}	1.80±1.077 ^{e}	4.10±1.773 ^{c}
S3W1	4.92±1.856 ^{d}	1.14±0.700 ^{c}	0.30±0.108 ^{f}	3.93±2.092 ^{b}	4.94±1.793 ^{a}
S3W2	11.60±4.864 ^{b}	0.34±0.118 ^{f}	0.36±0.200 ^{e}	3.97±0.811 ^{a}	4.64±1.956 ^{b}
P1S1W1	5.34±0.001 ^{l}	0.67±0.001 ^{i}	2.02±0.001 ^{d}	1.94±0.000 ^{j}	1.94±0.000 ^{o}

P1S1W2	5.76±0.001 j	3.88±0.000 b	1.70±0.001 e	5.02±0.002 b	0.09±0.000 u
P1S2W1	3.23±0.001 q	5.16±0.000 a	0.48±0.001i	0.57±0.001 o	3.16±0.001 j
P1S2W2	5.33±0.001 m	1.74±0.001 d	0.008±0.001 w	5.02±0.001 c	1.96±0.001 m
P1S3W1	5.18±0.000 n	3.24±0.002 c	0.14±0.001 r	9.44±0.000 a	9.60±0.002 b
P1S3W2	10.14±0.008 g	0.54±0.001 j	0.08±0.001 u	1.57±0.002 l	5.09±0.001 d
P2S1W1	1.65±0.001 r	0.19±0.001 s	0.43±0.001 j	3.13±0.000i	0.15±0.001 t
P2S1W2	5.66±0.001 k	0.34±0.001 q	0.09±0.000 t	1.57±0.000 l	0.19±0.001 s
P2S2W1	22.93±0.013 c	0.48±0.001 m	3.14±0.000 b	4.89±0.002 g	3.11±0.001 k
P2S2W2	23.55±0.005 b	0.92±0.001 g	5.20±0.001 a	0.42±0.006 r	3.17±0.001 j
P2S3W1	9.83±0.001 h	0.44±0.001 o	0.49±0.001 g	0.48±0.002 p	4.96±0.001 e
P2S3W2	24.06±0.007 a	0.53±0.003 j	0.33±0.001 l	4.37±0.000 h	3.22±0.002i
P3S1W1	4.98±0.000 o	0.98±0.001 f	0.15±0.001 q	1.92±0.000 k	1.95±0.001 n
P3S1W2	5.78±0.001i	0.53±0.002 k	0.17±0.000 o	0.15±0.001 s	4.43±0.001 f
P3S2W1	10.15±0.003 g	0.91±0.002 h	0.42±0.001 k	0.47±0.001 p	0.47±0.002 r
P3S2W2	0.48±0.003 u	1.61±0.002 e	2.05±0.000 c	0.89±0.001 m	1.93±0.001 p

P3S3W1	0.95±0.001 t	0.45±0.002 n	0.08±0.001 v	4.92±0.001 f	4.33±0.001 g
P3S3W2	0.35±0.002 v	0.21±0.003 r	0.09±0.002 s	4.98±0.000 d	9.77±0.001 a
CS1W1	1.12±0.001 s	0.50±0.001 l	0.09±0.000 u	1.93±0.001 j	3.09±0.000 l
CS1W2	1.12±0.001 s	0.10±0.002 t	0.21±0.001 m	0.43±0.001 q	4.31±0.001 h
CS2W1	11.25±0.011 f	0.08±0.000 u	0.19±0.001 n	4.92±0.001 f	9.35±0.002 c
CS2W2	11.63±0.005 e	0.09±0.001 u	0.16±0.002 p	0.88±0.001 n	9.35±0.006 c
CS3W1	3.73±0.001 p	0.42±0.002 p	0.48±0.000 h	0.88±0.001 n	0.88±0.001 q
CS3W2	11.86±0.006 d	0.07±0.000 v	0.94±0.000 f	4.95±0.001 e	0.48±0.003 r

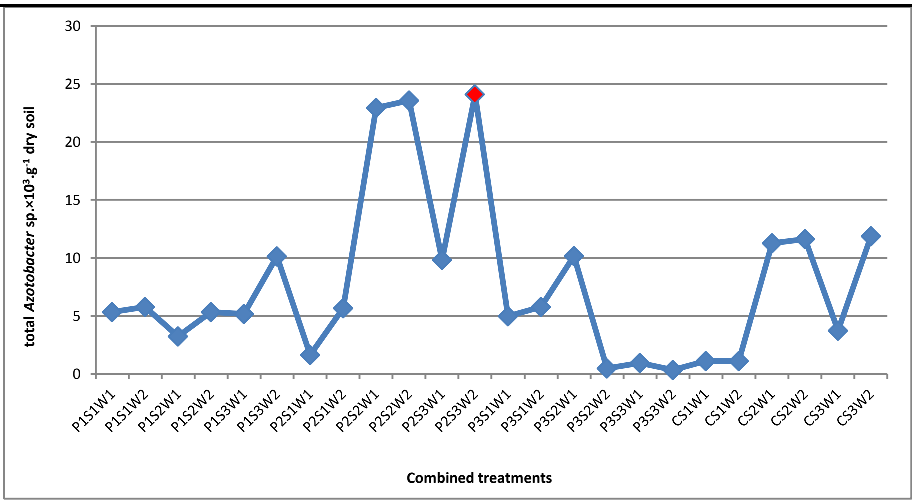

Figure-21: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during the 1st sampling period.

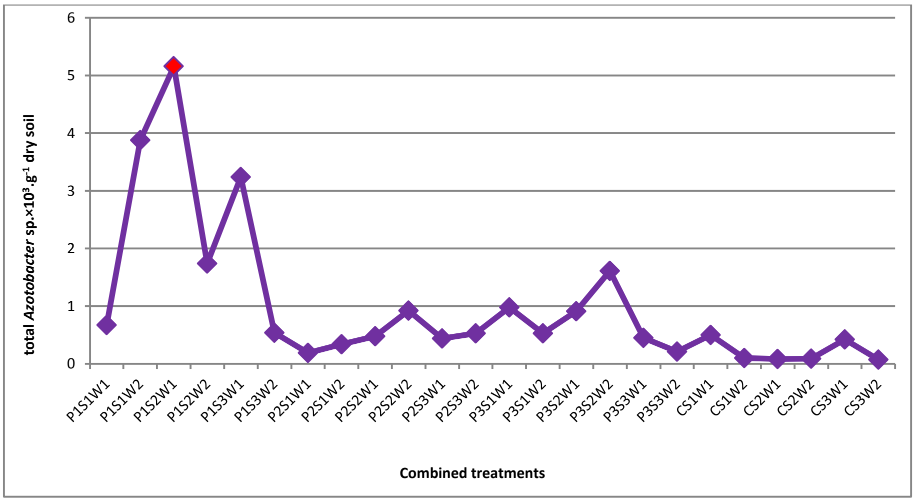

Figure-22: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during the 2ndsampling period.

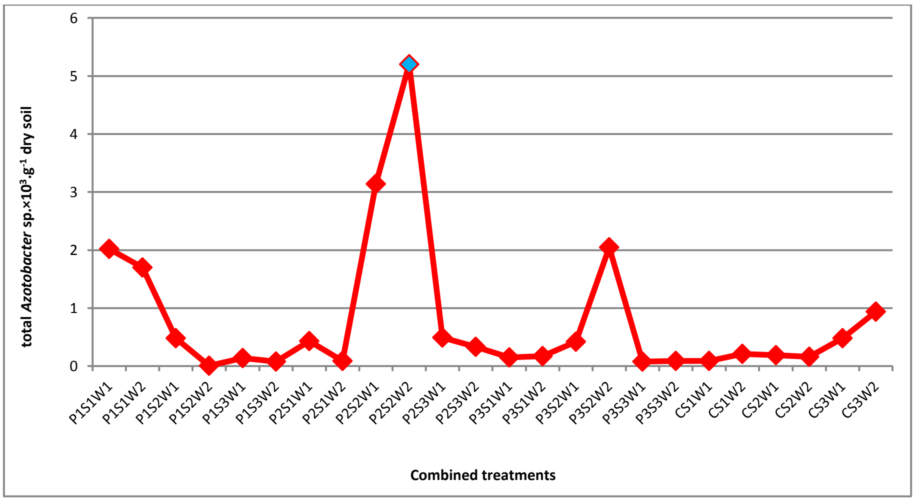

Figure-23: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during the 3rdsampling period.

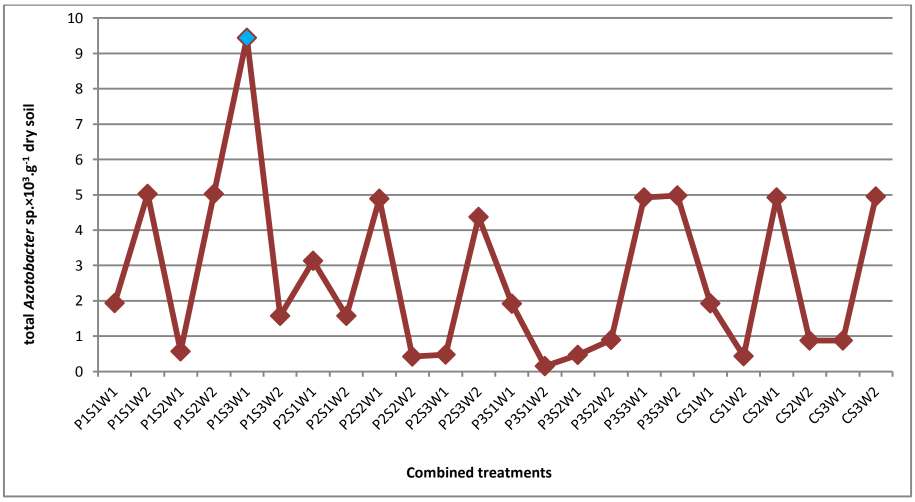

Figure-24: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total *Azotobacter*sp.×10^3.g^{-1} dry soil during the 4thsampling period.

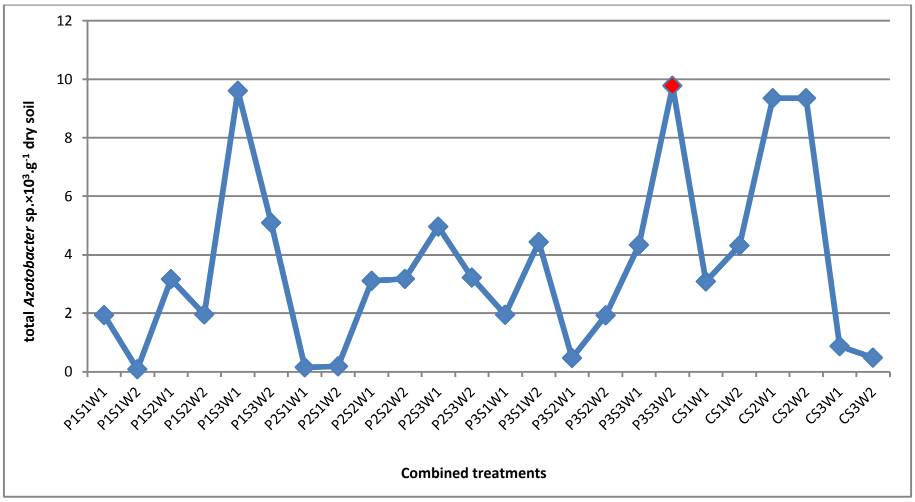

Figure-25: Effects of the combined treatments between pesticides, soil orders and moisture contents on soil total *Azotobacter*sp.$\times 10^3.g^{-1}$ dry soil during the 5^th^sampling period.

Discussion

Glyphosate caused the lowest total non-symbiotic nitrogen-fixing bacterial population during the 5th sampling period because glyphosate application has been found to reduce nitrogen fixation and make micronutrients less available [20] and [21]; during the 2nd sampling mancozeb showed the lowest total non-symbiotic nitrogen-fixing bacterial population, probably, because this bacterial group is regarded as a sensitive microbial indicator of environmental hazard in soil exposed to pesticides and other chemicals and the effects of these compounds on nitrogen fixation appear to be species-specific; during the 3rd and 4th sampling periods glyphosate showed the highest and diazinon showed the lowest population of total non-symbiotic nitrogen-fixing bacteria, in this regard [22] stated that non-symbiotic nitrogen-fixing bacteria and biological nitrogen fixation were more affected by pesticides than were other populations and activities of nitrogen cycle, as well as they stated that one pesticide could produce negative or positive effect on non-symbiotic nitrogen-fixing bacteria depend on the soil type.At the last sampling periods, 100% soil moisture content (W2) significantly reduced soil total non-symbiotic nitrogen-fixing bacteria and this may refer to higher temperature during these periods. The combined treatment CW2 showed the highest total non-symbiotic nitrogen-fixing bacterial count during the 1st and 2nd sampling periods because there was no pesticide treatment and typical water content was available for their growth. Diazinon and mancozeb decreased total *Azotobacter* sp. significantly during the last sampling periods and similar observations given by [23] when diazinon reduced *Azotobacter*population. Agholan soil showed the lowest number of total *Azotobacter*sp. during the experiment starting as observed by [23] when *Azotobacter*population reduced in clay-loam soil. At the last sampling periods Girdarasha soil showed the greatest number and Agholan showed the lowest number of total *Azotobacter*sp. and this may refer to higher organic matter and nitrogen content of Agholan which made *Azotobacter*sp. less inhabitant in such soil type. 100% soil moisture content showed the highest population of total *Azotobacter*sp. at the beginning and the end of the study and this may refer to proper humidity during this period.Mancozeb in Debaga soil (P2S2) revealed the highest population of total *Azotobacter*sp. during the 1st and 3rd sampling periods, probably because during mancozeb hydrolysis in the soil ethylenethiourea and ethyleneurea produced which in turn increased nitrogen content of soil and in the presence of excess nitrogen *Azotobacter*sp. use the available nitrogen for their cell synthesis [24]. The combination of P3W2 caused significant increasing in total *Azotobacter*sp. at the end of the study and this may due to the degradation of pesticide and reduced its concentration in the presence of sufficient water and thus the pesticide exhibited less effect. The combined treatment S1W1 decreased total *Azotobacter*sp. at the end of the study and this may due to the texture and composition of Agholan soil, in comparing with Debaga and Girdarasha soils, which has less pore size and oxygen content to be supplied for this aerobic group of bacteria.

Conclusion

Glyphosate and diazinon showed more positive effects on counts of soil total non-symbiotic nitrogen-fixing bacteria and total *Azotobacter* sp. than mancozeb. More increasing effect on counts of soil microbial population was shown by girdarasha soil. 100% soil moisture content (W2) showed more significant effects on soil total non-symbiotic nitrogen-fixing bacteria and total *Azotobacter*sp. counts.Different significant effects on soil characteristics were shown by the interactions between pesticides-soil orders, pesticides-soil moisture contents, soil orders-soil moisture contents and pesticides-soil orders-soil moisture contents.

Acknowledgment

Special thanks send to Dr. Abdul-Raheem Omer the director of Plant Protection in Erbil General Directorate of Agriculture who provided the studied pesticides. Special thanks to Dr. Aras Dashti and Miss. NareenQadrfor providing the used chemicals.

References

[1]Ellis, S. and A. Mellor. Soil and Environment. Routledge Publisher. Canada. (1995).

[2]Sang-Jun Kim, B.S., M.S.Bioaugmentation for the remediation of pesticide contaminated soil with microorganisms directly enriched in soil or compost. Ph.D. Thesis, University of Ohio State. (2003).

[3]Jiang, J. and N. Wan. A model for ecological assessment to pesticide pollution management. Ecol. Modelling. 220(15): 1844-1851. (2009).

[4]Meyers, R.A. Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation. John Wiley & Sons Ltd. 14484pp. (2008).

[5]Cycon, M; Z. Piotrowska-Seget and J. Kozdrój. Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. Int. Biodeterio. Biodeg. 64: 316-323. (2010b).

[6]Pozo, C.; M.V. Martinez-Toledo; B. Rodelas and J. González-López. Response of soil microbiota to the addition of 3,3-diaminobenzidine. Appl. Soil Ecol. 23: 119-126. (2003).

[7]Gopal, M.; A. Gupta; V. Arunachalam and S.P. Magu. Impact of azadirachtin, an insecticidal allelochemical from neem on soil microflora, enzyme and respiratory activities. Bioreso. Technol. 98: 3154-3158. (2007).

[8]Ababutain, M.I.; F.N. Barakah and A.M. Heggo. Effect of pesticides (lannate and diazinon) on growth and activity of soil free living nitrogen fixing bacteria. J. Saudi Soc. Agric. Sci. 8 (1): 40-52. (2009).

[9]Hang, M.; C. Zhongyun; Z. Yuhua and C. Meichi. Effects of trifluralin on soil microbial populations and the nitrogen fixation activities. J. Environ. Sci. Health, Part B. 36(5): 569-579. (2001).

[10]USAID. Soil testing. Perennial Crop Support Series. Jalalabad, Afghanistan. Publication No. 2008-001-AFG. February 8. (2008).

[11]Ryan, J.; Estefan, G. and Rashid, A. Soil and Plant Analysis Laboratory Manual. Second Edition. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. (2001).

[12]Pansu, M. and Gautheyrou, J. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods. Springer-Verlag Berlin Heidelberg. (2006).

[13]Bashour, I.I. and A.H. Sayegh. Methods of Analysis for Soils of Arid and Semi-Arid Regions. Food and Agriculture Organization of the United Nations, Rome. (2007).

[14]vanReeuwijk, L.P. Procedures for Soil Analysis, Technical Paper 9. Sixth Edition. International Soil Reference and Information Centre. The Netherlands. (2002).

[15]Hill, D.S. Pests of Crop in Warmer Climates and their Control. Springer Netherlands. XII Edition. (2008).

[16]Dashti, A.M. Isolation and characterization of Azotobacter spp. in Erbil soils, and study the effect of biofertilizers (Azotobacterchroococcum and transconjugant Lactobacillus plantarum) on nutrient uptake by wheat. Ph.D. Thesis, University of Salahaddin, Iraq. (2011).

[17]Atlas R.M. Handbook of Media for Environmental Microbiology. Second Edition. Taylor & Francis Group, LLC. New York. (2005).

[18]Pepper, I.L. and C.P. Gerba. Environmental Microbiology: A Laboratory Manual. Second Edition. Elsevier Academic Press. USA. (2004).

[19]Scharlau, C. Handbook of Microbiological Culture Media. Sixth Edition. Spain. (2001).

[20]Feng, J.C. and D.G. Thompson. Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. J. Agric. Food Chem. 38(4):118-1125. (1990).

[21]Gaines, T.A.; W. Zhang; D. Wang; B. Bukun; S.T. Chisholm; D.L. Shaner; S.J. Nissen; W.L. Patzoldt; P.J. Tranel; A.S. Culpepper; T.L. Grey; T.M. Webster; W.K. Vencill; R.D. Sammons; J. Jiang; C. Preston; J.E. Leach and P. Westra. Gene amplification confers glyphosate resistance in Amaranthuspalmeri. Proceedings of the National Academy of Sciences of the United States of America. 107(3): 1029-1032. (2010).

[22]Roger, P.A.; I. Simpson; R. Oficialc; S. Ardales and R. Jimenez. Effects of pesticides on soil and water microflora and mesofauna in wetland ricefields: a summary of current knowledge and extrapolation to temperate environments. Australian J. Exp. Agric. 34: 1057-1068. (1994).

[23]Barakah, F.N.; M.I. Ababutain and A.M. Heggo. Effect of lannate and diazinon pesticides on some soil microorganisms. Alexaneria Sci. Exch. J. 28(1): 38-53. (2007).

[24]McKinney, R.E. Environmental Pollution Control Microbiology. Marcel Dekker, Inc. 454pp.(2004).