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Full Length Research Paper

Effect of Thiobencab and Penoxsulam Herbicides on Soil Microbial Population and Weed Control in Transplanted Rice

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The present study was to investigate the effect of two herbicides, thiobencarb 50% EC and penoxsulam 24% SC at their recommended field rates on soil microbial population at regular intervals i.e. 4th to 28th day after treatment, control of weeds and crop yield of transplanted rice. From each plot, five soil sub-samples were collected randomly and thoroughly mixed to form one composite sample. Results showed that the counts of total aerobic bacteria dramatically decreased with lapse of time after treatment which decreased from 4th days (6.01 log CFU ml^{-1}), to 28th days (5.72 log CFU ml^{-1}), during 2016 season, and from 4th days (6.39 log CFU ml^{-1}), to 28th day (5.41 log CFU ml^{-1}), during 2017 seasons. Also, the higher enumeration of N_2-fixing bacteria, N_2-fixing cyanobacteria and sulphate reducing bacteria were recorded from 4th to 12th, then decreasing to 28th days after treatment. Also, the counts of nitrifies bacteria, showed increased up to 16th days after treatment, then decreased thereafter. Also, high significantly affected by weed control treatments. Application of penoxsulam recorded the lowest value of *Echnochlo crus-galli, Echnochlo colona* and total weeds dry weight and achieved the highest number of panicles and grain yield as compared to weedy check, which gave the highest value of total weeds dry weight and the lowest rice yield characters. penoxsulam was observed to be less toxic than thiobencarb against microbial enumeration, control of weeds and increase crop yield of rice plant.

Key words: Microbial populations; Thiobencarb; Penoxsulam; Weeds; Rice

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereals not only in Egypt but also all over the world, it is considered as the most important food for about the half of world population, contributing about 20% of cereal consumption.

The total cultivated area by rice in Egypt every year in average 0.562 million hectare and total productivity was 5.3 million ton RRTC (2016).

In agricultural systems, unwanted plants that are commonly referred to as "weeds". Weeds are the cause of serious yield reduction problems in rice production, its competitor of crop fields for light, nutrition, water, land spaces, carry insect pests and diseases, lower quality of

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production and sometimes causes complete failure of the main crop Abd El-Razek et al. (2014). In modern agriculture, weed control in rice fields by herbicides has been increased steadily and it has been expanding enormously worldwide over the past 20-40 years. Therefore, the goal of herbicide is to kill or stunt weed infestation allowing the rice to grow and gain a competitive advantage. So, every farmer must be provided with a great amount of useful scientific information for control these weeds and increasing yield (Yamamoto and Nakamura 2003 and Galhano et al. 2010).

Beside herbicides used to control unwanted plants in the ecosystem, also leave unwanted residues in soil due to reduce the performance of important soil functions and poses a risk to the entire ecological system such as (a) affecting plant growth regulators, (b) affecting protein synthesis, (c) affecting cellular membrane, and (d) changing their biosynthetic mechanism Ayansina and Oso (2006) and Riaz et al. (2007).

In the top layer soil (0-15 cm), most of the microbiological activities occur, especially during the application of the herbicides. These herbicides can cause qualitative and quantitative effects on soil microbial communities and change in enzyme activity Xia et al. (2012), Baboo et al. (2013), Das et al. (2015), Asad et al. (2017) and Chen et al. (2017). Carbonaceous substances including the accumulated herbicides in soil can degraded by microorganisms to derive their energy and other nutrients for their cellular metabolism due to microbial biomass increases Debnath et al. (2002) and Das et al. (2003). On the other hand, some herbicides are not easily utilized by soil microorganisms due to microbial biomass decreases (Nongthombam et al. 2009).

Many studies pointed out that application of herbicides in the rhizosphere soils of rice, increased the growth and activities of aerobic non-symbiotic N_2-fixing and phosphate solubilizing bacteria as well as greater uptake for nitrogen and phosphorus led to increasing yield Das and Debnath (2006). Similar results was observed by Das et al. (2015), when applied thiobencarb and pretilachlor in the rhizosphere soils of rice. Also, significantly increased the microbial biomass C, N and P, resulting in greater release of available plant nutrients in soil as reported by Bhowmick et al. (2014) and Barman and Das (2015).

The objectives of the present study were to investigate the effect of two post-emergence herbicides, penoxsulam and thiobencarb at their recommended field rates on soil microbial population at regular intervals i.e. 4^{th} to 28^{th} day after treatment, total weeds dry weight (g m^{-2}), number of panicles (m^{-2}) and rice grain yield (ton ha^{-1}), during 2016 and 2017 seasons.

MATERIALS AND METHODS

This study was conducted during 2016 and 2017 summer seasons in the experimental effects of farm of the Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt to study the two herbicides *viz*., thiobencarb 50% EC and penoxsulam 24% SC on soil microbial populations, control of weeds, growth and yield of rice plant under transplanting method.

Plant Material and Growing Conditions

Seeds of rice variety (Sakha 106) was obtained from the Rice Research and Training Center. The nursery area was prepared by plowing, dry and wet leveling after flooding. Rice seeds was soaked in fresh water for 24 hour and incubated for another 24 hour, then broadcasted at the rate of 120 kg ha^{-1} in the flooded nursery homogenously by hand. Fertilizers were applied for the nursery area as recommended. Thirty days old seedling was transplanted at the rate of 3-4 seedlings per hill with spacing 20 x 20 cm between hills and rows. While were plots flooded for 4 days and drained for 2-3 days for well establishment of the plants. Thereafter, seedlings were transplanted at 20^{th} of May in both seasons of study. Plot size was 12 m^2 (3 x 4) with four replicates following Randomized Complete Block Design (RCBD). The field experiment was conducted in clayey texture having the following characteristics as shown in Table 1.

All cultural practices; *i.e.,* land preparation, mineral fertilization and pest management were done as recommended in transplanted rice field. From analysis of the experimental soil Table 1, there is no need to phosphorus fertilizer, which there is enough amount for rice plant.

Herbicides and dosage

Two herbicides, viz., thiobencarb 50% EC (Saturn) at 2.38 kg a.i. ha^{-1} (Recommended dose) as pre-emergence herbicide was applied mixed with sand as well as penoxsulam 24% SC (Granite) at 0.02 kg a.i. ha^{-1} (Recommended dose) as post-emergence herbicide was sprayed at 4 days after transplanting (DAT) mixed with 300 liters water per hectare on flooded land then was kept the field flooded by water for 3 days after herbicidal application.

Sampling of soil

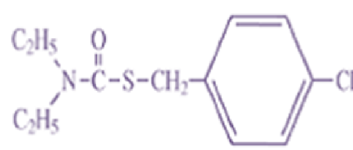
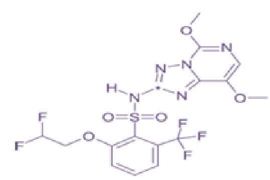
Five soil sub-samples were collected randomly from each plot at a depth of (0-15) cm, and thoroughly mixed to form

Table 1: Some mechanical, chemical and biological properties of the experimental soil sites during 2016 and 2017 seasons

Season	Mechanical analysis %			Texture	pH *	EC ** dSm^{-1}	OM ** %	Available elements (mg Kg^{-1})		
	Sand	Silt	Clay					N	P	K
2016	32.00	11.00	57.00	Clayey	8.05	2.00	1.65	13.5	14.00	366
2017	35.00	11.00	54.00	Clayey	8.20	2.05	1.50	12.60	12.00	350
Season	**Total count of bacteria (CFU ×10^{7} g^{-1} dry soil)**			**Total count of fungi (CFU ×10^{4} g^{-1} dry soil)**			**Total count of Actinomycetes (CFU ×10^{5} g^{-1} dry soil)**			
2016	13			27			82			
2017	20			17			52			

* 1:2.5 soil: water suspension, ** Soil past extract, CFU: Colony Forming Unit.

Table 2: Some characteristics of studied herbicides

Character \ Herbicide	Saturn 50% EC	Granite 24% SC
Active ingredient	Thiobencarb	Penoxsulam
Chemical group	Thiocarbamate	Sulfonamide or Triazolopyrimidine
Chemical name	[(S-(4-chlorophenyl) methyl) N,Ndiethylcarbamothioate]	2-(2,2-difluoroethoxy)-N-(5,8-diimethoxy [1,2,4]triazolo[1,5-c] pyrimidin-2-yl)-6-(trifluoromethyl) benzenesulfonamide
Molecular formula	$C_{12}H_{16}ClNOS$	$C_{16}H_{14}F_5N_5O_5S$
Mode of action	Systemic -photosynthesis inhibitors	Systemic – ALS inhibitors
Structure		
Rate (fed^{-1})	2 Lit.	35 ml.
Rate (Kg ai ha^{-1})	3.570	0.020
Target weeds	Grassy + sedges	Grassy + broadleaves + sedges

one composite sample and were analyzed for different parameters. The effect of studied herbicides in agricultural soil were analyzed in response to microbial enumeration with respect to control soil (without treatment) at regular intervals *i.e.* 4th, 8th, 12th, 16th, 20th, 24th and 28th day after treatment for a period of 4 weeks individually.

Microbial enumeration

Series of decimal dilutions were prepared in sterile physiological saline (8.5 g NaCl L^{-1}). 1.0 ml of the higher dilutions were used for the estimations of total aerobic bacterial and total nitrogen fixing bacterial counts using the standard plate counts (Allen, 1950) as well as determination of cyanobacteria, nitrifiers and sulphate – reducers using the most probable number (MPN) technique, Cochrane (1950).

Total aerobic bacteria

Total aerobic bacteria count was determined using the soil extract agar medium Mahmoud (1955), which containing g l^{-1}: soil extract, 500 ml; tap water, 500 ml; K_2HPO_4, 0.5 g; yeast extract, 0.5 g; glucose, 1.0 g; agar agar, 15.0 g. The medium is adjusted to pH 7 and autoclaved at 121°C for 15 minutes. The incubation period was four days at 28 °C.

Total nitrogen fixing bacteria

Total nitrogen fixing bacteria was count by combined carbon medium Free-living putative nitrogen-fixing bacteria Rennie (1981), which containing:
Solution I: K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g L-1; Na_2Fe EDTA, 28.0 mg; $Na_2MoO_4.2H_2O$, 25.0 mg; NaCl, 0.1 g; yeast extract, 0.1 g; mannitol, 5.0 g; sucrose, 5.0 g; Na-lactate

(60% v=v), 0.5 mL; distilled water, 900 mL; agar, 15.0 g. Solution II: $MgSO_4.7H_2O$, 0.2 g; $CaCl_2$, 0.06 g; distilled water, 100 mL. Solution III: Biotin, 5.0 mg mL^{-1}; p-aminobenzoic acid (PABA), 10:0 mg mL^{-1}. solutions I and II was adjusted to pH 7 and autoclaved at 121°C for 15 minutes, cool to 48°C and mix thoroughly, then add (filter-sterilized using a sterile 0:45 mm filter) 1 mL L^{-1} of solution III.

Nitrogen - fixing cyanobacteria

Nitrogen - fixing cyanobacteria was determined by the MPN using the Modified Watanabe medium El–Nawawy et al. (1958), which containing g l^{-1}: K_2HPO_4, 0.30 g; $MgSO_4.7H_2O$, 0.20 g; K_2SO_4, 0.20 g; $CaCO_3$, 0.10 g; glucose, 2.00 g; $FeCl_3$, 1% (freshly prepared) 0.20 ml, microelements solution 1.00 ml and distilled water up to 1000 ml. The microelements solution g l^{-1}: H_3BO_3, 2.80 g; $MnCl_2$, 1.80 g; $ZnSO_4.7H_2O$, 0.22 g; $CuSO_4.5H_2O$, 0.08 g; molybdic acid, 0.02 g; distilled water 1000 ml. The medium is adjusted to pH 7 and autoclaved at 121°C for 15 minutes.

Sulphate reducing bacteria

The Most Probable Numbers (MPN) of sulphate – reducing bacteria were counted using the modified Starkey's medium, Abd El-Malik and Risk (1958), which containing g l^{-1}: Sodium lactate, 3.5 g; NH_4Cl, 1.0 g; K_2HPO_4, 0.50 g; $MgSO_4.7H_2O$, 2.0 g; Na_2SO_4, 0.5 g; Mohr's salt (ferrous-ammonium sulphate) trace; Tap water 1000 ml. An iron nail of 4 cm length and 0.25 cm diameter was added as a reducing agent to secure the low oxidation-reduction potential needed for growth of the sulphate reducers. The medium is adjusted to pH 7 and autoclaved at 121°C for 15 minutes. The inoculated tubes were incubated at 30°C for two weeks. Positive tubes were recognized by the formation of bluish-black coloration of ferrous sulphide.

Nitrifiers bacteria

Nitrifiers bacteria was determined using the Stephenson's medium for autotrophic nitrifiers Stephenson (1950), which containing g l^{-1}: Ammonium sulphate, 2.00 g; K_2HPO_4, 0.75 g; FeSO4.$7H_2O$, 0.01 g; KH_2PO_4, 0.25 g; $MnSO_4.4H_2O$, 0.01 g; $MgSO_4.7H_2O$, 0.03 g; $CaCl_2$, 0.002 g; distilled water 1000 ml. The medium is adjusted to pH 7 and autoclaved at 121°C for 15 minutes. After autoclaved, a knife tip of sterile $CaCO_3$ was added to each tube. Incubation temperature was 30°C for 21 days. Diphenylamine in concentrated sulphuric acid was used for detection of the produced nitrate.

Estimation of weeds and crop yield

At 30 days after herbicide treatment (DAT), weeds were sampled by area of 50 x 50 cm quadrate replicated four times for each plot, weeds were cleaned, air dried then oven dried to stable weight, dry weight as g m^2 was recorded. Also, rice dry weight was measured in the same method. Before harvest, panicles were counted in two random quadrates of 50 x 50 cm and number of panicles per square meter was recorded. Panicle weight (g) was estimated by weighing ten panicles per plot and their average was recorded. After rice maturity, the central 5 m2 from each plot were manually harvested dried and grain yield then was recorded at 14% moisture content (t ha^{-1}). Thrashed and rice grains were estimated.

Statistical analysis

The results were subjected to staticall analysis of variance according to Snedecor and Cochran (1971). Weed data were transformed according to square-root transformation ($\sqrt{\square}$ + 0.5) then rice collected data and transformed data of weeds were statistically analyzed by MSTATC program. The means of both weeds and rice characters were compared using Duncan´s Multiple Range Test Duncan (1955).

RESULTS AND DISCUSSIONS

A. Microbial enumeration:

The abundance and distribution of different groups of soil microorganisms, included total count of aerobic bacteria and nitrogen fixing bacteria presented in terms of $\log_{10}$ transformed of CFU g^{-1} of soil. On the other hand, cyanobacteria; sulphate reducing bacteria and nitrifiers bacteria were enumerated and expressed in terms of colony forming units per gram of soil (CFU g^{-1}). Based on microbial enumeration, differences could be observed by the different herbicide treated soil profiles.

A.1. Total count of aerobic bacteria and N_2- fixing bacteria:

The variation of soil microbial population with respect to Penoxsulam and Thiobencarb herbicides with different times after application showed significant differences. Results in Table 3 indicated that the two herbicides attained a slight decrease in counts of total aerobic bacteria as compared to N_2-fixing bacteria. On the other hand, Penoxsulam herbicide recorded the highest

Table 3: Total count (Log CFU ml^{-1}) of aerobic bacteria and N_2- fixing bacteria as affected by weed control treatment and sampling times after application during 2016 and 2017 seasons

Treatments	**2016 season**		**2017 season**	
A- Weed control treatment	**Total aerobic bacteria**	**N_2-fixing bacteria**	**Total aerobic bacteria**	**N_2-fixing bacteria**
Control (un-treated)	6.03 a	5.22 c	6.15 a	5.16 c
Thiobencarb 50% EC	5.75 b	6.27 b	5.95 b	5.98 b
Penoxsulam 24% SC	5.78 b	6.36 a	5.98 b	6.06 a
F. test	**	**	**	**
B- Sampling times				
Control (Before application)	7.01 a	6.05 b	7.08 a	6.08 c
4 days	6.01 c	6.33 a	6.39 b	6.40 a
8 days	5.36 h	6.29 a	5.48 f	6.37 ab
12 days	6.06 b	6.27 a	6.24 c	6.31 b
16 days	5.73 d	6.03 b	5.91 d	6.11 c
20 days	5.68 e	5.30 c	5.84 e	5.83 d
24 days	5.86 f	4.79 d	5.86 e	5.45 e
28 days	5.72 g	4.80 d	5.41 g	5.04 f
F. test	**	**	**	**
Interaction A x B	**	**	**	**

In a column means followed by a common letter are not significantly different at 5% level by DMRT.

stimulated for N_2-fixing bacteria (6.36 log CFU ml^{-1}), as compared to Thiobencarb herbicide (6.27 log CFU ml^{-1}), during 2016 season. The same trend was exhibited at 2017 season. Therefrom, Penoxsulam herbicide was observed to be less effect harmful than thiobencarb herbicide. The counts of total aerobic bacteria dramatically decreased with lapse of time after treatment which decreased from 4th days (6.01 log CFU ml^{-1}), to 28th days (5.72 log CFU ml^{-1}), during 2016 season, and from 4th days (6.39 log CFU ml^{-1}), to 28th day (5.41 log CFU ml^{-1}), during 2017 seasons. Also, the higher enumeration of N_2-fixing bacteria was recorded from 4th to 12th, then decreasing to 28th days after treatment in the two growing seasons.

Various studies have revealed that herbicides can cause qualitative and quantitative change in soil microbial populations Bhowmick et al. (2014), Barman and Das (2015), Asad et al. (2017) and Chen et al. (2017). Many microorganisms have the ability to degrade herbicide for their growth, utilized as carbon source and energy production, while some others were adversely affected depending upon the agrochemicals (type/formulation and concentration), mode of action, groups of microorganisms and environmental conditions. The significant rise in soil microbial population during 4th–20th days after treatment followed by a gradual decrease up to 28th days due to biodegradation of herbicide and led to a change in microbial community structure and change in enzyme activity Das et al. (2015), Asad et al. (2017) and Chen et al. (2017). Also, many studies pointed out that vibration in soil microbial populations in the rhizosphere soils of rice, led to greater uptake for nitrogen and phosphorus and increasing yield as well as release of available plant nutrients in soil Bhowmick et al. (2014) and Barman and Das (2015).

B. Interaction effect of total count of aerobic bacteria and N_2-fixing bacteria as affected by weed control treatments and sampling times

B.1. Total count of aerobic bacteria:

Generally, total count of aerobic bacteria with respect to penoxsulam and thiobencarb herbicides as well as the number of days after treatment was found to be significant (p<0.05). The treated and untreated soil samples showed a decreasing trend in total count of aerobic bacteria from 4th day to 28th day as compared to the results of the soil samples before treatment (0 day) during 2016 and 2017 seasons. On the other hand, there are vibration in populations of total aerobic bacteria recorded decrease then increase values after treatment. The maximum values of total aerobic bacteria showed that 12th day after treatment recorded 6.07, 6.13 and 6.22, 6.26 log CFU ml^{-1} for penoxsulam and thiobencarb herbicides compared to control 5.98 and 6.23 log CFU ml^{-1} in 2016 and 2017 season, respectively (Figure 1).

At the end of the experiment, this vibration in populations of total aerobic bacteria resulted from drying and wetting conditions and through these different conditions can be utilized the herbicides as carbon source, energy production as well as other nutrients for their

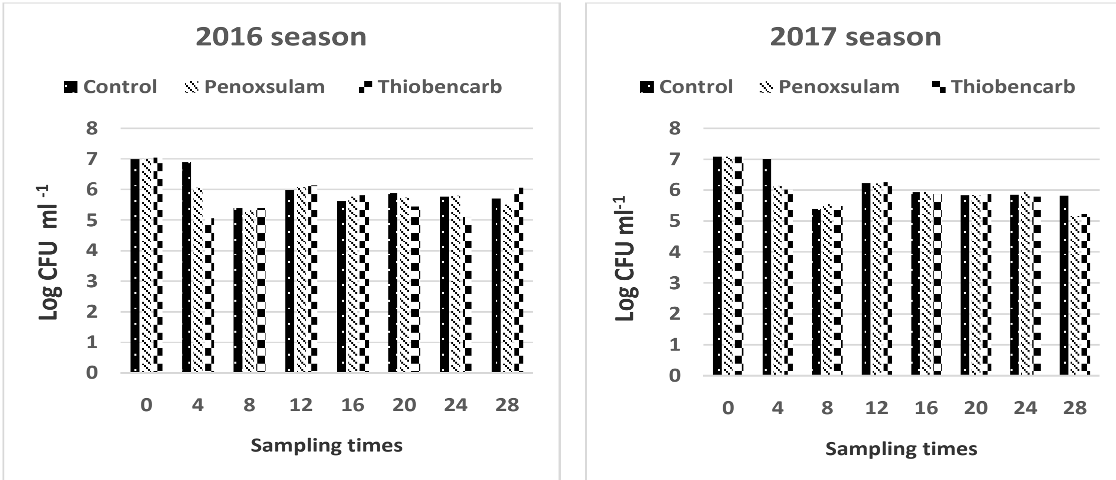


Figure 1. Log number of total count of aerobic bacteria as affected by the interaction between weed control treatments and times during 2016 and 2017 seasons.

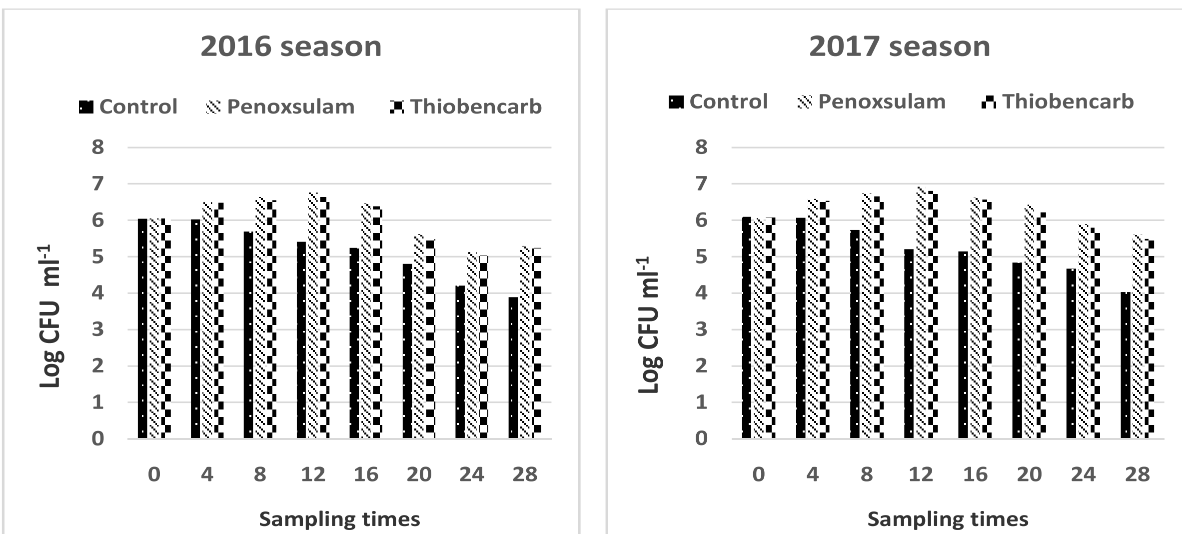


Figure 2. Log number of nitrogen fixing bacteria as affected by the interaction between weed control treatments and times during 2016 and 2017 seasons.

growth and metabolism especially in the herbicide-treated soils Das and Debnath (2006) and Chen et al. (2014, 2015). Many microorganisms have the ability to degrade herbicide for their growth, while some others were adversely affected depending upon the agrochemicals (type/formulation and concentration), mode of application, groups of microorganisms and environmental conditions Sebiomo et al. (2011), Baboo et al. (2013), Asad et al. (2017) and Chen et al. (2017). Besides, herbicides decomposition is frequently faster in soils that contain high organic matter, presumably because of more vigorous microbial activity. Various studies have revealed that herbicides can cause qualitative and quantitative change in soil microbial populations Pampulha et al. (2007), Latha and Gopal (2010), Bhowmick et al. (2014) and Barman and Das (2015).

B.2. Nitrogen fixing bacteria:

Data on total count of nitrogen fixing bacteria from the soil samples treated with herbicides during 2016 and 2017 seasons are presented in Figure 2. Significant differences

Table 4: N_2-fixing cyanobacteria, sulphate reducing bacteria and Nitrifiers bacteria (CFU×10^3 g^{-1} soil) as affected by weed control treatment and different times after application during 2016 and 2017 seasons

Treatments	2016 season			2017 season		
A- Weed control treatment	N_2-fixing cyanobacteria	Sulphate reducing bacteria	Nitrifiers bacteria	N_2-fixing cyanobacteria	Sulphate reducing bacteria	Nitrifiers bacteria
Control (un-treated)	9.14	0.99	0.63	9.91	1.31	0.68
Thiobencarb 50% EC	9.50	0.67	0.53	11.09	0.82	0.46
Penoxsulam 24% SC	13.23	0.63	0.66	14.02	0.91	0.75
B- Sampling times						
Control (Before application)	10.01	2.22	0.42	9.94	2.99	0.40
4 days	11.80	0.64	0.38	12.69	0.99	0.43
8 days	13.37	0.71	0.50	14.66	0.90	0.59
12 days	15.15	1.24	0.85	16.95	1.21	0.72
16 days	12.27	0.72	1.03	15.86	1.21	1.12
20 days	8.09	0.25	0.92	9.82	0.34	0.83
24 days	8.27	0.18	0.55	7.72	0.27	0.64
28 days	6.04	0.14	0.21	5.78	0.18	0.29

were observed in microbial population of nitrogen fixing bacteria at different days after the application of Penoxsulam and Thiobencarb herbicides. Concerning untreated control, results showed decreasing in counts of nitrogen fixing bacteria with different days after treatment. However, the treated soil showed an increasing trend of log number from 4^{th} day (6.50 and 6.48), to 12^{th} day (6.76 and 6.64), after treatment then gradually decreases and found to be minimum on 28^{th} day (5.29 and 5.24), for Penoxsulam and Thiobencarb herbicides at 2016 season, respectively. Similar trend was also exhibited in treated soil at 2017 season. Further, Penoxsulam herbicide was observed to be less toxic than Thiobencarb herbicide against microbial enumeration of nitrogen fixing bacteria. The significant rise in nitrogen fixing bacteria during 4^{th} to 12^{th} days followed by a gradual decrease up to 28^{th} days of sampling, pointed out that N_2-fixing bacteria preferably utilized the herbicide residues as their source of carbon and other nutrient elements for their cellular metabolism Debnath et al. (2002) and Das et al. (2012). Based on the agrochemicals formulation and concentration, application of herbicides, butachlor, fluchloralin, oxadiazon and oxyfluorfen showed a high stimulation of the population and activities of atmospheric nitrogen fixation and phosphate solubilization in the rhizosphere soils of the rice crop Das and Debnath (2006). In addtition, application of thiobencarb at lower concentration (1.5 kg ha^{-1}) increased of aerobic non-symbiotic N_2-fixing bacteria.

C. Effect of weed control treatments and sampling times after application on microbial population of nitrogen fixing cyanobacteria, sulphate reducing bacteria and nitrifiers bacteria

C.1. Nitrogen fixing cyanobacteria:

Results of microbial population of N_2-fixing cyanobacteria with application of herbicides (Thiobencarb 50% EC and Penoxsulam 24 % SC) as well as the results without this application are included in Table 4. Generally, population of N_2- fixing cyanobacteria were increased in 2017 season compared with 2016 season. Also, penoxsulam herbicide was observed to be less harmful effect than thiobencarb herbicide against microbial enumeration of N_2-fixing cyanobacteria. Based on the results of our research, the effect of herbicides on population of N_2-fixing cyanobacteria was found to be at its highest level on 12^{th} day (15.15×10^3 g^{-1} soil and 16.95×10^3 g^{-1} soil), then it gradually decreases on 28^{th} day (6.04 ×10^3 g^{-1} soil and 5.78×10^3 g^{-1} soil) during 2016 and 2017 seasons, respectively. The variation in soil N_2-fixing cyanobacteria was dependent on the composition and dose of herbicide with time. Therefore, biodegradation of herbicide by microorganisms may have led to a change in resource quality and caused a shift in microbial community structure. The significant rise in microbial population during 4^{th} –20^{th} days followed by a gradual decrease up to 28^{th} days of sampling, pointed out that N_2-fixing cyanobacteria preferably utilized the incorporated herbicide residues Baboo et al. (2013), Das et al. (2015), Asad et al. (2017) and Chen et al. (2017).

C.2. Sulphate reducing bacteria:

The variation in sulphate reducing bacteria with respect to studied herbicides and the number of days after treatment under field conditions are shown in Table 4. It could be observed that the untreated plots recorded the highest count figure for sulphate reducing bacteria followed by penoxsulam application, while Thiobencarb application recorded the least during the two seasons of study. In general, the treated and untreated soil showed a decreasing trend in total count of sulphate reducing bacteria with different days after treatment**.** The maximum enumeration was recorded 1.24×10^3 g^{-1} soil and 1.21×10^3 g^{-1} soil on 12th day after treatment during 2016 and 2017 seasons, respectively. A sharp decrease in total count of sulphate reducing bacteria was observed from 20th day until the end of the experiment (28th days after treatment).

Concerning the positive effects of herbicide application on vibration the populations of sulphate reducing bacteria through 4th to 16th days after treatment due to fast biodegradation of used herbicides which contains sulfur element (S) in molecular formula especially in wetting conditions. On the other hand, the lower counts of sulphate reducing bacteria after 20th days after treatment, due to the application of the chemical more or less sustained aerobic conditions in the system or direct effect of the material itself as an oxidizing agent as well as toxicity effects on the anaerobic sulphate reducers rather than the aerobic forms and /or a direct effect on various soil enzymatic activities produced by different microorganisms. These results are in agreement with those reported by Irisarri et al. (2001), Elsaadany (2006), Chen et al. (2015) and Chen et al. (2016).

C.3. Nitrifiers bacteria:

The application of herbicides for weed control in the soils in the presence of rice crop may resulted in stimulation of the population of nitrifiers bacteria (Table 4). Penoxsulam herbicide recorded the highest stimulated for nitrifies bacteria (0.66×10^3 g^{-1}), as compared to thiobencarb herbicide (0.53×10^3 g^{-1}) during 2016 season. The same trend was exhibited at 2017 season. So, Penoxsulam herbicide was observed to be less toxic than thiobencarb herbicide.

The higher enumeration of nitrifiers bacteria were stimulated on 16th days of treatment (1.03×10^3 g^{-1} soil and 1.12×10^3 g^{-1} soil), during 2016 and 2017 seasons, respectively, over control treatment then decreased thereafter at the end of the experiment (28th days), similar way in nitrifiers bacteria count was observed in penoxsulam and thiobencarb treated soil, which gave (9.75×10^3 g^{-1} soil) and (5.51×10^3 g^{-1} soil), compared with untreated soil control (2.85×10^3 g^{-1} soil), at 2016 season, respectively. In this regard, these results are compatible with Elsaadany (2006), Debnath et al. (2002), Chen et al. (2009) and Chen et al. (2017).

D. Estimation of weeds and rice yield

D.1. Estimation of weeds

The common weed species in the experimental field associated with transplanted rice crop during the two growing seasons were: Grassy weeds including; a- *Echinochloa crus- galli* (barnyard grass). b- *Echinochloa colona* (jungle rice) and c- Broad leaves. The presence % of weed species was (25, 49 and 26%) and 21, 50 and 29%) for the first and second season of the study, respectively.

Data on *E. crus-galli, E. colona* and total weeds dry weight as affected by weed control treatments in 2016 and 2017 seasons are presented in Table 5.

From data in Table (5), it could be detected that the application of both penoxsulam and Thiobencarb significantly reduced the dry weights of grassy, broadleaves and total weeds during the two seasons of study as compared to the untreated plots. Moreover, penoxsulam 24% greatly achieved more dry weights of E. crus-gally, E. colona, broadleaves and total weeds as compared to Thiobencarb in spite the non significant difference between both herbicides for dry weight of total weeds in the first season. On the other hand, weedy check plots exhibited the highest values of dry weights for all weed species and total weeds during 2016 and 2017 seasons. The reduction in dry weight of *E. crus-galli, E. colona* and total weeds may be related to the high efficiency of herbicide treatment at recommended dose against weeds by germination inhibition and growth reduction as compared to weedy check plots during the two seasons of study as cited by Pal et al. (2009) and Singh and Ghoshal (2010).

D.2. Estimation of rice yield

Data on rice dry weight, panicle weight, number of panicles and grain yield as affected by weed control treatments in 2016 and 2017 seasons are presented in Table 6.

Rice dry weight, panicle weight, number of panicles m^{-2} and grain yield (t ha^{-1}) were highly significantly affected by weed control treatments during the two seasons of study. The two chemical treatments resulted in considerable increases in dry weight of rice plants, panicle weight, panicles number pre unit area and rice grain yields as compared to weedy check plots. Additionally, highly significant increases in all these characters were obtained by the application of penoxsulam 24% as compared to Thiobencarb 50%. The same trend was detected during

Table 5: Dry weight (g.m^{-2}) of *E. crus-galli, E. colona* and total weeds as affected by weed control treatments during 2016 and 2017 seasons

Weed control treatment	Rate (Kg. ai. h^{-1})	Time of (application)	Dry weight (g.m^{-2})			
			E. crus-galli	*E. colona*	Broad leaves	Total weeds
			2016 season			
Thiobencarb 50% EC	3.570	4 DAT	15.93 (4.03 b)	66.30 (8.17 b)	8.23 (2.97 b)	90.47 (9.53 b)
Penoxsulam 24% SC	0.020	4 DAT	3.63 (2.03 c)	12.63 (3.60 c)	1.20 (1.30 c)	17.47 (9.53 b)
Control (untreated)	-	-	62.03 (7.90 a)	119.63 (10.97 a)	65.27 (8.10 a)	246.90 (15.73 a)
F. test	-	-	**	**	**	**
			2017 season			
Thiobencarb 50% EC	3.570	4 DAT	12.33 (3.53 b)	56.17 (6.70 b)	4.30 (2.13 b)	72.67 (8.57 b)
Penoxsulam 24% SC	0.020	4 DAT	2.33 (1.67 c)	8.33 (2.97 c)	0.30 (0.87 c)	10.97 (3.36 c)
Control (untreated)	-	-	41.93 (6.50 a)	103.20 (10.17 a)	58.00 (7.67 a)	203.13 (14.27 a)
F. test	-	-	**	**	**	**

In a column means followed by a common letter are not significantly different at 5% level by DMRT. DAT (Days after transplanting)

Table 6: Rice dry weight, panicle weight, number of panicles and grain yield as affected by weed control treatments during 2016 and 2017 seasons

Weed control treatment	2016 season			
	Rice dry weight (g. m^{-2})	Panicle weight (g)	Panicle number m^{-2}	Rice yield (t. ha^{-1})
Thiobencarb 50% EC	839.90 b	2.40 b	442.66 b	7.23 b
Penoxsulam 24% SC	1096.17 a	2.80 a	474.67 a	8.63 a
Control (un-treated)	454.73 c	1.60 c	259.00 c	2.76 c
F. test	**	**	**	**
	2017 season			
Thiobencarb 50% EC	903.28 b	2.48 b	458.66 b	7.78 b
Penoxsulam 24% SC	1232.62 a	2.92 a	490.67 a	9.29 a
Control (un-treated)	520.28	1.83 c	277.33 c	3.08 c
F. test	**	**	**	**

In a column means followed by a common letter are not significantly different at 5% level by DMRT.

the two seasons of study. These increases of abovementioned characters may be due the superiority application of penoxsulam 24% SC at 0.020 kg ai ha^{-1} which, may be resulted from the high efficiency of chemical treatment in controlling for both grassy and broadleaf weeds and thus allow rice to grow free of weed competition. These findings are confirmed with those reported by Singh and Ghoshal (2010), Kogan et al. (2011) and Kolo and Umaru (2012), they found that the highest grain yield was obtained with penoxsulam at 22.5 and 25 g h^{-1} at 3 days after transplanted rice.

CONCLUSION

The results of the present investigation confirmed that application of thiobencarb 50% EC and penoxsulam 24% SC herbicides, in general, attained a slight decrease in counts of total aerobic bacteria as compared to N_2-fixing bacteria at regular intervals from 4th to 28th day after treatment. On the other hand, N_2-fixing cyanobacteria and sulphate reducing bacteria were found to be at its highest level on 12th day then it gradually decreases on 28th day. For nitrifiers bacteria, the higher enumeration of nitrifiers bacteria were stimulated on 16th days of treatment, and these results are reflected in the significant reduction of weeds which is the main propose of the compounds and consequently increasing the crop yield.

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