# Influence of Herbicides on Population Densities of Certain Soil Microflora, Weed Control and Drill-Seeded Rice Productivity

Abdelgawad, Y. E.<sup>1</sup> and S. S. M. Abd El-Naby<sup>2</sup>

<sup>1</sup>Microbiology Dept., Sakha agricultural Research Station, Soil, Water and Environment Institute, ARC, Egypt

<sup>2</sup>Rice Dept., Field Crop Research Institute, ARC, Giza, Egypt



#### ARSTRACT

This study was conducted at the Experimental Farm of rice department, Sakha Agricultural Research Station, Egypt during 2015 and 2016 summer seasons to investigate the effect of recommended doses from Stomp (pendimethalin 50% EC) and Rainbow (penoxsulam 2.5% OD) herbicides on weed control, microbial activities and rice productivity under drill-seeded rice cultivation. Total bacterial count, N<sub>2</sub>-fixing bacteria, N<sub>2</sub>-fixing cyanobacteria, nitrifiers and sulfate-reducing bacteria were counted at different periods of crop growth. Dry weight of *Echinochloa crus-galli, Echinochloa colona, Ammania baccifera* and total weeds were measured at 45 and 60 DAS. Paddy rice dry weight at 45 and 60 DAS, number of panicles, panicle weight and grain yield were determined. The results revealed that total bacterial count, nitrogen-fixing bacteria, native N<sub>2</sub>- fixing cyanobacteria and nitrifying bacteria were increased with penoxsulam application, while sulphate reducers were decreased in both seasons compared with application of pendimethalin and the untreated control. Penoxsulam application significantly decreased dry weight of *Echinochloa crus-galli, Echinochloa colona* and total weeds. While increased rice dry weight, number of panicles/m<sup>2</sup>, panicle weight and grain yield of rice in the two growing seasons.

Keywords: Microflora, pendimethalin, penoxsulam, rice, weeds, yield

# INTRODUCTION

Rice crop is a staple food in the main dish in Egypt and more than half of the world countries. In later years, the area of direct seeded-rice was linearly increased in Egypt because of the high labor costs and irrigation water shortage. Drill-seeded rice as an upland crop, flooding is introduced 30-35 days after seeding (DAS). Under this rice practice management, weeds grow faster, harder and difficult to control, which necessitates the application of herbicides twice or maybe three times through the growing season (Abd El-Naby *et al.*, 2017).

Weeds are strong competitor of crop plants for nutrients, water, space and incident light which leads to reducing crop production (Kole and Dey, 1989).

Consequently, herbicides as hazardous materials have been increasing in recent years. Large quantities of herbicides accumulate in the top layer of soil, which leading to alterations in soil biota (Zaller et al. 2016, Mukherjee et al., 2016 and Usman et al., 2017). The increasing of herbicide use leads to significant changes in microbial activities, accompanied by higher dehydrogenase activity around the 16<sup>th</sup> day after butachlor application. This concomitantly influences microbial balance, soil nutrient status, productivity and soil health (Min et al., 2001 and Saeki and Toyota, 2004). In a previous study, for Elsadany (2006) under laboratory conditions, instance, found that application of the herbicide thiobencarb moderately to sharply decrease the cyanobacteria and nitrifiers count with no adverse effect on the total bacterial count. Recently, Omara and El-Ghandor (2018) found that penoxsulam application was effective in weed control and increased rice production while not toxic to microbial population. Some microbial species consume herbicides and can be used as bio-indicators of changes in the biological activity in the soil (Lee, 1994) and as sources of bio-genous elements (Milosevia and Govedarica, 2002). Microorganisms degrade herbicides for energy and use their nutrients for metabolism, which leads to increased growth and activities of free N2-fixing and phosphate solubilizing bacteria, which improved rice yield (Das and Debnath, 2006). Besides, application of herbicides resulted

in either enhancement or inhibition effects on soil microorganisms, depending on type and concentration of herbicide (Accinelli et al., 2004 and Willems et al., 1996). MCPA (2-methyl-4-chlorophenoxyacetic acid) herbicide was found degraded by 45 % on the 28th day of the application (Burns, 1995). Valle et al. (2006) detected the removal of herbicide azimsulfuron after 28 th day and bacterial community reaches to (76 %) in the soil. The half-life decomposition of penoxsulam in a flooded rice field was shown by Jabusch and Tjeerdema (2006) to be between 2<sup>nd</sup> and 13<sup>th</sup> day and 3.5-18<sup>th</sup> day of the application as stated by Cong et al. (2017). The presence of large amounts of microbial biomass leads to increase of the transformations of plant nutrients in the soil (Debnath et al., 2002). After application of the herbicide directly, the effect of this herbicide is strong and after that, the microorganisms degrade the herbicide partially as carbon and nitrogen source until the herbicide decomposes, this leads to an increase in the microbial population in the rhizosphere (Priya et al., 2017). The free-living microorganisms in the soil can reduce the herbicide effect within plant tissues or increase plant tolerance to herbicidal toxicity (Tétard-Jones and Edwards, 2015). On the contrary, in some cases, herbicides have no significant effects on the growth and nitrogenase activity of the diazotrophic Stenotrophomonas maltophilia under field conditions (Nahi et al. 2016). The total microbial population was influenced by different degrees with various herbicides (Kaur et al., 2014). Butachlor and fluchloralin applied showed a stimulatory effect on nitrification, nitrogenase, glutamine synthetase activities, while the N<sub>2</sub>-fixing cyanobacteria were found relatively tolerant to 2, 4-D under field conditions (Leganes and Valiente, 1992). Some herbicides cause a slight decrease of nitrifiers and an increase of sulfur oxidizers and no effect on enzymatic activities, which was not harmful with slight application rates (Tu, 1992). Herbicide glyphosate showed an increase in the growth and nitrogen fixation of native N<sub>2</sub>-fixing cyanobacteria in soil at lower concentrations, increased ammonification and nitrification compared with the control (Hart and Brookes 1996 and Seema and Tarar,

2016) and this herbicide is not dangerous in the presence of N fertilizer on nitrifying bacteria (Zabaloy *et al.*, 2017).

Cyanobacteria are sensitive to herbicides because they share many of the physiological properties of plants which form the site of herbicide action (Whitton, 2000). The differences in the effect of the herbicide on N<sub>2</sub>-fixing cyanobacteria are dependent on the herbicide application dose and type in addition to the cyanobacterial species, for example, Anabaena variabilis tolerates the herbicides arozin, alachlor, butachlor and 2,4-D, but with increasing of herbicides dose Nostoc punctiforme, Nostoc calcicola, A. variabilis, Gloeocapsa sp., Aphanocapsa sp. showed a gradual decline of growth, while arozin was more toxic to cyanobacterial growth (Singh and Datta, 2005). Anabaena sp. is a good cyanobacterium for biofertilizer in rice field, when is applied the herbicide, cyhalofop- butyl (Okmen et al., 2013). Applying herbicides were found by Das et al. (2015) safe for cyanobacteria and enhancers of rice production. High pendimethalin concentrations decreased growth rate, cell count, chlorophyll and dry weight of the green alga Protosiphon botryoides (Shabana et al., 2001 and Zvir et al., 2015). However, a positive correlation was found between sulphate reducing and nitrogen-fixing activity in flooded soils (Sidorenko, 1989).

The objective of this study is to detect the effect of Stomp (pendimethalin 50% EC) and Rainbow (penoxsulam 2.5% OD) application on weed control, microbial count and productivity of drill-seeded rice in the Nile delta.

#### MATERIALS AND METHODS

Two field experiments were conducted during the rice growing seasons 2015 and 2016 in the Experimental Farm of Rice Research Department, Sakha Agricultural Research Station, Egypt. Giza 178 rice cultivar was planted in field plots of 14 m<sup>2</sup> each using the drilling machine at the seed rate of 100 kg ha<sup>-1</sup> on 15<sup>th</sup> and 20<sup>th</sup> May in both seasons, respectively. Randomized complete

block design (RCBD) with four replications were used. Weed control treatments were randomly allocated over the plots. The rest of rice cultural practices were applied as recommended for drill-seeded rice.

Table 1. Some mechanical and chemical properties of the experimental soil sites during 2015 and 2016 seasons.

6-9 -1	Seasons			
Soil characteristics	2015	2016		
Soil texture (%)	Cla	yey		
clay %	57.00	54.00		
Sand %	11.00	11.00		
Silt %	32.00	35.00		
pH (1: 2.5 water suspension)	8.05	8.1		
EC (dSm <sup>-1</sup> )	2.0	2.0		
Organic matter	1.65	1.50		
Available P mg Kg <sup>-1</sup>	14.00	12.00		
Available NH <sub>4</sub> mg Kg <sup>-1</sup>	13.5	12.60		
Available NO <sub>3</sub> mg Kg <sup>-1</sup>	10.0	11.80		
Available K mg Kg <sup>-1</sup>	366	350		
Cations (meq L. <sup>-1</sup> )				
Ca <sup>++</sup>	7.20	6.00		
$Mg^{++}$	2.60	1.50		
$Na^+$	12.00	13.00		
$K^{+}$	0.50	0.50		
Anions (meq L1)				
HCO <sub>3</sub>	5.60	5.00		
Cl	14.00	14.00		
SO <sub>4</sub>	2.70	2.00		
CO <sub>3</sub>	0.00	0.00		

#### Weed control treatments were as follows:

T<sub>1</sub>- Pendimethalin 50% EC (Stomp) at 2.023 kg ai ha<sup>-1</sup>.

T<sub>2</sub>- Penoxsulam 2.5% OD (Rainbow) at rate of 0.0238 kg ai ha<sup>-1</sup>

T<sub>3</sub>- Weedy check (untreated control).

The Chemical structure of the herbicides Stomp and Rainbow show in Table (2)

Table 2. Chemical structure of the herbicides Stomp (pendimethalin 50% EC) and Rainbow (penoxsulam 2.5% OD)

Herbicide	Stomp 50% EC	Rainbow 2.5% OD
Active ingredient	Pendimethalin	Penoxsulam
Chemical group	Dinitroaniline	Triazolopyrimidine
Chemical name	<i>N</i> -(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine	2-(2,2-difluoroethoxy)- $N$ -(5,8-dimethoxy[1,2,4]triazolo[1,5- $c$ ]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide
Formula	$C_{13}H_{19}N_3O_4$	$C_{16}H_{14}F_5N_5O_5S$
Structure	CH <sub>3</sub> CH <sub>2</sub> CH—CH <sub>2</sub> CH—CH <sub>2</sub> CH—CH <sub>3</sub> CH CH <sub>3</sub> CH—CH <sub>2</sub> CH—CH <sub>3</sub>	H <sub>3</sub> C O F F F H <sub>3</sub> C O F F F
Site of action	Microtubule assembly inhibitor	Systemic – ALS inhibitors
Target weeds	Grassy and broadleaves	Grassy, sedges and broadleaves
Rate / fed	1.7 Lit.	400 ml
Rate (kg ai ha <sup>-</sup> )	2.023	0.0238

ai: active ingredient

#### Herbicides application

Pendimethalin 50% EC was sprayed in 300 liters water per hectare on a wetland at 4 days after seeding (DAS) by using a knapsack sprayer, then the soil was flush irrigated after 24 hours from herbicidal application. While penoxsulam 2.5% OD was applied at 15 DAS by the same method of pendimethalin application.

# Culturing and enumerating bacteria from soil samples

Five soil samples were collected randomly from each plot from a depth of (0-5) cm. Soil samples was sieved through a 2 mm mesh sieve and mix thoroughly. Count of soil microorganisms may be accomplished by the plate count technique or most probable number (MPN) technique. The effect of the herbicides on soil microorganisms was counted at the regular intervals: 6, 12, 18, 24 and 30 days after treatment (DAT).

Series of decimal dilutions were prepared in sterile physiological solution (8.5 g NaCl  $L^{-1}$ ). Aliquots one ml of the highest dilutions were used for the estimations of the total aerobic bacterial count and total nitrogen-fixers using the standard plate counts (Allen, 1950) as well as the determination of  $N_2$ -fixing cyanobacteria, nitrifiers bacteria and sulphate – reducers bacteria using the most probable number (MPN) technique, (Cochrane, 1950).

#### Total bacterial count

Total bacterial count was determined using the soil extract agar medium (Mahmoud 1955).

# Total nitrogen fixing bacteria

Total nitrogen fixing bacteria was counted by combining carbon medium Free-living putative nitrogen-fixing bacteria (Rennie 1981).

# Native nitrogen - fixing cyanobacteria

Nitrogen - fixing cyanobacteria was determined by the MPN using the Modified Watanabe medium (El-Nawawy *et al.* 1958).

#### Nitrifiers bacteria

Nitrifiers bacteria were determined using the Stephenson's medium for autotrophic nitrifiers (Stephenson 1950).

# Sulphate reducer

The Most Probable Numbers (MPN) of Sulphate – reducing bacteria were counted using the modified Starkey's medium, (Abd El-Malik and Risk 1958).

#### Weed and rice data collection

At 45 and 60 days after seeding (DAS), weeds and rice plants were sampled using an area of 50 x 50 cm quadrate replicated four times for each plot. The collected weeds were cleaned, air dried, and then oven dried to a constant dry weight. Also, the rice dry weight of each quadrate was measured after dryness. Before harvest, panicles were counted in four random quadrates of 50 x 50 cm for each plot and the number of panicles/m<sup>-2</sup> was calculated. After rice maturity, the central 5 m<sup>2</sup> from each plot were manually harvested, air dried and thrashed. Grain yield was estimated and recorded after adjustment to 14% moisture content.

#### Data analysis

The collected data were subjected to proper statistical analysis of variance according to Snedecor and Cochran (1971). Weed data were transferred according to square-root transformation ( $\sqrt{[x+0.5]}$ ), then statistically analyzed by a MSTATC program, while rice data were directly analyzed according to MSTATC program. The means of weeds and rice data were compared by using the Duncan's Multiple Range Test (DMRT).

# RESULTS AND DISCUSSION

#### A- Microbial enumeration

The abundance and population densities of nitrogen-fixing bacteria, cyanobacteria, sulphate-reducing bacteria and nitrifying bacteria are expressed in terms of colony forming units per gram dry soil (CFU g<sup>-1</sup>).

# 1. Influence of weed control treatments on $N_2$ -fixing bacteria and total bacterial count in 2015 and 2016 seasons.

Data in Table (3) show that in both seasons 2015 and 2016, the application of penoxsulam 2.5% OD positively affected N<sub>2</sub>-fixing bacteria and total bacterial count compared with the corresponding non treated control due to the ability of the microorganisms to degrade the herbicides in the soil as a source of energy and carbon. These results are in agreement with Das and Debnath 2006, and Debnath *et al.* 2002.

Table 3. Effect of weed control treatment and sampling date on  $N_2$ -fixing bacteria CFU×10<sup>5</sup> /g soil and total bacterial count (CFU ×10<sup>5</sup>/g dry weight soil in 2015 and 2016 seasons.

Factor	2015	5 season	2010	6 season
ractor	N <sub>2</sub> -fixing bacteria	Total bacterial count	N <sub>2</sub> -fixing bacteria	Total bacterial count
Weed control:	_			
1- Pendimethalin 50% EC	4.982 b	5.335 b	4.940 b	5.251 b
2- Penoxsulam 2.5% OD	5.754 a	5.871 a	5.733 a	5.733 a
3- Control (un-treated)	4.841 c	5.164 c	4.816 c	5.101 c
Sampling time (DAT):				
Control (Before applying)	5.041 d	2.359 e	5.047 d	2.343 e
6 DAT	5.552 b	6.018 bc	5.537 b	5.892 c
12 DAT	5.482 c	5.997 c	5.439 c	5.857 c
18 DAT	5.908 a	6.578 a	5.851 a	6.348 a
24 DAT	4.823 e	6.104 b	4.781 e	6.023 b
30 DAT	4.348 f	5.684 d	4.323 f	5.707 d

Means of each factor within each column, Means followed by the same letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT), DAT = days after treatment.

Generally, the count in the season 2016 was lower than 2015 season may be because of climate changes and plant-microbial interactions (Wookey *et al.*, 2009, van der Putten 2012 and Classen *et al.*, 2015). The count in the two seasons increased with lapse of time up to 18 days from the herbicidal applications and decreased thereafter.

# Effect of the interaction between weed control treatment and sampling date on the total bacterial count in 2015 and 2016 seasons.

Total count of bacteria as affected by pendimethalin and penoxsulam herbicides as compared with the control are shown in Table (4). In both seasons, the total bacterial counts were increased up to the  $18^{th}$  day after application and decreased thereafter up to the  $30^{th}$  days after treatment compared with the corresponding control. The maximum numbers were recorded on  $18^{th}$  after treatment (6.117, 7.933 and  $6.093\times10^5, 7.313\times10^5\,\text{CFU/g}$  soil dry weight) in cases of pendimethalin and penoxsulam application , respectively. Penoxsulam induced higher figures compared

with pendimethalin and untreated plots. There may be certain microbial groups (primary microorganisms) started to degrade herbicides a few days after application (Jabusch and Tjeerdema, (2006 and Cong et al., 2017). On the other side, there might be secondary microorganisms which degraded enzymes during their adaptation (Milosevia and Govedarica, 2002) and microorganisms utilized the herbicides as sources of energy and nutrients for growth and metabolic activities (Priya et al., 2017, Das and Debnath 2006, Debnath et al., 2002). On the other hand, the herbicidal harmful effects were recorded to be affected with concentration of the herbicide, physical and chemical soil properties, soil moisture content and temperature (Willems et al., 1996), decline of soil microbial biomass carbon (MBC), soil respiration rate (BSR), substrate induced soil respiration (SIR) (Mukherjee et al., 2016), alteration of soil microbial biomass, decrease of enzyme activities and dramatic changes in biodiversity (Usman et al., 2017).

Table 4. Effect of weed control treatment and sampling date on total bacterial count (CFU×10<sup>5</sup>/g dry weight soil) in 2015 and 2016 seasons.

Weed control		2015 season		2016 season			
Sampling date (DAT)	Pendimethalin	Penoxsulam	Control	Pendimethalin	Penoxsulam	Control	
	50% EC	2.5% OD	(un-treated)	50% EC	2.5% OD	(un-treated)	
Control (Before applying)	2.357 g	2.360 g	2.360 g	2.350 f	2.343 f	2.337 f	
6 DAT	6.077 c	6.143 c	5.833 de	5.793 d	6.180 c	5.703 d	
12 DAT	5.873 d	6.250 c	5.867 de	5.760 de	6.160 c	5.650 de	
18 DAT	6.117 c	7.933 a	5.683 e	6.093 c	7.313 a	5.637 de	
24 DAT	5.830 de	6.660 b	5.823 de	5.750 de	6.573 b	5.747 de	
30 DAT	5.757 de	5.877 d	5.420 f	5.760 de	5.830 d	5.530 e	

Means followed by a common letter within a season are not significantly different at 5% level, using Duncan's Multiple Range Test. DAT = days after treatment.

 N<sub>2</sub>-fixing cyanobacteria, nitrifying and sulfate reducing bacteria as influenced by weed control treatment and sampling date during 2015 and 2016 seasons.

The data in Table (5) show the effect of herbicidal applications on indigenous  $N_2$ -fixing cyanobacteria, nitrifiers and sulfate reducers in the two seasons. At the  $18^{th}$  and  $30^{th}$  days after herbicidal applications, penoxsulam increased the counts of  $N_2$ -fixing cyanobacteria and

nitrifiers but decreased sulphate reducers compared with both pendimethalin and the corresponding control. This is possible because the herbicide is not harmful (Omara and El-Ghandor, 2018), completely decomposed (Burns 1995, Jabusch and Tjeerdema 2006, Valle *et al.* 2006 and Cong *et al.*, 2017) and/or used as a source of energy and nutrients (Milosevia and Govedarica, 2002 and Das and Debnath, 2006).

Table 5. Effect of weed control treatment and sampling date on N<sub>2</sub>-fixing cyanobacteria, nitrifying bacteria and sulphate reducers(CFU×10<sup>3</sup>/g dry weight soil) in 2015 and 2016 seasons.

Factor Weed control	2	2015 season		2		
	N <sub>2</sub> -fixing cyanobacteria	Nitrifying bacteria	Sulphate reducers	N <sub>2</sub> -fixing cyanobacteria	Nitrifying bacteria	Sulphate reducers
1- Pendimethalin 50%	1.327	24.600	0.828	1.337	24.215	0.817
2- Penoxsulam 2.5%	1.943	27.135	0.617	1.918	26.995	0.582
3- Control (un-treated)	0.592	17.348	1.248	0.588	17.263	1.227
Sampling date (DAT):						
Control (before application)	0.020	3.00	0.010	0.020	3.030	0.010
6 DAT	0.720	17.400	1.133	0.703	17.190	1.110
12 DAT	1.253	21.800	0.763	1.233	21.657	0.807
18 DAT	1.7130	29.733	1.433	1.713	29.303	1.327
24 DAT	1.890	32.367	0.930	1.893	32.267	0.887
30 DAT	2.127	33.867	1.117	2.123	33.500	1.110

DAT = days after treatment.

4. Native  $N_2$  –fixing cyanobacteria as affected by the interaction between weed control treatment and sampling date in 2015 and 2016 seasons.

The data in Table (6) shows that in both seasons, the count of  $N_2$  -fixing cyanobacteria increased under

pendimethalin and penoxsulam application compared with the control up to 30 days from herbicidal applications, penoxsulam increased the cyanobacterial count compared with pendimethalin. These results are in agreement with Okmen *et al.* (2013), Shabana *et al.* (2001) and Das *et al.*  (2015). The maximum figures after 30 days of application were 3.30 and 3.29 CFU×10<sup>3</sup>/g dry weight soil for the two seasons, respectively. These results are suggested to be due to differential tolerance of cyanobacteria towards

herbicides (Whitton, 2000) and dose as well as type of herbicide and the cyanobacteria species (Singh and Datta, 2005 and Priya *et al.*, 2017).

Table 6. Effect of interaction between weed control treatment and sampling date on  $N_2$  –fixing cyanobacteria (CFU×10³/g dry weight soil in 2015 and 2016 seasons.

Weed control Sampling date (DAT)		2015 season		2016 season			
	Pendimethalin 50% EC	Penoxsulam 2.5% OD	Control (un-treated)	Pendimethalin 50% EC	Penoxsulam 2.5% OD	Control (un-treated)	
Control(Before applying)	0.02	0.02	0.02	0.02	0.02	0.02	
6 DAT	0.69	0.94	0.53	0.68	0.91	0.52	
12 DAT	1.48	1.62	0.66	1.45	1.60	0.65	
18 DAT	1.67	2.78	0.69	1.76	2.70	0.68	
24 DAT	1.90	3.00	0.77	1.92	2.99	0.77	
30 DAT	2.20	3.30	0.88	2.19	3.29	0.89	

DAT = days after treatment.

# Effect of interaction between weed control treatment and sampling date on Nitrifiers in 2015 and 2016 seasons.

Data of Table (7) show that in both seasons the count of nitrifiers increased with application of both pendimethalin and penoxsulam comparing with the corresponding untreated control. They registered 40.90 and  $40.50\times10^3$  CFU/g soil with penoxsulam application at 30 DAT in the two seasons, respectively. While the lowest numbers of nitrifiers (14.59 and  $14.57\times10^3$  CFU/g soil) were recorded at 6 DAT for the corresponding control

treatments. These proliferations in numbers may be due to the forth mentioned increases in cyanobacterial population, which produce oxygen through their photosynthetic activities, and ammonical N by the nitrogen fixation process. However, some herbicides have no negative effects on the nitrifiers populations (Leganes and Valiant 1992, Singh and Tiwar 1988, Hart and Brookes, 1996, Seema and Tarar 2016 and Zabaloy *et al.*, 2017), while Tu (1992) showed decreasing in nitrifiers activities due to herbicides application.

Table 7. Effect of interaction between weed control treatment and sampling date on nitrifying bacteria (CFU×10<sup>3</sup>/g dry weight soil in 2015 and 2016 seasons.

Weed control		2015 season		2016 season			
Sampling date (DAT)	Pendimethalin	Penoxsulam	Control	Pendimethalin	Penoxsulam	Control	
	50% EC	2.5% OD	(un-treated)	50% EC	2.5% OD	(un-treated)	
Contro(Before applying)	3.00	3.00	3.00	3.09	3.00	3.00	
6 DAT	18.60	19.01	14.59	18.00	19.00	14.57	
12 DAT	22.40	24.00	19.00	22.30	23.67	19.00	
18 DAT	33.20	36.00	20.00	31.90	36.00	20.01	
24 DAT	34.00	39.90	23.20	34.00	39.80	23.00	
30 DAT	36.40	40.90	24.30	36.00	40.50	24.00	

DAT = days after treatment.

# Effect of interaction between weed control treatment and sampling date on sulphate reducers in 2015 and 2016 seasons.

Table (8) shows that numbers of sulphate reducers fluctuated all over the experimental period. Generally, the

penoxsulam decreased the counts compared with Pendimethalin and the non-treated control. This trend was suggested by Sidorenko (1989) and Tu (1992) to be related to types and chemical composition of the herbicide.

Table 8. Effect of interaction between weed control treatment and sampling date on sulphate reducing bacteria (CFU×10<sup>3</sup>/g dry weight soil in 2015 and 2016 seasons.

Weed control		2015 season		2016 season			
Sampling date (DAT)	Pendimethalin 50% EC	Penoxsulam 2.5% OD	Control (un-treated)	Pendimethalin 50% EC	Penoxsulam 2.5% OD	Control (un-treated)	
Control(Before applying)	0.01	0.01	0.01	0.01	0.01	0.01	
6 DAT	1.63	0.23	1.54	1.62	0.19	1.52	
12 DAT	0.50	0.30	1. 49	0.53	0.45	1.44	
18 DAT	1.27	1.13	1.90	1.10	1.03	1.85	
24 DAT	0.56	0.93	1.30	0.46	0.91	1.29	
30 DAT	1.20	0.90	1.25	1.18	0.90	1.25	

DAT = days after treatment.

However, the proliferation of the photosynthetic cyanobacteria and release of oxygen is strongly justifying

the decrease in the population densities of the anaerobic sulphate reducers. Sulfate-reducing bacteria were then expected to proliferate under un-treated control comparing with treated ones which, as mentioned, proliferates the cyanobacterial populations.

However, the lowest population densities were shown with application of the penoxsulam than the other tested herbicidal treatments.

#### **B- Weeds:**

1- Effect of weed control treatment on dry weights of *E. crus-galli, E. colona, A. baccifera* and total weeds at 45 and 60 DAS in 2015 and 2016 seasons.

Data of dry weight of *Echinochloa crus-galli*, *Echinochloa colona*, *Ammania baccifera* and total weeds at 45 and 60 DAS as affected by weed control treatments in 2015 and 2016 seasons are shown in Table (9).

Table 9. Dry weights of *Echinochloa crus-galli, Echinochloa colona, Ammania baccifera* and total weeds at 45 and 60 DAS as affected by weed control treatment in 2015 and 2016 seasons. Weed data were subjected to square-root ( $\sqrt{|x+0.5|}$ ) transformation before analysis; transformed values are shown in parentheses.

Square Tool ( ) [x			m <sup>-2</sup> ) at 45 DA				m <sup>-2</sup> ) at 60 DA	
Weed control	E.	E.	Ammania	Total	E. crus-	E.	Ammania	Total
weed control	crus-galli	colona	baccifera	weeds	galli	colona	baccifera	weeds
				2015	season			
1. Don dim othelin 500/ EC	245.33	96.00	4.83	346.17	360.2	156.1	12.2	528.5
1- Pendimethalin 50% EC	(15.67 b)	(9.80  b)	(2.27 b)	(18.63 b)	(18.99 b)	(12.51 b)	(3.56 b)	(23.0 b)
2. Danavaylam 2.59/ OD	207.33	56.00	3.67	267.00	280.1	80.8	9.6	370.5
2- Penoxsulam 2.5% OD	(14.43 b)	(7.50 c)	(2.03 b)	(16.33 c)	(16.75 b)	(9.02 c)	(3.18 b)	(19.26 c)
2. Waady abaals	454.00	320.00	132.00	906.00	744.6	524.2	208.0	1476.8
3- Weedy check	(21.27 a)	(17.80 a)	(11.50 a)	(30.07 a)	(27.30 a)	(22.91 a)	(14.44 a)	(38.44 a)
F test	**	**	**	**	**	**	**	**
				2016	season			
1- Pendimethalin 50% EC	84.00	213.33	5.33	302.67	176.3	354.3	7.2	537.8
1- Fendimenianii 30% EC	(9.17 b)	(14.63 b)	(2.40 b)	(17.43 b)	(13.3 b)	(18.84 b)	(2.8 b)	(23.2 b)
2. Beneverlans 2.59/ OD	50.67	186.67	4.47	241.80	98.2	212.7	6.8	317.7
2- Penoxsulam 2.5% OD	(7.17 c)	(13.67 c)	(2.20 b)	(15.53 c)	(9.93 c)	(14.6 c)	(2.7 b)	(17.84 c)
2. Woody about	229.33	386.67	114.67	730.67	324.6	482.8	174.3	981.7
3- Weedy check	(15.13 a)	(19.67 a)	(10.73 a)	(27.03 a)	(18.03 a)	(21.98 a)	13.22 a)	(31.34 a)
Ftest	**	**	**	**	**	**	**	**

Means of transforming data followed by the same letter within a column for a season are not significantly different at the 5 % level, using Duncan's Multiple Range Test. DAS = days after seeding.

At 45 DAS, the results revealed that penoxsulam 2.5% OD application at 15 DAS recorded the lowest dry weight of Echinochloa crus-galli, Echinochloa colona and total weeds in both seasons of study with no significant differences between pendimethalin 50% EC at 4 DAS in the first season for E. crus-galli. While, the highest dry weights of E. crus-galli, E. colona and total weeds were obtained by weedy check (untreated) plots in both seasons, the same trend was observed in the second sample (at 60 DAS). For Ammania baccifera, the data in Table (9) also revealed that both of penoxsulam and pendimethalin herbicides achieve the best control as compared with weedy check plots which gave the highest dry weight of this weed through in the two samples through both seasons of study. The high efficiency of penoxsulam may be due to the high killing ability to germinated weeds, moreover, presence of flooding water after penoxsulam application by 10-15 days under drilling system as compared with 30 days in case of pendimethalin which gave the chance for weed seeds to germinate under these conditions. Similar results were obtained by Abd El-Naby et al., 2017.

# C- Rice:

1- Effect of weed control treatment on dry weight at 45 and 60 DAS, number of panicles/m², panicle weight and grain yield of rice in 2015 and 2016 seasons.

As shown from the data in Table (10), weed control treatments significantly affected rice dry weight at 45 and

60 DAS, panicles .m<sup>-2</sup>, panicle weight and grain yield of rice in the two growing seasons.

Penoxsulam application at 15 DAS achieved the best rice dry weight in the two sampling times, number of panicles. m<sup>-2</sup>, panicle weight and grain yield in the two seasons of study. On the other hand, the lowest values of rice studied traits were obtained by weedy check plots in both seasons. It may be due to the high efficiency of chemical weed control in controlling weeds which gave the chance to the good optimum vegetative growth of rice resulting in increasing yield and its components as mentioned by Singh et al. (2006). Moreover, N2- fixing microorganisms (Diazotrophs) contributes to the supply of the plant part of the fertilizer requirements, especially nitrogen, the release of growth substances and increase the count and activities of microorganisms. In turn this may reflected in crop growth and productivity. The natural endophytic bacteria can remove the toxicity of herbicide and the increase tolerance of the plant to stress. There are a large number of detoxification mechanisms available in free-living microorganisms used in phytoremediation by biotransformation pathways, such as those acting on nitrile and halogen functions or degradation aromatic ring (Tétard-Jones and Edwards, 2015).

Table 10. Rice dry weight at 45 and 60 DAS, number of panicles, panicle weight and grain yield as affected by weed control treatment in 2015 and 2016 seasons.

Weed control	Rice dry weight (g.m <sup>-2</sup> ) at 45 DAS	Rice dry weight (g.m <sup>-2</sup> ) at 60 DAS	Number of panicles (m <sup>-2</sup> )	Panicle weight (g)	Grain yield (ton. ha <sup>-1</sup> )
		•	2015 season	•	•
1- Pendimethalin 50% EC	756.7 b	1006.67 b	422.67 b	1.72 b	5.662 b
2- Penoxsulam 2.5% OD	912.1 a	1283.33 a	496.00 a	2.00 a	7.050 a
3- Weedy check	308.4 c	476.33 c	186.67 c	1.22 c	1.330 c
F test	**	**	**	**	**
			2016 season		
1- Pendimethalin 50% EC	796.2 b	1085.33 b	452.00 b	1.80 ab	5.855 b
2- Penoxsulam 2.5% OD	988.2 a	1367.33 a	510.67 a	2.10 a	7.193 a
3- Weedy check	364.6 c	508.00 c	181.33 c	1.31 b	1.387 c
F test	**	**	**	**	**

In a column for a season, means followed by the same letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT). DAS = days after seeding.

#### CONCLUSION

The results of this study showed that spraying penoxsulam 2.5% OD at 15 DAS by the recommended dose enhanced the total bacterial counts,  $N_2$ - fixing bacteria, indigenous  $N_2$ - fixing cyanobacteria and nitrifiers counts, while it decreased sulfate-reducing bacteria in the rice plant rhizosphere, the results also showed that penoxsulam application achieved the best weed control, which was reflected on growth and productivity of rice. It could be concluded that use of penoxsulam has no negative effects on the soil microbial diversity in addition to effective weed management and increased rice productivity.

#### REFERENCES

- Abd- El- Malek, Y. and Rizk, S. G. (1958). Counting of sulphate reducing bacteria in mixed bacterial populations. Nature London, 182.
- Abd El-Naby, S. S. M.; Abou El-Darag, I. H. and El-Ghandor, A. M. A. (2017). Weed management in broadcast-seeded hybrid rice (*Oryza sativa L.*). J. Plant Production, Mansoura Univ., 8 (10): 1021 1028.
- Accinelli, C.; Dinelli, G. and Vicari, A. (2004). Metolachlor and linuron soil degradation after repeated applications under laboratory conditions. Agrochimica. 48(3/4): 132-140.
- Allen, O. N. (1950). Experiments in soil bacteriology. Burgess Publishing Co.
- Burns, R. G. (1995). Enumeration, Survival, and Beneficial Activities of Microorganisms Introduced into Soil. In: Environmental Impact of Soil Component Interactions, Metals, Other Inorganics, and Microbial Activities (eds., Huang, P. M., Berthelin, J., Bollag, J.-M., McGill, W. B., Page, A. L.), 145—164, CRC Press. Inc.
- Classen, T. I.; Sundgvist, K. M., Henning, A. J.; Newman, S. G.; Moore, A.M. G.; Cregger, A. M.; Moorhead, C. L. and Patterson, M. C. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere. 6(8): 1-21.
- Cochrane, W. G. (1950). Estimation of bacterial densities by means of the «most probable number». Biometrics. 6: 105-116.

- Cong, L.; Jian, Y. H.; Fei, L. Y.; Li, L.; Xiang, R. X. and Wei, L. (2017). Residue analysis and degradation dynamics of penoxsulam in water and soil of rice field by QuEChERS-high performance liquid chromatography. Journal of Food Safety and Quality. 8(2):380-385.
- Das, A. C. and Debnath, A. (2006). Effect of systemic herbicides on N<sub>2</sub>-fixing and phosphate solubilizing microorganisms in relation to availability of nitrogen and phosphorus in paddy soils of West Bengal. Chemosphere. 65:1082-86.
- Das, N. P.; Kumar, A. and Singh, P. K. (2015). Cyanobacteria, pesticides and rice interaction. Biodivers Conserv. 24:995–1005.
- Debnath, A.; Das, A. C. and Mukherjee, D. (2002). Rhizosphere effect of herbicides on nitrogen fixing bacteria in relation to availability of nitrogen in rice soil. J. the Ind. Soc. of Soil Sci. 50: 463-66.
- El- Nawawy, A. S.; Lotfi, M. and Fahmy, M. (1958). Studies on the ability of some blue- green algae to fix atmospheric nitrogen and their effect on growth and yield of paddy. Agric. Res. Rev. Min. Agric., Cairo 36:308-320.
- Elsadany A. Y. (2006). Some alterations in microbiological and chemical properties of flooded soils inoculated with dinitrogen-fixing cyanobacteria. M. Sc, Fac. Agric. Mansoura, Univ., Egypt.
- Hart, M. R. and Brookes, P. C. (1996). Soil microbial biomass and mineralization of soil organic matter after 19 years of cumulative field applications of pesticides. Soil Biol. Biochem. 28 (12): 1641— 1647.
- Jabusch, T. W. and Tjeerdema, R. S. (2006). Microbial Degradation of Penoxsulam in Flooded Rice Field Soils. J. Agric. Food Chem.54 (16): 5962–5967.
- Kaur, S.; Singh, S. and Phutela, R. P. (2014). Effect of herbicides on soil microorganisms in direct-seeded rice. Indian Journal of Weed Science. 46(3): 229– 233
- Kole, S. C. and Dey, B. K. (1989). Effect of aromatic amine herbicides on microbial population and phosphate solubilizing power of the rhizosphere soils of groundnut. Indian Agriculturist. 33, 1–8.

- Lee, K. E. (1994). The functional significance of biodiversity in soils. 15<sup>th</sup> World Congress of Soil Science, Acapulco., Mexico. 4: 168—182.
- Leganes, F. and Valiente, F. E. (1992). Effects of phenoxyacetic herbicides on growth, photosynthesis, and nitrogenase activity in cyanobacteria from rice fields. Arch. Environ. Contam. Toxicol. 22:130-134.
- Mahmoud, S. A. Z. (1955). A study on spore formers occurring in soils. Their germination and biochemical activity. Ph. D. Thesis, Leads Univ. England, UK.
- Milosevia, A. N. and Govedarica, M. M. (2002). Effect of herbicides on microbiological properties of soil. Proceedings for Natural Sciences. 102: 5-21.
- Min, H.; Ye, Y. F.; Chen, Z. Y.; Wu, W. X. and Yufeng, D. (2001). Effects of Butachlor on microbial populations and enzyme activities in paddy soil. Journal of Environmental Science and Health. 36(5): 581-95.
- Mukherjee, S.; Tripathi, S.; Mukherjee, A. K.; Bhattacharyya, A. and Chakrabarti, K. (2016). Persistence of the herbicides florasulam and halauxifen-methyl in alluvial and saline alluvial soils, and their effects on microbial indicators of soil quality. Eur. J. Soil Biol. 73: 93–99.
- Nahi, A.; Othman, R.; Omar, D. and Ebrahimi, M. (2016). Effects of Selected Herbicides on Growth and Nitrogen Fixing Activity of *Stenotrophomonas maltophilia* (Sb16). Polish Journal of Microbiology. 65 (3): 377–382.
- Okmen, G.; Turkcan, O. and Erdal, P. (2013). Effect of Herbicides on Chlorophyll-a, β- Caroten, Phycocyanin and Allophycocyanin Content of Anabaena sp. Journal of Applied Biological Sciences. 7 (2): 20-27.
- Omara, A. and El-Ghandor, A. (2018). Effect of Thiobencab and Penoxsulam Herbicides on Soil Microbial Population and Weed Control in Transplanted Rice. Global Advanced Research Journal of Microbiology. 7(5): 084-094.
- Priya, R. S.; Chinnusamy, C.; Arthanari, P. M. and Janaki, P. (2017). Microbial and dehydrogenase activity of soil contaminated with herbicide combination in direct seeded rice (*Oryza sativa L.*). Journal of Entomology and Zoology Studies. 5 (5): 1205-1212.
- Rennie, R. J. (1981). A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. Can. J. Microbiol., 27: 8–14.
- Saeki, M. and Toyota, K. (2004). Effect of bensulfuronmethyl (a sulfonyurea herbicide) on the soil bacterial community of a paddy soil microcosm. Biological Fertility Soils. 40: 110- 118.
- Seema S. B. and Tarar, J. L. (2016). Impact of herbicide Glyphosate on metabolic activities of Cyanobacterial species. Phykos. 46 (1): 1-6.
- Shabana, E. F.; Battah, M. G.; Kobbia, A. I. and Eladel, H. (2001). Effect of Pendimethalin on Growth and Photosynthetic Activity of Protosiphon botryoides in Different Nutrient States. Ecotoxicology and Environmental. 49(2): 106-110.

- Sidorenko, O. D. (1989). Role of Sulphate-Reducing Bacteria in Rhizosphere of Rice and Flooded Soil in Rice Fields. Developments in Soil Science. 18:349-354.
- Singh, J. L. and Tiwar, D. N. (1988). Effects of selected rice-field herbicides on photosynthesis, respiration, and nitrogen assimilating enzyme systems of paddy soil diazotrophic cyanobacteria. 31(2): 120-128.
- Singh, S. and Datta, P. (2005). Growth and survival potentials of immobilized diazotrophic cyanobacterial isolates exposed to common rice field herbicides. World Journal of Microbiology & Biotechnology. 21(4):441-446.
- Singh, S.; I. Bhushan; J. K. Ladha; R. K. Gutpa; A. N. Rao and B. Sivaprasad (2006). Weed management in dry-seeded rice (*Oryza sativa*, L.) cultivated in the furrow-irrigated raised-bed planting system. Crop Prot. 25:487–495.
- Snedecor, G. W. and Cochran, W. G. (1971). Statistical Methods. 6<sup>th</sup> ed., Iowa State Univ. Press Ames, USA.
- Stephenson, M. (1950). Bacterial metabolism, 3rd ed., Longmans, Green &Co. London.
- Tétard-Jones, C. and Edwards, R. (2015). Potential roles for microbial endophytes in herbicide tolerance in plants. Pest Manag. Sci., 72: 203–209.
- Tu, C. M. (1992). Effect of some herbicides on activities of microorganisms and enzymes in soil. Journal of Environmental Science and Health, Part B. 27 (6): 695-709.
- Usman, S.; Kundiri, A. M. and Nzamouhe, M. (2017). Effects of organophosphate herbicides on biological organisms in soil medium-a mini review. J. Ecol. Toxicol. 1(1):3-5.
- Valle, A.; Boschin, G.; Negri, M.; Abbruscato, P.; Sorlini, C.; D'Agostina, A. and Zanardini, E. (2006). The microbial degradation of azimsulfuron and its effect on the soil bacterial community. Journal of Applied Microbiology. 101: 443–452.
- Van der Putten, W. H. (2012). Climate change, aboveground-belowground interactions and species range shifts. Annual Review of Ecology, Evolution, and Systematics. 43:365–383.
- Whitton, B.A. (2000). Soils and rice-fields. In: The Ecology of Cyanobacteria: Their Diversity in Time and Space, Whitton, B.A. & Potts, M. (Eds.), pp. 233-255, Kluwer Academic Publishers, ISBN: 0-7923-4735-8, Dordrecht.
- Willems, H. P. L.; Lewis, K.J.; Dyson, J. S. and Lewis, F. J. (1996). Mineralization of 2,4-D and Atrazine in the unsaturated zone of a sandy loam soil. Soil Biol. Biochem. 28 (8): 989—996.
- Wookey, P.; Aerts, R.; Bardgett, R. D.; Baptist, F.; Brathen, K. A.; Cornelissen, J. H. C. and Gough, L. (2009). Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. Global Change Biology. 15:1153–1172.

- Zabaloy, M. C.; Allegrini, M.; Tebbe, A. D.; Schuster, K. and Gomez, V. E. (2017). Nitrifying bacteria and archaea withstanding glyphosate in fertilized soil microcosms. Applied Soil Ecology. 117-118: 88-95.
- Zaller, J. G.; König, N.; Tiefenbacher, A.; Muraoka, Y.; Querner, P.; Ratzenböck, A.; Bonkowski, M. and Koller, R. (2016). Pesticide seed dressings can affect the activity of various soil organisms and reduce decomposition of plant material. BMC Ecol. 16 (37): 1-11.
- Zvir, G. I.; Moroz, O. M. and Hnatush, S.O. (2015). Dissimulator sulfate reduction in bacteria Desulfovibrio desulfuricans IM V K -6 upon influence of Uragan and Raundup herbicides. Visn. Dnipropetr. Univ. Ser. Biol. Med. 6(1): 40-44.

تأثير مبيدات الحشائش علي بعض ميكروبات التربة ومكافحة الحشائش وإنتاجية الأرز التسطير عبي بعض ميكروبات التربة ومكافحة الحشائش وإنتاجية الأرز التسطير عبدالنبي أخيدالجواد يوسف السعدني و صبري صبحي محمد عبدالنبي أقسم بحوث الزراعية بسخا - مركز البحوث الزراعية - مصر. مصر. أقسم بحوث الأرز معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية - الجيزة - مصر.

أجريت تجربة حقلية بالمزرعة البحثية بقسم بحوث الأرز – محطة البحوث الزراعية بسخا حكفر الشيخ خلال الموسم الصيفي لعامي ٢٠١٥ و ٢٠١٦ بهدف دراسة تأثير استخدام مبيدي مكافحة حشائش الأرز بينوكسو لام وبنديميثالين على الأعداد الكلية للميكر وبات،البكتريا المثبتة لازوت الهواء الهواء الجوي، السيانوبكتيريا المثبتة للازوت الجوي – بكتريا التأزت البكتريا المختزلة للكبريتات وذلك علي فترات زمنية بعد إضافة كل من المبيدين. كما تناولت الدراسة أيضاً تأثير استخدام كلا المبيدين علي مكافحة الحشائش ونمو وانتاجية الصنف جيزة ١٧٨ المنزرع بطريقة التسطير وكانت أهم النتلج المتحصل عليها كالاتي: أدي استخدام مبيد بينوكسو لام (رينبو) إلى زيادة أعداد البكتريا الكلية البكتريا المثبتة لأزوت الهواء الجوي – بكتريا التأزت مقارنة باستخدام مبيد بينداميثالين وغير المعامل (الكنترول). أثرت المبيدات موضع الدراسة تأثيرا معنويا علي مكونات المحصول في كلا الموسمين فكان لاستخدام مبيد بينداميثالين وغير المعامل (الكنترول). تحصول الأرز وتأثيرا سلبيا علي المكانس والكنترول). تحت ظروف هذه سلبيا علي الحشائش في كلا الموسمين مقارنة باستخدام مبيد بينداميثالين أو في حالة غير المعامل بمبيدات الحشائش (الكنترول). تحت ظروف هذه الدراسه يمكن استخلاص أن استخدام مبيد بينوكسو لام في مكافحة الحشائش النامية في الأراضي المنزرعة بصنف أرز جيزة ١١٧٨ آمن على مكوبات التربة المفيدة لنبات الأرز وفي نفس الوقت له كفاءة عالية في مكافحة الحشائش وزيادة مكونات وانتاجية محصول الأرز المنزرع بطريقة التسطير.