Journal of Soil Sciences and Agricultural Engineering

Journal homepage & Available online at: www.jssae.journals.ekb.eg

Using *Pseudomonas aeruginosa* to control some kinds of weeds and its effect on soil microbes

Ghada A. Z. Ibrahim*

Department of Soil Fertility and Microbiology – Desert Research Center, Cairo – Egypt

ABSTRACT

Biological control of weeds is the use of natural enemies to reduce the density to a tolerable level. The target of this is not removal but the reduction of the weed population to an economically low. *Pseudomonas aeruginosa* used here as bio control agent. In vitro, used as addition in two bioassay experiments on weeds compared to control. First experiment was using *Pseudomonas* broth culture. As results, there was significant reduction reached to (40–85%) in growth parameters (lengths, fresh & dry weights and germination %) of weeds (*Echinochloa crus galli*, *Phalaris minor*, *Beta vulgaris* L and *Pennisetum purpuremum schumach Gramineae*). The second experiment was using *Pseudomonas* ethyl acetate crude extract (organic extract). There were reductions in weeds growth parameters reached to (55-100%). In vivo, using *Pseudomonas* organic extract compared to control, there were significant reductions of weeds growth parameters reached to (21-82%). There was no negative effect on *Zea mays* growth parameters. There found unclear interactions between weed roots and soil microbes. It caused reductions in weeds rhizosphere microbes' counts. After weeds removed and Maize cultivated in same pots, soil microbes' counts increased during a month of Maize life in soil. *Pseudomonas* organic extract was identified by HPLC-MS to: Quinoline and Quinoline derivatives (Quinic acid, Quinolone 2-heptyl-4- hydroxyquinolone-N-oxide, 3-Quinolinecarboxylic acid, 1-ethyl-1, 4-dihydro-7-methoxy-4-oxo- and Quinolinediol. We recommend use *Pseudomonas aeruginosa* to reduce weeds especially with *Zea mays* cultivation and add biofertilizers. Effect of weed roots on soil microorganisms is unclear and needs more future studies.

***Keywords*:** *Ps. aeruginosa*, Quinoline, biocontrol of weeds.

INTRODUCTION

Weeds drastically lower food production, have an adverse effect on animal and human health. Chemical herbicides are employed to control weeds primarily, but their detrimental impacts on the environment and food safety are a significant issue. The development of microorganisms as bio-herbicides for weed control has taken a lot of time and effort. Plant-associated bacteria (PAB) are common in the weeds, crops, and the rhizosphere. They are also common inside plants. To prevent the growth of weeds, several PAB species produce phytotoxic metabolites, auxins, hydrogen cyanide, and other substances. The efficiency of PAB herbicides is influenced by several factors, including crop management plans, crop surfactants, additives, and formulation types. The differences between field performance and the outcomes of in vitro screening may be explained by these factors, but more research is required. Successful bio-herbicides need to be specific to the target weeds or related weeds in order to be successful. In-depth studies on factors like formulation, application tactics, and coordination with cultivation techniques should be done to maximize the effectiveness of PAB-based bio-herbicides, Fang *et al.*, (2022).

Chemical herbicides can be replaced with bioherbicides based on microorganisms. Due to their high levels of specificity and selection, they frequently have little impact on crops that are close to certain weeds. Compared to chemical herbicides, bioherbicides are less toxic and hazardous to the environment and human health. Because bioherbicides have a shorter half-life than chemical compounds, they degrade more quickly, do not build up in the environment, and do not harm ecosystems. Some naturally occurring allelochemicals dissolve readily in water and do not need chemical surfactants. Low production costs are a result of the rapid proliferation and accessibility of the microorganisms utilized to make bioherbicides. Bioherbicides can successfully stop the growth of weeds, even at modest doses.

Additionally, bioherbicides successfully eradicate plant species that have become resistant to chemical herbicides. Natural phytotoxins operate in numerous ways, lowering the likelihood of resistance. Also, using bioherbicides in organic farming is beneficial, Kubiak *et al.*, (2022).

In biological weed control, we acquired four of bacteria is an ecofriendly way to preparation of main strong bacterial isolates under genera *Pseudomonas*, *Xanthomonas* and *Bacillus* from Wadi El Natroun region. All the examined bacterial isolates caused large significant reductions in seed germination and seedling growth of *Convolvulus arvensis* and *Portulaca oleracea*. The isolates Bioassaying ethyl acetate crude extracts showed that *Pseudomonas* sp. (isolate1) was the highest active against seedling stage of *Portulaca oleracea*. Pseudomonas aeruginosa has high possibility to be used in broadleaf weed control, Tawfik *et al.*, (2019).

*** Corresponding author.**
E-mail address: ghada_micro@yahoo.com
DOI: 10.21608/jssae.2023.215816.1164

The objective of this work was reduction the density of weeds with less negative effects on *Zea mays* and soil rhizosphere microbes.

MATERIALS AND METHODS

1. Microbial strain

Pseudomonas aeruginosa was isolated from olive mill wastewater sample and identified by Bergy's Manual of Determinative Bacteriology (1994), it was used to identify bacteria to species. According to Berg *et al.* (2002), the bacterial isolates were also identified using a partial 16S rRNA gene sequence analysis. PCR was used to amplify bacterial 16S rRNA gene sequences (Lane, 1991), (Ibrahim *et al*., 2016).

2. Biochemical analysis of *Pseudomonas aeruginosa*

A. Catalase enzyme assay: Catalase and Glutathione peroxides were determined in Central Lab. of Desert Research Center - Egypt, Cairo, by HPLC Ultimate 3000 Thermo dionex, Germany. Catalase is an antioxidant enzyme found in all aerobic cells. It's one from the body's defensive mechanisms against H_2O_2, a strong oxidant that can cause cellular damage. The catalase test joins the Antioxidant family, according to (Paglia and Valentine 1967) and (Iwase *et al*., 2013).

B. Glutathione Peroxidase assay: It is a member of family of the glutathione peroxides enzymes which detoxify peroxides in the cell. Peroxides can breakdown into reactive compounds. Free radicals can damage the cell. The processing of H_2O_2 to water was catalyzed by peroxides enzymes. This estimated according to (Aebi, 1984).

C. Anti-oxidant assays

The bacterial sample was determined in Egypt, Cairo - Desert Research Center, Central Lab., by Spectrophotometrecally. Anti oxidants were determined in Ten ml of broth inoculated medium of *Pseudomonas* according to the method of De Marco *et al*. (2007). Radical scavenging activity of broth culture against stable $DPPH^0$ (2, 2-diphenyl- 2- picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany). The changes in colour (from deep—violet to light—yellow) were measured at 515nm on a Shimadzu Spectrophotometer (UV-1601 PC). Radical scavenging activities of plants were measured by method of (Brand-Williams *et al.*, 1995), as described below. The radical scavenging activity of the samples (antioxidant activity) was expressed as percent inhibition of $DPPH^0$ radical as following:

$$\% \text{ Inhibition} = [(A_{control} - A_{treatment}) / A_{control})] \times 100$$

Where:

$A_{control}$: is the absorbance of the control.

$A_{treatment}$: is the absorbance of the treatment.

Butylated hydroxyl anisol (BHA) and tert-butylated hydroxyl qunione (TBHQ) were used as reference compounds.

3. Bioassay experiment in vitro

Seeds were surface sterilized by submerge them in 95% ethanol for a few seconds followed by several washing with sterilized distilled water, (Russel *et al*., 1982). The weeds growth parameters determined were: length/ cm, fresh & dry weights/ gm and germination %, according to (Black *et al*., 1965).

A. Bioassay with *Pseudomonas* broth culture

Ten seeds of every kind of weeds or plants separated planting on 3 replicates of Petri dishes. The control was irrigation with water only. 100 ml of King's medium according to (King *et al*., 1954) put in 250 ml Erlenmeyer flask. After sterilization, inoculated with 5 ml of *Pseudomonas aeruginosa* then incubated at 30° C for 5 days. Treat the seeds in Petri dishes for 10 days. Then take results of growth parameters as above.

B. Bioassay with *Pseudomonas* organic extract

Pseudomonas ethyl acetate crude extract (organic extract) was used in this experiment. Ten seeds of every kind of weeds or plants separated planting on 3 replicates of Petri dishes. The control was irrigation with water only. Equal volume of filtrated microbial broth culture and ethyl acetate solvent were shacked in separation funnel to separate the secondary metabolites. Microbial broth culture centrifuged to remove *Pseudomonas* cells and then filtrated through filter paper Whatman no1. The organic phase was separated from the aqueous phase then evaporated ethyl acetate to dryness by using a rotary evaporator. Then being re-suspended the organic extracted in a tiny volume of 70 % (v/v) ethanol and put in a glass vial for use in HPLC-Mass spectrometry analysis, "Gealy and Gurusiddaiah, (1996)" and Balah (2012). The extract was used and evaluated on weeds and Maize after dissolved in distilled water. It was prepared with a concentration (2500 ppm). Growth parameters results were taken after 10 days as above, (Kamruzzaman *et al*., 2013).

4. Pots experiment in vivo

Bioassay experiment with *Pseudomonas* organic extract

Pots experiments were conducted in greenhouse of Department of Soil Fertility and Microbiology, Desert Research Center (30°47 0 and 30°49 0 N and Longitudes 32°22 0 and 32°25 0 E), season (2019). The seeds of weeds and *Zea mays* seeds were throughout this work provided by Agriculture Research Center, Ministry of Agriculture and Land Reclamation (MALR), Cairo – Egypt.

The seeds of weeds (*Echinochloa crus galli*, *Pennisetum purpuremum schumach Gramineae*, *Phalaris minor* and *Beta vulgaris L*) were used in weeds biological control experiment. The seed of *Zea mays* used to evaluate the effect of organic extract on the crop. Twenty seeds of every kind of weeds or Maize separated planting on 3 replicates of pots. The control was irrigation with water only. In the same pots, Maize cultivated after weeds removed. The addition of bacterium organic extract on weeds was pre-emergence on soil, while the first addition in Maize was after 10 days from planting. Then the second dose of addition was after 15 days from first one in both. The crude extract effect was also studied on soil microorganisms. 50 ml organic extract/ pot (has 1 kilo of soil) on twice. All growth parameters were taken after 30 days from planting, according to (Black *et al*., 1965). The soil which used in pots experiments was from Baloza station, Desert Research Center, Table (1).

Table 1. The Physical and chemical properties of the experimental soil from Baloza station

Particle size distribution (%)			Texture	EC (dS/m)	pH	Nutrients content c molc/Kg				Water soluble ions c molc/Kg				
Sand	Silt	Clay				P	Na^+	K^+	Ca^{++} (mg/l)	Mg^{++} (mg/l)	$CO3^-$	HCO_3^- (mg/l)	$SO4^-$	Cl^-
89.42	4.81	5.77	Sand	1.38	8.5	0.96	4.59	0.68	5.47	3.06	-	2.32	6.58	4.90

5. Microbiological determination

Nutrient agar medium (Jacobs and Gerstein, 1960) was used for counting of total microbial densities. Modified Ashby's medium (Abd- El – Malek and Ishac, 1968) for counting of nitrogen fixers by M.P.N technique and calculated using Cochren's tables, (Cochran, 1950) for

isolating and counting of nitrogen fixers, used (Bunt and Rovira, 1955) to determine Phosphate dissolving bacteria counts and King's medium used to cultivate or count *Ps. aeruginosa* (King *et al*., 1954).

6. Determination of bacterial metabolism by HPLC (HPLC–Mass spectrometry)

Extraction of broth metabolic culture of *Ps. aeruginosa* with ethyl acetate crude extract (organic extract) by LC–Mass spectrometry: Broth culture of bacterial supernatant mixed with same equal volumes of ethyl acetate to extract the active compounds from the aqueous phase. The organic phase was separated from the aqueous phase and evaporated to dryness, re-suspended in a small volume of ethanol 70% v/v (1ml), and placed in a glass vial for use in bioassays against weeds, then analyzed that extracts by High- Performance Liquid Chromatography HPLC [Ultimate 3000, Thermo Dionx], Germany, HPLC equipped with photodiode array detector and software for data analysis. An efficient gradient of acetonitrile-o-phosphoric acidified bi-distilled water (pH = 2.6) was used with an Interchrom C_{18}, 5µm reversed phase column. Wavelength: 254.0 were used during the elution, and data collection and integration were performed with software. Identified of sample was in Central Lab. of Faculty of Pharmacy-Ain Shams University, Cairo – Egypt.

Statistical analysis

The present work data was statistically analyzed and the differences between the means of the treatments were important, as they were more than the least significant differences (L.S.D) at the 5% level by using computer program of Statistix version 9 (Analytical software, 2008).

RESULTS AND DISCUSSION

1. Microbial strain

Pseudomonas aeruginosa is a Gram negative short rods bacterium. It has many different roles in agriculture. It used as growth promoting bacteria, pest control agent, biodegradation agent and others. In this study it used as a weed control agent.

2. Biochemical analysis of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa gave negative results of Catalase, Glutathione peroxides and Antioxidants.

3. Bioassay experiment in vitro

A. Bioassay with *Pseudomonas* broth culture

Table (2) showed the significant reduction in weeds growth parameters, by using *Ps. aeruginosa* broth culture compared with control. There was reduction in lengths of (*Echinochloa crus galli*, *Phalaris minor*, *Beta vulgaris L* and *Pennisetum purpuremum schumach Gramineae*) up to (75.7, 77.5, 59.8 and 64.7%, respectively). The reduction in fresh weights were up to (55.5, 76, 54.5 and 48.27%, resp.), *Echinochloa crus galli* dry weight reduction was up to (72.6%) and other weeds were no significant in dry weight. and the germination reductions were up to (40, 85, 80 and 70%, resp.). These agree with Lawrance *et al*., (2019) who observed the germination inhibitions of selected weeds were shown by metabolites of the strain *Pseudomonas aeruginosa* H6.

Table 2. Growth parameters of weeds in vitro treated with *Ps. aeruginosa* broth culture

Weeds	Treatments	Growth Parameters			
		Length/cm	Fresh weight/gm	Dry weight/gm	Germination (%)
Echinochloa crus galli	Control	3.5a	0.09a	0.073a	100a
	Pseudomonas	0.85b	0.04b	0.02b	60b
$LSD_{(0.05)}$		1.4435	0.0227	0.0167	10.264
Phalaris minor	Control	4a	0.225a	0.0064	100a
	Pseudomonas	0.9b	0.054b	0.0038	15b
$LSD_{(0.05)}$		1.611	0.0103	NS	5.7796
Beta vulgaris L	Control	3.56a	0.055a	0.01	100a
	Pseudomonas	1.43a	0.025b	0.008	20b
$LSD_{(0.05)}$		2.267	0.0216	NS	4.5339
Pennisetum purpuremum schumach Gramineae	Control	6.6a	0.29a	0.096	100a
	Pseudomonas	2.33b	0.15b	0.054	30b
$LSD_{(0.05)}$		2.3012	0.0227	NS	5.7796

B. Bioassay with *Pseudomonas* organic extract

Table (3) showed the high significant reductions in weeds growth parameters by using *Ps. aeruginosa* organic extract compared with control. There was reduction in lengths of (*Echinochloa crus galli*, *Phalaris minor*, *Beta vulgaris* L and *Pennisetum purpuremum schumach Gramineae*) up to (78.4, 84.7, 85 and 73.5 %, respectively). The reduction in fresh weights was up to (70.8, 81, 84.9 and 65.6 %, resp.), the reduction of dry weights was up to (87.5, 60, 100 and 58.75 %) and the germination reductions were up to (55, 88, 84 and 77%, resp.). These agree with Mustafa *et al*., (2019) who evaluated the combined application of *Pseudomonas aeruginosa* strain PAO1 and *Trichoderma harzianum* T-MN6 reduced the shoot length of *Phalaris minor* up to 30 % and *Avena fatua* 40 %, root length 22 % and 28 %, fresh biomass 29 % and 31 % respectively over their sole application. Kruh *et al*., (2020) who isolated *Pseudomonas* sp. strain (PhelS10) from tissue of tomato plant and *Ps. aeruginosa* strain (PAO1) were reducing weeds parasitism (*Phelipanche aegyptiaca*), that it might be used as a bio-control agent of weeds. Our findings demonstrated that quinolone signal (PQS) production from *Ps. aeruginosa* was 2.1 times more than that of the traditional *Ps. aeruginosa* strain (PAO1), resulting in a 22% higher biofilm forming potential,

4. Pots experiment in vivo

Bioassay experiment with *Pseudomonas* organic extract

Table (4): showed that the pots at greenhouse effect, the reduction percent of weeds growth parameters by using *Ps. aeruginosa* organic extract compared to control. The significant reductions of (*Echinochloa crus galli*, *Phalaris minor*, *Beta vulgaris* L and *Pennisetum purpuremum schumach Gramineae*) in lengths reached to (60, 33.9, no significant and 67.8%, respectively). The reduction in fresh weights reached to (68, 60, 20.75 and 62.85%, resp.) and the dry weights reductions reached to (55.5, 82.19, NS and 69.6%). These results agree with Cheng *et al*., (2022) who predicted that natural agents that limit seed germination or stop seedling growth might enable inventive weed seed bank control solutions. Additional natural compounds may be discovered through research on bacteria that contribute to weed control in the field. This is in line with Hasan's findings from (2021), who investigated how bioherbicides are created

from either plants that contain phytotoxic allelochemicals or certain disease-carrying microorganisms that can reduce weed populations. Only a few in vitro studies have been conducted on the physiological reactions' weeds elicit in response to bioherbicides, even though they have shown significant promise in inhibiting weed seed germination and growth. By interfering with normal cell function and causing the bioherbicidal agent to secrete harmful compounds, weed populations are reduced. The inhibition of cell division, food uptake, pigment synthesis, and plant growth-promoting regulators occurs with the regulation of weed germination and growth by stress-mediated hormones, erratic antioxidant activation, and other metabolites. These also agree with Juan *et al*., (2014) who reported the shoot and root parameters of *Digitaria sanguinalis* by metabolites of *Ps. aeruginosa* CB-4 were significantly inhibited. The IC_{50} of the filtrate extracts of culture for the *radicula* and *coleoptile* of *D. sanguinalis* were 0.299 and 0.210 mg mL^{-1}, respectively.

Table 3. Growth parameters of weeds in vitro treated with organic extract

Weeds	Treatments	Growth Parameters			
		Length/cm	Fresh weight/gm	Dry weight/gm	Germination (%)
Echinochloa crus galli	Control	3.2a	0.12a	0.08a	100a
	Pseudomonas	0.69b	0.035b	0.01b	45b
$LSD_{(0.05)}$		1.6110	0.0324	0.0227	3.5844
Phalaris minor	Control	5.1a	0.202a	0.005a	100a
	Pseudomonas	0.78b	0.038b	0.002b	12b
$LSD_{(0.05)}$		1.6031	1.603E-03	2.267E-03	5.7796
Beta vulgaris L	Control	5.4a	0.073a	0.04a	100a
	Pseudomonas	0.8b	0.011b	0.00b	16b
$LSD_{(0.05)}$		1.6110	2.267E-03	0.0160	8.1736
Pennisetum purpuremum schumach Gramineae	Control	5.3a	0.32a	0.08a	100a
	Pseudomonas	1.4b	0.11b	0.033b	23b
$LSD_{(0.05)}$		2.2670	0.0227	2.2670	3.5844

Table 4. Growth parameters of weeds under greenhouse effect treated with organic extract

Weeds	Treatments	Growth Parameters		
		Length /cm	Fresh weight/gm	Dry weight/gm
Echinochloa crus galli	Control	5.5a	2.5a	0.09a
	Pseudomonas	2.2b	0.8b	0.04b
$LSD_{(0.05)}$		2.052	1.45	0.0227
Phalaris minor	Control	9.33a	0.5a	0.0073a
	Pseudomonas	6.16b	0.2b	0.0013b
$LSD_{(0.05)}$		2.23	0.2267	2.267
Beta vulgaris L	Control	4.1	0.53a	0.07
	Pseudomonas	3.5	0.42b	0.056
$LSD_{(0.05)}$		NS	0.0227	NS
Pennisetum purpuremum schumach Gramineae	Control	14.3a	0.7a	0.033a
	Pseudomonas	4.6b	0.26b	0.01b
$LSD_{(0.05)}$		1.634	0.1611	0.0358

Fig. (1): showed that there is no significant effect in length, fresh & dry weights of *Zea mays* by using bacterium organic extract. ($LSD_{0.05}$) values were no significant. From below results, we found increased in soil microbes in number and activity which positively reflects on Maize, especially with increase the life of the crop. This agree with Dahiya *et al*., (2019) who evaluated the production of aminolevulinic acid, indole acetic acid, hydrogen cyanide and toxins has been correlated with the growth different weeds. Thus, inoculation of plants with bio weeds control agent has been found to increase seedling vigor, germination percentage, shoot and root growth, seed weight and increased grain, fodder and fruit yields. These environment-friendly biocontrol strategy for manage of weeds are high compatible with the sustainable agriculture. Theses also agree with these findings are consistent of Lakshmi *et al.*, (2014), who discovered the possibility of using *Pseudomonas fluorescent* as a weed bio-control agent, which can limit the development and vigour of weed seedlings, while having no negative effects on the targeted crop plants.

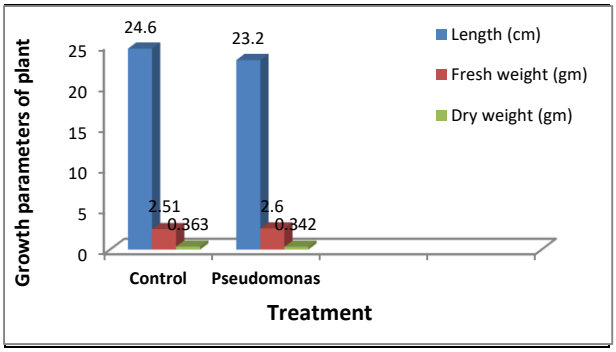

Fig. 1. Growth parameters of treated *Zea mays* with *Pseudomonas* organic extract under greenhouse effect

5. Microbiological determination

Table (5) showed that after 30 days from planting, the reduction percent on rhizosphere microbiological counts by using *Ps. aeruginosa* organic extract on weeds compared to control. Nitrogen fixer's count reduction in these weeds (*Echinochloa crus galli*, *Phalaris minor*, *Beta vulgaris L* and *Pennisetum purpuremum schumach Gramineae*) reach to (36.2, 16.6, 46.39 and 14.28, respectively), *Pseudomonas* count reduction reached to (56.5, 61.45, 25.13 and 38.16%, resp.). Total microbial count reduction reached to (29.9, 60, 34.6 and 38.3%, resp.) and PDB count reduction reached to (59.4, 75, 83.16 and 57.83%). As the same principle in bioherbicides, according to Shao and Zhang (2017) who studied the fact that biopesticides are increasingly used to replace artificial pesticides in pest control, it is needed to estimate their ecotoxicity and their non-target effects on microorganisms of soil, which is in generaly unknown. In this research, the effects of the artificial pesticide (carbendazim) and the biopesticides (norcantharidin and cantharidin) on microbial parameters in soil were estimated. After about several days, the hurtful effects owing to the application of pesticides phased out and eventually became comparable with control samples. The degradation of biopesticides was fast than artificial pesticide in the soil. This study presents an overall assessment of the toxicity of soil

microbial of these biopesticides for reasonable and effective use. The biopesticides (Cantharidin and norcantharidin) substantially decreased the fungal community diversity on the 3rd and 7th days compared to controls. At higher concentrations, non-target effects are more cleared. . However, the diversity of microflora of soil gradually increased after incubation of 15 days and was compared with or even overrun those values of control samples on day 35. Yang (2022) who studied that is unclear how plant species, especially those used as pasture and weeds, affect the variety, composition, and relationship of soil microbes. The North China Plain has five prevalent weed species (*Echinochloa crusgalli, Portulaca oleracea, Digitaria sanguinalis, Acalypha australis, and Chenopodium album*), as well as native lucerne plant called *Medicago stativa*. In this study, we look at the soil's physical and chemical characteristics and bacterial and fungal communities in this agroecosystem. In comparison to *M. stativa*, the Shannon diversity of the communities of fungi and bacteria in the five weeds was much lower. Our knowledge of how weeds affect the soil microbiome in agroecosystems is improved by our analysis of the microbial ecological network. In an *M. stativa* field in the NCP, the effects of five common weeds on the community composition, diversity, and microbial co-occurrence networks were examined. When compared to *M. stativa*, the diversity of bacteria and fungi was significantly reduced by these weeds. The five weeds were closely linked to alterations in edaphic factors (soil pH, NH_4^+-N, and NO_3^--N), as well as plant characteristics (shoot and root biomass, and R/S ratio). This led to changes in the structural community compositions. Additionally, weeds simplified the network of microbial co-occurrences and may have helped to promote increased assembly between microbial species. How weed species and soil microorganisms collaborate in the weed-crop competition will be further understood with the help of an integrated understanding of community assemblage. This also agree with Olanrewaju *et al*., (2019) who studied the relationship between the rhizosphere-microorganisms and plant hosts able to be beneficial, non-effective, or pathogenic depending on the microorganisms and the involved plant. This relationship, determines the destiny of the host plant's duration. Many effective proteins are activated in plants when contact with outer factors. These proteins may promote growth promoting or growth repressing responses from the plants.

Table 5. Microbial counts of weeds rhizosphere which treated by organic extract of *Pseudomonas*

Weeds		*Pseudomonas* sp. (10^3X CFU)	Nitrogen fixers (10^3X CFU)	Total counts (10^3X CFU)	PDB (10^3X CFU)
Echinochloa crus galli	Control	283	58	214	170
	Ps. aeruginosa	123	37	150	96
Phalaris minor	Control	275	60	295	180
	Ps. aeruginosa	106	50	118	45
Beta vulgaris L	Control	183	97	260	297
	Ps. aeruginosa	137	52	170	50
Pennisetum purpuremum schumach Gramineae	Control	283	70	287	166
	Ps. aeruginosa	175	60	177	70

Fig. (2): showed the results of microbial rhizosphere counts of *Zea mays* by using *Pseudomonas* organic extract compared to control. As results, Nitrogen fixer's, *Pseudomonas* sp. count and total microbial counts were increased up to (5.6, 1.75 and 100 %, respectively). PDB counts in Maize rhizosphere increased compared to PDB counts in weeds rhizosphere. Each type of soil microbes in Maize has been increased compared to the same type in soil microbes in weeds.

From the results, the negative effect on soil microbial counts in weeds wasn't due to used crude extract, but attributed to interactions between weed roots and soil microbes. These interactions were not clear.

From a reference above and from our showed results, the organic extract compounds were gradually degraded in soil after about 30 days from second dose on weeds. After weeds removed and Maize cultivated in the same pots, the Maize soil microbial counts were increased with Maize life in soil were increased. In addition, the unclear interactions between weed roots and soil microbes were finished after weeds removed. So, this gave soil microbial counts in Maize a big chance to increase within a month of Maize life in soil. These results agree with a study of Hu *et al*., (2023) who evaluated that the weeds develop in fields of crops, and could affect microorganisms correlated with crops through a neighbor-hood effect. This also agree with Motamedi *et al*., (2022) performed a study to validate the impact of three native plant growth-promoting bacteria derived from the Medicago sativa rhizosphere, involving (*Pseudomonas putida (B) and Serratia rubidaea (A), Serratia sp. (C) plus Synorhizobium meliloti* (R)) and their mixtures on microbial population, antioxidant enzymes (APX, CAT, and GPX) actions, plant biomass, and malondialdehyde and hydrogen peroxide contents at the existence and absence of the herbicide. The findings demonstrated that herbicide application reduced plant biomass. The plant biomass, microbial population, and antioxidant actions were lowered under CR, BCR, BC, and ABCR inoculations.

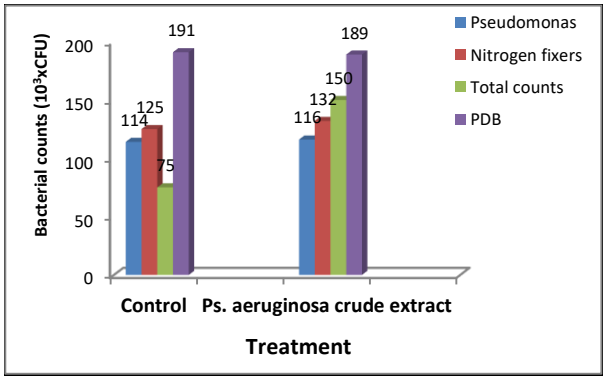

Fig. 2. Microbial counts of *Zea mays* rhizosphere treated with organic crude extract

6. Determination of bacterial metabolism by HPLC (LC–Mass spectrometry)

The most effective phytotoxic metabolites compounds in ethyl acetate crude extract of *Ps. aeruginosa* were evaluated and identified by using High-Pressure Liquid Chromatography-mass spectrometry electrospray analysis as: first identified compound with molecular weight 270.47 deduced from m/z 271.47 [M+1] might be coronatine which have the molecular formula $C_{18}H_{23}$ it

might be Quinoline. The second phytotoxic compound corresponding to molecular weight 190.14 deduced from m/z 191.14 [M+1] might be coronatine which have the molecular formula $C_7H_{12}O_6$ it might be Quinic acid. Compound with molecular weight 245.14 deduced from m/z 246.14 [M+1] might be coronatine which have the molecular formula $C_{16}H_{21}NO_2$ it might be Quinolone (2-heptyl-4- hydroxyquinolone-N-oxide). Compound with molecular weight 247.29 deduced from m/z 248.29 [M+1] might be coronatine which have the molecular formula $C_{13}H_{13}NO_4$ it might be 3-Quinolinecarboxylic acid, 1-ethyl-1,4-dihydro-7-methoxy-4-oxo- and compound with molecular weight 260.18 deduced from m/z 261.18 [M+1] might be coronatine which have the molecular formula $C_{18}H_{23}$ it might be 2,4-Quinolinediol.

In this study, Quinoline and Quinoline derivatives was the main reason of weeds inhibition. This agree with Saalim *et al* (2020) who estimated the alkyl-4-quinolones (AQs) are a class of metabolites produced originally by genus of the *Pseudomonas*, consisting of a 4-quinolone core substituted by a range of pendant groups, most commonly at the C-2 position. It was isolated a range of alkylquinolones with properties of antibiotic from *Pseudomonas aeruginosa*. More lately, it was discovered that a derivative of alkylquinolone, the *Pseudomonas* Quinolone Signal (PQS) plays a role in bacterial connection and quorum sensing in *Pseudomonas aeruginosa.* Lawrance *et al*., (2019) who determined the most active component metabolites produced by *Pseudomonas aeruginosa*, identified by GC- MS analysis which can control some weeds. Many chemical compounds were identified in Ethyl acetate crude extract of bacterial metabolite broth culture.

CONCLUSION

Pseudomonas aeruginosa was used as control against of weeds. There were significant reductions in growth parameters of these weeds (*Echinochloa crus galli*, *Phalaris minor*, *Beta vulgaris* L and *Pennisetum purpuremum schumach Gramineae*), by using *Pseudomonas* ethyl acetate crude extract in vitro and vivo. No negative effect on *Zea may* growth parameters by using organic extract in vivo. Rhizosphere microbial counts in weeds were decreased by *Pseudomonas* organic treatment compared to control. About 30 days, the organic extract was gradually degraded in soil. After remove weeds, the unclear interactions between weed roots and soil microbes would end. Therefore, the numbers of soil microbes increased with planting Maize in same pots after weeds removed. It happened during a month of Maize life in soil. The compounds in the organic extract identified by HPLC/MS to: Quinoline and Quinoline derivatives (Quinic acid, Quinolone 2-heptyl-4- hydroxyquinolone-N-oxide, 3-Quinolinecarboxylic acid, 1-ethyl-1,4-dihydro-7 -methoxy-4-oxo- and Quinolinediol.

REFERENCES

Abd-El-Malek, Y. and Ishac, Y.Z. 1968. Evaluation of methods used in counting *Azotobacter*. J. App. Bacterio., 31:267 – 275.

Aebi, H., 1984. Methods Enzymol 105, 121 – 126 Fossati, P., *et.al*. 1980. Clin.Chem. 26, 227 – 231.

Al-Daamy, A. A. H., Ahmed, A., & Mohammad, G. 2018. Antimicrobial agents' production by fungi isolates from the whisperers. Scientific Journal of Medical Research, 2(6), 104-107

Analytical software (2008). Statistix Version 9, Analytical Software, Tallahassee, Florida, USA.

Bergy's Manual of Determinative Bacteriology. 1994. John G. Hol, Noel R. Kriey, Peter H.A. Sneath, Jamest-Staley T. Williams (ed) (Nint 1 edition) Williams and Wilkins, Baltimore London.

Berg, G.; Roskot, N.; Steidle, A.; Eberl, L.; Zock, A. and Smalla, K. 2002. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. App. Enviro. Microbio., 68: 3328–3338.

Bunt, J. S. and Rovira, A. D. 1955. Microbiological studies of some sub –ataractic soils. J. Soil Sci., 6,119.

Black, C. A.; Evans, D. D.; Ensminger, L. E.; White, J. L. and Clark, F. E. 1965. Methods Of Soil Analysis. part 1. Chap. 18, Water Capacity by klute A., A.S.A. Monograph series No. 9, Amer. Soc. Agron. Madison, Wissconson, U.S.A. rom different *Verticillium* host plants. App. Enviro. Microbio., 68: 3328–3338.

Brand-Williams, W.; Cuvelier, M. E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. Lebens. Wisse Tech., 28: 25–30.

Cheng, L.; DiTommaso, A. and Kao-Kniffin, J. 2022. Opportunities for Microbiome Suppression of Weeds Using Regenerative Agricultural Technologies. Review article. Front. Soil Sci., Sec. Soil Biology, Ecosystems and Biodiversity. Vol. 2 - 2022 | https://doi.org/10.3389/fsoil.2022.838595.

Cochran, W. G. 1950. Estimation of bacterial densities by mean of the (Most Probable Number) Biometrics, 6:105-115.

Dahiya, A.; Chahar, K. and Sindhu, S. S. 2020. The rhizosphere microbiome and biological control of weeds: A review. Spanish Journal of Agricultural Research, 17(4), e10R01. https://doi.org/10.5424/ sjar/2019174-15073.

De Marco, E.; Savarese, M.; Paduano, A. and Sacchi, R. 2007. Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. Food Chem., 104: 858-867.

Fang, F.; Liu, F.; Wu, Zh.; Zhang, Zh. And Wang, K. 2022. Plant-Associated Bacteria as Sources for the Development of Bioherbicides. *Plants* 2022, *11*(23), 3404; https://doi.org/10.3390/ plants11233404.

Hasan, M.; Hamdani, M.S.A.; Rosli, A. M. and Hamdan, H. 2021. Bioherbicides: An Eco-Friendly Tool for Sustainable Weed Management. Plants (Basel). 10 (6): 1212. doi: 10.3390/plants10061212.

Ibrahim, Gh. A. Z.; El-Tayeb, T. S.; Omar, A. M.; Balah, M. A. and Ramadan, E. M. 2016. Effect of Fermented Olive Mill Waste Water on The Growth and Productivity of Sorghum Grown Under Field Conditions. J. Environ. Sci. Institute of Environmental Studies and Research – Ain Shams University. Vol.33, No.1, 187:211.

Iwase, T.; Tajima, A.; Shinya Sugimoto; Ken-ichi Okuda; Ippei Hironaka; Kamata, Y.; Takada, K. & Mizunoe, Y., 2013. A Simple Assay for Measuring Catalase Activity: A Visual Approach. *Sci Rep* 3, 3081. https://doi.org/10.1038/srep03081.

Jacobs, M.B. and Gerstein, M.J. 1960. Hand Book of Microbiology. De. Van Nostranal Co. Inc., New York, 139 – 202.

Juan,Y.; CAO Hong-zhe ; Wei, W.; Li-hui, Z. and Jin-gao, D. 2014. Isolation, Identification, and Herbicidal Activity of Metabolites Produced by *Pseudomonas aeruginosa* CB-4. Journal of Integrative Agriculture 2014, 13: 1719-1726.

King, E.O.; Wand, M.K. and Raney, O.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med., 44: 301 – 307.

Kruh, L. I.; Bari, V. K.; Abu-Nassar, J.; Lidor, O. and Aly, R. 2020. Characterization of an endophytic bacterium (*Pseudomonas aeruginosa*), originating from tomato (*Solanum lycopersicum* L.), and its ability to inhabit the parasitic weed *Phelipanche aegyptiaca*. Plant Signal Behav. 2020; 15(7): 1766292. doi: 10.1080/ 15592324.2020.1766292.

Kubiak, A.; Wolna-Maruwka, A.; Niewiadomska, A. and Pilarska, A. A. 2022. The Problem of Weed Infestation of Agricultural Plantations vs. the Assumptions of the European Biodiversity Strategy. Agronomy 2022, 12(8), 1808; doi.org/10.3390/ agronomy 12081808.

Lakshmi V.; Kumari S.; Singh A. and Prabha, Ch. 2014. Isolation and characterization of deleterious *Pseudomonas aeruginosa* KC1 from rhizospheric soils and its interaction with weed seedlings. Journal of King Saud University - Science. Vol. 27, Pages 113-119. DOI: 10.1016/j.jksus. 2014.04.007. License CC BY-NC-ND 3.0.

Lane, D.J. 1991. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, editors. Nucleic acid techniques in bacterial systematics. Chichester: John Wiley & Sons.,115–175.

Lawrance, S.; Varghese, Sh.; Varghese, E. M.; Aju, K. ; Asok and Jisha M. S. 2019. Quinoline derivatives producing *Pseudomonas aeruginosa* H6 as an efficient bioherbicide for weed management. Biocatalysis and Agricultural Biotechnology

Volume 18, March 2019, 101096. doi.org/10.1016/ j.bcab . 2019.101096.

Motamedi, M.; Zahedi, M.; Karimmojeni, H.; Motamedi, H. and Mastinu, A. 2022. Effect of rhizosphere bacteria on antioxidant enzymes and some biochemical characteristics of *Medicago sativa* L. subjected to herbicide stress. Acta Physiologiae Plantarum volume 44, Article number: 84.

Mustafa, A.; Naveed, M. ; Abbas, T; Saeed, Q; Hussain, A; Ashraf, M. N. and Xu Minggang. 2019. Growth response of Wheat and associated weeds to plant antagonistic rhizobacteria and fungi. Italian Journal of Agronomy 14.

Olanrewaju, O.S.; Ayangbenro , A. S.; Glick, B. R. and Babalola, O. O. 2019. Plant health: feedback effect of root exudates-rhizobiome interactions. Applied Microbiology and Biotechnology volume 103, pages1155–1166.

Paglia, D.E. and Valentine, W. N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase J. Lab. Clin. Med. 70: 158 – 169.

Pena, G., Pereyra, C., Armando, M., Chiacchiera, S., Magnoli, C., Orlando, J., Cavaglieri, L. 2010. *Aspergillus fumigatus* toxicity and gliotoxin levels in feedstuff for domestic animals and pets in Argentina. Letters in Applied Microbiology, 50(1), 77-81.

Russel, A.D., Hugo, W.B., Aylif, G.A.J. 1982. Principles and Practices of Disinfection, Preservation and Sterilization, Blackwell Scientific, London.

Saalim, M.; Moreno, J. V. and Clark, B. R. 2020. Bacterial Alkyl-4-quinolones: Discovery, Structural Diversity and Biological Properties. Molecules. 2020 Dec; 25(23): 5689.doi: 10.3390/molecules25235689.

Shao, H. and Zhang, Y. (2017). Non-target effects on soil microbial parameters of the synthetic pesticide carbendazim with the biopesticides cantharidin and norcantharidin. Sci Rep. 17;7(1):5521. doi: 10.1038/ s41598-017-05923-8.

Tawfik, M. M. ; Ibrahim, N. A.; Balah, M. A. and Abouzeid, M. M. 2019. Evaluation of Bacteria from Soil and Rhizosphere as Herbicidal Candidates of Some Broadleaf Weeds. Vol. 59: 283-291.

Yang, Ch.; Tang. W.; Sun, J.; Guo, H.; Sun, Sh.; Miao, F.; Yang, J.; Zhao, Y.; Wang, Z. and Sun, J. 2022. Weeds in the Alfalfa Field Decrease Rhizosphere Microbial Diversity and Association Networks in the North China Plain. Sec. Terrestrial Microbiology. Vol. 13 – 2022. doi.org/10.3389/fmicb.2022.840774.

استخدام سيدوموناس إريجينوزا للتحكم في بعض أنواع الحشائش وتأثيرها على ميكروبات التربة

غادة أمين زكى إبراهيم

قسم خصوبة وميكروبيولوجيا الأراضى – مركز بحوث الصحراء – القاهره – مصر

الملخص

التحكم البيولوجي فى الأعشاب الضارة هو استخدام الأعداء الطبيعية لتقليل الكثافة إلى مستوى مقبول. الهدف من هذا ليس الإزالة ولكن تقليل عدد الأعشاب إلى حد الإنخفاض الإقتصادي. سيدوموناس إريجينوزا تستخدم هنا كعامل تحكم حيوي. وتستخدم كإضافات في تجربتين للمقاومة الحيوية بالحشائش مقارنة مع الكونترول. في المختبر، كان أول تجربة هى استخدام المزرعة السائلة للسيدوموناس. من النتائج ، كان هناك خفض للحشائش (دنيبة، فلارس، سلق وعلف الفيل) بنسبة (40-85 ٪) في الأطوال، والأوزان الطازجة والجافة ونسبة الإنبات. وكانت التجربة الثانية هى استخدام مستخلص خلات الإيثيل الخام للسيدوموناس. أدى إلى انخفاض للحشائش بنسبة (55-100٪). في تجربة الأصص بالصوبة، باستخدام مستخلص السيدوموناس العضوى مقارنةً بالكونترول ، كان هناك انخفاض في قياسات نمو الأعشاب بنسبة (21-82 ٪). لم يكن هناك انخفاض في قياسات نمو الذرة. وجدت هناك تفاعلات غير واضحة بين جذور الحشائش وميكروبات التربة تسببت في انخفاض أعداد ميكروبات ريزوسفير الحشائش. وبعد إزالة تلك الحشائش وزراعة الذرة في ذات الأصص، زادت أعداد ميكروبات التربة خلال شهر من حياة الذرة في التربة. تم تعريف المركبات الموجودة فى مستخلص السيدوموناس الخام بواسطة HPLC-MS وكانت النتائج هى: مشتقات الكينولين والكينولين (حمض الكينك، الكينولون 2-4-Heptyl- هيدروكسيكينولون-أكسيد، حمض 3 كينولينيكسيليك، 1-إيثيل--4-ديهيدرو-7 ميثوكسي- 4-اوكسو- و كوينولينيديول). نوصي باستخدام سيدوموناس إريجينوزا لتقليل الحشائش خاصة مع زراعة الذرة، وإضافة الأسمدة الحيوية. تأثير جذور الحشائش على الكائنات الحية الدقيقة في التربة غير واضح ويحتاج إلى مزيد من الدراسات المستقبلية.