An-Najah National University

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Enhanced Phytoremediation Of Olive Mill Wastewater "Zibar" Using Plant Growth Promoting Rhizobacteria (PGPR) With Barley and Clover

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Dedication

All praise and thanks be to Almighty "Allah"

I would like to Thank Allah for all the blessings He gave us, thank you Allah for providing us with your guidance, and under His name, I dedicated this work to all knowledge seekers.

I dedicated this work also to my late father may Allah rest his soul in peace as he was always my guide and teacher in life, the one who first taught me how to step and how to never give up the one who I owe him my success and every single beautiful moment in my life, I would like also to thank my husband Eng. Mohammad Mosleh as he's been always an inspiration for me, my mother and my siblings too(especially my brother sameer), and Eng. Naeem budair, who helped me accomplish my goals and encouraged me through good and bad times, my son and daughters Marah, Meera, Tala and Abdullah without them, I wouldn't have stepped ahead in life. Additionally, I would like to send my thanks and gratitude to Dr Hassan abu Qaoud who didn't keep any effort in encouraging us to do a great job, providing our group with valuable information to be better each time. Thanks for the continuous support and kind communication which had a great effect regarding to feel interesting about what we are working on. Finally, we convey our warmest thanks to our friends and all the people who helped, supported and encouraged us to successfully finish the project whether they were in the university or out of it. I extend my thanks to my teacher and supervisor Dr.Shehdeh Jodeh, and Co- Supervisors Dr. Raed Alkowni

I hope this research meets your expectations.

Best regards,

Samar Omar Sameer Abed Alqader

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Lastly, I wish to express my sincere gratitude to my family for their encouragement and moral support.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل عنوان:-

Measuring of Photosynthesis Rate of Rhizobacteria Enhanced Barley and clover Irrigated with Olive Mill Wastewater "Zibar"

اقر بان ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص, باستثناء ما تمت الإشارة إليه حيثما ورد وان هذه الرسالة ككل أو جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

ACC	1-Amino Cyclopropane-1-Carboxylate
AL	Actinic Light
AMP	Ampicillin Antibiotic
ARF	Auxin Responses Factor
ATP	Adenosine Triphosphate
BOD	Biochemical Oxygen Demand
C^3	Cubic Meter
COD	Chemical Oxygen Demand
dd H_2O	De-ionized Distilled water
FRL	Far Red light
IAA	Indole-3-Acetic Acid
Kc	Crop Coefficient
LHCI	Light Harvesting Chlorophyll Protein Complex I
LHCII	Light Harvesting Chlorophyll Protein Complex II
MCP	Methyl Cellulose Polymer
MML	Modulated Measuring Light
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NAP	Nitrogen – Activated Protein
NARC	National Agriculture Research Center
FS	Fytoscope
NM	Nano Meter
OD	Optical Density
OMWW	Olive Mill Waste Water
PAM	Pulse Amplitude Modulated
PGPR	Plant Growth Promoting Rhizobacteria
Pheo	Pheophytin
PPM	Part Per Million
PSI	Photosystem I
PSII	Photosystem II
R.P.M	Round Per Minute
ROS	Reactive Oxygen Species
S-AdoMet	S- Adenosyl Methionie
SAM	S-Adenosyl-Methionine
SP	Saturating Pulse
TDS	Total Dissolved Solids
TS	Total Solid
TSB	Tryptic Soy Broth
TSS	Total Suspended Solids

UW3	*Pseudomonas putida*
UW4	*Pseudomonas putida*

Chlorophyll Fluorescence Nomenclature

F	Actual fluorescence intensity at any given time.
F’	Fluorescence at any light level and induction state. Some PSII closed ($0 \leq qP \leq 1$, $0 \leq qN \leq 1$), some ΔpH
F_o	Minimal fluorescence in dark-adapted tissue; fluorescence intensity with all PSII reaction centers open while the photosynthetic membrane is in the non-energized state (qP = 1 and qN = 0); ΔpH. It can also be used for the O level in Kautsky nomenclature.
F_m	Maximal fluorescence in dark-adapted tissue; fluorescence intensity with all PSII reaction centers closed (qP = 0), all non-photochemical quenching processes are at a minimum (qN = 0); no ΔpH
F_v	Variable fluorescence in dark-adapted tissue; maximum variable fluorescence in the state when all non-photochemical processes are at a minimum ($qP = 1 \rightarrow 0, qN = 0$), i.e. Fm-Fo
F_s	Fluorescence in steady states; defined by an author as a period within which the fluorescence intensity does not change while the external circumstances remain constant
F_s’	Steady-state fluorescence at any light level. Some PSII closed ($0 \leq qP \leq 1$, $0 \leq qN \leq 1$), some ΔpH
F_v/F_m	Exciton transfer efficiency in dark-adapted tissue; (Fm-Fo)/Fm
F_o’	Minimal fluorescence in light-adapted tissue (quick application of Far-Red PSI light); fluorescence intensity with all PSII reaction centers open in any light adapted state (qP = 1 and $qN \geq 0$), some ΔpH
F_m’	Maximal fluorescence in light-adapted tissue; fluorescence intensity with all PSII reaction centers closed in any light adapted state (qP = 0 and $qN \geq 0$)
F_v’	Variable fluorescence in light-adapted tissue; maximum variable fluorescence in any light adapted state, i.e Fm’ – Fo’, caused by closure of PSII in the light ($qP = x \rightarrow 0$, $0 < qN \leq 1$)
F_v’/F_m’	Exciton transfer efficiency in light-adapted tissue; (Fm’ – Fo’)/Fm’
qP	Photochemical quenching; (Fm’ –F)/(Fm’ – Fo’)
qN	Non-photochemical quenching; 1-(Fm’-Fo’)/(Fm-Fo)
Yield	Effective quantum yield of PSII; (Fm’-Fs)/Fm’

Enhanced Phytoremediation Of Olive Mill Wastewater "Zibar" Using Plant Growth Promoting Rhizobacteria (PGPR) With Barley and Clover

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Abstract

Olive mill wastewater (OMWW) has negative environmental impact. Utilization of OMWW in irrigation is difficult due to the toxic effect. Different phytoremediation methods were used to improve the use of OMWW in irrigation.

In this research, PGPRs were implemented to investigate their efficiency of improving the phytoremediation technique for plants irrigated with olive mill waste water. Two strains of PGPR (UW3, *Pseudomonas putida*(A). UW4, *Pseudomonas putida*(C) with unassigned one (B)) were used with Barley (*Hordeum valgare* L.) and clover plants (*Trifolium* sp.). All trials were carried in a designed green house in faculty of agriculture at An-Najah national university in Tulkarem for 30 days. Plants irrigated with different concentration of OMWW (0%, 10%, 25%, and 50%). Seeds of both barley and clover irrigated by different concentration of olive mill waste water, showed significant differences in germination among the concentration levels of OMWW. It was notable that the OMWW has negative impact on seed germination of both plants.

Neither barely nor clover plants treated with PGPRs had significant improvement in biomass compared with those irrigated with fresh water. Root length was decreased significantly with the increase of OMWW levels (57.8 and 58.5cm respectively). The OMWW application significantly reduced the shoot length. OMWW at 50% reduced the stem length (15.5 cm). A similar trend was observed with other measures (both fresh and dry weight of the plant). OMWW application was highly reduced both weights of stems and roots of both plant species.

For clover plants, root length, shoot length, wet weight (P=(0.0057-0.0001), were reduced, however, total dry weight, dry weight of roots, and dry weight of shoots ($p < .05$). The higher root length was observed with the control and 10% OMWW (25.22and 23.98 cm, respectively). Regarding shoot, shoot length was reduced, the lowest shoot length was observed (4.879) at 50% zibar application. Wet weight of clover was differs significantly among the different concentration of OMWW and different type of bacteria used.

Pulse Amplitude Modulation (PAM) fluorometry showed no improvement in photosynthesis. Barely plants their values of **Fv/Fm** were ranged from (0.55 -0.68), which mean that plant is under stress, and its photosynthesis not proceed as it should be, and NPQ values ranged (0.11-0.17). The same was for clover plants treated with PGPR (UW3), irrigated with fresh water, 10% concentration of OMWW, values of **Fv/Fm** are closed to 0.8 and NPQ are decreased to .07.Other Trails of clover plants values of **Fv/Fm** were

ranged from (0.62 -0.70), and NPQ values ranged (0.04-0.16).Which mean that plant is under stress, and its photosynthesis not proceed as it should.

Chapter One

Introduction

1.1 Background

The olive oil extraction process produces huge amounts of liquid waste called olive mill wastewater (OMWW) Zibar, which are produced within few months (October to December). The annual OMWW production in worldwide approximately is 1880000 ton. With the majority in the Mediterranean basin (El-Khtib et al,2009), The annual OMWW production of the Mediterranean olive growing countries is estimated to amounts ranging from 7 to over 30 million m3 (Niaounakis et al, 2004). Olive mills in the West Bank generate about 200 thousand m3/year of OMWW (Subuh, 1999).

The farming and processing of olives for olive oil production are among the most important industries in Mediterranean countries. Olive production is the base of Palestinian agriculture. Olive farms cover almost half of the cultivated area in The West Bank, and oil production contributes around 28.7% of the agriculture domestic income(El-Khatib,et al, 2009).

OMWW is a mixture of vegetation water and soft tissues of the olive fruit and the added water used in the various stages of the oil extraction process. Typical OMWW composition by weight is 83-94% water, 4-16% organic compounds and 0.4-2.5% inorganic compounds (mineral salts) (Davies et al., 2004).

OMWWs contain an enormous supply of organic matter very rich in phenolic compounds, which are toxic and very low quantity of heavy metals. Wastewater from the different olive-mills sited in and around the different villages in Palestine is being inclined of into the wadies. There, it is mixed with the unprocessed flowing municipal wastewater or with rainwater. The resulting high organic polluted wastewater affects the soil and water receiving bodies (Shaheen, 2007).

Recent research revealed that the olive oil production process is a major contamination for the environment. Olive mills wastewater OMWW (known in Palestine as Al-zibar), was found to be the highest source of environmental pollution in the countries of Mediterranean region (Jodeh et al, 2014).

Phytoremediation is an emerging green technology which uses plants to remove, transfer, stabilize, and destroy contaminants in soil and sediment. Contaminants may be either organic or inorganic. Research in the field of phytoremediation is aiming to develop modern, economical and environmentally well-suited approaches to remove heavy metals from the environment (Mathew, 2001).

Plant Growth Promoting Rhizobacteria (PGPR) were found to improve plant growth by lowering production of stress ethylene compound within plants, thereby increasing the biomass and photosynthetic activity (Hamed, 2014).

PGPRs were implemented to investigate the efficiency of phytoremediation techniques for treatment of generated olive mill waste water. *valgare* L.).

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There are several treatment processes and technologies employed to reduce the negative environmental impact of OMWW. The effectiveness, feasibility and sustainability of the treatment processes to remove organic matters must be taken into consideration when making a decision on the most suitable treatment of OMWW (Adham, 2012). All the traditional methods that were used until now to remove the organic contaminants from OMWW were inefficient.

1.2 Objectives of Study

Reducing the negative environmental impact of olive mill wastewater is a great importance to protecting the biophysical environment. To improve the plants' resistance to the toxicity of OMWW, three strains of Rhizobacteria were combined with cereal and legume selected plants (Barley, Clover) to assess their impact on phytoremediation. Specifically, the following tasks were analyzed and discussed:

- The use of barley and clover in the phytoremediation to remove organic compounds and heavy metals from olive mill wastewater(Zibar).
- To study if the rate of photosynthesis in barley and clover affected by using olive mill waste water (Zibar) enhanced
- To study if the Zibar will be used as fertilizers for some plants.
- Study the effect of Plant Growth Promoting Rhizobacteria (PGPR) on plants in terms of biomass production and photosynthetic activity under olive mill wastewater (Zibar).

Chapter Two

Literature Review

2.1 Olive mill wastewater characteristics

The characteristics of OMWW in terms of its quantity and quality are highly reliant on the withdrawal process (Shaheen, 2007). The composition of OMWW is rather variable depending on the crop, the variety of fruit and in exacting on the technological system used for oil extraction (press, centrifugation or filtration) (Lopez & Ramos-Cormenzana, 1996).

OMWW contain a vast supply of organic matter very rich in phenolic compounds, which are toxic. phenolic compounds divided into low-molecular weight (caffeic acid, tyrosol, hydroxytyrosol, p-cumaric acid, ferulic acid,syringic acid, protocatechuic acid etc.) and high molecular weight compounds (tannins, anthocyanins, etc) (Davies et al., 2004).

OMW contains very low quantity of heavy metals and regular supply of $50m^3$/ha/an provides 30 to 100 times less of heavy metals than the limits allowed by the EU standards for the environment (Naija et al 2014).

The amount of heavy metals naturally present in the olive mill wastewater , Zn was the predominant metal (3.907 ppm) followed in decreasing order by Cu (1.376 ppm), Mn (1.359 ppm), Ni (0.545 ppm), Pb (0.180 ppm), Co (0.075 ppm), and Cd (0.036 ppm), as reached by Fatih Vuranand and Mustafa Demir.

Table (2.1):- General characteristics of olive mill wastewater.

Parameter	Value	Parameter	Value
COD (total)	40,000 – 220,000	Potassium	2,800-11,600
COD (soluble)	32,000-176,000	Polyphenols	5,000-80,000
BOD5	23,000 – 100,000	Carbohydrates	3,000-24,000
pH	3.0-5.9	Oil Content	1,000-23,000
Alkalinity as CaCO3 (total)	1,170	Total Solids	30600-58200
Organic Nitrogen	154-1106	Total Volatile Solids**	21300-45900
Phosphorous	100-900	Total Suspended Solids**	1400-36000
Sodium	100-500	Total Bacteria * (106 col/ml)	5
Magnesium	200-900	Total yeasts and fungi* (106 col/ml)	5
Calcium	100-700		

Source: (Naser et al., 2007; * Gonzalez-lopez et.al., 1994; **Esra et al., 2001), values are in mg/l unless indicated.

All the above parameters must be taken into consideration in the drawing of a well-included treatment process of OMWW.

According to Ruba Al Adham the characteristic of OMWW in the West Bank are showen in table (2,2).

Table (2.2):- General characteristics of OMWW in the Northern West Bank.

Parameter	**Jenin**	**Nabluse**	**Tulkarem**	**Salfit**	**Qalqilya**	**Average**
BODs	8830	8755	13698	12580	13010	11375
CODs	136750	130625	145000	136750	138500	137525
Total	5276.0	4032.4	6232.7	3179.1	4239.7	4592.0
TS(mg/l)	73970	46250	87800	62450	66920	67478
TSS(mg/l)	58070	38150	68600	45680	49570	52014
TDS	15900	8100	19200	16770	17350	15464
pH	4.8	4.9	4.9	4.6	4.9	4.8

Source: (Adham, 2012)

2.2.1 Polyphenols

The olive oil extraction process leads to detachment of the olive fruit phenolic content into two main groups; group of polyphenols that has higher similarity to the aqueous phase and endorsed to antimicrobial and phytotoxic effect of OMWW (Davies et al., 2004) , and the other group of polyphenols that possess useful antioxidant effect and which ends up in the olive oil. The strong antioxidant nature of phenols renders them principally durable and non-biodegradable, thus complicating any biological treatment process for the OMWW.The large fraction of polyphenols is lost in OMWW, in the range from 0.5 to 2.4 g/l (Sorlini et al., 1986).

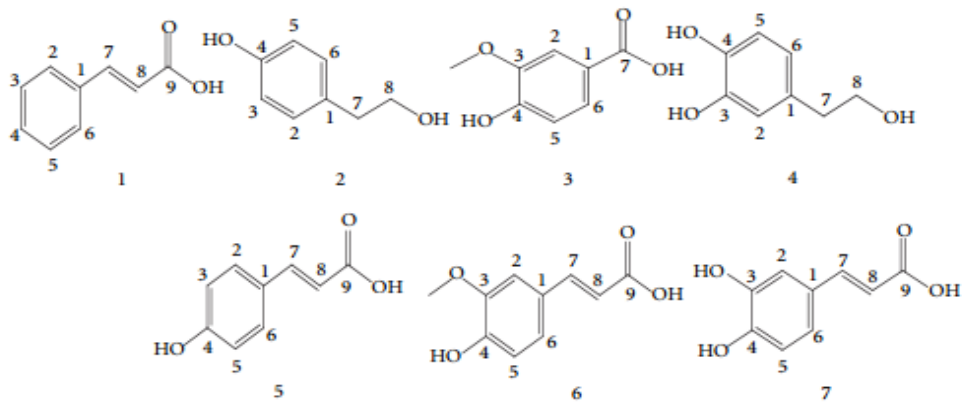


Figure (2.1): Structures of the phenolic compounds (Deep, et. al. 2012).

2.2.2 Removal of poly-phenols from olive mill wastewater

There are several treatment processes and technologies employed to reduce the negative environmental impact of OMWW. The efficiency, feasibility and sustainability of the following treatment processes must be taken into consideration when making a decision on the most suitable treatment of OMWW (Adham, 2012). All traditional methods used until now to remove

these organic contaminants that are harmful to nature, human being, and animals from OMWW were ineffective. One of the methods to remove phenol from OMWW is adsorption (Jodeh et al, 2014).

Few researches in Palestine concerned with the removal of phenol from OMWW. Jodeh et al, (2014) studied the removal of phenol from OMWW by using cross-linked polytaconic acid (x-PIA) as phenol adsorbent. Ruba Adham (2012) studied the removal of phenol from OMWW by using activated olive stones as phenol adsorbent. According to El-Khatib et al., (2009), 84% of COD removal from diluted OMWW was achieved using Up-Flow Anaerobic Sludge Bed (UASBA)

2.3 Phytoremediation

Phytoremediation (from Ancient Greek φυτο (phyto), meaning "plant", and Latin remedium, meaning "restoring balance") describes the treatment of environmental problems (bioremediation) through the use of plants that mitigate the environmental problem without the need to hollow out the contaminant material and dispose of it elsewhere. Phytoremediation is a rising green technology to remove, degrade, or contain toxic chemicals (metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants) in soils, sediments, groundwater, surface water, and air by using plants (Wikipedia).

Phytoremediation of organic contaminants primarily occurs by one or more of the following five mechanisms:

Table (2.3):- Different types of phytoremediation.

Phytotechnique	Description	Route of contaminant uptake	Mechanism
Phytoextraction	Translocates and concentrates contaminants from soil via plant roots into harvestable plant parts e.g. shoots	Uptake by plant roots	Absorption or uptake via dissolution in water or through cation pumps accumulation or sequestration
Phytofiltration	Utilizes plants with extensive root systems for removal of pollutants from water	Uptake by plant roots (rhizofiltration), young plant seedlings (blastofiltration) or excised plant shoots (caulifiltration)	Filtration, sorption or precipitation of pollutants surrounding the root zone
Phytostabilization	Immobilizes pollutants and reduces their bioavailability	Uptake by plant roots	Sorption Precipitation or complexation in rhizosphere
Phytotransformation	Breakdown organic contaminants through plant metabolic activities or plant enzymes	Uptake by plant roots or metabolism within root zone	Absorption by root system resulting in metabolic or enzymatic transformation within or external to plants
Rhizodegradation	Degrades pollutants by soil dwelling microbes in rhizosphere due to simulation of microbial activity by plant secretions	Transformation within root zone	Secretion of root exudates or enzymes around root zones and subsequent microbial degradation of xenobiotics
Phytovolatilization	Transforms pollutants into volatile form or gas phase and their subsequent release in atmosphere through transpiration	Uptake of water soluble pollutants by plant roots	Modification of pollutants during vascular translocation from roots to leaves

Source**:** (Schwitzguebel, 2004)

To achieve a major reduction of contaminants within one or two decades, it is necessary to use hyper accumulators (plants capable of accumulating >100 mg Cd kg-1, >1000 mg Pb kg^{-1}, and >10 000 mg Zn kg^{-1} in the dry matter of their shoots when growing in their natural habitats) or crops with a metal bioconcentration factor (which is the ratio of metal concentration in the shoot tissue to the metal concentration in the soil) of 20 and a biomass production of 10 tons per hectare, or with a metal bio-concentration factor of 10 and a biomass production of 20 $ton.ha^{-1}$ (Poniedziałek 2010).

There are some studies on the phytoremediation of organics and metals from olive mill wastewater, but phytoremediation of heavy metals from the soil and water are discussed by many researchers (Poniedzialek et al, 2010; Dahbia et al, 2013; Pivetz, 2010; Pant et. al, 2011;). Few researches concerned with phytoremediation of organically contaminated soils (Chen et al, 2013; Kamath et al, 2016). And phytoremediation of organic-polluted soils (Daraghmeh, 2016**)** studied the Use of Plant Growth-Promoting Rhizobacteria (PGPR) to Improve Plant Growth in Heavy Metal Contaminated Soil for Phytoremediation.(S.abdallah, 2015) studied the Phytoremediation of organics and metals from olive Mill Wastewater**.**

In general, there are two approaches based on the difference in remediation mechanism. First, taken up organic pollutants can be directly by plants, resulting in the appropriation or degradation of pollutants inside of plants, which is called phytoextraction. Second, degraded organic pollutants by plant-secreted enzymes or plant-modified microbial community in the

rhizosphere, which is called plant-assisted rhizoremediation (Chen et al., 2013).

2.4 Photosynthesis

Photosynthesis is a physiological process in plant uses energy to form O2, carbohydrates and ATP (adenosine triphosphate). The process starts with the absorption of light and change of photon energy to an electron. Then electron agitated to higher energy levels through electron transport chain in the thylakoid membrane, ended with change NADP+ to NADPH form and adenosine triphosphate (ATP) [Baker,2008; Flexas et al. , 2004].

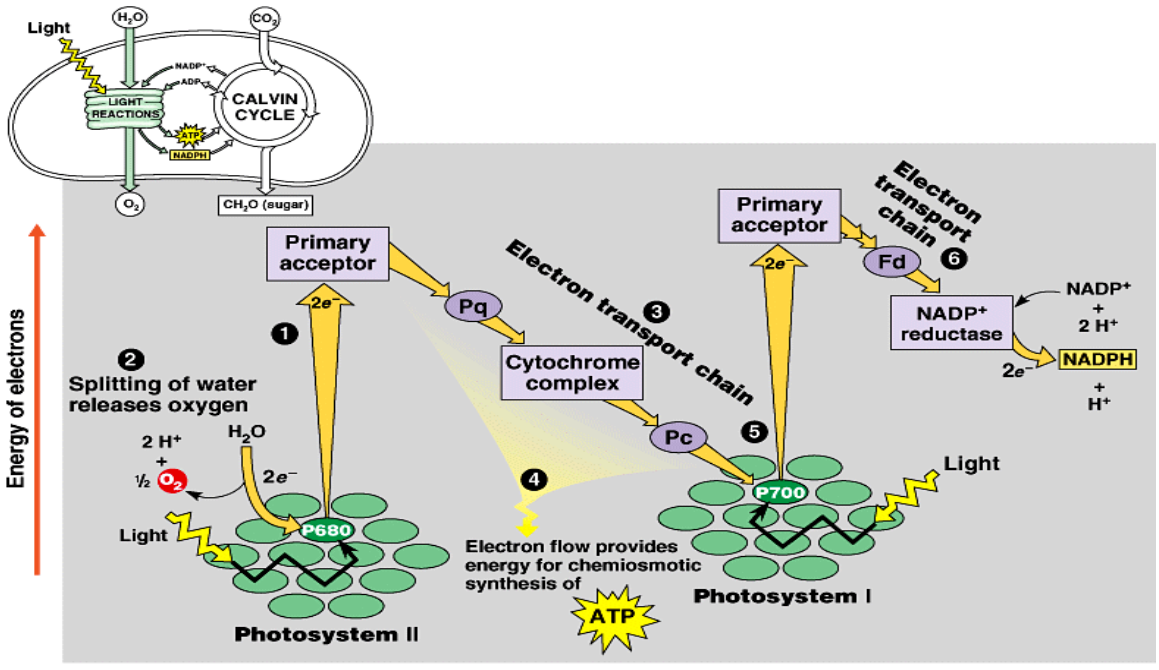


Figure (2.1): Photosystems and electron transport.

2.5.1 Plant Growth Promoting Rhizobacteria (PGPR)

Plant Growth Promoting Rhizobacteria is naturally occurring bacteria which is abbreviated to PGPR term. It is useful free living soil bacteria that exist in association with the roots of many different plants. The narrow zone of soil direct surround around the root system of the plant is the Rhizosphere(Kloepper and Schroth, 1978).The effect of PGPR on crop productivity varies on greenhouse and laboratory and field trials. because the soil is an impulsive environment and an intended result is sometimes difficult to achieve, also climate variations have a great impact on the efficiency of PGPR but sometimes poor growth conditions in the field are to be expected as normal functioning of agriculture (Zaidi et al., 2009). PGPR can be divided into two major groups due to the relationship between Rhizobacteria and its host plant :(1) Symbiotic Rhizobacteria, which may occupy the inside of cells and stay alive inside the cell (also called intracellular PGPR, e.g., nodule bacteria), (2) Free-living Rhizobacteria that live outside plant cells (called extracellular PGPR, e.g., Bacillus, Pseudomonas, Burkholderia, and Azotobacter) (Khan, 2005; Babalola and Akindolire, 2011)

Table (2.4):- Plant Growth Promoting Rhizobacteria mechanisms [Munees and Mulugeta, 2014].

PGPR action through directly and indirectly mechanism	1- Nitrogen Fixation
	2- Hormone Production
	3- Helps in Nodulation
	4- Nutrient Uptake
	5- Siderphores production bio control

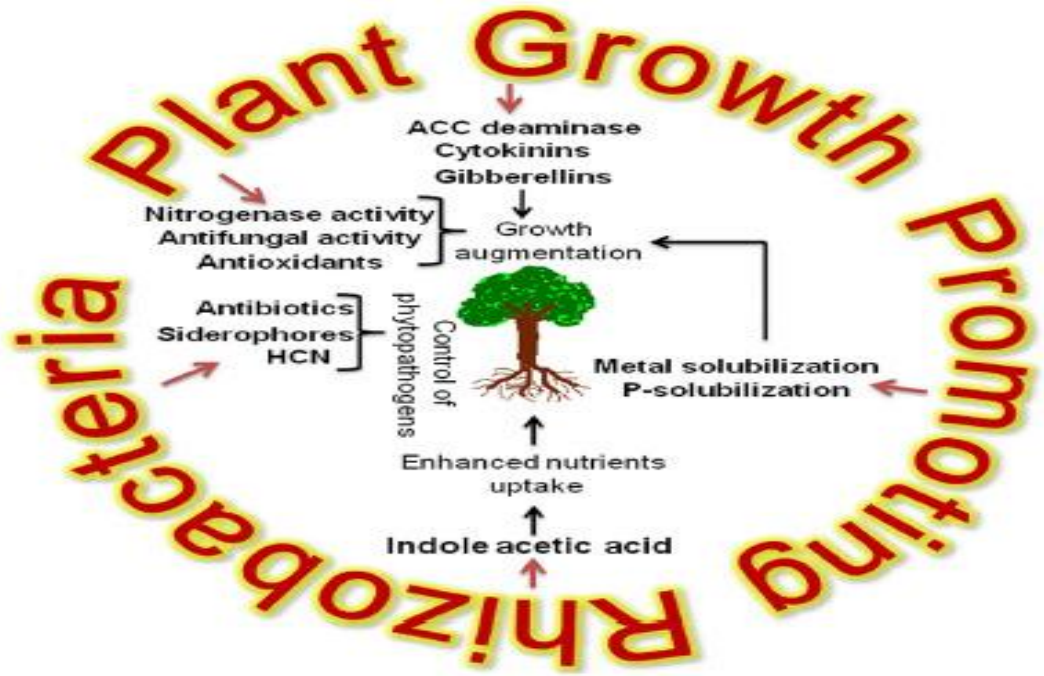
These microbes naturally forced plant growth support through direct and indirect mechanisms shown in (Table 2.5), and (Table 2.6) shows examples of some of these strains via its functions [Munees and Mulugeta , 2014 ; Wu,2009].

Table (2.5): Plant Growth Promoting Rhizobacteria strains [Munees and Mulugeta, 2014].

PGPR	Plant Growth Promoting Rhizobacteria Strains
Pseudomonas putida	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Pseudomonas aeruginosa	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Klebsiella sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Enterobacter asburiae	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Pseudomonas sp. A3R3	IAA, siderophores
Psychrobacter sp. SRS8	Heavy metal mobilization
Bradyrhizobium sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides
Pseudomonas aeruginosa 4EA	Siderophores
Bradyrhizobium sp. 750, *Pseudomonas* sp., *Ochrobactrum cytisi*	Heavy metal mobilization
Bacillus species PSB10	IAA, siderophores, HCN, ammonia
Paenibacillus polymyxa	IAA, siderophores
Rhizobium phaseoli	IAA
Stenotrophomonas Maltophilia	Nitrogenase activity, phosphate solubilization, IAA, ACC Deaminase

2.5.2 Mechanism of plant growth promotion by rhizobacteria

PGPR-mediated plant growth promotion occurs by the adjustment of the entire microbial community in rhizosphere position through the production of various substances (Kloepper and Schroth, 1981). In general, PGPR promote plant growth directly by either modulating plant hormone levels or facilitating resource gaining (nitrogen, phosphorus and essential minerals), or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Glick, 2012)(Fig.2.3)



Fig(2.3): Mechanism of plant growth promotion by rhizobacteria (Ahmed et al., 2013).

2.5.3. ACC deaminase And Ethylene

ACC deaminase (E.C. 3.5.99.7) is the enzyme of a pyridoxal phosphate that is able of cleaving ACC, the direct precursor of ethylene in all higher plants, to α-ketobutyrate and ammonia (Honma and Shimomura 1978), then lowering the ethylene levels in plant tissues (Glick et al. 1998, 2007a). consecutively, decreased ethylene levels help the plant to be more opposed to a wide variety of environmental stresses, and stimulate root growth, all of which stimulate the plant to increase its endogenous level of ethylene (Li et al. 2000; Glick et al. 2007b). Bacteria produced ACC deaminase have been promoted plant growth under different environmental stress comprise: - metal and organic contaminants, salt stress, water logging, heavy metals drought, petroleum exposure, Consequently, PGPR effect on plant appear in longer root length shoot length and are more resistant to growth inhibition by a variety of ethylene- inducing stresses. [Glick, 2004; Glick, 1995; Glick and Bashan, 1997; Glick and Penrose, 1998].

Ethylene is produced almost in all plants. It is important for normal growth and development in plants (McKeon and Yang 1987), and it is essential components for seed germination of many plants. But high levels of it can hinder plant growth. PGPR are proficient to slow down production of high concentration of ethylene through hydrolyzed ethylene precursor ACC [Glick, 1995].

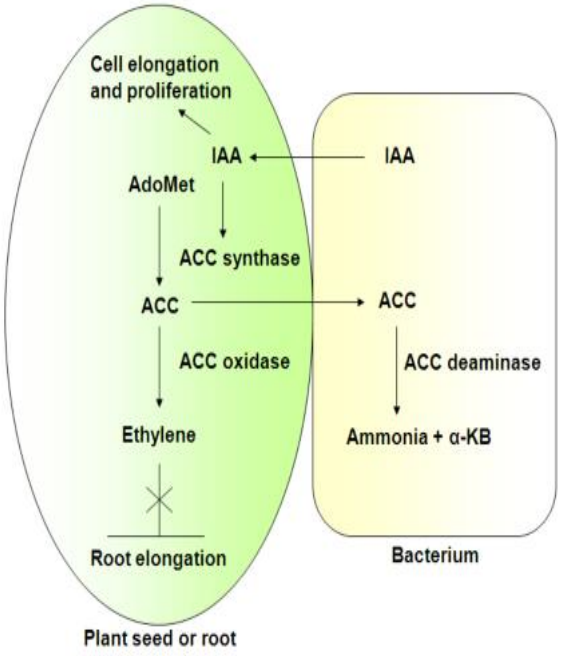
Some strains of PGP (e.g., Pseudomonas sp. strain ACP and 6G5 R.strains UW1, UW2 and, UW3of P. putrid)(Shah et al.1998)produced ACC deaminase defines as 1-aminocyclopropane-1-carboxylate (ACC)

deaminase. Under stress inside plant cell ACC synthase converts S-adenosyl methionine (AdoMet) into ACC which convert after that to ethylene by ACC oxidase, where high concentration of ethylene cause stress to plant and growth inhabitation, so extinction of PGPR on the rhizosphere of roots exuded ACC, and by the enzyme ACC deaminase its hydrolyzed to ammonia and α-ketobutyrate, this lead to take another pathway in the reaction result in decrease in amount of ethylene and thus mitigate ethylene–induced stress and prevent inhabitation of root elongation. [Glick, 2004; Munees and Mulugeta, 2014; Mac neill, 2011; Wu, 2009].

Indo-3-Acetic Acid (IAA) is secreted by some strains of PGPR such as pseudomonas putida UW3 and pseudomonas putida UW4, which consider as a regulator for plant growth and it goes in plant cells to encourage the growth of root and elongation, or it can. Also, it stimulates enzyme ACC synthase that catalyzes the formation of ACC, as a result, the concentration of ethylene depends on the balance of the IAA and ACC deaminase [Glick and Penrose, 1998; Munees and Mulugeta, 2014].

The decrease in levels of ethylene by ACC deaminase not only controls plant stress responses. Also mitigate ethylene-suppressed Auxin Responses Factor (ARF) synthesis lead to plant growth promotion resulted from both stress mitigation and growth stimulation [Glick, 2004]. The pathways are shown in (Figure 2.4).

However, ACC Synthesis is also simulated to produce more ACC and ethylene, with an increase in ARF synthesis. This suppresses ARF synthesis. In this way, ethylene confines its own production.



Figure(2. 4) : Schematic diagram of PGPR containing ACC deaminase lower the ethylene hormone, ACC [Shan, 2009].

2.6 Pulse Amplitude Modulated (PAM) Fluorometry

Pulse amplitude modulated (PAM) fluorometers can potentially be used for measurements of photosynthesis rates, It is a form of signal modulation where the importance information is programmed in the amplitude of a sequence of signal pulses (Wikipedia).

Chlorophyll fluorescence has long been used as a noninvasive means to evaluate photosynthetic execution in plants. Pulse-amplitude modulated (PAM) fluorometry is one of the most widespread techniques used to study the induction and quenching of chlorophyll fluorescence in physiological studies (Brooks MD, Niyogi KK,2011)

Chlorophyll (Chi) *a* fluorescence originates in close area to the sites where light energy is changed into chemically unchanging energy. The same

excitation states that give rise to fluorescence production also contribute in photochemical energy translation. These features make Chi fluorescence only one of its kind marker of photosynthesis (Ulrich.Schreiber,2004).

Chlorophyll inside a leaf exists as pigment–protein complexes in PSII, PSI, and inside the light-harvesting complexes (LHCs) connected with each of these reaction centers. Light energy absorbed by chlorophyll molecules can be (1) drive photosynthesis (photochemistry). (2) be re-emitted as heat

(3) be re-emitted as light (fluorescence).

These three processes do not exist in separation but quite in opposition with each other. therefore the yield of chlorophyll fluorescence emission gives us valuable information about the quantum effectiveness of photochemistry and heat indulgence. This is important for plant photosynthesis and eventually efficiency because photochemistry is used to supply energy and reducing power for CO2 absorption. At room temperature, we believe the variations in the fluorescence signal arise from PSII only and we disregard emission from PSI largely because the signal does not make an important involvement below 700 nm (Butler, 1978; Pfündel,1998; Baker, 2008).

2.6.1Principles of chlorophyll fluorescence analysis

The biochemical proceedings that occur inside the thylakoid membrane that are related for considerate chlorophyll fluorescence analysis, The condition of reduction and oxidation (redox) state of type electron carriers is significant when sympathetic the actions that guide to changes in chlorophyll fluorescence. and this is described in Fig. (2.5).

(A) a schematic figure:- viewing electron transport inside the PSII reaction centre complex. Energy engrossed by chlorophyll inside the light-harvesting complex can be dissolute via photochemistry, by heat (non-photochemical quenching), or as fluorescence. The competition between these processes allows us to determine the efficiency of PSII. (B) a distinctive fluorescence trace made on dark-adapted leaf material showing how *F* o and *F*m are produced. The measuring ray excites chlorophyll but is not of a sufficient intensity to induce electron transport through PSII (i.e. 'charge separation' when Pheo is reduced). This gives *F*o, the minimal level of fluorescence, and reaction centers are said to be open. A short saturating pulse of light results in the formation of the maximum' probable yield of fluorescence, *F*m. During this pulse reaction centers are efficiently closed. (C) a schematic figure explanation the move of energy and electrons inside PSII that result in open and closed centers and the formation of *F*o and *F*m states, correspondingly. The excited state P680* and following transfer of an electron to the primary acceptor QA gives increase to a closed centre. QA– cannot agree to another electron in anticipation of it has approved its electron onto the next electron acceptor, QB **(Murchie1, Lawson,**2013).

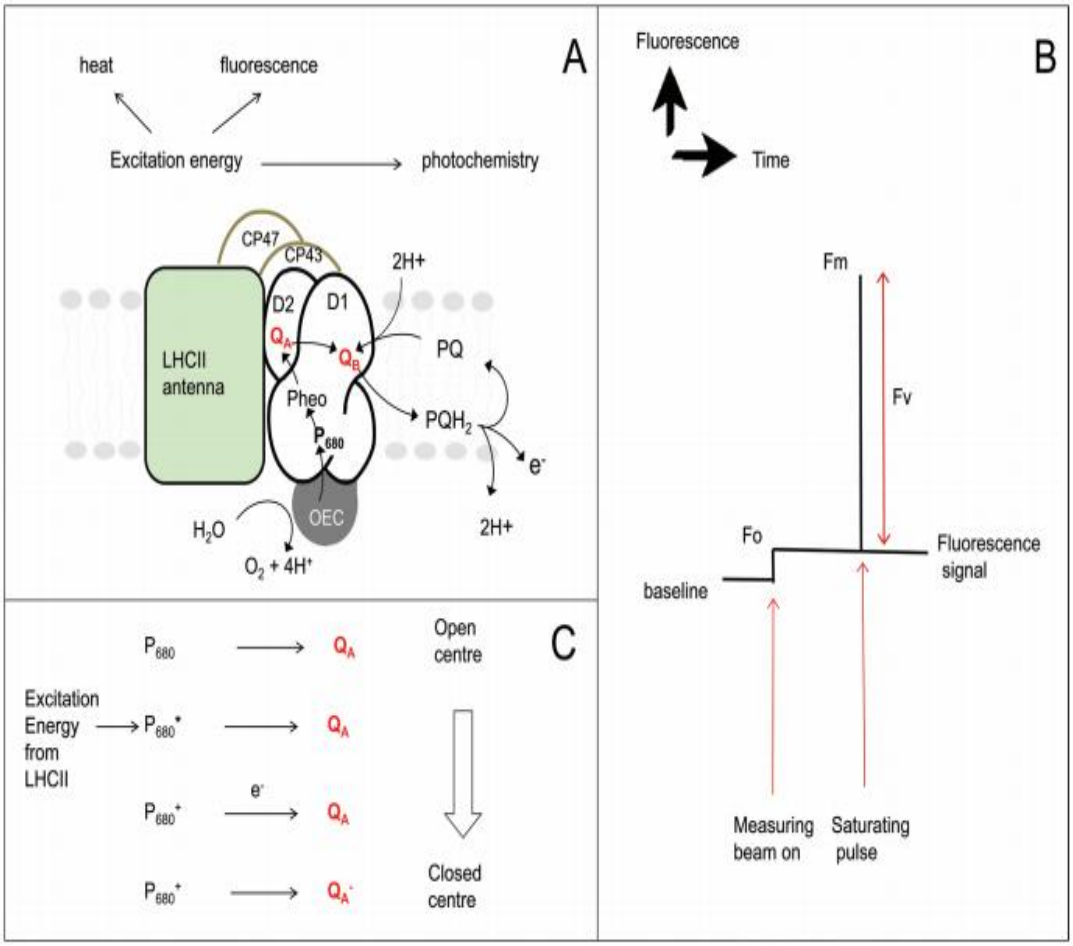


Figure (2.5):- A simplified depiction of events in PSII that lead to identification of key parameters in fluorescence analysis (Murchie1, Lawson,2013).

In this research, (PAM) fluorometry measured chlorophyll a fluorescence. Recoding information from instrument indicates functionally of PSII as flow of electron, rate of photosynthesis by emitted light from the pulse, and measured light. Taken heat corruption is comparatively stable during measurements. The following charts indicate several chlorophyll fluorescence parameters, as: Fv/Fm, yield, Qp, Qn, as shown in Figure (2.6) [Mac Neil, 2011].

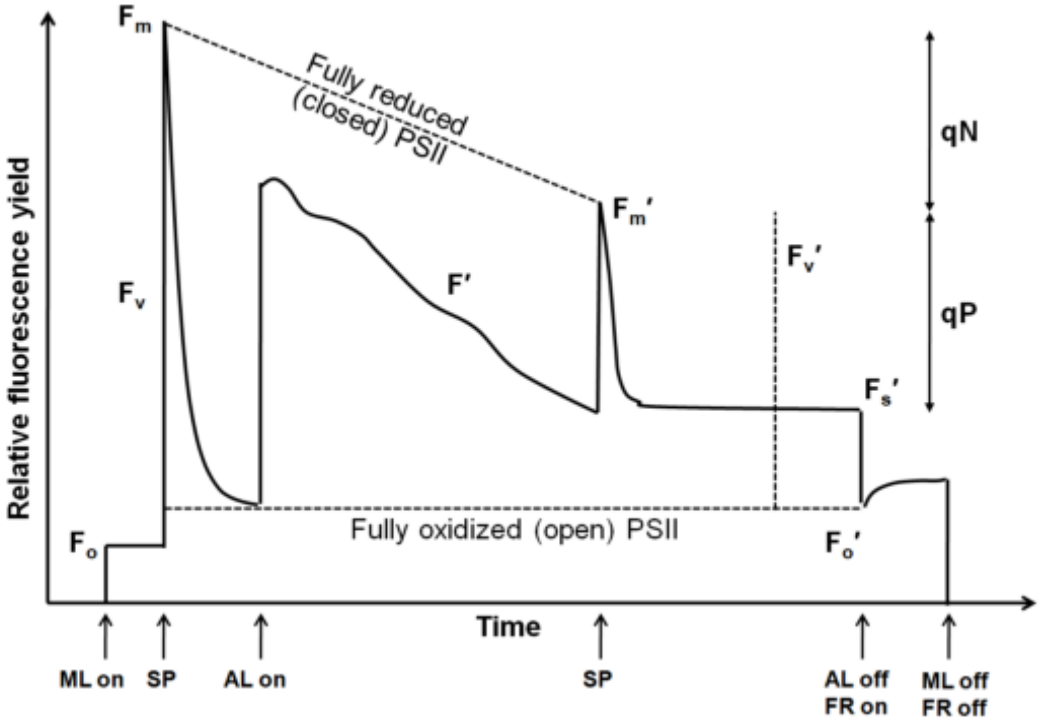


Figure (2.6): Nomenclature of PAM fluorescence parameters for dark-adapted leaf [Mac Neil, 2011].

These parameters used to evaluate the efficient of photochemistry in plants. Beside in this study they are as indication effect of olive mill waste water on photosynthetic electron transports.

Each term abbreviated for the following:

ML term: refer for modulated measuring light.

SP term: saturating pulse.

AL term: for incident light.

FR term for: far-red light.

Fv term: is the variable difference fluorescence between Fm and F0.

Fm term: is the maximal fluorescence of dark –adapted tissue.

Fm term: is the maximal fluorescence of light –adapted tissue.

Fo term: is the minimal fluorescence.

Fs term: is s the stead –state fluorescence.

Yield parameter equal to:

Yield = Fv / Fm ..……Equation 4

Fv =Fm-Fo……..……Equation 5

It represent maximum quantum yield of PSII center when it's open.

Y is another calculation of yield at steady state photosynthesis and represented by:

Y = [Fm –Fs / Fm]……..……..Equation 7

Optimal values for yield ranges between 0.5 to 0.75, lowered value indicates that plant is stressed.

q_p term is photochemical quenching represented as

q_p =[(Fm' –Fs) /(Fm'-Fo)] ……..……Equation 8

q_n term is non-photochemical quenching of fluorescence which is represented by

[1- (Fm'-Fo) / (Fm- Fo)]……..……Equation 9

Value of qp indicate PSII reaction center that are open and equal the approximate oxidation of PSII, while qn parameter related to the dissipation of energy as heat and photo inhabitation [Shan,2009].

Chapter Three

Material and Methods

3.1 Selecting and culturing PGPR

Plant growth promoting Rhizobacteria (PGPR): (Pseudomonas putida) UW3 giving number130-A (Glick et al.1995). and (Pseudomonas putida) UW4 giving number131 (referred as c) (Glick et al.1995) (Pseudomonas putida) unassigned (referred as B),. These strains were used in coating seeds separately, or in combination. These two bacterial strains: *Pseudomonas putida*, UW3 and UW4; had been chosen and brought from Professor Glick lab; at Waterloo University; in Canada, were grown in Troptic Soy Growth (TSB) media. The three strains were grown in Troptic Soy Broth (TSB) media in the laboratories of An-Najah National University. For preparing (TSB) 9g added to 300ml distilled water after sterilization, Divided equally 300ml of the solution in 6 Erlenmeyer flask(250ml) each two Erlenmeyer flask give letter (A,A.B,B.C,C). After autoclaving, 100 µg/ml Ampicillin antibiotic (AMP) was added to the media in the two Erlenmeyer flasks have(UW4) C,. Then 500 µl selected bacterial strains were cultured in 50ml of sterile Troptic Soy Broth (TSB)medium contained in a250ml Erlenmeyer flask and grown at 30 ºC, on a rotary shaker at 80 rpm for 24-36 hours.

3.2 Seed treatment with PGPR

Seed treatment with PGPR following the published methods of Greenberg et al. 2008 and Greenberg et al 2007:

The bacterial culture grown in TSB was transferred aseptically into a sterile 50 ml Flacon tube, centrifuged at 2000 rpm for 20 minutes. The supernatant was discarded, and the cell pellets were washed resuspended with 50 ml of 0.1% (w/v) sodium pyrophosphate to take away secondary metabolites and centrifuged once more at 2000 rpm for 20 minutes. The final bacterial pellet was resuspended in sterile ddH2O (de- ionized and distilled water) to an absorbance of (1.5 for bacteria A and B) and (2.0 for bacteria C) at 600 nm, the optical density was measured using a spectrophotometer.

Methylcellulose polymer (Sigma Aldrich, Oakville, ON), which is used to facilitate adhesion of the bacterial cells to the seed surface. It was prepared at 1.5% w/v by assimilation on a stir plate for 1 hour waiting the entire clumps had broken separately, and then the solution autoclaved for 131 ºC and for 30 psi for 40 minutes, after which a gelatinous solid formed. Upon cooling, the gel liquefied (reverse gelatinization) into a slurry form.

A commercial non-toxic blue colorant (color coat blue, Becker Underwood, Saskatchewan), was added to the bacterial-polymer slurry at a ratio (1.75%). The existence of colorant was essential to get together safety and regulations requiring all treated seeds to be obviously colored to avoid use for animal consumption. The polymer was added to the bacterial suspension at a rate of (1:5) of bacterial suspension, forming "blue bacterial-polymer slurry".

An aliquot of the blue bacterial-polymer slurry were applied to equivalent of barley seeds volumes at a rate of (1:10), and for clover seeds at rate (1:5) using a seed theater or closed bag / Petri dish with vigorous shaking for one minute (seeds were obtained from National Agriculture Research Center

(NARC) of Ministry of Agriculture, Jenin.). Then the seeds were left to dry at room temperature (for one hour). After that, the seeds placed immediately into sealed plastic bags and stored at 4 ºC for a maximum of two weeks prior to usage.

3.3 Seed germination test

Seeds treated by PGPR placed on sterile cotton in sterile Petri dishes, the seeds of barely 10 seeds per Petri dish, and for clover, the seeds number around 20 seeds per Petri dish. The solution was olive mill waste water in dH2O for treatments, in different concentration (0%, 25%,50%,75%). Seeds were watered once daily with 50mL of solution per Petri dish for one week. Each Petri dish contained **(**untreated seeds, seeds treated by bacteria (A,B,C)**)** as it is in table (3.1). Petri- plates were placed in incubator at room temperature with artificial light. After one week the percentage of germination was calculated.

Table (3.1): Chart for seed germination test in vitro for barely and clover seeds.

Type of bacteria	**Concentration**			
Zero bacteria (control)	**0%**	**25%**	**75%**	**50%**
Bacteria A(UW3)	**0%**	**25%**	**75%**	**50%**
Bacteria B(unassigned).	**0%**	**25%**	**75%**	**50%**
Bacteria C.(UW4)	**0%**	**25%**	**75%**	**50%**

3.4 Soil Preparation

Soil samples were collected and filled in bags and autoclaved to ensure removal of any bacteria and fungi suspensions. Then, allowed to dry to remove moisture. Soil samples were filled in plastic pots of 17* 16*15 cm (length*width* height), 200-300 gram of soil was filled in the plastic pots which have holes at the bottoms to enable water drainage from soil samples. all pots were contained in a tray(with holes) for leaching of solution from the soil . The soil aggregation from the top 0-20 cm layer and mixed to ensure homogeneity, 48 pots were filled with soil for planting barley seeds, also other 48 pots were filled with soil for planting clover seeds. All the pots were labeled and placed in greenhouse conditions were artificial light for 12 hours, day time temperature ranged from 25-35 ºC, and the night time temperature ranged from 18-27 ºC.

3.6 planting

The soil which in the pots was soaked in water before planting the seeds. Then, barley and clover seeds were cultivated in those pots and seeded in a similar way like seeding in fields(approximately one seed per cm).15 seeds were planted in each pot and covered with a thin layer of soil. The pots were distributed into 4 groups, and each group consist of seeds (zero bacteria, bacteria A, bacteria B, bacteria C). The first group were watered by fresh water as a control group. The second group were watered by a diluted sample of OMWW with 50% concentration to knowing if the acidity is very high as to avoid killing the barley. The third group were watered by a diluted

sample of OMWW with 25% concentration. The fourth group were watered by a diluted sample of OMWW with 10% concentration.

For seven days after cultivation each group were irrigated by fresh water until seed germination, then it was irrigated by100 ml of solution with different concentration of zibar (0%.10%,25%,50%) for each group, this process was repeated every day for one month, under natural sunlight and green houselights, with temperature ranging from 25-35 ºC during the day and 20-27 ºC during the night. Germination of plants was monitored daily. Every week, the length of the shoot was measured, and the number of leaves was taken for all plants on each replicate.

During growth stages plants had been photographed and the shoot length was measured before it reached crop coefficient (Kc) end cycle of its life. After 30 days all plants were taken from pots and subjected to tests.

All pots were planted in early February in 2016; and maintained in a greenhouse in An- Najah National university in Tulkarem city. All pots were placed inside in 3 rows and distributed randomly and labeled with 4 colors represented different concentration to make it easy for irrigation.

Table (3.2):- Trials Schemes.

Plant/ Trials	NO.	**Control trials**	NO.	**Seeds pots germinated withUW3 (A)**	NO.	**Seeds pots germinated withUW4 (C)**	NO.	**Seeds pots germinated with Unassigned (B)**
Barely	1	**Irrigation with** -Fresh water	2	**Irrigation with** : -Fresh water	3	**Irrigation with** : -Fresh water	4	**Irrigation with** : -Fresh water
	5	-10 ml/L of olive mill waste water solution	6	-10 ml/L of olive mill waste water solution	7	-10 ml/L of olive mill waste water solution	8	-10 ml/L of olive mill waste water solution
	9	-25 mم/L of olive mill waste water solution	10	-25 mم/L of olive mill waste water solution	11	-25 mم/L of olive mill waste water solution	12	-25 ml/L of olive mill waste water solution
	13	-50 mم/L of olive mill waste water solution	14	-50 mم/L of olive mill waste water solution	15	-50 mم/L of olive mill waste water solution	16	-50 ml/L of olive mill waste water solution
Clover	1	**Irrigation with** -Fresh water	2	**Irrigation with** : -Fresh water	3	**Irrigation with** : -Fresh water	4	**Irrigation with** : -Fresh water
	5	-10 ml/L of olive mill waste water solution	6	-10 ml/L of olive mill waste water solution	7	-10 ml/L of olive mill waste water solution	8	-10 ml/L of olive mill waste water solution
	9	-25 ml/L of olive mill waste water solution	10	-25 ml/L of olive mill waste water solution	11	-25 ml/L of olive mill waste water solution	12	-25 ml/L of olive mill waste water solution
	13	-50 ml/L of olive mill waste water solution	14	-50 ml/L of olive mill waste water solution	15	-50 ml/L of olive mill waste water solution	16	-50 ml/L of olive mill waste water solution

Table(3.3):- Distribution trial schemes in greenhouse(for each of barely and clover).

1	10	19	28	9	41	17	35	26	14	5	48	32	23	39	43
4	13	22	31	37	44	27	2	36	18	30	12	21	8	45	40
7	16	25	34	11	46	20	38	6	33	24	29	15	3	42	47

3.6 Plant Analysis

Shoot length and number of leaves for each plant were measured after 30 days of planting. Then, the plants were extracted from pots. After that, the roots of plants were washed with fresh water to remove the soil, then it was dried by tissue to remove the water and any residue, and the length of each root was measured. The wet weight of each plant was measured, then the roots and shoots of plants were cut and each of them was weighted separately. One day later after drying the plants in an oven in temperature 105 ºC, the roots and shoots were re-weighted to get the dry weight. Then statistical analysis(SAS) were done. The dry weight was compared with the wet weight to assess the photosynthesis and to compare the plant response to different concentrations of olive mill waste water.

3.7 Measurement of Photosynthesis with (PAM) Fluorometry

Barley and clover Plant trials were measured for their photosynthesis actions using pulse amplitude modulated fluorometry (Fytoscope with Fluorescence imaging FS-FI-2200). Chlorophyll fluorescence imaging allows multiple plants to be monitored at the same time under identical conditions, Chlorophyll fluorescence in a straight line relates to the rate of energy stream by means of the electron transport chain and consequently any

perturbation that impacts on plant metabolism will impact on fluorescence parameters yet if not directly connected to photosynthesis (Barbagallo *et al.*, 2003). Super saturating light intensity = 4500uE providing an ideal screening platform Samples were dark adapted for 1;20 minutes, at Temperature = 22C by turning off all lambs in the device before pulse amplitude modulated analysis were carried out to make sure the PSII centers were open. Analyses were done for all plants at each trial with no other light interference to ensure only fluorescence light were measured. The **actinic light off** and with the **auto mode off**, Then, switch on the actinic light (Actinic light intensity = 800uE) and follow the changes in the brightness of the leaf image on the computer screen. The fluorescence signal will rise during the first second and decline thereafter.

A. Measurement of Fv/Fm

The leaf material was adapted in dark condition for a minimum of 10 min. The leaf must be kept darkened continuously for the whole process of measuring *F*v/*F*m. The fluorescence detector was applied with the measuring beam off and ensure the reading is zero. Switch on the measuring beam. make sure for quenching induced by the measuring beam and fine-tune the measuring beam intensity consequently. Usually, this is only necessary once for a given set of plants/plant material. Then Measure *F*o. Apply saturating pulse, typically 0.8s at an intensity of at least 4000μmolm–2s–1. Achieve *F*m value.

B. To measure F q′/Fm′ (ϕPSII) and NPQ at a known light intensity. If following on from (A) above, apply the actinic light immediately after measurement of *F*v/*Fm*. From the dark-adapted state, this will usually take a minimum of several minutes and should be monitored using the *F′*. From a

non-dark adapted state using ambient light, it is sensible to wait until the *F′* signal is stable. Then the saturating pulse is applied. For NPQ it is necessary to start with a dark-adapted material (a measurement of *Fo′* is not required). Following the steps in (A) and (B) above, NPQ is calculated as in Table (3:4).

C.To measure Fq′/Fv′(qP)

As in (B), but a measurement or calculation of *Fo′* is essential. Following the saturating pulse, the actinic light is switched off and *F* o′ measured after few seconds. correctness is enhanced with the use of an FR light source to oxidize PSI, QA, and electron transport intermediates. Then all resulted parameters (Fv/Fm, yield, qPN) were measured and marked on graphs.

Table (3:4): List of commonly used abbreviations and equation.

Parameter	Also known as	Formula (see Fig. 2)	Description
F_v/F_m		$(F_m–F_o)/F_m$	Maximum quantum efficiency of PSII photochemistry.
F_v'/F_m'		$(F_m'–F_o')/F_m'$	Maximum efficiency of PSII photochemistry in the light, if all centres were open.
F_q'/F_m'	ϕPSII, $\Delta F/F_m'$	$(F_m'–F')/F_m'$	PSII operating efficiency: the quantum efficiency of PSII electron transport in the light.
F_q'/F_v'	qP	$(F_m'–F')/(F_m'–F_o')$	Photochemical quenching: relates PSII maximum efficiency to operating efficiency. Non-linearly related to proportion of PSII centres that are open. See qL.
NPQ		$(F_m–F_m')/F_m'$	Non-photochemical quenching: estimates the rate constant for heat loss from PSII.
qL		$(F_q'/F_v')/(F_o'/F')$	Estimates the fraction of open PSII centres.

Note that this is include to identify only the most common parameters, and we refer the reader to comprehensive reviews of Baker (2008) and Maxwell and Johnson (2000).

Chapter Four

Results

4.1 Seed germination test

The Barley and clover" PGPRs treated seeds were germinated in plates that irrigated with a diluted OMWW (Zebar 75%), a diluted OMWW (Zebar 50%), a diluted OMWW (Zebar 25%), and fresh water (FW) as a control. The germination test had been recorded after eight days of planting (Table 4.1).

Table (4.1):- Percentage of seed germination for barley and clover seeds treated with PGPR after watering by different concentration of olive mill waste water solution.

	OMWW concentration							
Treatment	**0%**	**25%**	**50%**	**75%**	**0%**	**25%**	**50%**	**75%**
	Clover				**Barley**			
Without bacteria (control)	**97.57**	**66.67**	**8.54**	**4.41**	**96.30**	**39.63**	**0**	**0**
Bacteria A(UW3)	**100**	**61.54**	**50**	**41.67**	**22.23**	**0**	**0**	**0**
Bacteria B (un assigned)	**96**	**70**	**55.56**	**41.18**	**60**	**0**	**0**	**0**
Bacteria C (UW4)	**94.74**	**84.22**	**41.67**	**29.17**	**44.45**	**0**	**0**	**0**
Pearson Chi square	**244.893**				**529.745**			
P-value	**.000**				**.000**			

The results revealed significant differences among the different levels of zibar for the different measured variables (P= 0).For each barely and clover plants.

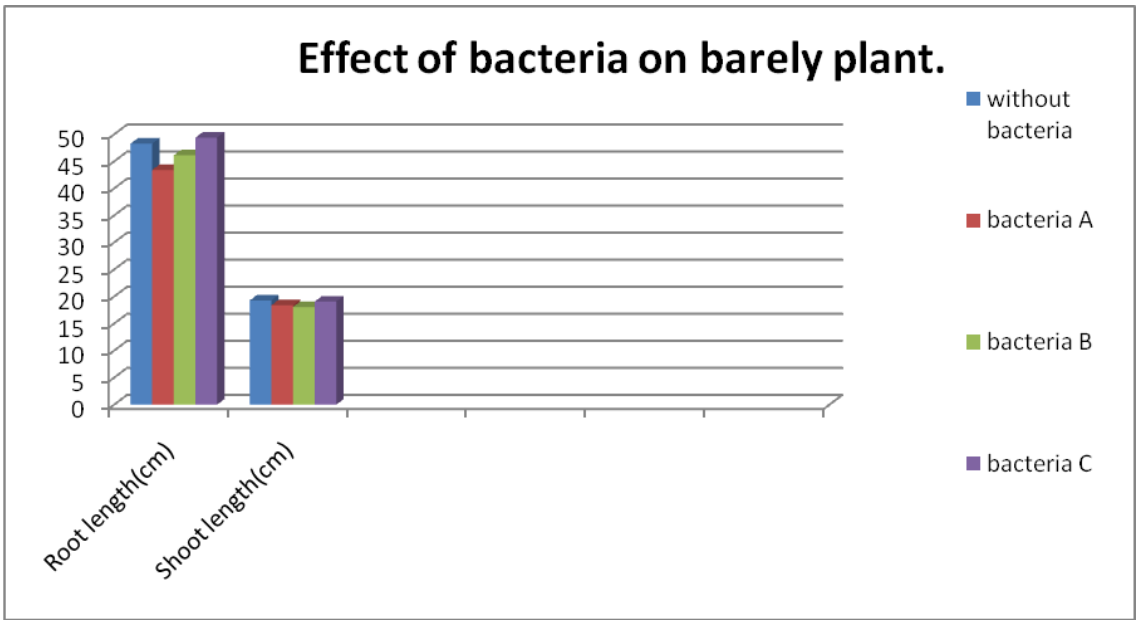
Seeds germination was decreased significantly with increasing the OMWW levels, the higher number of germinated seeds was observed with the control and 25% OMWW, respectively, however, the lowest was obtained when 50%and 75% OMWW was used. As regards of clover, germinated seeds is higher than germinated seeds of barely in a different level of OMWW, in addition to, the clover seeds treated with bacteria shows high seeds germination at (25,50,75%) of OMWW, In comparison to the clover seeds not treated with bacteria at (25,50,75%) of OMWW. (Table4. 1)

4.2 Effects of bacteria on barely plant

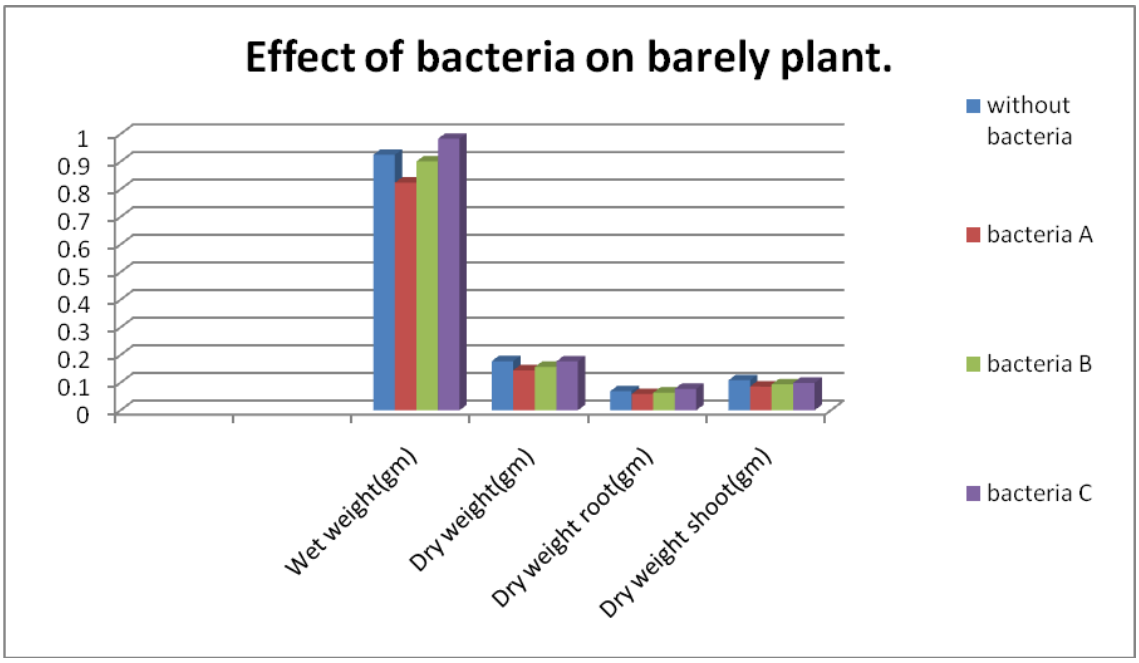
The results revealed no significant differences among the different types of bacteria for the different measured variables (P> 0.05) (Table.4: 2)

Table (4.2) :- Effect of bacteria on barely plant that was irrigated with water.

Bacteria	Root length(cm)	Shoot length(cm)	Wet weight(gm)	Dry weight(gm)	Dry weight root(gm)	Dry weight shoot(gm)
1 Without bacteria	48.264	19.256	0.925	0.178	0.069	0.109
2 With bacteria(A)	43.394	18.371	0.824	0.145	0.059	0.086
3 With bacteria(B)	46.112	18.062	0.901	0.157	0.064	0.094
4 With bacteria(C)	49.396	19.064	0.983	0.177	0.078	0.099
P-value	*0.635*	*0.529*	*0.849*	*0.704*	*0.667*	*0.744*



Figure(4.1):- Effect of bacteria on root and shoot length of barely plant.

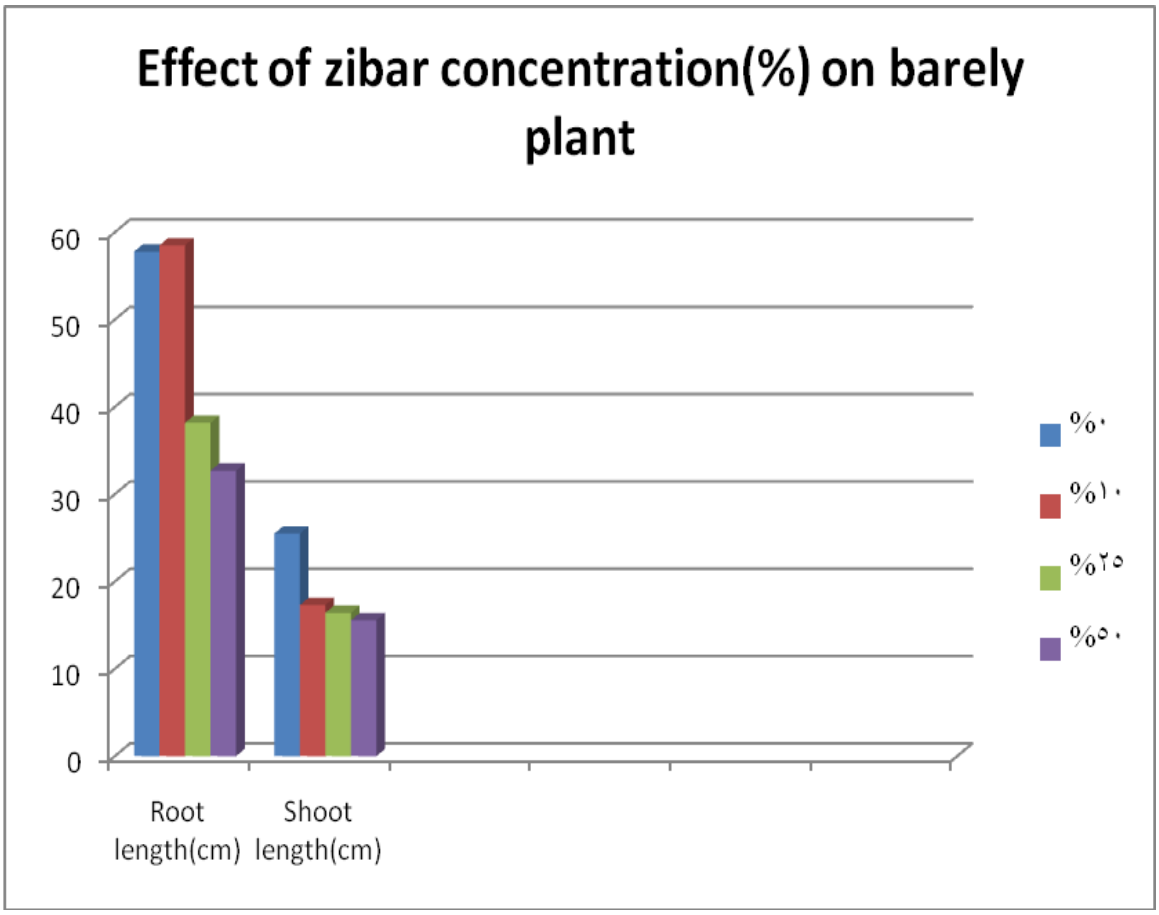


Figure(4.2):- Effect of bacteria on wet and dry weight and wet and dry weight for root and shoot of barely plant.

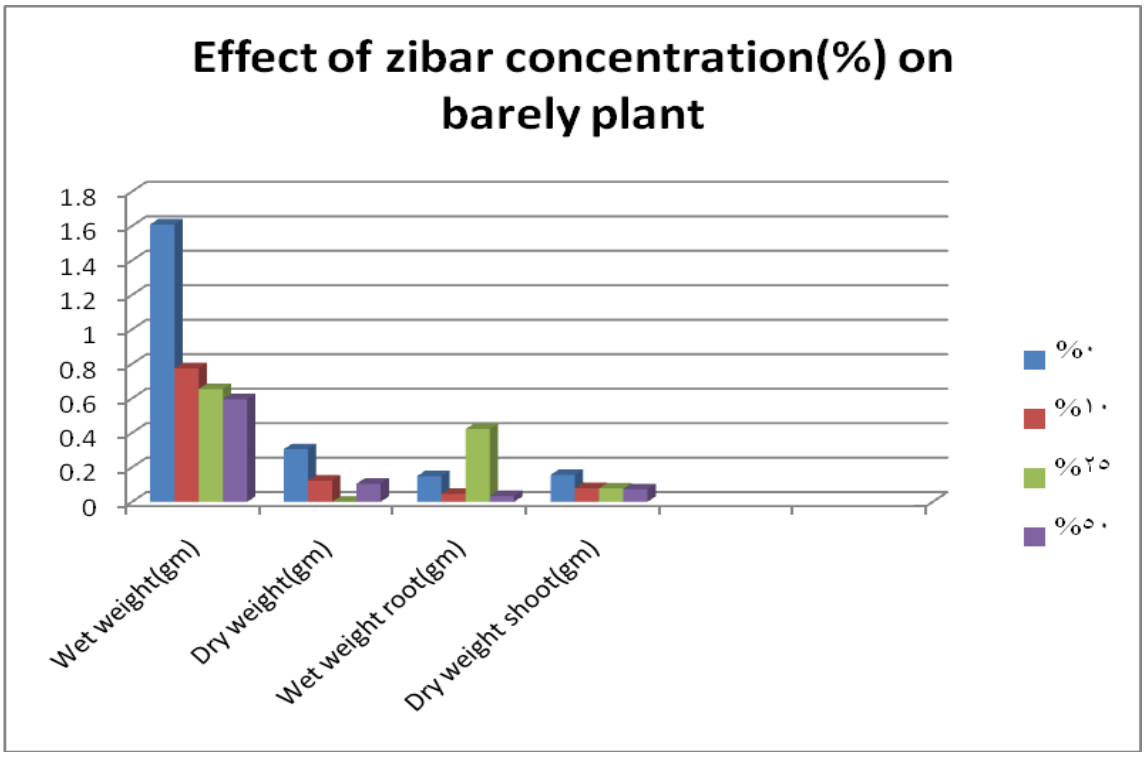
4.3 Effect of zibar concentration (%) on barely plant

Table.4.3:- Effect of zibar concentration(%) on barely plant

Zibar concentration(%)	Root length(cm)	Shoot length(cm)	Wet weight(gm)	Dry weight(gm)	Wet weight root(gm)	Dry weight shoot(gm)
0%	57.776a	25.489a	1.609a	0.305a	0.149a	0.156a
10%	58.514a	17.304b	0.774b	0.123b	0.046b	0.079b
25%	38.181b	16.413b	0.654b	.0.122b	0.422b	0.078b
50%	32.695b	15.547b	0.595b	0.105b	0.033b	0.073b
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001



Figure(4.3):- Effect of zibar concentration(%) on root and shoot length of barely plant.



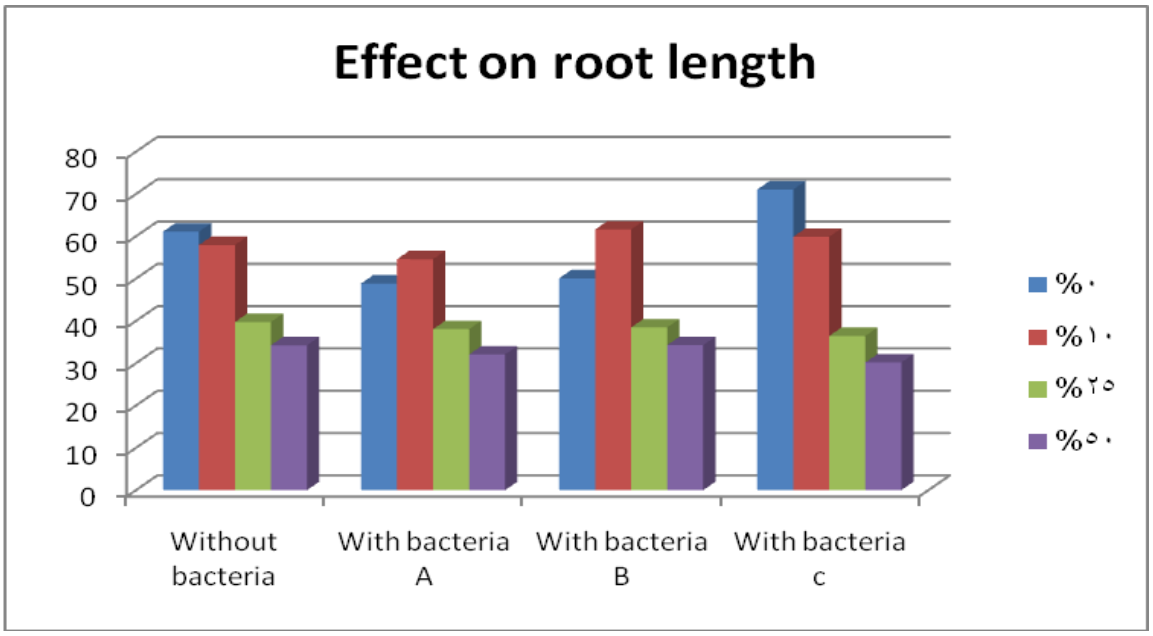
Figure(4.4):- Effect of zibar concentration(%) on wet and dry weight and wet and dry weight for root and shoot of barely plant.

The results revealed significant differences among the different levels of zibar for the different measured variables (P= 0.0001). Root length was decreased significantly with increasing the OMWW levels, the higher root length was observed with the control and 10% OMWW (57.8 and 58.5cm), respectively, however, the lowest was obtained when 50% OMWW was used. Regarding shoot length, OMWW application significantly reduced the shoot length, the higher stem length was obtained without OMWW application (25.5 cm), when OMWW was used at 50%, lower stem length was observed (15.5 cm). A Similar trend was observed with another measured variable (both fresh and dry weight of the plant, OMWW application highly reduced both fresh and dry weight of both stem and root, the higher it was obtained without OMWW application (Table 4:3).

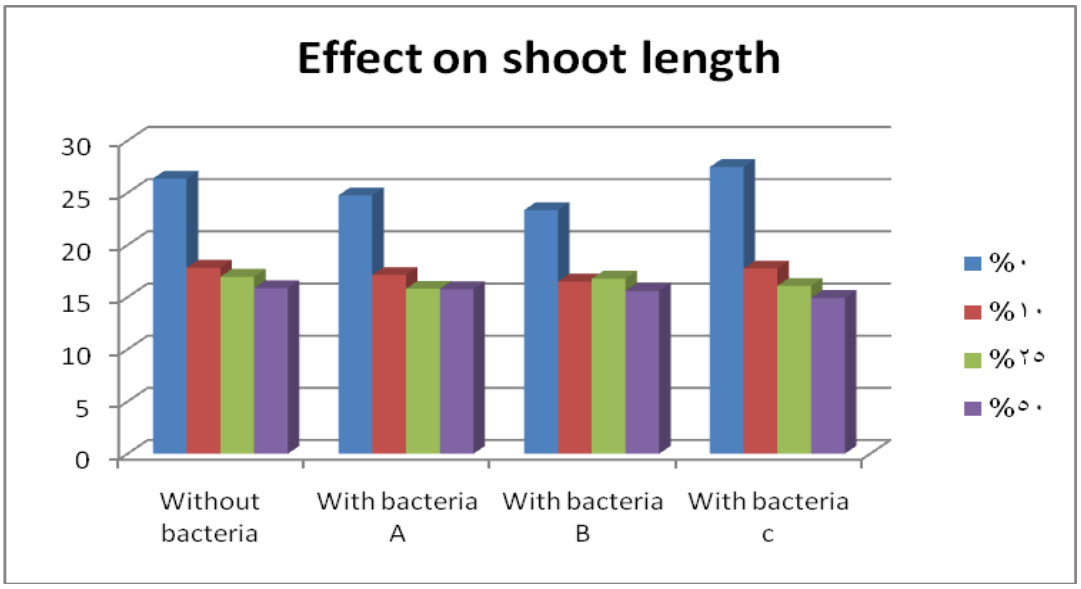
4.4 Effect of both bacteria and OMWW concentration(%) on barely plant growth.

Table(4.4):- Effect both of bacteria and OMWW concentration(%) on barely plant

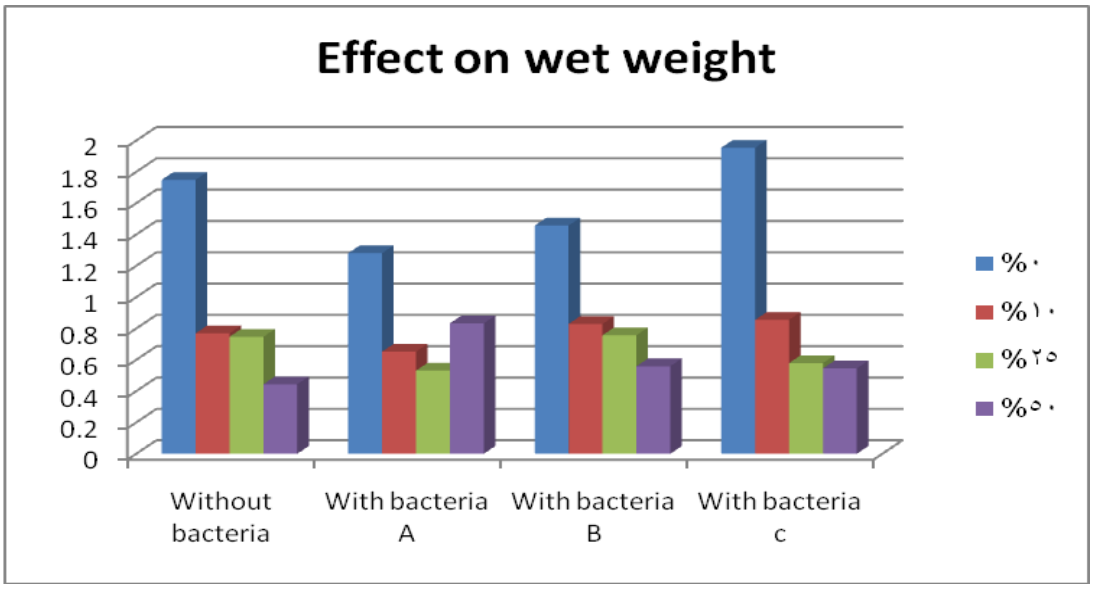
Bacteria / OMWW concentration	Barely plant	1 Without bacteria	2 With bacteria A	3 With bacteria B	4 With bacteria c	p-VALUE
0%	Root length(cm)	61.102	48.852	50.089	71.062	0.739
10%		57.951	54.603	61.613	59.893	0.739
25%		39.743	38.056	38.467	36.458	0.739
50%		34.259	32.069	34.282	30.169	0.739
0%	Shoot length(cm)	26.368	24.765	23.349	27.479	0.816
10%		17.819	17.137	16.504	17.754	0.816
25%		16.962	15.817	16.784	16.088	0.816
50%		15.874	15.766	15.612	14.936	0.816
0%	Wet weight(gm)	1.749	1.281	1.457	1.954	0.769
10%		0.769	0.652	0.829	0.855	0.769
25%		0.747	0.531	0.757	0.579	0.769
50%		0.444	0.833	0.559	0.545	0.769
0%	Dry weight (gm)	0.347	0.246	0.269	0.359	0.954
10%		0.127	0.109	0.124	0.133	0.954
25%		0.125	0.123	0.125	0.116	0.954
50%		0.115	0.099	0.108	0.097	0.954
0%	Dry weight root (gm)	0.149	0.124	0.123	0.201	0.633
10%		0.044	0.038	0.053	0.049	0.63
25%		0.048	0.043	0.046	0.033	0.633
50%		0.039	0.032	0.032	0.028	0.633
0%	Dry weight shoot (gm)	0.197	0.122	0.147	0.159	0.981
10%		0.084	0.072	0.071	0.084	0.981
25%		0.078	0.079	0.079	0.0829	0.981
50%		0.076	0.069	0.076	0.069	0.981



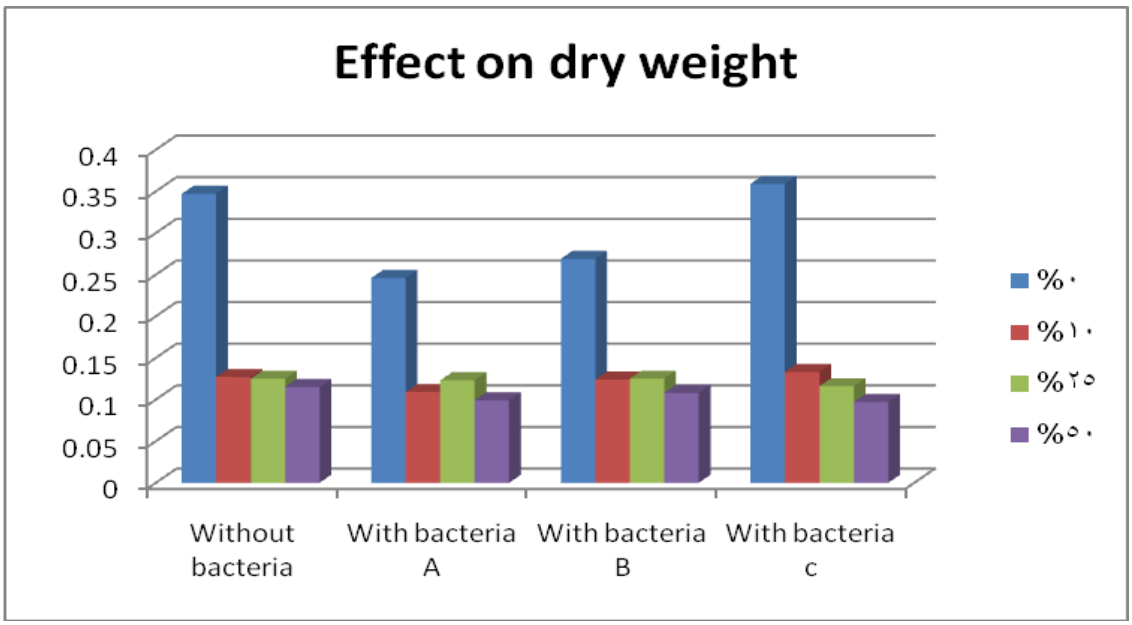
Figure(4.5):- Effect of bacteria and OMWW concentration(%) on root length of barely plant.



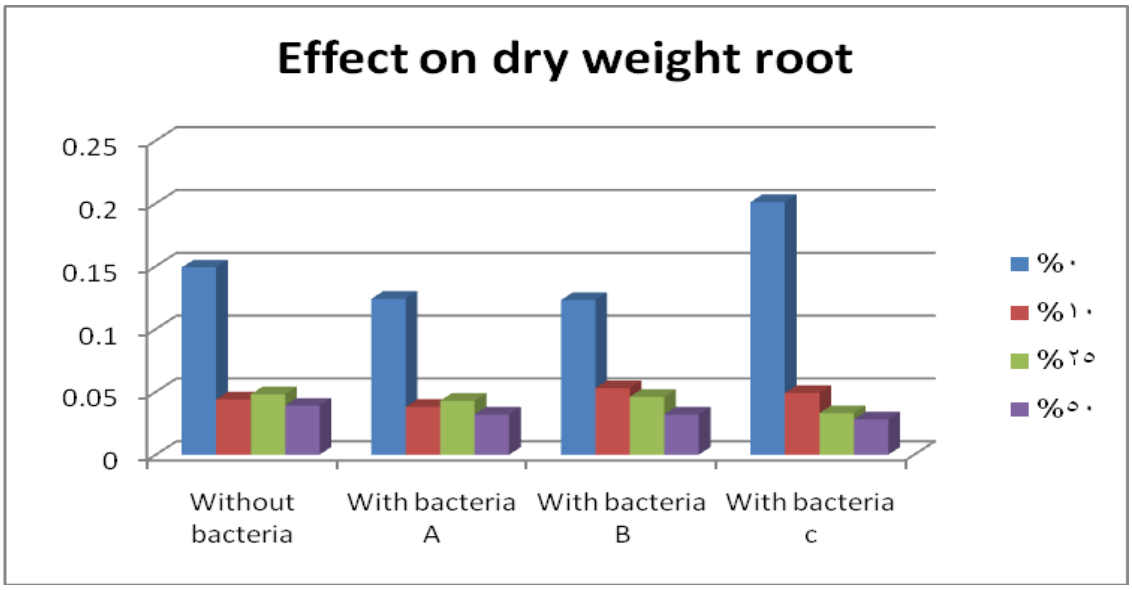
Figure(4.6):- Effect of bacteria and OMWW concentration(%) on shoot length of barely plant.



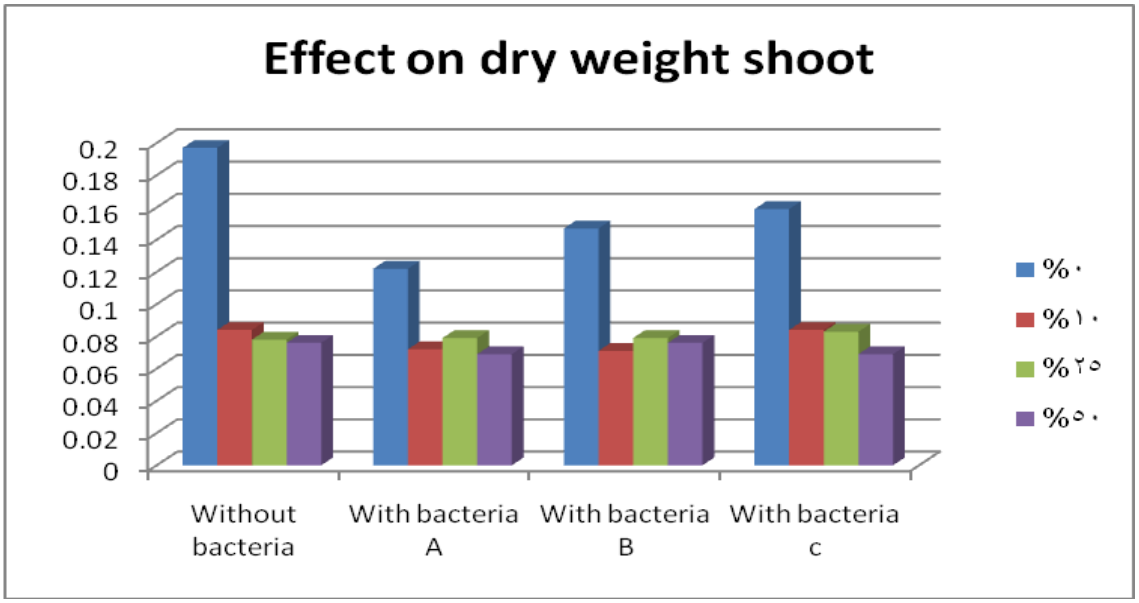
Figure(4.7):- Effect of bacteria and OMWW concentration(%) on wet weight of barely plant.



Figure(4.8):- Effect of bacteria and OMWW concentration(%) on dry weight of barely plant.



Figure(4.9):- Effect of bacteria and OMWW concentration(%) on dry weight root of barely plant



Figure(4.10):- Effect of bacteria and OMWW concentration(%) on dry weight shoot of barely plant.

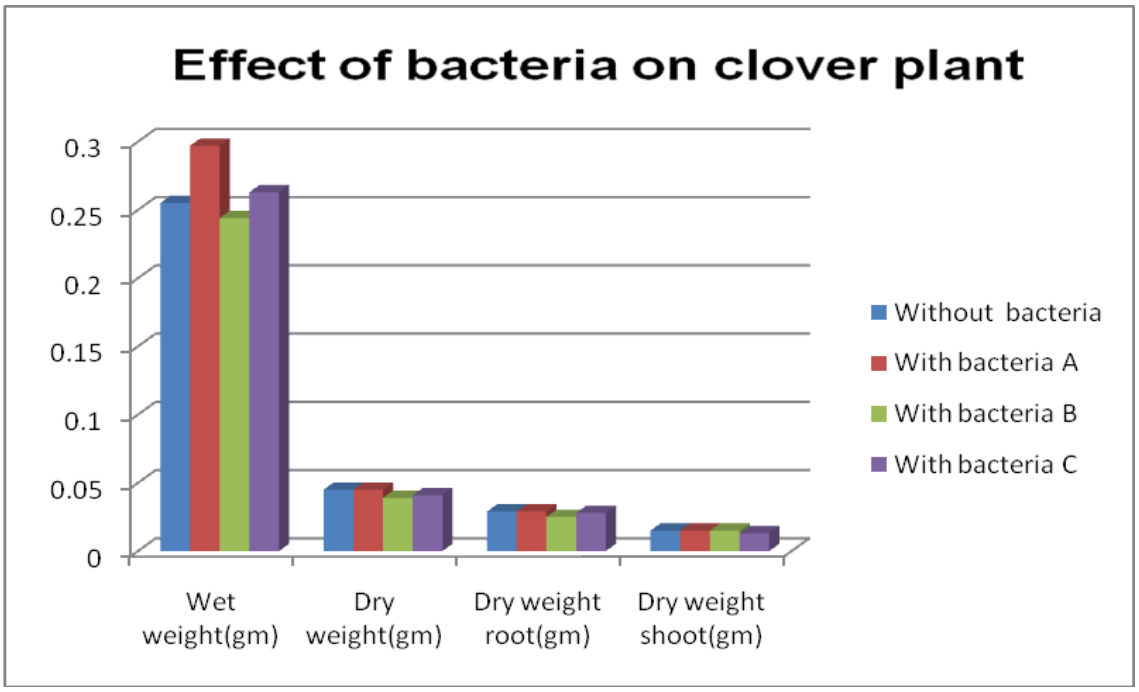
The results show no significant differences among the different levels of OMWW and the different types of bacteria for the different measured variables ($p > .05$). Root length was decreased significantly with the

increasing of the OMWW levels, the higher root length was observed with the control and 10% OMWW at different type of bacteria, respectively, however, the lowest was obtained when 50% OMWW was used. Regarding shoot length, OMWW application significantly reduced the shoot length, the higher stem length was obtained without OMWW application, when OMWW was used at 50%, lower stem length was observed. Similar inclination was observed with other measured variable (both fresh and dry weight of the plant, OMWW application highly reduced both fresh and dry weight of both stem and root, the higher wt was obtained without OMWW application (Table 4:4)

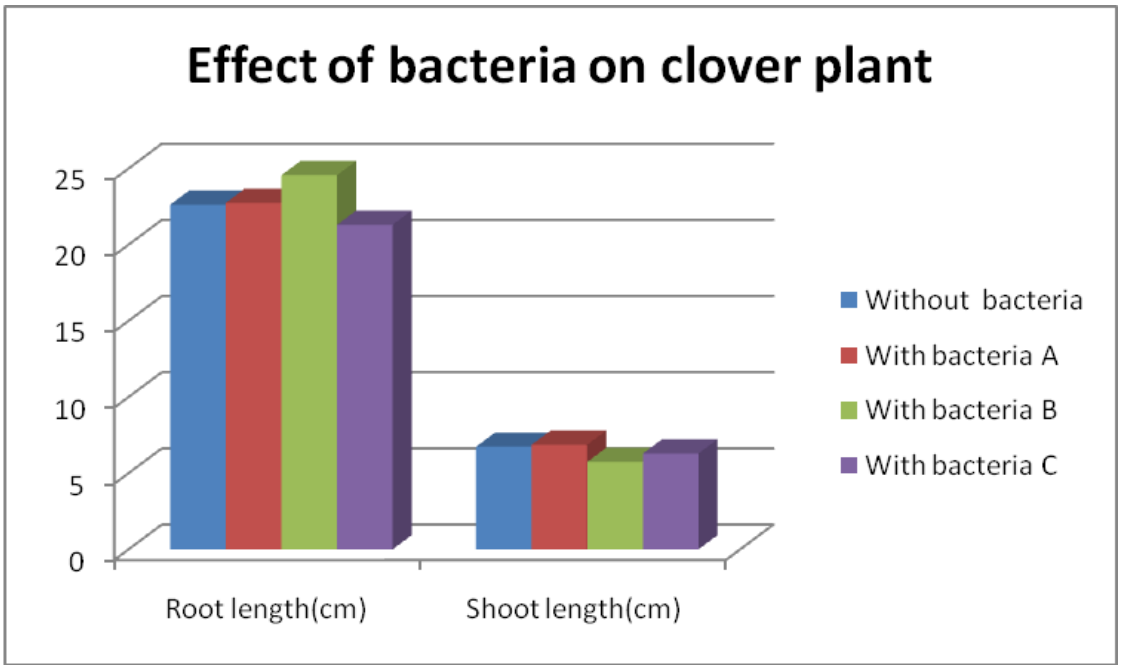
4.5 Effect of bacteria on clover plant.

Table(4.5):- Effect of bacteria on clover plant that was irrigated with water.

Bacteria	Root length(cm)	Shoot length(cm)	Wet weight(gm)	Dry weight(gm)	Dry weight root(gm)	Dry weight shoot(gm)
1 Without bacteria	22.631a	6.737a	0.255ab	0.045a	0.029a	0.015a
2 With bacteria A	22.750ab	6.867a	0.297a	0.045a	0.029a	0.015a
3 With bacteria B	24.564a	5.741b	0.244b	0.039a	0.025a	0.015a
4 With bacteria C	21.307b	6.289ab	0.263ab	0.041a	0.028a	0.013a
P-value	0.230	0.065	0.189	0.948	0.952	0.961



Figure(4.11):- Effect of bacteria on wet and dry weight and wet and dry weight root and shoot of clover plant.



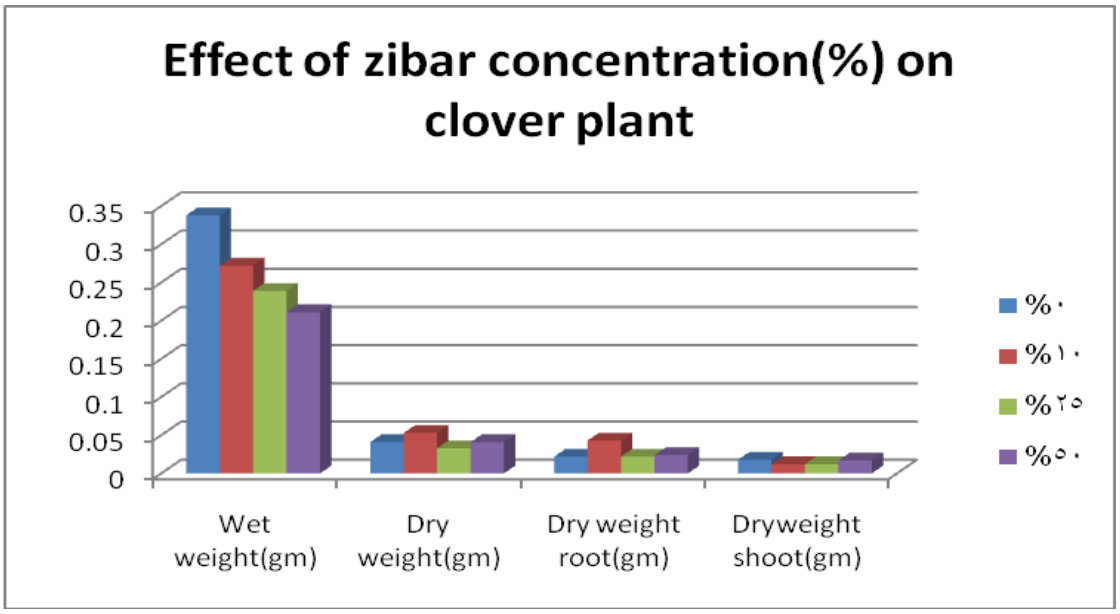
Figure(4.12):- Effect of bacteria on root and shoot length of clover plant.

The results revealed no significant differences among the different types of of bacteria for the different measured variables (P> 0.05). (Table.4: 5)

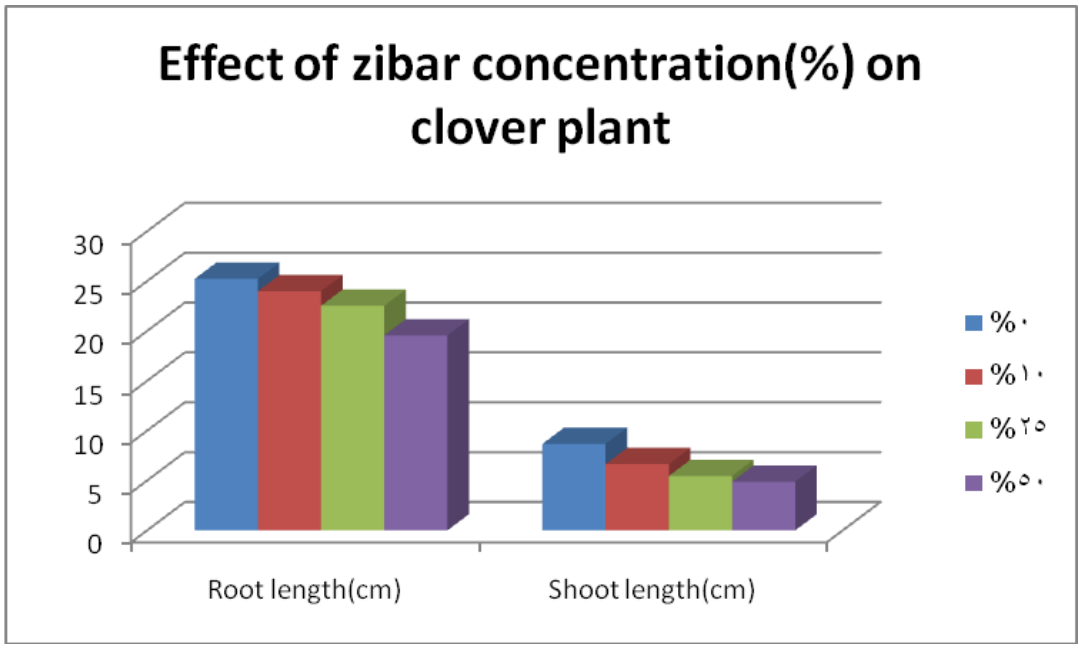
4.6 Effect of OMWW concentration (%) on clover plant

Table (4.6):- Effect of OMWW concentration(%) on clover plant.

OMWW concentration (%)	Root length(;8 cm)	Shoot length(cm)	Wet weight(gm)	Dry weight (gm)	Dry weight root (gm)	Dry weight shoot (gm)
0%	25.22a	8.669a	0.338a	0.041a	0.022a	0.018a
10%	23.98a	6.638b	0.272b	0.053a	0.043a	0.012a
25%	22.52ab	5.449c	0.239bc	0.033a	0.022a	0.012a
50%	19.55b	4.879c	0.211c	0.041a	0.024a	0.017a
P-value	0.0057	0.001	0.001	0.398	0.187	0.2983



Figure(4.13):- Effect of OMWW concentration on wet and dry weight and wet and dry weight root and shoot of clover plant



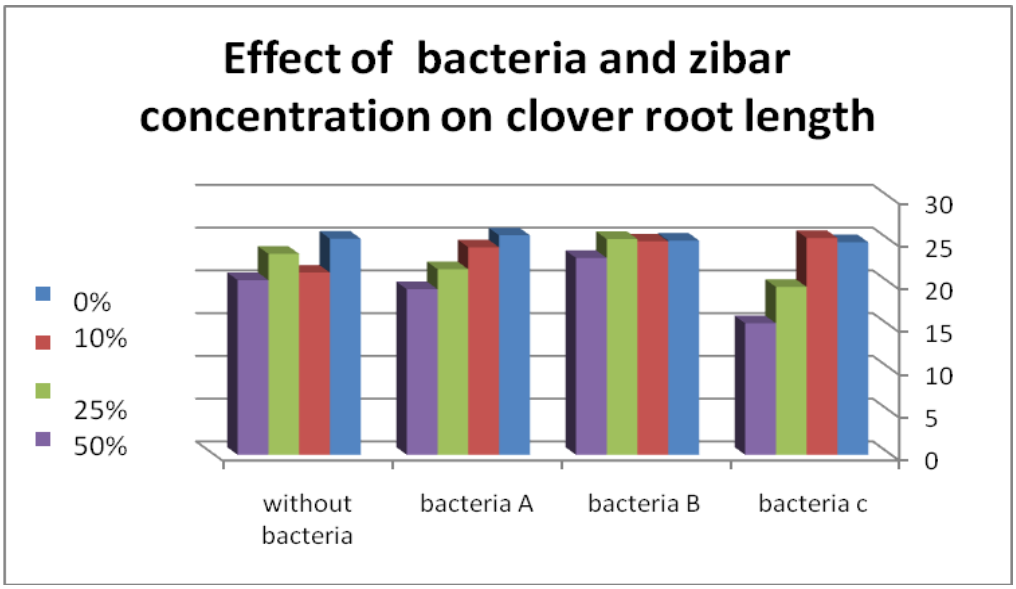
Figure(4.14):- Effect of OMWW concentration on root and shoot length of clover plant

The results revealed significant differences among the different levels of OMWW for the root length, shoot length, wet weight (P=(0.0057-0.0001)).But in the other variables , dry weight, dry weight root, dry weight shoot($p > .05$) so there are no significant differences for this variables. Root length was decreased significantly with increasing the OMWW levels, the higher root length was observed with the control and 10% OMWW(25.22and 23.98 cm), respectively, however, the lowest was obtained when 50% OMWW was used. Regarding shoot length, OMWW application significantly reduced the shoot length, the higher shoot length was obtained without OMWW application(8.669 cm), when OMWW was used at 50%, lower shoot length was observed (4.879). Similar trend was observed with another measured variable (fresh weight of the plant, OMWW application highly reduced both fresh and dry weight of both stem and root, the higher wt was obtained without OMWW application (Table .4:6)

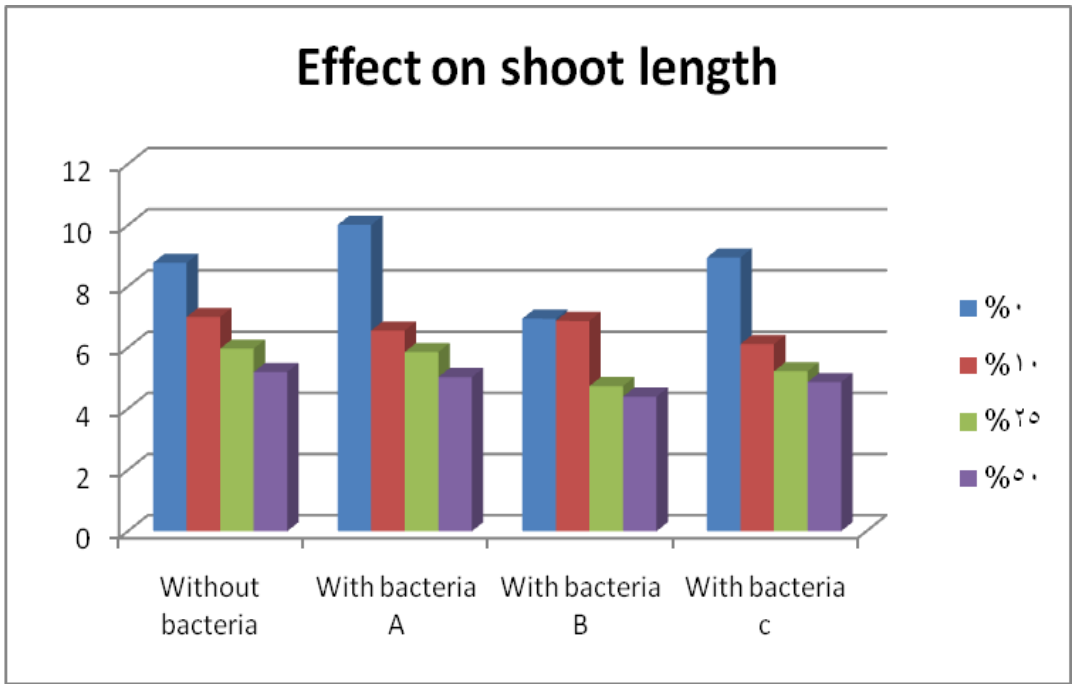
4.7 Effect both of bacteria and OMWW concentration(%) on clover plant

Table.(4.7):- Effect both of bacteria and OMWW concentration(%) on clover plant

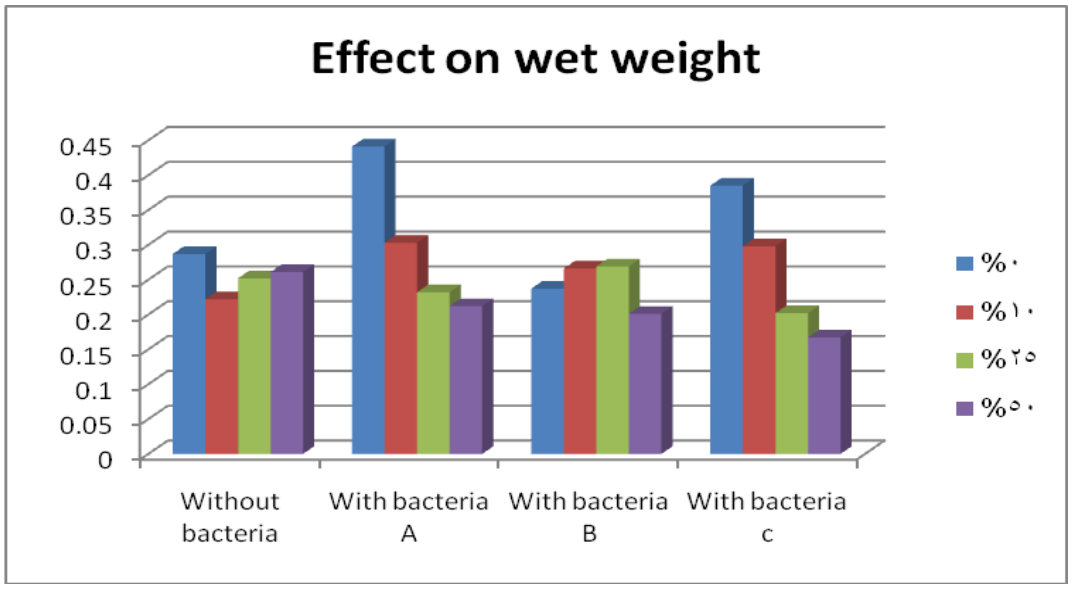
Bacteria / OMWW Concentration	clover plant	1 Without bacteria	2 With bacteria A	3 With bacteria B	4 With bacteria c	p-VALUE
0%	Root length(cm)	25.284	25.672	25.056	24.868	0.570
10%		21.308	24.271	24.952	25.361	0.570
25%		23.499	21.699	25.227	19.627	0.570
50%		20.433	19.359	23.021	15.375	0.570
0%	Shoot length(cm)	8.771	10.012	6.948	8.947	0.448
10%		7.003	6.559	6.874	6.117	0.448
25%		5.969	5.859	4.743	5.226	0.448
50%		5.204	5.038	4.400	4.873	0.448
0%	Wet weight(gm)	0.287	0.441	0.237	0.385	0.019
10%		0.222	0.303	0.266	0.298	0.019
25%		0.252	0.232	0.269	0.202	0.019
50%		0.261	0.212	0.201	0.167	0.019
0%	Dry weight (gm)	0.034	0.062	0.025	0.044	0.935
10%		0.066	0.048	0.051	0.049	0.935
25%		0.038	0.029	0.039	0.026	0.935
50%		0.040	0.039	0.039	0.045	0.935
0%	Dry weight root (gm)	0.016	0.041	0.012	0.026	0.937
10%		0.054	0.039	0.039	0.037	0.937
25%		0.024	0.014	0.028	0.023	0.937
50%		0..025	0.025	0.018	0.027	0.937
0%	Dry weight shoot (gm)	0.018	0.021	0.013	0.019	0.799
10%		0.013	0.009	0.012	0.013	0.799
25%		0.015	0.017	0.012	0.004	0.799
50%		0.015	0.014	0.021	0.018	0.799



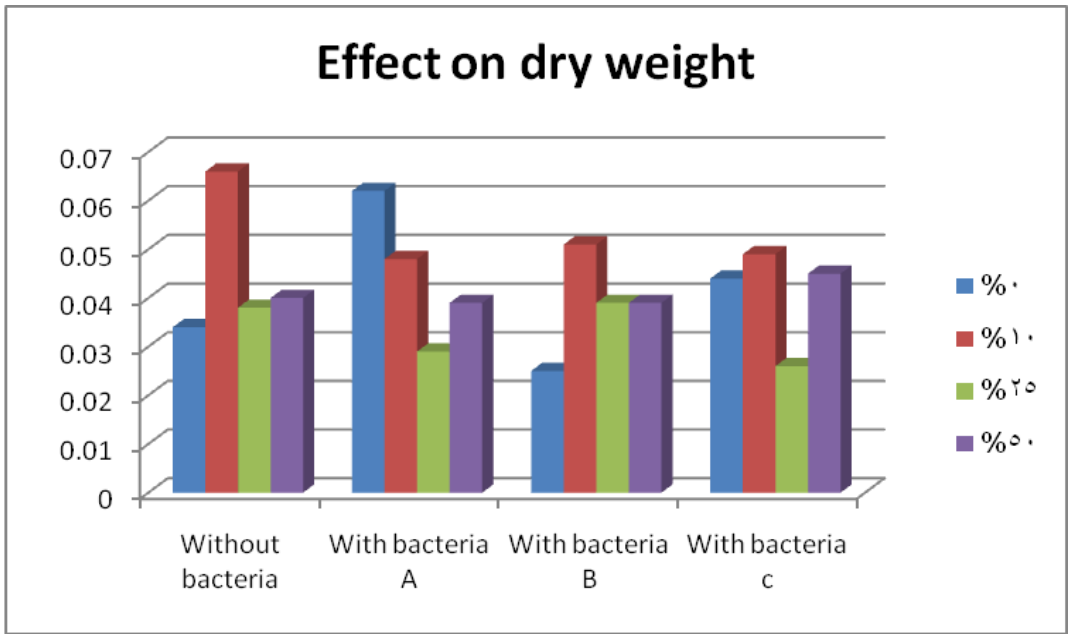
Figure(4.15):- Effect of bacteria and zibar concentration on root length of clover plant



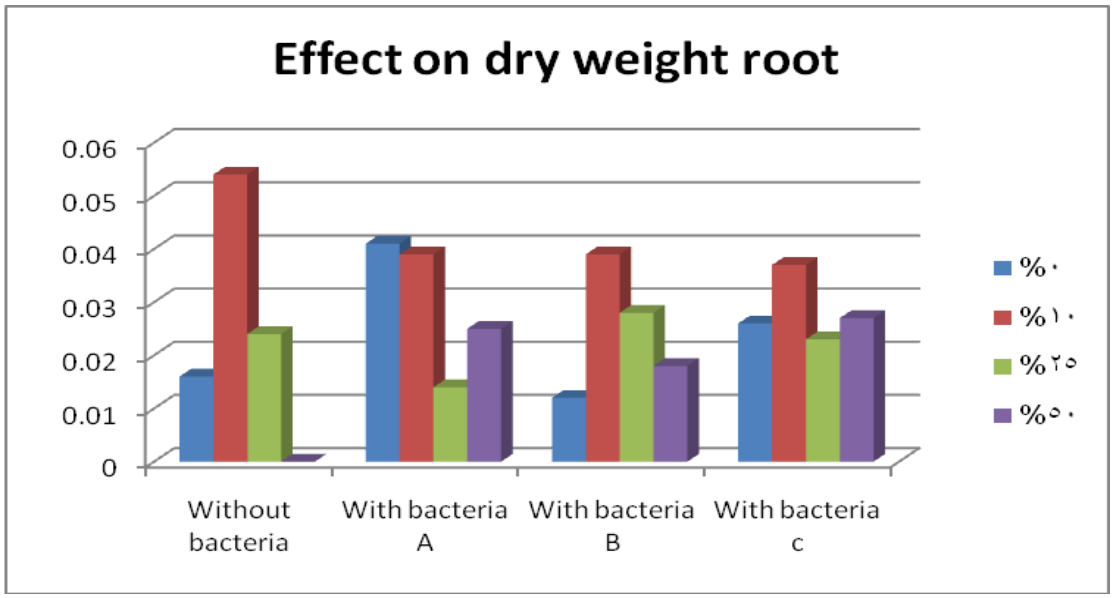
Figure(4.16):- Effect of bacteria and OMWW concentration on shoot length of clover plant.



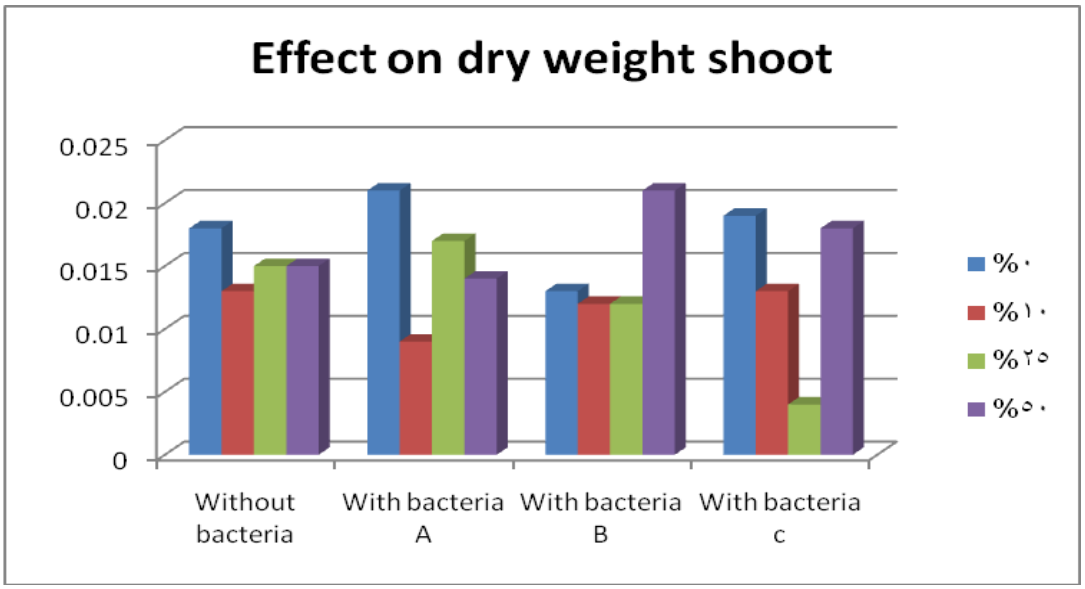
Figure(4.17):- Effect of bacteria and OMWW concentration on wet weight of clover plant.



Figure(4.18):- Effect of bacteria and OMWW concentration on dry weight of clover plant.



Figure(4.19):- Effect of bacteria and OMWW concentration on dry weight root of clover plant.



Figure(4.20):- Effect of bacteria and OMWW concentration on dry weight shoot of clover plant.

The results show no significant differences among the different levels of OMWW and different types of bacteria for the different measured (p > .05) variables. Except for the wet weight, there are significant differences among the different levels of OMWW and different types of bacteria for the different measured (p < o.o5). Root length was decreased significantly with

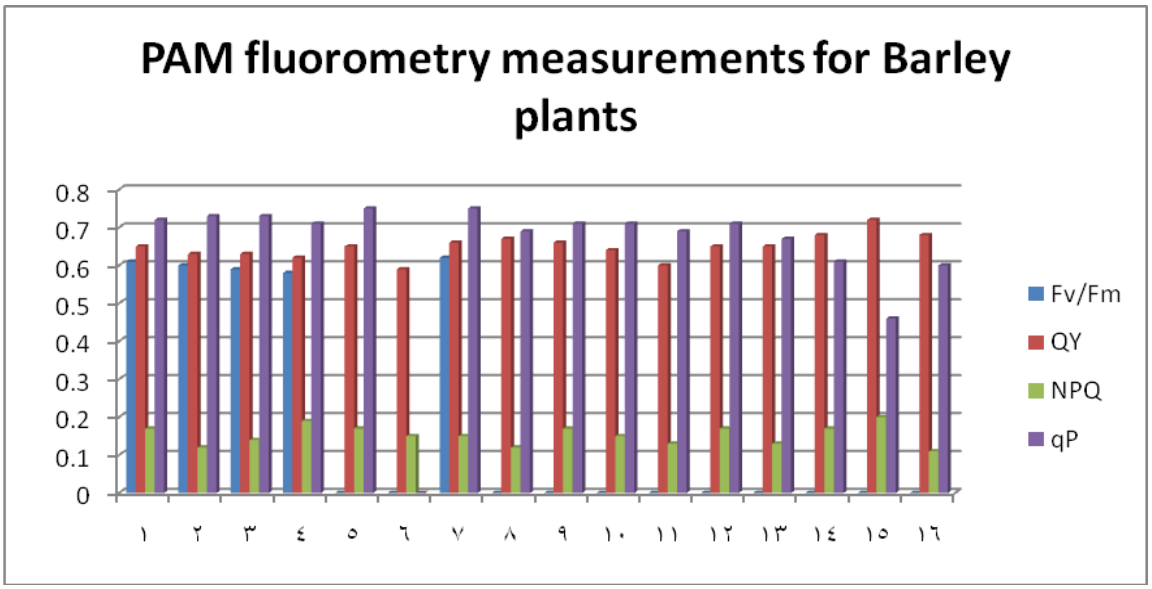
increasing the OMWW levels, the higher root length was observed with the control and 10% OMWW at a different type of bacteria, respectively, however, the lowest was obtained when 50% OMWW was used. Regarding shoot length, OMWW application significantly reduced the shoot length; the higher stem length was obtained without OMWW application. When OMWW was used at 50%, lower stem length was observed. A Similar inclination was observed with another measured variable dry weight of the plant dry weight of shoot and root, OMWW application highly reduced dry weight of plant and dry weight of both shoot and root, the higher weight was obtained without OMWW application (Table 4:7).

4.8. Measurements of photosynthesis with (PAM) fluorometry:

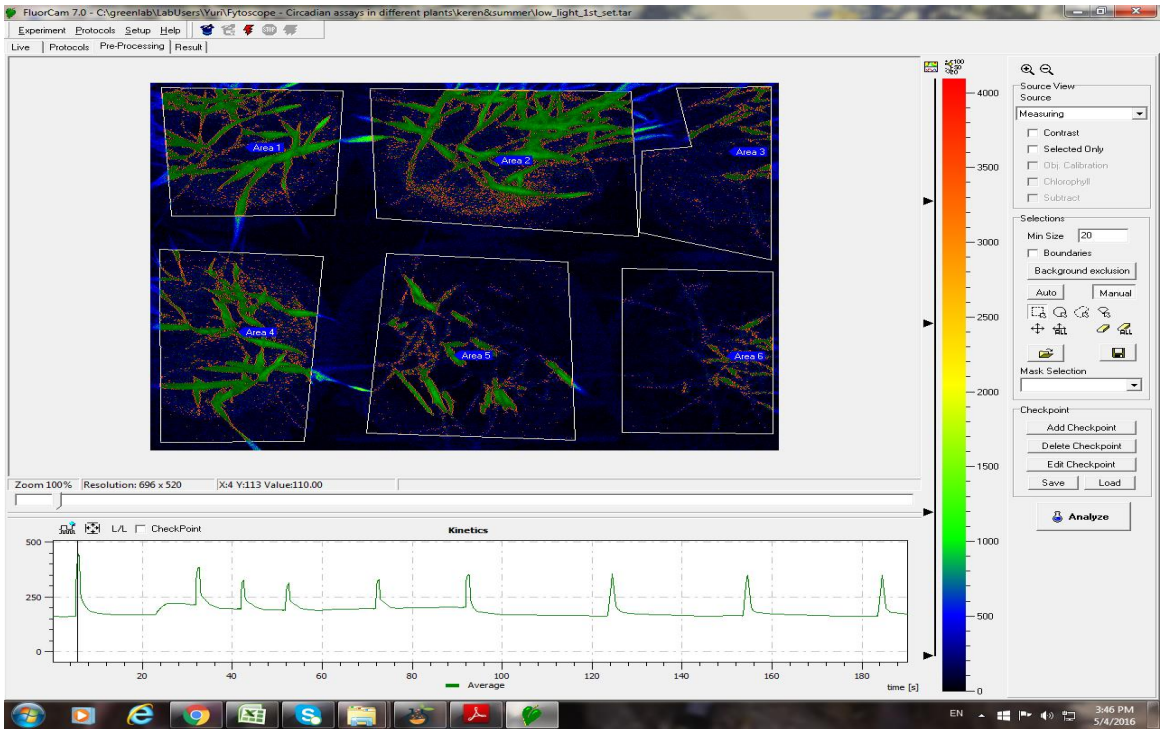
Photosynthesis activities of Barley and clover plant trials were measured using (PAM).

Table (4.8):- PAM fluorometry measurements for Barley plants, each trial repeated in 3 replicates.

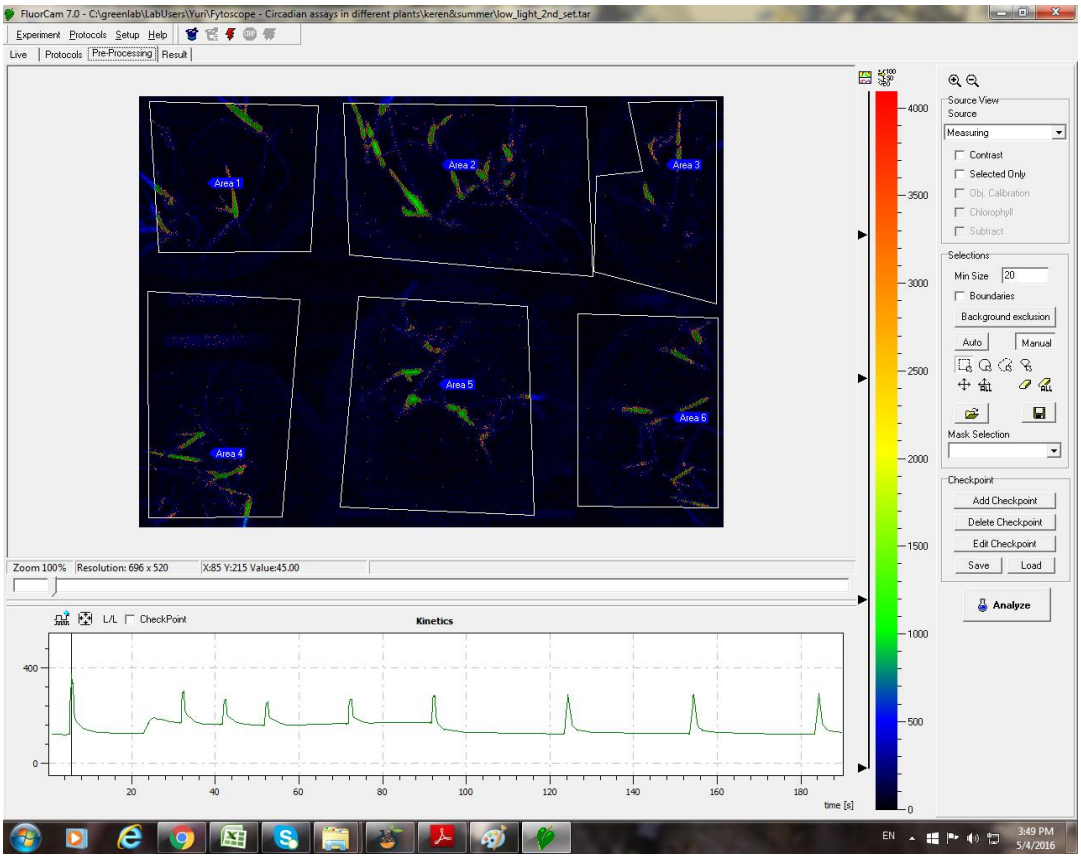
No.	Type of plant treatment(barely)	Fv/Fm	QY	NPQ	qP
1	**Control trials** -Fresh water	0.61	0.65	0.17	0.72
2	**Seeds pots germinated withUW3 (A)**-Fresh water	0.60	0.63	0.12	0.73
3	**Seeds pots germinated with Unassigned (B)**-Fresh water	0.59	0.63	0.14	0.73
4	**Seeds pots germinated with UW4(C)**-Fresh water	0.58	0.62	0.19	0.71
5	**Control trials** -10 mg/L of olive mill waste water solution	0.61	0.65	0.17	0.75
6	**Seeds pots germinated withUW3 (A)** -10 mg/L of olive mill waste water solution	0.55	0.59	0.15	B0.75
7	**Seeds pots germinated with Unassigned (B)** -10 mg/L of olive mill waste water solution	0.62	0.66	0.15	0.75
8	**Seeds pots germinated with UW4 (C)** -10 mg/L of olive mill waste water solution	0.64	0.67	0.12	0.69
9	**Control trials** -25 mg/L of olive mill waste water solution	0.62	0.66	0.17	0.71
10	**Seeds pots germinated with UW3 (A)** -25 mg/L of olive mill waste water solution	0.61	0.64	0.15	0.71
11	**Seeds pots germinated with Unassigned (B)** -25 mg/L of olive mill waste water solution	0.57	0.60	0.13	0.69
12	**Seeds pots germinated with UW4 (C)** -25 mg/L of olive mill waste water solution	0.61	0.65	0.17	0.71
13	**Control trials** -50 mg/L of olive mill waste water solution	0.62	0.65	0.13	0.67
14	**Seeds pots germinated with UW3 (A)** -50 mg/L of olive mill waste water solution	0.65	0.68	0.17	0.61
15	**Seeds pots germinated with Unassigned (B)** -50 mg/L of olive mill waste water solution	0.68	0.72	0.20	0.46
16	**Seeds pots germinated with UW4 (C)** -50 mg/L of olive mill waste water solution	0.65	0.68	0.11	0.60



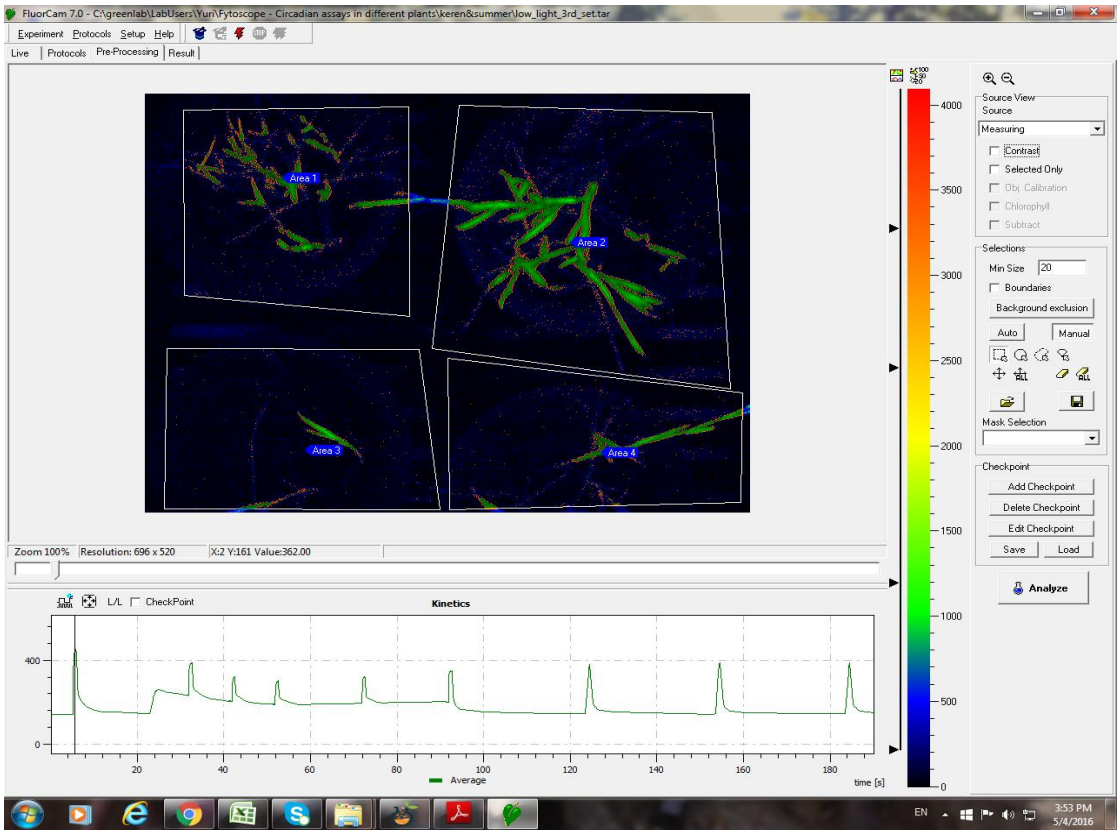
Figure(4.20): PAM fluorometry measurements for Barley plants.



Figure(4.21):-[Image1 of barely plants by (PAM)] (**Area 1**) Control trials +Fresh water,(**Area2**) Seeds germinated withUW3(A) +Fresh water,(**Area 3**) Seeds germinated with Unassigned(B) +Fresh water, (**Area 4**) Seeds germinated with UW4(C) +Fresh water, (**Area 5**) Control trials +25ml/L of olive mill waste water solution ,(**Area 6**)) Seeds germinated withUW3(A)+ 25 ml/L of olive mill waste water



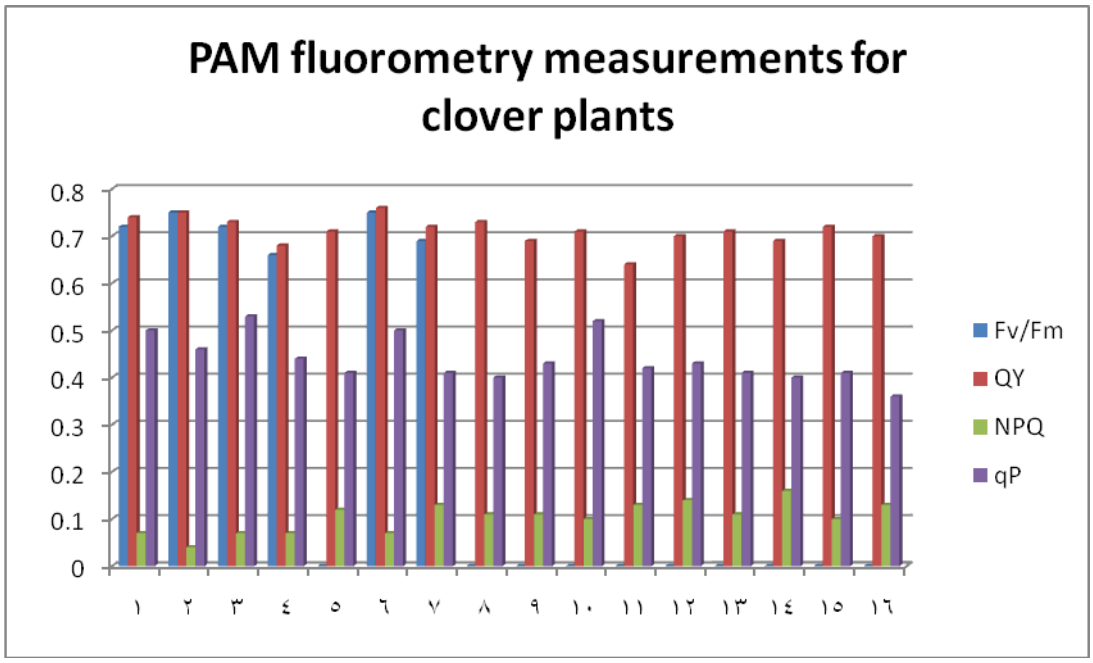
Figure(4.22):-[Image2 of barely plants by (PAM)] (**Area 1**) Seeds germinated Unassigned(B) + 25 ml/L of olive mill waste water,(**Area2**) Seeds germinated with with UW4(C) + 25 ml/L of olive mill waste water,(**Area 3**)Control trail +50 ml/L of olive mill waste water solution, (**Area 4**) Seeds germinated withUW3(A)+ 25 ml/L of olive mill waste water, (**Area 5)** Seeds germinated Unassigned(B) 25 ml/L of olive mill waste water,(**Area6**) Seeds germinated with UW4(C) +25 ml/L of olive mill waste water.



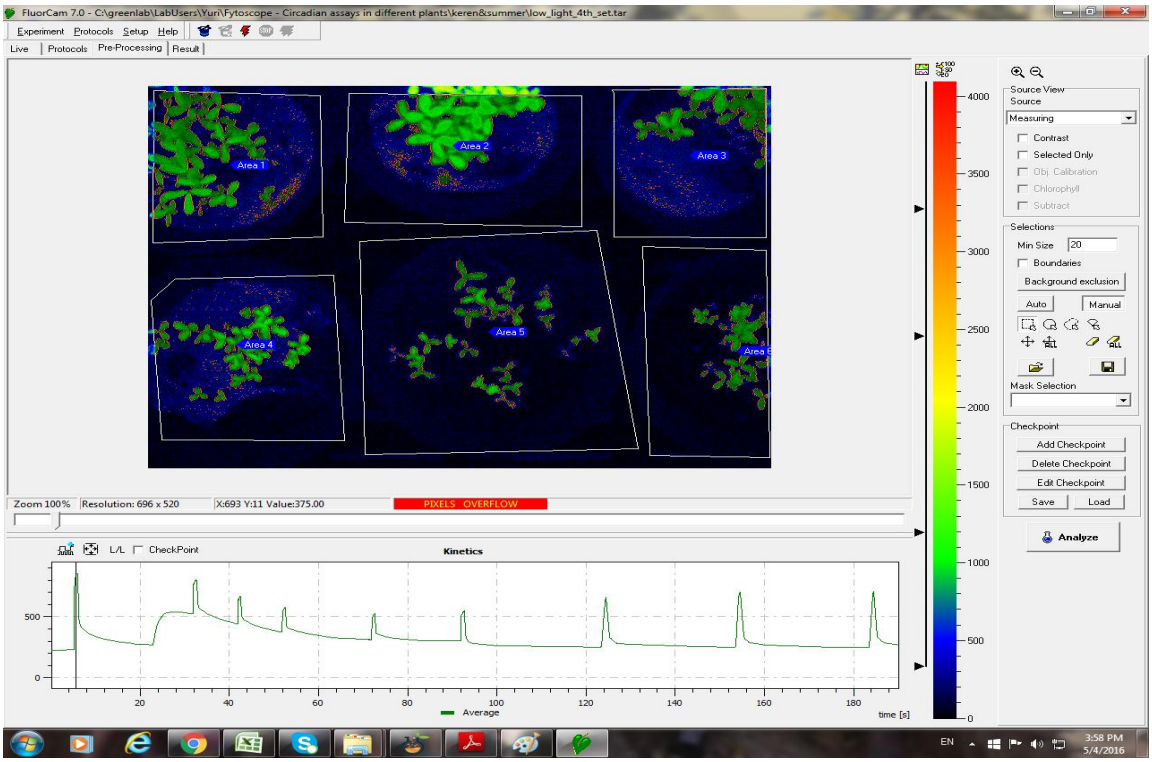
Figure(4.23):-[Image3of barely plants by (PAM)] **(Area 1**)Control trail +10 mg/L of olive mill waste water solution, (**Area 2**) Seeds germinated withUW3(A)+ 10 ml/L of olive mill waste water, (**Area 3)** Seeds germinated with Unassigned (B)+ 10 ml/L of olive mill waste water,(**Area4**) Seeds germinated with UW4 (C) +10 ml/L of olive mill waste water.

Table (4.9): PAM fluorometry measurements for clover plants, each trial repeated in 3 replicates.

No.	Type of plant treatment(clover)	Fv/Fm	QY	NPQ	qP
1	**Control trials** -Fresh water	0.72	0.74	0.07	0.50
2	**Seeds pots germinated withUW3 (A)** -Fresh water	0.75	0.75	0.04	0.46
3	**Seeds pots germinated with Unassigned (B)** -Fresh water	0.72	0.73	0.07	0.53
4	**Seeds pots germinated with UW4(C)** -Fresh water	0.66	0.68	0.07	0.44
5	**Control trials** -10 ml/L of olive mill waste water solution	0.66	0.69	0.11	0.43
6	**Seeds pots germinated with UW3 (A)** -10 ml/L of olive mill waste water solution	0.69	0.71	0.10	0.52
7	**Seeds pots germinated with Unassigned (B)** -10 ml/L of olive mill waste k2water solution	0.62	0.64	0.13	0.42
8	**Seeds pots germinated with UW4 (C)** -10 ml/L of olive mill waste water solution	0.67	0.70	0.14	0.43
9	**Control trials** -25 ml/L of olive mill waste water solution	0.68	0.71	0.11	0.41
10	**Seeds pots germinated with UW3 (A)** -25 ml/L of olive mill waste water solution	0.65	0.69	0.16	0.40
11	**Seeds pots germinated with Unassigned (B)** -25 ml/L of olive mill waste water solution	0.70	0.72	0.10	0.41
12	**Seeds pots germinated with UW4 (C)** -25 ml/L of olive mill waste water solution	0.67	0.70	0.13	0.36
13	**Control trials** -50 ml/L of olive mill waste water solution	0.69	0.71	0.12	0.41
14	**Seeds pots germinated withUW3 (A)** -50 ml/L of olive mill waste water solution	0.75	0.76	0.07	0.50
15	**Seeds pots germinated with Unassigned (B)** -50 ml/L of olive mill waste water solution	0.69	0.72	0.13	0.41
16	**Seeds pots germinated with UW4 (C)** -50 ml/L of olive mill waste water solution	0.71	0.73	0.11	0.40

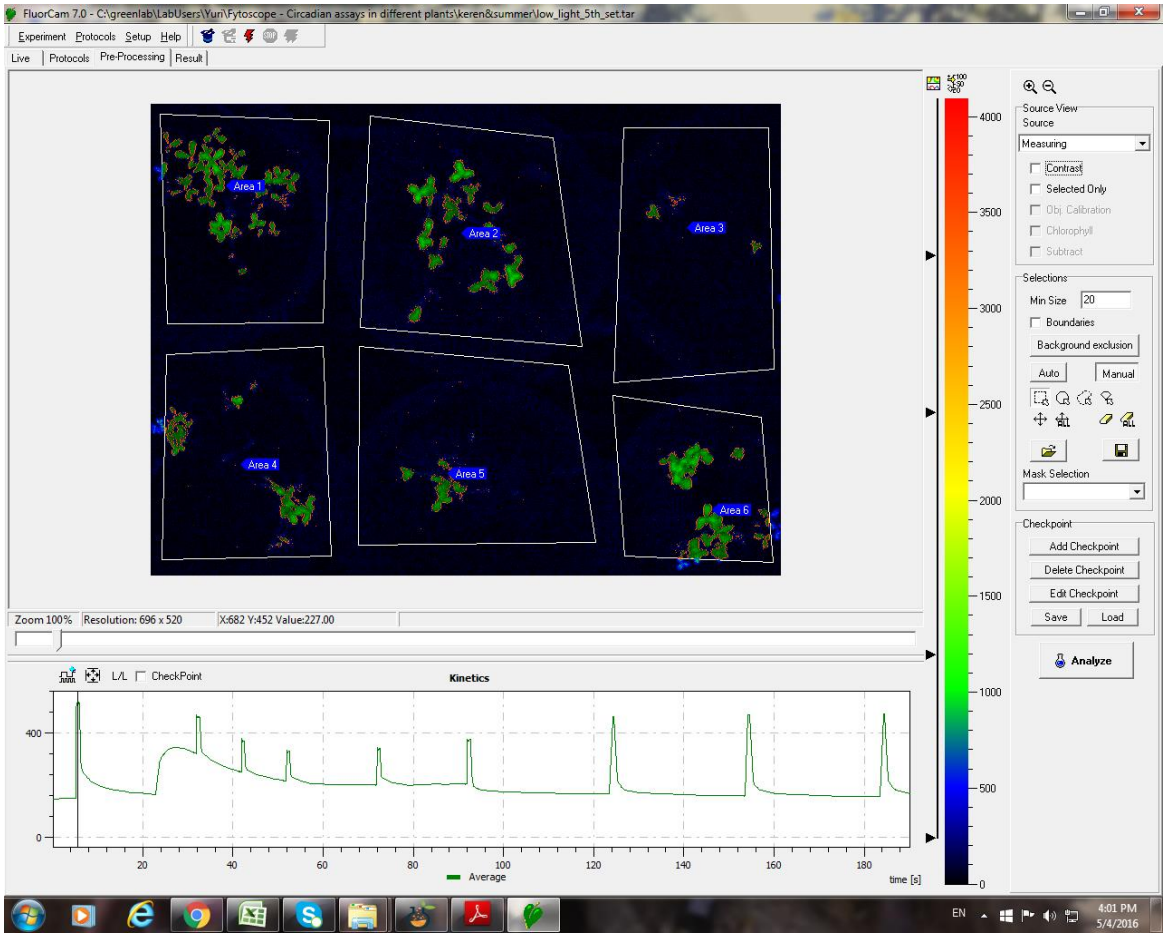


Figure(4.24):- PAM fluorometry measurements for clover plants

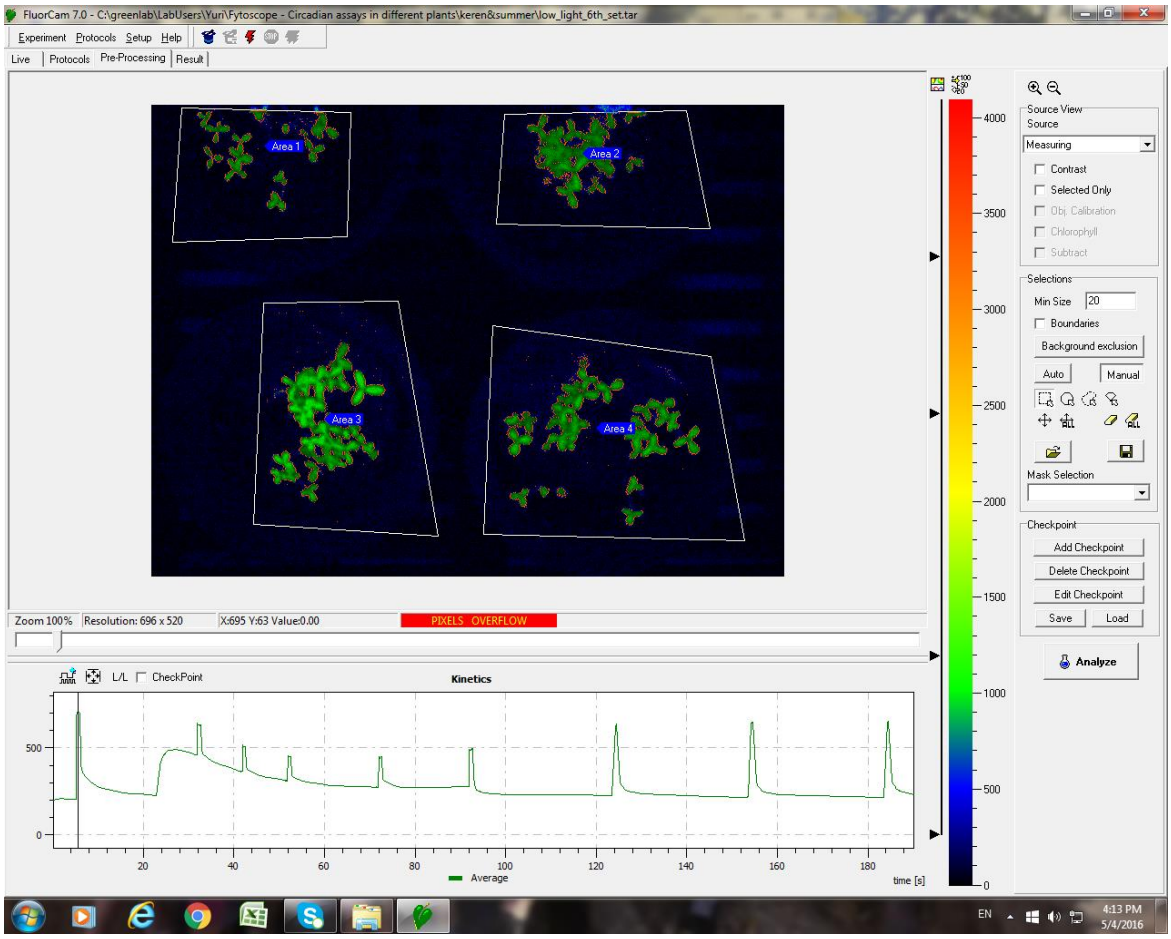


Figure(4.25):- [Image1 of clover plants by (PAM)] **.(Area 1**) Control trials +Fresh water,(**Area2**) Seeds germinated withUW3(A) +Fresh water,(**Area 3**) Seeds germinated with Unassigned(B) +Fresh water, (**Area 4**) Seeds germinated with UW4(C) +Fresh water, (**Area 5**)

Control trials +25ml/L of olive mill waste water solution ,(**Area 6**)) Seeds germinated withUW3(A)+ 25 ml/L of olive mill waste water



Figure(4.26):-[Image2 of clover plants by (PAM)] **.** (**Area 1**) Seeds germinated Unassigned(B) + 25 ml/L of olive mill waste water,(**Area2**) Seeds germinated with with UW4(C) + 25 ml/L of olive mill waste water,(**Area 3**)Control trail +50 ml/L of olive mill waste water solution, (**Area 4**) Seeds germinated withUW3(A)+ 25 ml/L of olive mill waste water, (**Area 5)** Seeds germinated Unassigned(B) 25 ml/L of olive mill waste water,(**Area6**) Seeds germinated with UW4(C) +25 ml/L of olive mill waste water.



Figure(4.27):-[Image3 of barely plants by (PAM)] **. (Area 1**)Control trail +10 mg/L of olive mill waste water solution, (**Area 2**) Seeds germinated withUW3(A)+ 10 ml/L of olive mill waste water, (**Area 3)** Seeds germinated with Unassigned (B)+ 10 ml/L of olive mill waste water,(**Area4**) Seeds germinated with UW4 (C) +10 ml/L of olive mill waste water

Chapter Five

Discussion

It is noticed that PGPR combined with OMWW doesn't affect significantly the growth of both barley and clover. However, OMWW significantly affected biomass and growth of both plant species. On the other hand, seed germination of clover was significantly higher with OMWW application, however, clover seeds treated with bacteria showed higher seeds germination. Our findings are inconsistent with other researchers. The OMWW inhibit seeds vegetation and plant growth (Della Greca et al., 2001)

Parameter	Jenin	Nablus	Tulkarem	Salfit	Qalqilya	Average
BODs	8830	8755	13698	12580	13010	11375
CODs	136750	130625	145000	136750	138500	137525
Total	5276.0	4032.4	6232.7	3179.1	4239.7	4592.0
TS(mg/l)	73970	46250	87800	62450	66920	67478
TSS(mg/l)	58070	38150	68600	45680	49570	52014
TDS	15900	8100	19200	16770	17350	15464
PH	4.8	4.9	4.9	4.6	4.9	4.8

Source: Adham, R,(2012)

Phytotoxicity may be due to the characteristics of OMWW, mainly their phenolic content as well as some organic acids, such as acetic acids, produced during storage(medpan, 2007). Table (2.3) shows the general characteristics of OMWW in the Northern West Bank especially in Tulkarem city from where the OMWW was obtained; the values indicated toxicity and hazards of OMWW to the environment. According to(Soliman,2015), It was noticed that the concentration of polyphenol, Fe

and Zn decreased in soil. The concentrations of polyphenol and Fe that absorbed by barley plants were the most significant. Absorption ratio of polyphenol was 0.25 and 0.26 in the samples which irrigated with Zibar 50% and fresh water respectively. So the increase of polyphenolic compound in plant tissue may affect plant activity and decrease plant growth and photosynthetic activity. Despite the presence of PGPR there is no significant effect on both clover and barely plant in the greenhouse. The temperature in the greenhouse during the experimental period may affect the activity of bacteria. Also, the type of plants may be not suitable for this type of phytoremediation.

During the experiment, many changes were observed on the plants with different levels of OMWW especially at 50%, changes occur in the color and number of leaves , number of plants, length of shoot and root, in addition to the presence of organic layer on the soil surface which prevented evaporation of water from soil and accumulation of water between soil particles in spite of the presence of holes at the bottom of the trail .

The effect of PGPR in harvest productivity varies under laboratory, greenhouse and meadow trials, soil is an unpredicted able environment that will affect the activity of the PGPR. Climatic variations also have a large impact on the efficiency of PGPR but sometimes unfavorable growth conditions in the field are to be predictable as normal functioning agriculture

(Zaidi et al., 2009). Plant growth promoting trait do not work separately of each other but additively as it was optional in the ‘‘additive hypothesis,’’ that multiple

mechanisms, such as nitrogen fixation, ACC deaminase and antifungal activity, phosphate solubilization, IAA and siderophore biosynthesis etc. are responsible for the plant growth promotion and increased yield (Bashan and Holguin.1997)

Measurements of photosynthesis with (PAM) fluorometry:

According to Eq. (4) and Eq. (5) the maximal yield of PSII (Fv/Fm) ratio was intended, where delegate value of it is equal to 0.8 [Mac Neil, 2011].

Trails of barly plants treated with PGPR irrigated with different concentration of OMWW their values of **Fv/Fm** were ranged from (0.55 - 0.68), which mean that plant is under stress, and its photosynthesis does not proceed as it should. and the trail of control barely plant with fresh water the value is 0.61, and NPQ values ranged (0.11-0.17)

Trails of clover plants treated with PGPR(UW3) irrigated with fresh water, 10% concentration of OMWW, values of **Fv/Fm** are closed to 0.8 and NPQ are decreased to .07 , while other Trails of clover plants treated with PGPR irrigated with different concentration of OMWW their values of **Fv/Fm** were ranged from (0.62 -0.70), and NPQ values ranged (0.04-0.16).Which mean that plant is under stress, and its photosynthesis does not proceed as it should.

Measurements showed several chlorophyll fluorescence parameters which are: (qP ,QY, NPQ).These parameters were measured at minimal fluorescence in a dark–adapted plant tissue (F_0) and at a maximal fluorescence (Fm), steady-state fluorescence (Fs) exposed in each spectrum, use of a saturating pulse to adark-adapted leaf induces a maximum value of fluorescence by closing reaction centers. At this position, in a healthy non-stressed plant there is no NPQ because the material has been fully dark adapted, and The level of NPQ is the only measurement that requires knowledge of the dark-adapted values of *F*o and *F*m. consequently, if the dark-adapted *F*v/*F*m value is significantly lower than 0.83, this value should be treated with caution and in particular, NPQ in leaves with differing *F*v/*F*m values should not be compared (**Murchie.2011**). The reason for the decrease in photosynthesis in trials without PGPR can be correlated with the increase of the high concentration of OMWW in tissue that responsible for photosynthesis process. It could be as a result of destroying of thylakoids, and deformation of chloroplast membrane; which lead to disorder all process in plant [Mac Neil, 2011]. Barley and clover Plant leaves was light green color , this indicated that there were no complete photosynthesis processes and didn’t confirm positive response to PGPR treatment as estimated.

Figure(4.28):- barely plants in greenhouse

Figure(4.29):- clover plants in greenhouse

Conclusion:

1- Specifically, trials treated with PGPRs has shown no significant improvements in the plant growth for the plants (Barley and clover) that used in these experiments, indicated that these two plants cannot be used in this type of phytoremediation process in combination of the PGPRs (*Pseudomonas pituda* UW3 and/or UW4).

2- Results from pulse amplitude modulated fluorometry (PAM) studies indicated that these plants which treated with PGPR have no effect on the rate of photosynthesis.

3- Biomass measurements showed no significant effect on mass for those plants treated with PGPRs compared with those control (untreated); so there is no phytoremediation efficiency.

4- Concentration of OMWW less than 10% more suitable for this type of phytoremediation

Recommendation

OMWW at low concentration (less than 10%) could be used in irrigating barley and clover treated with *Pseudomonas pituda* UW3 and/or UW4. In addition, we recommend using other plant species with these PGPRs and comparing their responses to conditions, besides testing other strains combined with other plant species.

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Annexes

جامعة النجاح الوطنية

كلية الدراسات العليا

استخدام الرايزوبكتيريا لمعالجة تأثير المياه العادمة من معاصر الزيتون"الزيبار" على نباتي الشعير والبرسيم

إعداد

سمر عمر سمير عبد القادر

إشراف

أ.د. شحده جودة

د. رائد الكوني

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس- فلسطين.

2017

استخدام الرايزوبكتيريا لمعالجة تأثير المياه العادمة من معاصر الزيتون "الزيبار" على نباتي الشعير والبرسيم

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اشراف

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د. رائد الكوني

الملخص

تعتبر مياه الصرف الصحي لعصارات زيت الزيتون ذات تأثيرات بيئة سلبية حادة. لذلك فإنه من الصعب استخدام هذه المياه في الري نظراً لسميتها. تم استخدام طرق مختلفة لعلاج النبات لتحسين استخدام مياه الصرف الصحي لعصارات زيت الزيتون في الري إن كلا من سلالتي الرايزوبكتيريا (اليو دبليو3، البكتيريا الزائفة (أ) والـــ يو دبليو 4، البكتيريا الزائفة (ج) بالإضافة إلى أخرى غير معينة (ب)) تم استخدامها مع نباتات الشعير والبرسيم وقد أجريت جميع التجارب في بيت بلاستيكي مصمم في كلية الزراعة في جامعة النجاح الوطنية في مدينة طولكرم لمدة 30 يوما. وقد شملت جميع التجارب علاج هذه النباتات باستخدام الرايزوبكتيريا المعززة لنمو النباتات تارة وبدون استخدامها تارة أخرى والري باستخدام تركيزات مختلفة من مياه صرف عصارات الزيتون (0% ، 10% ، 25% و 50%). إن كلا من بذرتي البرسيم والشعير والتي تم ريها باستخدام تركيزات متفاوتة من مياه صرف عصارات الزيتون أظهرت فروق ذات دلالة إحصائية بين مستويات تركيز مياه صرف عصارات الزيتون على كلٍ من نبتتي الشعير والبرسيم بعد أسبوع واحد من الإنبات. حيث كان من الملاحظ إن مياه صرف عصارات الزيتون لها تأثيرات سلبية في إنبات بذور كلتا النبتتين. لم يكن هناك تحسن كبير في الكتلة الحيوية للبرسيم ولا حتى للشعير المعالجة بالرايزوبكتيريا المعززة لنمو النباتات والمروية باستخدام تركيزات متفاوتة من مياه صرف عصارات الزيتون بالمقارنة مع تلك المروية بالمياه. وجد إن طول الجذر يتناقص بشكل ملحوظ مع زيادة تركيز ومستوى مياه صرف العصارات

(سم57.8 و 58.5سم على التوالي). إن استعمال مياه صرف عصارات الزيتون قلل بشكل ملحوظ من طول الساق وعندما استخدمت مياه صرف العصارات بنسبة 50%، لوحظ اقصر طول للساق بواقع (15.5 سم). كما ولوحظ اتجاه مماثل مع مقاييس أخرى (كل من وزن النبات الطازج والجاف) حيث إن استعمال مياه صرف العصارات قلل وبشكل كبير من وزن كل من الجذور والسيقان.

كشفت النتائج عن فروق ذات دلالة إحصائية بين المستويات المختلفة من مياه صرف العصارات لنباتات البرسيم لطول الجذور، طول الساق، وزن الطازج عند.((P=(0.0057- 0.0001) ولكن في المتغيرات الأخرى، مجموع الوزن الجاف، الوزن الجاف للجذور، والوزن الجاف للبراعم عند المتغير (p< .05) لم تكن هناك فروق ذات دلالة إحصائية. وقد لوحظ أن أطوال الجذر انخفضت بشكل ملحوظ مع زيادة مستويات مياه صرف العصارات. وقد لوحظ أن اكبر طول للجذر مع الضبط و10٪ من مياه تصريف العصارات (25.22 و 23.98 سم، على التوالي) ومع ذلك، تم الحصول على أدنى مستوى عند 50٪ من تركيز مياه تصريف العصارات. فيما يتعلق بطول الساق، إن استعمال مياه تصريف عصارات الزيتون خفض بشكل كبير في طول الساق، تم الحصول على أعلى طول للساق دون استعمال مياه الصرف (8.669سم) وفي الوقت نفسه، عندما استخدمت مياه تصريف عصارات الزيتون بنسبة 50٪ لوحظ أدنى طول للساق (4.879 سم). وأظهرت النتائج أن الوزن الرطب من البرسيم له فروق ذات دلالة إحصائية بين التراكيز المختلفة من مياه صرف العصارات والنوع المختلف من البكتيريا لمختلف المقاس (o.o5> p). بالإضافة إلى ذلك فإن معاملات استشعاع الكلوروفيل المختلفة(II) Fv/Fm, Y, و QN تم الحصول عليها من تعديل مدى النبض(PAM) أظهرت أن النباتات المعالجة بالرايزوبكتيريا المعززة لنمو النبات ليس لديها أي تحسن في عملية التمثيل الضوئي الخاصة بها.

إن مسارات نبات الشعير المعالجة بالرايزوبكتيريا المعززة لنمو النباتات والمروية باستخدام تراكيز متفاوتة من مياه صرف العصارات كانت قيم الضغط فيها تتراوح ما بين – 0.55 (0.68) مما يعني أن النبتة كانت تحت الضغط وعملية التمثيل الضوئي لا تمضي قدما كما

يجب أن تكون، بالمقارنة مسارات نبات الشعير المروية بالماء العذب فان القيمة كانت 0.61 وقيم التكييف غير الضوئي كانت تتراوح بين .(0.17–0.11). كذلك الأمر مع نبات البرسيم المعالجة باستخدام الرايزوبكتيريا المعززة لنمو النباتات (يو دبليو 3) والمروية بالمياه العذبة مع 10% من مياه تصريف عصارات الزيتون فإن قيم الضغط تكون قريبة من 0.8 وقيم التكييف غير الضوئي تقل ل0.7. بينما مسارات نبات البرسيم المروية بتركيزات مختلفة من مياه تصريف العصارات تتراوح قيم الضغط فيها بين ,(0.70– 0.62) وقيم التكييف غير الضوئي تتراوح بين .(0.16–0.04) مما يعني أن النبتة كانت تحت الضغط وعملية التمثيل الضوئي لا تمضي قدما كما يجب أن تكون.