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Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 26(1), 397-412, 2018

REUSE OF WASTE DRAINAGE WATER AFTER ITS TREATMENT USING PGPRS TO IRRIGATE SOME HORTICULTURAL CROPS

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Keywords: Bioremediations; Phytoremediation; Heavy metals; Pathogenic bacteria; PGPRs; *Mentha viridis* cv, *Gladiolas grandiflorus* cv.

ABSTRACT

Natural sources of water are limited in Egypt. The rapidly growing populations necessitate continuous expansion of the cultivated area. This means an increase of the gap between the demands of water for irrigation and the limited water supply. Looking for other sources such as low quality water like (industrial effluent, drainage and sewage) must be used in irrigation of some garden and wood plants. This investigation was conducted on agricultural drainage wastewater from El Mohete drain (Marioteya Canal) west of Cairo; the samples were collected from different places during (Summer and Winter seasons). The wastewater contaminated with pathogenic microorganisms, the excess of fertilizers (inorganic & organic), heavy metals, and the residuals of pesticides. Plant Growth Promoting Rhizobacteria (PGPR) could remediate the wastewater as biological bioremediation to remove some pollutants such as pathogenic microorganisms, heavy metals and pesticides. Chemical remediation was used as nitrification inhibitor to stop transformation of ammonia to nitrate. This work was conducted to study the ability of PGPR strains e.g. *Bacillus megaterium, Bacillus subtillis, Bacillus circulans, Paenibacillus polymyxa, Pseudomonas floresense, Serratia sp. and Azotobacter chroococcum* 5, 9 and 23 to treat the drainage water for irrigation the *Mentha viridis* cv. and *Gladiolas grandiflorus* cv. plants.

Two pot experiments were conducted in greenhouse. The treatments were applied as follows; Natural water, treated water and drainage water to irrigate the plant. Use the PGPR as inoculants and thiourea as nitrification inhibitor, Heavy metal treated was (Copper, Cobalt, Zinc, Cadmium and Mercury) the result showed us heavy metals removal by PGPR from drainage water.

The characterizations of PGPRs as shown in the obtained results are they could enhance plant growth by using their own metabolism (solublizing phosphate, producing hormones or fixing nitrogen) as well as correlation of them with the potenit of effects on the growth of plants in unfavorable conditions in order to improve the efficiency of phytoremdiation of contarinated soils. The removal of heavy metals and the elimination of pesticides residues were markedly noticed in this investigation. Results also confirmed the ability of PGPRs in suppressing the effect of pathogenic bacteria like *Salmonella*sp and *E.coli.* These abilities are of great importance in terms of plant and soil health. Consequently, the role of PGPRs bacteria associated with plant rhizosphere in remediation of water and soil contaminations due to its biochemical activity and thus, stimulate plant growth is a great important subject in phytoremediation process nowadays.

INTRODUCTION

Water pollution is stated as “a change in the chemical, physical, biological, and radiological quality of water that is injurious to its existing, intended, or potential uses” (Water Pollution, 2002). The implications of pollution on the Earth began many years ago with the inconsiderate behaviors and careless attitude toward the environment.

A major concern for reuse of agricultural waste drainage water is the bioaccumulation of hazardous wastes especially heavy metals and pesticides

(Received 25 July, 2017)
(Revised 27 July, 2017)
(Accepted 29 July, 2017)

in food chain. Also heavy metals affect the enzyme activity within the plant with subsequent reduction in yield **(Teisseire and Guy, 2000)**. In aquatic systems, heavy metals can be found in different forms **(Cheung et al 2003)**.The problem of heavy metal pollution is basically associated with: (i) acute toxicity linked with particular metals (Cu, Hg or Cd), even in lower concentration; (ii) the fact that heavy metals are not degraded or destroyed and tend to circulate; thus, they remain for a long time in nature and are accumulated through the food chain, which leads to serious ecological and health problems **(Eman R. Zaki 2014)**.

Agricultural wastewater contains pathogenic microorganisms such as bacteria, viruses, and parasites, which have the potential to cause diseases. In particular, human parasites such as protozoa and helminthes eggs are of special significance in this regard as they prove to be most difficult to remove by treatment processes and have been implicated in a number of infectious gastrointestinal diseases in both developed and developing countries. The use of untreated agricultural wastewater for irrigation, no doubt, poses a high risk to human health in all age groups. However, the degree of risk may vary among the various age groups **(Feenstra et al 2000)**. Treatment of pollutants in agricultural wastewater is the process of removing contaminations from wastewater and effluents. It includes physical, chemical, and biological processes to remove the contaminants. Its objective is to produce an environmentally-safe fluid treated effluent and solid waste suitable for reuse usually as farm fertilizer **(Emane R. Zaki 2014)**.

Bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances in to less toxic or non-toxic substances". Microbial degradation of pesticides has been recognized as the most important process **(Karpouzas and Singh, 2006)**. therefore, biodegradation using native microorganisms for pesticides removal from the environment is quite attractive **(Maya et al 2011)**.

Heavy metal resistant bacteria have significant role in bioremediation of heavy metals in wastewater. The heavy metals Hg and Cu were removed by *Bacillus* sp. The average Hg reduction was 45% and Cu reduction was recorded as 62%. The heavy metals Cd, As and Co were removed by *Pseudomonas* sp. The average Cd reduction was 56%, average As reduction was 34% and average Co reduction was recorded as 53%. **(Manisha et al 2011)**.

The PGPRs can indirectly or directly affect plant growth. Indirect plant growth promotion includes the prevention of the deleterious effects of phytopathogenic organisms. This can be achieved by the production of siderophores, i.e. small iron-binding molecules. In soils, iron is found predominately as ferric ions, a form that cannot be directly assimilated by microorganisms **(Heba M. Hewait, 2010)**. Another mechanism by which PGPR can inhibit phytopathogens is the production of hydrogen cyanide (HC) and/or fungal cell wall degrading enzymes e.gchitinase and β-1, 3-glucanase **(Friedlender et al 1993)**.

Symbiotic and non-symbiotic PGPRs may also promote plant growth directly through production of plant hormones such as auxins **(Tien et al 1979)**, gibberellins **(Gutiérrez-Mañero et al 2001)** and ethylene **(Lynch, 1990 and Heba M. Hewait 2010).**

The objective of this study is to use some PGPRs in removing some organic, inorganic and biological pollutants in a agricultural waste water to be used in irrigation of some horticultural crops (Mint and Gladiolas).

MATERIALS AND METHODS

Bioremediation of agricultural wastewater become an urgent necessity nowadays to face the challenges in water resources shortage. Using microbial strains have the ability to eliminate the pollutants in agricultural wastewater is considered a promising way as it is safe and cheap. The ability of some bacterial species to degrade the toxic effect of pollutants in agricultural wastewater and at the same time its use as plant growth promoters is our main target in this study.

Agricultural wastewater collection

Ten samples were collected from different localities along Marioteya (El-Mohate drainage canal).

The samples were collected in sterilized high-density polyethylene bottles and immediately brought to the laboratory in an ice box and stored at 4 ^{o} C to carryout physical, chemical and biological analysis 100 ml from each bottle were taken and mixed thoroughly to reach 1 liter of homogenized sample which represents the 10 sites and be used for analysis.

Bacterial strains

Some plants growth promoting rhizobacteria via; *Bacillus megaterium* , *Bacillus subtilis* , *Bacillus circulans, Paenibacillus polymyxa* , *Pseudomonas flouresens* and *Serratia* sp. were kindly obtained from the department of agricultural microbiology, Soils, water and environment Research Institute (SWERI), Agricultural Research center (ARC) Giza, Egypt whereas three *Azotobacter* strains*; Azotobacter chroococcum* (*Azo*.3), (*Azo*.9) and (*Azo*.23) were obtained from the department of microbiology, Faculty of agriculture , Ain shams university, Cairo, Egypt.

These bacterial strains were grown and maintained on their specific media for each as follows;

Bacillus megaterium strain was cultivated in pikoveskay's medium **(Pikoveskaya, 1948)** for 3 days at 30^{o} C to reach 1x10^{8} clle /ml culture.

Bacillus subtilis was cultivated in nutrient broth medium **(Difco, 1985)** and incubated for 48h at 28$^{\pm 2}$ ^{o}C to reach 1x10^{8} cell/ml culture.

Bacillus circulans strain was grown and maintained on Alexandrov's broth medium **(Zahara, 1969)** and incubated for 4 weeks at 28^{o}C to reach maximum growth 1x10^{8} cell/ ml culture.

Three nitrogen fixers strains belong to genius *Azotobacter* via, *AZtobacter chroococcum* (*Azo*.3), *Azo*.9 and *Azo*.23 were grown on modified Ashby's medium **(Abdel-Malek and Ishac, 1968)** at 30^{o}C for 7 days to reach the finial desity of 10^{8} cfu/ml culture according to **(Cochran, 1950)**.

An active strain of *Pseudomonas fluorescens* was cultured and maintained on King's broth medium **(King et al 1954)** at 28±2^{o}C for 7 days. The biomass of *P. fluoresens* was prepared by inoculating 1 ml of pre-culture of *Pseudomonas fluoresens* (1x10^{8} cfu ml^{-1}) in a 500 ml Erlenmeyer flask containing 200 ml of King's broth medium, then incubated on a rotatory shaker (120 rpm) for 72 hour at 28±2^{o}C. The density of bacterial cell culture was adjusted to 10^{8} cell/ml. *peanibacillus polymyxa* strain was grown and maintained on nutrient broth medium **(Difco, 1985)**. This strain was cultured on this medium for 48h at 28±2^{o}C to reach 10^{8} cell/ml culture.

Serratia spp: An active strain was obtained from the department of agricultural microbiology, soils, waters and environment research Institute (SWERI), agriculture research center (ARC), Giza, Egypt.

This strain was cultured in 100 ml nutrient broth medium **(Difco, 1985)** in a 250 conical flasks. The flasks were incubated for 48 h at 28±2^{o} C to reach the density 1x10^{8} cell/ml.

Antagonistic action of the used strains against pathogenic microbial pollutants

In vitro Inhibition test

The antimicrobial activity of the used PGPR's (cell free filtrate) against (*Escherichia coli* and *Salmonella* spp.,), that isolated from agricultural wastewater samples by the well diffusion assay. The pathogenic bacteria were incubated in Brain heart Infusion (BHI) broth at appropriate temperature for 24 hrs. Petri dishes containing 20 ml of Muller Hinton agar were prepared previously and inoculated with 0.1 ml of 24 hrs broth culture of pathogenic bacteria. Once solidified the dishes were stored for 2 hrs in a refrigerator. Four wells were made and filled using 100 µl of cell-free filtrate of each PGPRs according to **(Lee, S.W., et al 2010)**. Incubation of the Petri dishes at 37°C for 42 hrs then the diameter of the inhibition zone was measured with calipers in mm. The antimicrobial activity was determined by measuring the clear zone around the wells **(Lee, S.W., et al 2010) and (Lee and Wendy (2013).**

The antagonistic activity against *E. coli* and *Salmonella* sp. was carried out by using the PGPR's individually, in dual and in groups.

Agricultural wastewater analysis

Microbiological examinations

The examination of wastewater samples microbiologically was conducted to determine the populations of some pathogenic microorganisms Total Coliform, Fecal Coliform, *Salmonella* and *Shigella* (SS) were counted according to **(Apha, 1989)**.

Chemical analysis of untreated and treated drainage wastewater included; PH, EC, COD, BOD, Total phosphorus, Total nitrogen, Total potassium, Heavy metals, Hydrocarbons, $NH4^{+}$, $NO2^{-}$ and $NO3^{-}$N analysis were determined according to **(Apha, 1989)**.

Pot experiments

Two pot experiments were conducted to study the effect of the treated agricultural wastewater biologically by using the different strains of PGPRs (*Bacillus megaterium*, *Bacillus subtillus*, *Bacillus circulans, Paenibacillus polymyxa*, *Pseudomonas flouresens* and *Serratia* sp. and three *Azotobacter* strains*; Azotobacter chroococcum* (*Azo*.3), (*Azo*.9) and (*Azo*.23). and chemically through nitrification inhibitors via, thiourea when used to irrigate Gladiolas as Horticultural crops and Mint as an ornamental plant.

Table 1. Chemical analysis of Homogenized agricultural wastewater

Sample	EC	PH	COD	BOD	TS	TSS	Fe	Cu	Zn	Mn	K	NA	P
Homogenized	3.1 (dSm-1)	7.8 (dSm-1)	6.9 mg/L	9.8 mg/L	2.59 g/ 100m	1.75 g/ 100m	0.03 Ppm	0.29 ppm	0.12 ppm	0.0	17.8 ppm	0.04 ppm	5.7 Ppm

Table 2. Different counters standers of heavy metals **(El-Tohamy, S.A. et al 2015)**

Elements	Cd	Cr	Cu	Ni	Pb	Zn	Mn
Eu	1-3	100-150	50-140	30-75	50-300	150-300	-

Soil: Clay soil was used to fill plastic pots (30 cm diameter) with 5 kg soil for Gladiolas experiment. And (15 cm diameter) with 2.5 kg soil for mint experiment. The physic-chemical analysis of soil was illustrated in **Table (3)**.

Table 3. Physic-chemical analysis of the used soil

Soil characteristics	**Value**	**Soil characteristics**	**Value**
Particle size distribution%:		*Soluble cations (soil paste mmole$_c$L^{-1}):*	
Sand	13.5	Ca^{2+}	7.35
Silt	25.8	Mg^{2+}	4.15
Clay	60.7	Na^{+}	58.50
Textural class	Clayey	K^{+}	0.60
Soil chemical properties:		*Soluble anions (soil paste mmole$_c$L^{-1}):*	
pH (soil paste extract)	8.15	CO_3^{2-}	0.00
$CaCO_3$ %	3.15	HCO_3^{-}	4.50
Organic carbon %	1.12	Cl^{-}	4.50
ECe (dS/m, soil paste extract)	6.92	SO_4^{2-}	24.70
Soil physical properties:			
Bulk density g cm^{-3}	1.37	Soil moisture at wilting point %	8.69
Soil moisture at field capacity %	21.00	Avail. Water %	12.31

Available Nutrients mg kg^{-1}						
N	P	K	Cu	Fe	Mn	Zn
27.40	4.12	374.50	0.65	3.84	0.95	0.78

Plant material

Bulbs of Gladiolas and seedlings of Mint were kindly obtained from Horticulture Research Institute, ARC, Giza, Egypt. The two pot experiments were conducted in a greenhouse located at wheat research institute, ARC, Giza, Egypt. Each treatment has four replicates.

Irrigation: The two experiments were irrigated twice a week with the filtration of Homogenized treated agricultural wastewater.

Fertilization: The recommended dose of nitrogen fertilizer (nitrogen 120 Kg/fed) as ammonium sulphate (20.6% N) was applied in three equal doses at 1st, 2nd and 3rd irrigation, 200Kg super phosphate/fed (15.5% P2O5) and 100 Kg/fed as potassium sulphate (48% K_2O) were used before cultivation (Omar et al, 2014). The experiments were planted on the 10th of November, 2016.

First experiment: Data recorded on *Gladiolas grandiflorus cv*.

After 110 days from planting when the flowers reached the second floret stage of blooming. Plant samples were collected, and the following data were recorded:

1-Vegetative growth parameters

Plant height (cm) and Number of leaves/plant.

2-Flowering parameters

Length of cut spike (cm), Spike stem diameter (cm), Rachis length (cm), Number of florets/spike, Diameter of the first floret (cm), Fresh weight of cut spike (g) and Dry weight of cut spike (g).

3- Chemical constituents

Pigments content in leaves

Chlorophyll (a), Chlorophyll (b) and Carotenoids.

Second experiment: Data recorded on *Mentha viridis* cv.

After 70 days from planting plant samples were collected and the following data were recorded:

1-Plant morphology

Plant height (cm) and Number of leaves/plant.

2-Plant parameters

Stem diameter (cm), Fresh weight of plant (g) and Dry weight of plant (g).

3-Root growth parameters

Root length (cm)

4-Chemical constituents

a-Pigments content in leaves

Chlorophyll (a), Chlorophyll (b) and Carotenoids.

b- Mineral % of Oil Mintol (ml/100g plant)

Determination of percentage and oil yield / plant:

Essential oil percentage in the fresh herb was determined according to **(British Pharmacopoeia, 1963)**.

Chemical analysis

a- Leaf pigments (chlorophyll a, b and carotenoids)

The concentration (mg/g fresh matter) of pigments (chlorophyll a, b and total carotenoids) were determined in leaf samples, according to **Wettestein (1957)** a five discs (one cm in diameter) of fresh tissues (flag leaf) were weighed, cut into small pieces and mixed in to a mortar with 80% (v/v) acetone in water.

Weighed 0.1 g of fresh plant with 10 ml acetone, add 5ml acetone throw grinding. To prevent transformation of chlorophyll to pheophyten the homogenate was filtrated through a glass funnel of final porosity and residue was washed with 5 ml of acetone, in order to isolate the pigments. The filtrate its optical density was spectrophotometerically (Spectronic 21 D Milton Roy Company) measured at wave lengths of 663.2, 646.8 and 470 nm. The concentration of photosynthetic pigments was measured as follows:

Ch-a=12.25 x A663.2 – 279 x A646.8 = mg/g
Ch-b=21.5 x A646.8 – 5.1 x A663.2 = mg/g
Carotenoids= ((1000 x A470) – (1.82Ca – 85.02Cb))/198 = mg/g

Where: Chl.a, b and carot. = concentrations of chlorophyll, a, b and carotenoids in mg/g fresh weight.
A = optical density at the wave length indicated.

Dehaydroginas activity in the used soil

The dehydrogenase activities (DHA) in rhizosphere soil, non rhizosphere soil either waterlogged or normally irrigated as well as in washed rise roots were estimated as indication of the respiratory activity of soil micro-organisms in each system using 2, 3, 5- Triphenyl tetrazolium chloride (TTC) in tris- buffer according to the method described by **(Thalmann, 1967)**.

Statistical analysis: Data collected from the two experiments were subjected to (One way analysis of variance) using the (**MSTAT-C)** program **(SAS, 2006. Statistical Analysis System)**. Mean separation was performed using the New Multiple Range Test at the 5% level of significance, as described by **Duncan (1955) and Steel et al (1997)**.

RESULTS AND DISCUSSION

Table 4. Heavy metals (mg/L) concentration in *Homogenized sample of agricultural wastewater using growing PGPR for 48 hr at 30 ^{o} C.

Microbes (PGPR)	Copper	Zinc	Nickel	Cobalt
Control	301.7	50	50	50
Azotobacter chroococcum 9	242.4	40	48	47
Serratia marcescens	239.6	43	49	Not Detected
Azotobacter chroococcum 23	50	45	45	44
Pseudomonas Flourecens	47	48	44	42
Bacillus Circulans	45	45	49	45
Bacillus Megatherium	44	46	45	43
Bacillus Sabtilis	48	44	42	41
Paenibacillus Polymyxa	40	40	46	45
Azotobacter chroococcum 5	141.6	41	47	47
Mix (*Azo*9, *Sr*, *Bs*, *Bm*,*Pf*)	Not Detected	40	40	Not Detected

*Homogenized sample is a mixture of 10 samples collected from ten sites along EL-Mohet canal.
*Mercury and Cadmium were not detected.

As shown in **Table (4)** the concentration of heavy metals in the untreated homogenized agricultural waste water sample varied from element to another.

The concentration of heavy metals as in control treatment (untreated) exceeded the critical limit with Copper 301.7 mg/L according to Urbian Standers whereas the concentrations of Zinc, Nickal and cobalt were the same and were below the critical range 50 mg/L. There is No detection for both mercury and cadmium. The PGPRs could reduce the concentration of Copper compared to control where the average Cu reduction was 19.7 % by (Azo.3) and 20.6 % by Serratia and 53.6 % by (Azo.5) and ranged between 85.42 to 86.74 with other PGPRs.

For Mercury and Cadmium there is no detection in control and other treatments. The reduction of Zinc, Nickal and cobalt ranged between 2-20 % compared to control by all other Treatments. The mixture of PGPR's treatment is considered the best one as it agricultural waste water sample.

The PGPRs facilitated the removal of metals from water and this based on the ability of these microorganisms to complex and precipitate metals and the specific interactions for metal removal include metal binding to microbial cell surfaces and expolymer layers intracellular uptake, metal volatilization and metal precepitions via, microbially facilitated metal redoxreactions **(Scholz and Xu, 2002)**.

Data in **Table (5)** show the ability of the used PGPRs to degrade and remove the pesticide residues from the polluted agricultural waste water that originally found in little amounts.

The organophosphours pesticides like, Atrazine, Propamocarb, Piperonyl butoxide, Chloropyrifors are found in untreated waste water sample in concentrations, 0.1, 0.14 and 0.05 mg/kg for Atrazine, Propamocarb and Chlorpyrifos respectively. All PGPRs could induce biodegration of pesticides and B.*megaterium, B.subtilius, Serratia, Azotobacter* 3 and *Azotobacter* 5 could completely degrade and remove the residues of Atrazine whereas *Bacillus polymyxa, B.circulans and Azotobacter* 9 partiolly degraded Atrazine comparing to control in different ration.

Regarding to propanocarb, all PGPRs could totally degrade and renoue this pesticide and changing it to save products, whereas *pseudomonas floursens* reduced the concentration to 0.13 mg/kg compared to control. The same trend was observed with chlorpyrifs as *B.polymxa, B.subtilis* and *B.circulans* reduced the concentration to 0.05, 0.02 and 0.02 mg/kg respectively. Other PGPRs could completely reduce and remove the residues effect of this pesticide.

Table 5. Pesticide residues in homogenized agricultural wastewater sample using PGPRs for 48 hr at 30^{o} C.

Microbes (PGPR)	Atrazine	Propamocarb	Piperonyl butoxide	Chlorpyrifos
Control	0.1	0.14	0.0	0.05
Bacillus megateriuom	0.0	0.0	0.01	0.0
Paenibacillus polymyxa	0.05	0.0	0.0	0.05
Bacillus subtilis	0.0	0.0	0.0	0.0
Bacillus circulans	0.03	0.0	0.0	0.02
Pseudomonas fluorocens	0.0	0.13	0.0	0.0
Azotobacter chroococcum 23	0.02	0.0	0.0	0.0
Serratia marcescens	0.0	0.0	0.0	0.0
Azotobacter chroococcum 9	0.0	0.0	0.0	0.0
Azotobacter chroococcum 5	0.0	0.0	0.01	0.0
Mix (*Azo*9,*Sr*,*Bs*,*Bm*,*Pf*)	0.0	0.0	0.0	0.0

*Data are expressed as mg/Kg.

Bacteria were the most enormously available decomposers and were able to degrade the pesticide residues. These pesticide residues can last for long time in the environment depending on the initial concentration of these pesticides and the biodegradation rate **(Nawaz et al 2011)**.

The PGPRs degrade the pesticides as a source of C, N and P **(Awed et al 2011)**. Various researches have reported the pesticides are degraded Co metabolically and that depended on the concentration, solubility and availability of pesticides as essential factors affecting the rate and extent of bioremediation **(Eissa et al 2014)**.

Culture technique was employed in vitro to analyze whether the five bio-agent PGPRs when they used in a mixture form showed inhibition patterns towards both *E.coli* and *Salmonella* spp. Through the antagonistic as in **Table (6)**. The mixture of PGPRs exhibited antibacterial potential by inhibiting the growth of both pathogens. The highest inhibition action of (*Serratia sp. + Azotobacter chroococcum 9 + B.megaterium + P.flouresens + B.subtilius*) 94.4 % was detected against *E.coli* and 96.6 % against *Salmonella* spp. Microscopic study showed that the mixture of PGPRs was able to degrade and utilize the two pathogens cells through production of secondary metabolities and antibiotics that could suppress the pathogens growth and also secreting cell-wall degrading enzymes as mentioned by **(Viterbo et al 2002)**.

The pathogenic bacterial counts: As shown in **Figs (1, 2)** Mohate water is polluted by Fecal coliform, Total coliform and salmonella. Finding these pathogenic microorganisms may be due to some people have bad behavior, they throw sludge from trench into running water canal specially in rural environment. The numbers were higher in water after treatment than in soil. The plant growth promoting Rhizobucteria could markedly reduce the numbers of these Pathogenic organisms. This owing to the antagonistic action of these PGPRs specially *Azotobacter chrococcoum* strains (*Azo*. 5, *Azo*. 9 and *Azo*.23) they all produce cellulase, polygalacturonase and pectin lyase enzymes suppress the action of pathogenic microorganisms through pathogen cell wall lysis and degradations and yet, the death of pathogenic cells this was obivous shown with treatments 8B (T8= Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 50%) in irrigation water filterate and in soil respectively. Our findings matched with **(Son et al 2014)** who found that the selected PGPRs strains could suppress the action of pathogenic bacteria and have inhibitory effects on them **(Grobelak et al 2015)**.

As recorded in **Table (7)**, soluble nitrogen in soil and in the drainage irrigation filtrates vary from one treatment to another where in general a remarkable increase of NH_4 in the drainage irrigation filtrate than in soil and the highest ammonium.

Table 6. Zone inhibition among mixture of five PGPRs strains and some pathogenic bacteria

PGPRs	Growth of *E.coli* (cm)	% Inhibition	Growth of *Salmonella* sp(cm)	% Inhibition
Serratia sp. + *Azotobacter chroococcum* 5 + *Azotobacter chroococcum* 9+ *Bacillus megaterium* + *Pseudomonas flouracens*	2.4	73.3	2.8	68.8
Serratia sp. + *Azotobacter chroococcum* 23+ *Bacillus megatheriuom* +*Pseudomonas flouracens* + *Bacillus subtilius*	0.5	94.4	0.3	96.6
Azotobacter chroococcum 3+ *Azotobacter chroococcum* 9+ *Bacillus megaterium* + *Pseudomonas flouracens* + *Bacillus polymexa*	3	66.6	3	66.7

Inhibition%= [(D1-D2)/D1] x100. Where: D1=Colony diameter in control, D2=Colony diameter in treatment.

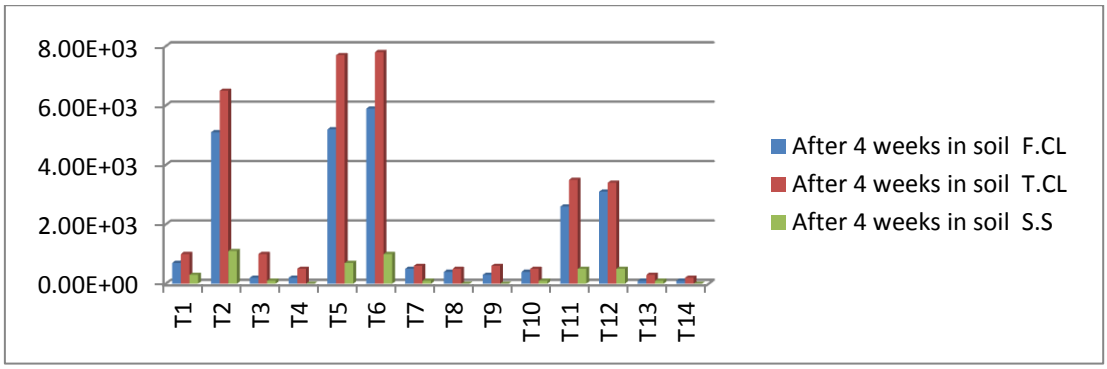

Fig. 1. The Pathogenic bacterial counts (fecal coliform after 48 hr at 44° C, total coliform and salmonella and shigella after 24 hr at 30° C. in soil

[**Treatments (T1** = Control (tap water), **T2** = Wastewater (drainage), **T3**= Wastewater microbial treatment with PGPR and using fertilizer (NPK) 50%., **T4**= Wastewater microbial treatment with PGPR and using fertilizer (NPK) 100%., **T5**= Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 50%., **T6**= Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 100%., **T7**= Wastewater microbial and chemical treatments using fertilizer (NPK) 50%., **T8**= Wastewater microbial and chemical treatments using fertilizer (NPK) 100%., **T9**= Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 50%., **T10**= Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 100%., **T11**= Wastewater diluted with water 50:50 chemical treatment with Nitrification inhibitors fertilizer (NPK) 50%., **T12**= Wastewater diluted with water 50:50 chemical treatment with Nitrification inhibitors fertilizer (NPK) 100%., **T13**= Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%., **T14**= Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%.]

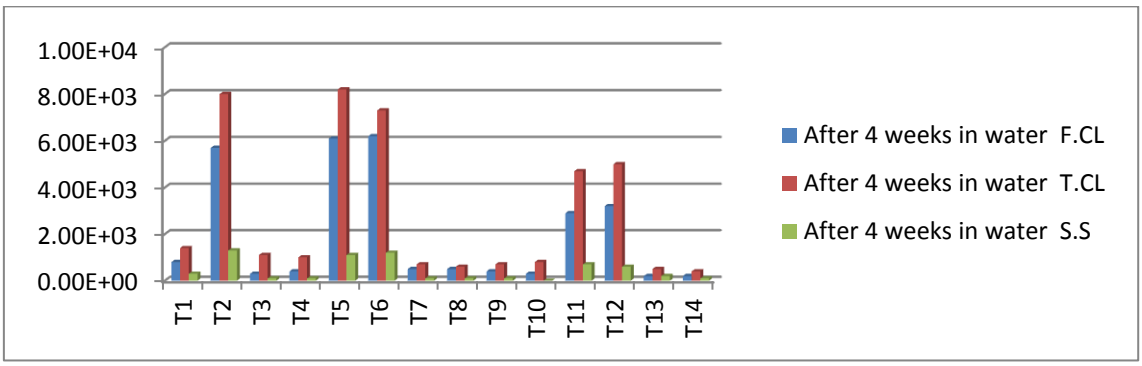

Fig. 2. The Pathogenic bacterial counts (fecal coliform after 48 hr at 44° C, total coliform and salmonella and shigella /ml after 24 hr at 30° C

Table 7. Determination of NH_4, NO_2 and NO_3 in soil and in the drainage irrigation filterate (ppm)

Treatments	Soluble nitrogen in soil			Soluble nitrogen in drainage irrigation filtrate		
	NH_4	NO_2	NO_3	NH_4	NO_2	NO_3
T1	0.27	9.318	2.129	1.811	4	39.77
T2	0.04	3.773	2.193	2.308	4.590	46.54
T3	1.34	41.27	1.838	8.105	31.545	16.45
T4	2.91	24.05	0.387	8.377	32.863	20.09
T5	0.55	21.59	0.838	1.05	33.409	55.51
T6	0.86	43.55	0.58	1.33	34.590	62
T7	1.48	27.59	0.516	3.7	45.181	58.12
T8	4.16	6.955	1.45	4.6	58.863	58.41
T9	1.1	21.23	0.451	5.6	28.363	10.35
T10	6.51	50.59	0.516	8.6	31.863	15.09
T11	1.81	27.95	1.225	0.87	31.954	60.03
T12	2.11	12.59	2.22	1.21	32.954	60.45
T13	3.7	18.68	0.87	2.39	44.681	52.32
T14	5.7	14.05	2.70	3.22	53.863	58.03

values were obtained with the treatments T3 8.105, T4 8.377, T9 5.6, T10 6.51 in soil and 8.6 in irrigation filtrate and T14 5.7 in soil and 3.22 ppm in irrigation filtrate.

Treatment T10 and T8 exhibited the optimum NO_2 where they recorded 50.59 in soil and 58.86 in irrigation filtrate ppm respectively. In concern, Total nitrogen in soil T14 obtained higher valve 2.7 ppm than all other treatments whereas in the drainage irrigation filtrate Total nitrate T6 treatment recorded 62 ppm more than all other treatments. The increase of NH_4-N in the drainage filtrate more than it in soil may be due to re-mineralization of nitrogen immobilized at a stage of plant growth **(Omar, 1980)**. The increase of ammonium in irrigated soil was due to ammonification processes.

There are major factors affecting the soluble nitrogen like bacterial inoculation, ammonium sulphate and nitrification inhibitors and these factors led to the increase of NO_2 and NO_3 in the drainage irrigation water filtrate than in soil. These findings are matched with those obtained by **(Nelson and Huber 2001)**.

Dehydrogenase activity in soil

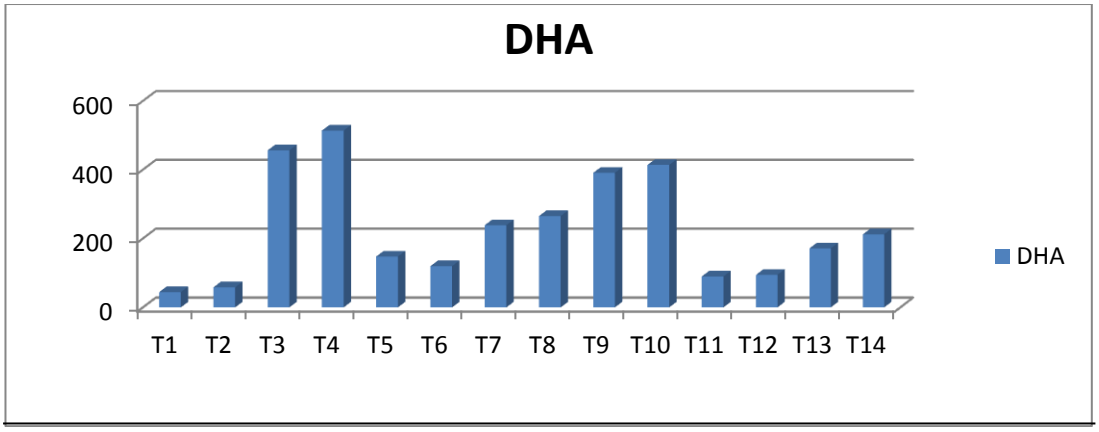

Fig. 3. Dehydrogenase activity in soil (µg Tpf/g dry soil/24 hr).

It is clear from **Fig. (3)** that dehydrogenase activity is strongly affected by rhizosphere soil. The dehydrogenase values increased with increasing the populations of viable microbial groups in soil. The activity of this enzyme steadly increase with increasing the root volume of plants, therefore after 4 weeks the activity increased to maximum values specially with treatment 6B (Wastewater microbial and chemical treatments using fertilizer (NPK) 50%) that recorded 1.52 µg Tpf/g dry soil/day. Dehydrogenase indicates the microbial activity in soil and root surfaces. The increase of dehydrogenase activity as shown in **Fig. (5)** with 6B (Wastewater microbial and chemical treatments using fertilizer (NPK) 50%) treatment more than other treatments relied on the viability of PGPRs and other native beneficial microorganisms and the existence in high populations that could colonize the rhizosphere, which led to the increase of CO2 evolution and carbonic acids formation that decreased soil PH slightly and enhanced the growth of any plant cultivated in this soil **(EL-Gamal et al 2015)**.

Results in **Figures (4, 5)** show the effect of inoculation with plant growth promoting rhizobacteria on the photosynthetic pigments in both mint and gladiolas plants.

In mint, treatment 6B (Wastewater microbial and chemical treatments using fertilizer (NPK) 50%) obtained higher chlorophyll a content 1.030 mg, chlorophyll b 0.796 mg and carotenoids 1.95 mg than all other treatments besides controls.

In gladiolas, treatment 8A (Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 50%) was the superior one as it obtained the highest content of chlorophyll a 0.92 mg , chlorophyll b 0.811 mg and carotenoids 1.760 mg.

PGPRs could enhance plants and improve the growth that reflect on the healthy state of plants were the PGPRs provide plants with the available nutrients such as N and P where the two essential elements enter in the stracture of chlorophyll and also, producing phytohormons (biostinulants). IAA (Indole acetic acid), Cytokines and, Gibberellins these compounds has a direct influence an plants as they could increase cell division, cell elongation, increase the surface area of fire roots and yet increasing the availability of nutrients and let the plants in healthy state through the increase of leaves area and number all that positively reflected on improving photosynthes and pigmint content in mint and gladiolas **(Grobelak et al 2015)**.

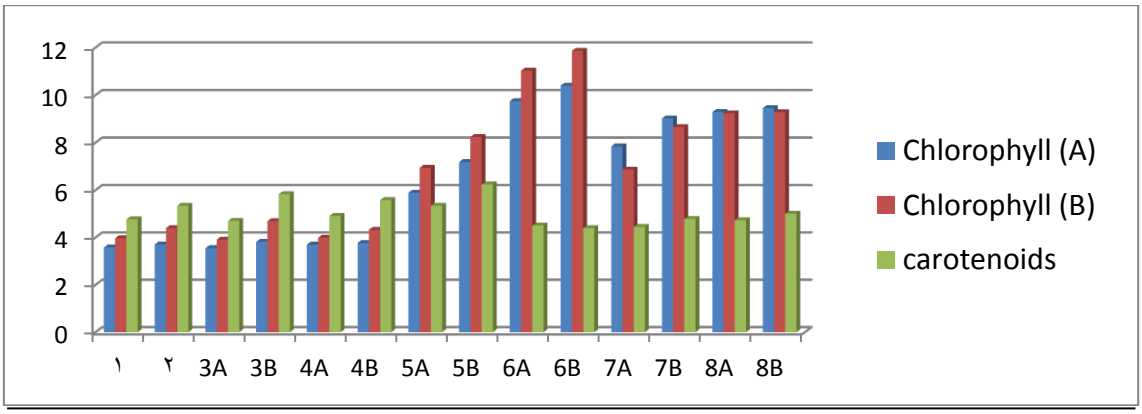

Fig. 4. Determination of Chlorophyll a, b and carotenoids (mg/g fresh leaves of *Mentha viridis* cv)
Treatments (1 = Control (tap water), **2** = Wastewater (drainage), **3A**= Wastewater microbial treatment with PGPR and using fertilizer (NPK) 50%., **3B**= Wastewater microbial treatment with PGPR and using fertilizer (NPK) 100%., **4A**= Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 50%., **4B**= Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 100%., **5A**= Wastewater microbial and chemical treatments using fertilizer (NPK) 50%., **5B**= Wastewater microbial and chemical treatments using fertilizer (NPK) 100%., **6A**= Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 50%., **6B**= Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 100%., **7A**= Wastewater diluted with water 50:50 chemical treatment with Nitrification inhibitors fertilizer (NPK) 50%., **7B**= Wastewater diluted with water 50:50 chemical treatment with Nitrification inhibitors fertilizer (NPK) 100%., **8A**= Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%., **8B**= Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%.

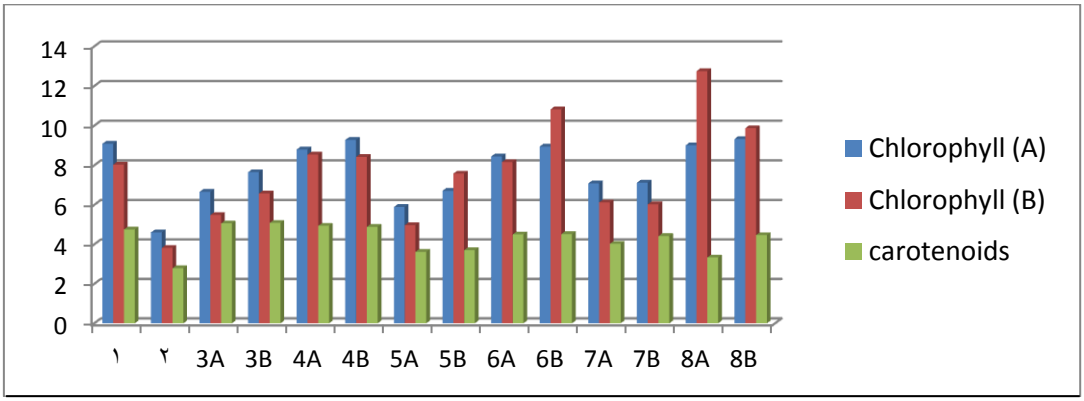

Fig. 5. Chl.a, b and carotenoids (mg/g fresh leaves of *Gladiolas*)

Morphological prametars

Table 8. Morphological parameters of *Mentha viridis* cv plant

Treatments	number of leaves	plant height (cm)	stem diameter (cm)	fresh weight (g)	Dry weight (g)	Root length (cm)
C	35.33c	30.667abcd	0.327b	9.03ab	2.143b	42.667a
T1	52bc	16.33e	0.22b	7.73b	1.8b	23a
T2	60bc	29bcd	0.8937a	14.27ab	3.2b	38.33a
T3	42,67bc	28.33cd	0.327b	11.47ab	2.2b	32.33a
T4	54bc	29.33bcd	0.377b	15ab	3.5b	28.667a
T5	43.33bc	37.66a	0.367b	9.83ab	2.4b	31a
T6	56bc	30.3abcd	0.367b	13.77ab	2.987b	30.3a
T7	64bc	29.66abcd	0.327b	15.47ab	3.243b	33.3a
T8	156ab	36abc	0.327b	36.67a	7.587ab	38a
T9	203.33a	27d	0.4b	30.57ab	12.9a	34a
T10	34c	27.3d	0.293b	8.7ab	1.9b	40.3a
T11	34.67c	36.66ab	0.383b	15.8ab	3.7b	33.667a
T12	162ab	29.667abcd	0.407b	30.27ab	6.267b	33.667a
T13	45.67bc	23,667d	0.310b	18.37ab	3.020b	43a
Duncan	3782.35	18.47619	15.6197	203.447	10.117	128.428

Treatments (C = Control (tap water), **T1** = Wastewater (drainage), **T2**= Wastewater microbial treatment with PGPR and using fertilizer (NPK) 50%., **T3**= Wastewater microbial treatment with PGPR and using fertilizer (NPK) 100%., **T4**= Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 50%., **T5**= Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 100%., **T6**= Wastewater microbial and chemical treatments using fertilizer (NPK) 50%., **T7**= Wastewater microbial and chemical treatments using fertilizer (NPK) 100%., **T8**= Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 50%., **T9**= Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 100%., **T10**= Wastewater diluted with water 50:50 chemical treatment with Nitrification inhibitors fertilizer (NPK) 50%., **T11**= Wastewater diluted with water 50:50 chemical treatment with Nitrification inhibitors fertilizer (NPK) 100%., **T12**= Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%., **T13**= Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%.

Inoculation with plant growth promoting rhizobacteria significantly increased the morphological parameters of mint plants **(Table 8)** T9 (Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 100%) possessed the highest leaves number 203.33 and plant dry weight 12.9 g respectively.

T5 (Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 100%) recorded optimum mint height 37.66 cm. highest root length 43 cm recorded with T13 (Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%). In concern, the morphological parameters in Gladiolus.

Table 9. Morphological parameters of *Gladiolus grandiflorus cv.*

Treatments	Plant height (cm)	Length of cut spike (cm)	Diameter of the first floret (cm)	Number of florets	Number of leaves	Fresh weight of cut spike(g)	Dry weight of cut spike(g)
C	95.5^{a}	81^{ab}	1.57^{a}	11.5^{bc}	9.5^{ab}	74.17^{abc}	10.25^{abc}
T1	69^{b}	56.5^{c}	0.705^{b}	11.5^{bc}	8.5^{ab}	26^{d}	4.85^{c}
T2	90^{ab}	76^{ab}	0.8350^{b}	12.5^{abc}	9^{ab}	58.4^{abc}	8.25^{bc}
T3	94^{a}	83^{ab}	0.98^{ab}	12.5^{abc}	9.5^{ab}	70.55^{abc}	10.25^{abc}
T4	95^{a}	82^{ab}	0.92^{ab}	10^{c}	10.5^{a}	55.6^{bc}	9.1^{bc}
T5	81.5^{ab}	68.5^{bc}	0.835^{b}	11^{c}	8.5^{ab}	47.86^{cd}	7.2^{bc}
T6	95.5^{a}	81.5^{ab}	1.135^{ab}	11^{c}	8.5^{ab}	57.09^{bc}	9.5^{bc}
T7	96.5^{a}	81^{ab}	0.85^{b}	11^{c}	9.5^{ab}	59.7^{abc}	8.95^{bc}
T8	98.667^{a}	80.667^{ab}	0.9267^{ab}	13.33^{abc}	9^{ab}	63.07^{abc}	10.067^{abc}
T9	92.5^{a}	79.5^{ab}	0.94^{ab}	14.5^{ab}	9.5^{ab}	71.9^{abc}	12^{ab}
T10	97^{a}	90a	0.95^{ab}	12.5^{abc}	9^{ab}	49.83^{cd}	8.9^{bc}
T11	92.5^{a}	79^{ab}	0.83^{b}	11.5^{bc}	9^{ab}	52.6^{bcd}	8.65^{bc}
T12	101.5^{a}	86.5^{a}	1.1^{ab}	15.5^{a}	7.5^{b}	86.93^{a}	15.4^{a}
T13	103^{a}	91^{a}	0.995^{ab}	15^{a}	9^{ab}	80.87^{ab}	12.05^{ab}
Duncn	**92.14**	**47.54**	**0.0794**	**2.044**	**1.1**	**146.412**	**5.69**

The trend differs from it in mint plants where T3 (Wastewater diluted with water 50:50 microbial and chemical treatments using fertilizer (NPK) 50%) exhibited better plant height 103 cm and length of cut spike 91 cm than all other treatments control treatment recorded the biggest diameter 1.97 cm of the first floret. T4 (Wastewater chemical treatment with Nitrification inhibitors fertilizer (NPK) 50%) obtained the highest leaves namber 10.5 whereas T12 attained the highest floret number 15.5, fresh wt of cut spike 86.9 mg and dry weight of cut spike 15.4 cm respectively.

In general addition of bacteria as PGPRs increased plant leaves number, stem biomass, plant height and plant dry weight. PGPRs properties maybe result of synthesis of phytohormons, solubilization of minerals and fungal activities **(Grobelak et al 2015)**.

The percentage of oil content /100 g mint plant **(Table 9 , Fig. 6)** was marked higher in both T8 (Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 50%) and T9 (Wastewater dilution with water 50:50 microbial treatment with PGPR and using fertilizer (NPK) 100%) where they both obtained the highest percent 1.6 %. All other treatments exhibited less percentage.

The role of PGPRs as rhizobacteria is considered very important where these PGPRs could promote plant growth by improving the nutrient status of host plants besides their ability to synthesize and secrete plant hormones like indole-3-acetic acid (IAA) Gibberellins (Gas), cytokinins and certain volatiles that altered in increase of oil content in ornamental plants **(Perez et al 2014)**.

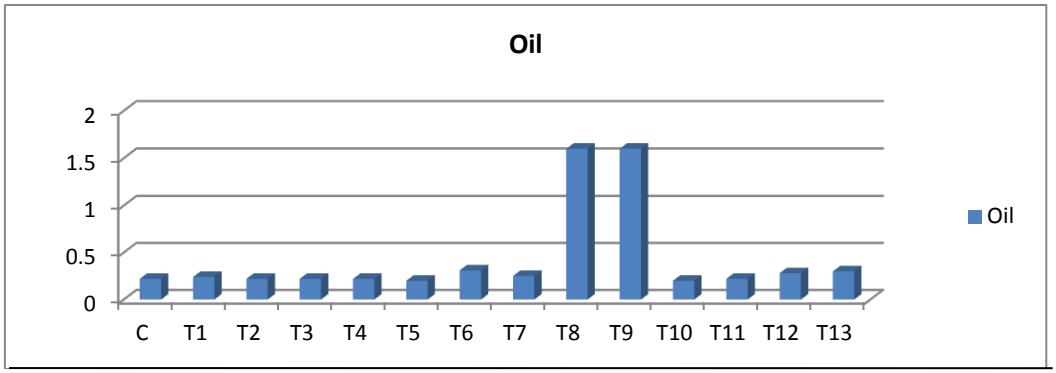

Fig. 6. The titration (%) in Mint/100g fresh *Mentha viridis* cv plant

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