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Influence of Soil pH on *Azotobacter* Population With Using Microbiological Characteristics as Bio-Measurement in Arable Lands of Tripoli. N. W. Libya

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Abstract: In the present study, 15 samples of soil were collected to isolate *Azotobacter* from the rhizosphere in different regions of Tripoli. LG specified medium was used for the isolation of bacteria and were purified on the same medium for identification and characterisation.The colonies were identified through microscopical and biochemical tests and the results obtained were classified as *Azotobacter* sp. Subsequently, the microbial population was calculated by colony count method. The soil pH, total nitrogen content (N), total phosphorus content (P) and organic carbon (OC) in soil were determined. The results of this study indicated to effects positive and negative of soil pH levels on *Azotobacter* population. In the estimation of above chemical properties of all soil samples it was showed that bacterial population differs significantly among the different soil samples.

Key words: *Azotobacter* population, Soil properties**.**

INTRODUCTION

The genus *Azotobacter* has a great attention to stimulate plant-growth-promoting rhizobacteria (PGPR), and their role in rising the growth and health of plants. Moreover, many other species have the ability to produce compounds with antimicrobial activity. The genus *Azotobacter* was discovered by Martinus Beijerinck in 1901. *Azotobacter* belongs to the phylum proteobacteria, class: Gammaproteobacter order: pseudomonadales, family Azotobacteraceae, comprises more species among them: *Azotobacter vinelandii*, *A. chroococcum*, *A. salinestris*, *A. nigricans*, *A. beijerinckii*, *A. paspali*, and *A. armeniacus* (Kennedy *et al*. 2005). *Azotobacter* is an aerobic free living diazotrophic bacteria generally distributed in different soils. *Azotobacter* play an important role in the nitrogen cycle in nature. In addition, the bacteria are the most significant genera found in rhizosphere gramineae (Dart and Day 1975). The plant growth is improved, both directly through nitrogen fixation, excretion of growth promoting and producing plant growth substances such as indole-3-acetic acid (IAA), increasing solubilization of mineral phosphates and indirectly through producing hydrogen cyanide, siderophore and antifungal antibiotics by means of the bacteria (Benizri *et al.* 2001). Several studies mentioned nitrogen fixation, production of phytohormones, vitamins and increasing of food uptake as the reasons for yield increase of inoculated maize with *Azotobacter* (Gonzalez-Lopez *et al.* 1991). *Azotobacter* inoculation with oak seedlings results in positive growth responses was suggested by (Pandey *et al.* 1986). Moreover the inoculation of barley grains with *Azotobacter* in leads to growth of plant

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length, dry matter, soil nitrogen content in sand and nitrogen deficient lands (Shehata *et al.* 2005). *Azotobacter* can produce antifungal antibiotics which inhibit *Rhizoctonia solani* growth (Zarrin *et al*. 2009), *Azotobacter* is found in many environments such as soil, water, surfaces of roots (rhizosphere) and leaves (phyllosphere). Also, some species appear in the tropical and polar regions. Their frequency is different in various soils. They are frequent in neutral to alkaline soils and rarely found in acidic soils (Jensen and Petersen 1955) *Azotobacter* is gram–negative, nitrogen–fixing soil bacteria that have extremely high respiration rates. *Azotobacter* can fix at least 10 mg nitrogen per gram of carbohydrate (Becking 1992). This bacterium is an obligate aerobic. Nitrogen fixation is achieved by the enzyme nitrogenous, which reduces $N_2$to NH_3. However, this enzyme is extremely sensitive to oxygen in *Azotobacter* species. High respiration rates and conformational protection of the enzyme are suggested as two factors which make nitrogen fixation possible in an aerobic environment (Hill and Sawers, 2000) Reduction of O_2 by *Azotobacter* species occur at such a high rate that large amounts of superoxide radicals are produced (Vikhe 2014). *Azotobacter* is a free-living fixing bacteria and related to soil organic components, and the amount of nitrogen fixation is lower in *Azotobacter* compared to the associative and symbiotic bacteria as reported by (Hammad 1998).The ecological distribution of *Azotobacter* is a complex subject and related to a variety of factors which determine the presence or absence of this bacterium in soil. It has been demonstrated soil properties and climate conditions are two most important factors that affect the distribution of this microorganism (Dobereiner and Pedrosa 1987). These characteristics include organic matter content, moisture, pH and C/N ratio (Gonzalez-Lopez *et al.* 1991). Different studies showed that some *Azotobacter* mutants can fix N_2 in the presence of excess NH_4^+ which is related to *Azotobacter* industrial applications(Terzaghi 1980). The mutants are of industrial significance, because they hinder mobilization in alginate beads and provide the opportunity to produce ammonia (which can be used as plant fertilizer). So *Azotobacter* is used in biofertilizer and biotechnological processes (Tejera *et al.* 2005).

As well as, this study aimed to address the effect of the chemical properties of different soil samples in different regions of Tripoli as soil pH, total nitrogen content (N), total phosphorus content(P) and organic carbon (OC) on *Azotobacter* population.

MATERIALS AND METHODS

Collection of Soil samples : This experiment was conducted in Soil microbiology laboratory at Faculty of Agriculture, University of Tripoli at the end of Winter season of 2016, Fifteen soil samples were collected from the different cultivated and uncultivated regions in Tripoli area. 1 kg of soil was collected randomly from the rooting zone at a depth of (5- 30 cm) below the surface with three replicates of each of soil samples. Prior to commencement of the experiment, bulk soil samples were air-dried, cleaned and passed through a 5 mm sieve to determine particles chemical analysis.

Measuring of Soil chemical properties

microbiological properties: The chemical properties of soil mean most chemical interactions with or between minerals in soil environment. Such as soil ph, Cation Exchange Capacity, Basic Saturation…ect. While microbiological properties of soil belong biological activity in soil, such as N-fixation, humus formation. Which include microorganisms activity in soil environment. The pH of soil was measured using pH meter. Organic carbon was observed by using the method of (Walky and Black 1934) and Seeley and Vandemark (1981). The estimation of total nitrogen was done by using the Kjeldahl method and the total phosphorus content (P) was analysed using Olsen method by extracting soil samples with 0.5M $NaHCO_3$ (pH 8.5) at a solid to solution ratio 1:20 for 30 min (Olsen 1954) and using Spectrophotometer at 660nm wavelength (Table1).

Table (1). Chemical and microbiological properties of soil samples

Soil samples	*Azotobacter population (1gram soil x 10.0000)*	pH	Total N%	Total P%	OC%
1	4.98	7.3	5.87	7.96	2.14
2	6.5	7.1	57.16	11.9	3.63
3	4.37	7.3	33.53	22.88	1.88
4	1.17	8.4	5.23	23.4	1.63
5	2.84	8	8.25	20.96	1.52
6	1.13	8.2	1.35	14.9	2.68
7	5.33	7.8	34.75	19.3	2.34
8	5.7	7.23	33.08	14.19	2.79
9	3.8	7.5	27.58	19.85	1.42
10	5.65	7.5	44.62	15.66	3.22
11	5.63	7.4	47.04	19.90	0.76
12	6.13	7.2	59.75	23.2	4.7
13	5.49	7.17	37.98	5.70	2.79
14	4.23	7.4	32.58	22.89	3.08
15	1.95	8.1	3.8	12.56	1.736

Isolation of *Azotobacter:* The soil paste–plate method of (Becking 1981) was used to Isolate of *Azotobacter* from soil samples. Each soil sample was mixed thoroughly with approximately 0.5 g of mannitol, 0.5 g of $CaCO_3$, 0.12 ml of 10% aqueous K_2HPO_4 solution, 0.12 ml of 10% aqueous $MgSO_4$ solution, and some extra distilled water was also added in order to obtain a soil paste, and then incubated at 30°C for 48h. Then brown, glistening, slimy *Azotobacter* colonies were grown on the soil surface. Subsequently, brown blots of soil paste surface were placed on Jensen medium and purified (Subba Rao, 1993). Bacterial colonies were transferred to plates of the same medium.

Identification of bacteria: Isolates were cultured on plates of N-free LG medium for identification and characterization. In gain isolates from each soil samples were Gram-stained using standard procedures. Morphology characterization was determined using a compound microscope in oil immersion (1000 x) about 100 colonies were chosen at random at all the colonies from the rhizosphere of soil samples whatever their size, shape and color were transferred onto other plate to check for purity. All the colonies grown on the plates were about 1mm diameter and white with flat margins initially glossy and gummy but turned into glistening colonies with clear slime upon further growth (Brenner *et al.* 2004). The following biochemical tests were used: catalase, oxidase, nitrate reduction and movement (Seeley and Vandemark 1981). Moreover, the carbon sources utilization test was determined by using the phenol red medium and dispensed into sterile test tubes. Then, 0.5% (w/v) of the glucose, fructose, malonate, mannitol, caproate, inositol, malonate, rhamnose and starch were separately added to 24 h old inoculated culture and incubated at 30°C for 24 h. Temperature is perhaps the most important environmental factor determining the activity of microorganisms in soil. The effect of temperature on the growth rate was determined by patching the bacteria on to the LG medium and incubated at different temperatures 15, 18, 21, 32, 37°C. The growth of bacteria colonies until 5 days after the incubation indicated their ability to grow in the cited temperatures. Motility was assessed using a Craigie tube with a semi-solid medium Nitrate reduction was tested by inoculating trypticase-nitrate tubes with the colonies and then incubating at 27°C for 48 h. One ml of sulfanilic acid was added to each tube,

and then 1 ml of dimethyl 1-naphthylamine solution (Seeley and Vandemark (1981). Some of the pure isolates from each soil samples were defined by direct use of microscopic morphological characteristics and compared to some of the known and available cultures and then were characterized using the criteria of (Brenner *et al*. 2004).

Estimation of *Azotobacter* population:To estimate numbers of *Azotobacter* in each soil sample the colony count method was used (Cappuccino and Sherman 1987). Ten grams of soil sample was transferred into the 250 ml of the conical flask containing 90 ml of sterilized distilled water and was shaken for 30 min at 150 rpm, and 1 ml of this solution was added to the test tubes containing 9 ml sterilized distilled water to prepare 10^{-2} dilution. The latter solution was mixed and one ml of this solution was transferred to another test tube containing 9 ml sterilized distilled water to prepare 10^{-3} dilution again and the same method was followed to prepare 10^{-5} dilution. Subsequently, 0.1 ml each of the dilutions was transferred to a plate containing Jensen medium and was dispensed to the above medium equally. Three replicates were maintained for each sample. 50 mg cycloheximide was added to medium as fungal growth inhibitor. The plates were incubated at 30°C for 3-7 days and *Azotobacter*–like colonies were counted. The dilutions with colony number between 10 – 60 colonies were accepted. The average colony number was calculated in the three replicates multiplied in ten and the reverse of appropriate dilution.

Statistical Analysis: The data were subjected to correlation analysis of variance using statistical program (SPSS software) Table (2). The differences among various treatment means were compared using Tukey's family error test (standard deviation) at a probability of P = 0.05.

RESULTS AND DISCUSSION

Isolation of *Azotobacter:*The pure isolates of bacterial colonies were sub cultured from the 60 isolates on the LG medium for further studies. The colonies formed by these bacteria on the LG medium were small, transparent, circular, flat, and slimy with regular border (Fig 1).

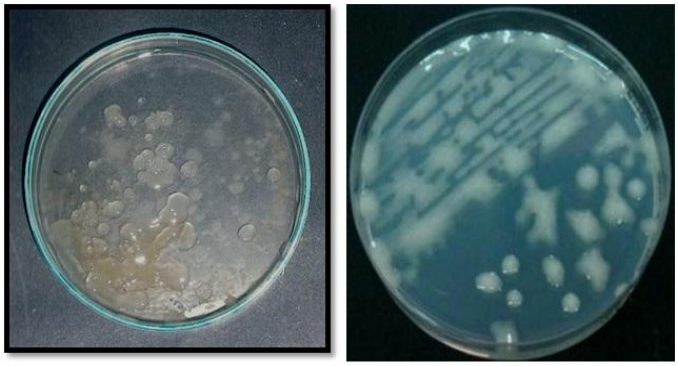


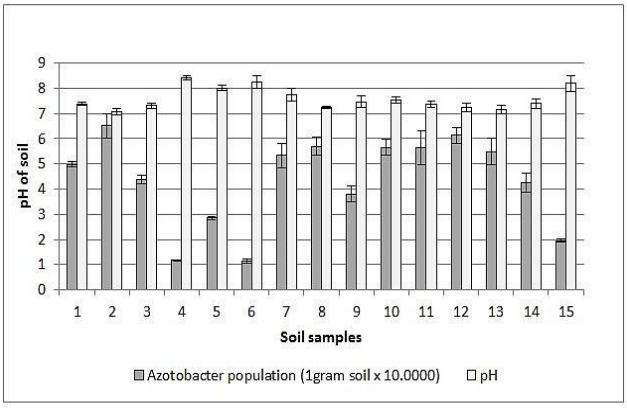
Fig (1). Colonies of *Azotobacter* on LG medium Incubated at 30°C for 3-7 days

Bacteria were Gram-negative with rounded ends. Also, the isolates produced yellow-green and brown pigments and were put in one group. Biochemical and morphological characteristics of these bacteria included the following: motile, catalase positive, oxidase activity positive and Nitrate reduction positive. The utilization of glucose, fructose, malonate, mannitol, caproate, inositol, malonate, rhamnose but not starch was detected. Bacteria grew well in LG medium with 15, 18, 21, 32, 37°C temperatures. On the basis of cultural, morphological and biochemical characteristics a total of 15 soil isolates were classified according to (Brenner *et al.* 2004) as *Azotobacter* sp. It is in agreement with the obtained results by (Ahmad *et al.* 2008).

Relationship of chemical properties of soil with *Azotobacter* population:

Soil pH : The soil pH are definition as the negative logarithm of the hydrogen ion concentration pH = -log (Bashan).The soils are referred to as being acidic, neutral or alkaline, depending on their PH values, also these categories of soils are dividing to group of classes according to degrees of acidity of soil. Among these classes soil neutral is 6.5-7.5 PH, and soil slightly alkaline is 7.5-8.0 PH, while soil moderately alkaline is 8-8.5 PH. In this study the

Azotobacter population was determined in different 15 soil samples. The result showed that all samples contained *Azotobacter* and the high population of *Azotobacter* was observed in soil samples with the range of pH 7 - 7.5. whereas *Azotobacter* population relatively continue in range PH of soil slightly alkaline, while *Azotobacter* population was decline as soon as commence at a zone of moderately alkaline soil as in fig.(2). Also observed through soil samples 4,5,6 and 15 from table (1) and fig.(2) decrease in the amount of total N% in soil with decrease in the *Azotobacter* population while happen increasing in soil alkaline levels, on other hand the opposite was happen in soil samples 2 and 12. This explain an existence increasing relationship between *Azotobacter* population and total N% in neutral soils.



Error bars represent the standard deviation ± SD

Fig (2). The relationships between soil pH with *Azotobacter* population

Several studies indicated that the soil pH value influences the *Azotobacter* population (Jensen and Petersen 1955). The studies showed that all soils with pH of above 7.2 (pH range 7.3 - 8.5) contained *Azotobacter* and, in the pH ranges of 7.0 - 7.4, 6.5 - 6.9, and 6.0 - 6.4, the percentage of *Azotobacter* was 90, 58, and 35%, respectively (Gonzalez-Lopez *et al.* 1991, Kanungo *et al.* 1997) has indicated that the optimum pH for the growth of *Azotobacter* sp. is near to 7. Also, (Becking 1981) noted that *Azotobacter* population in tropical soils with pH of above 7.5 differs between 10^2 and 10^4 per gram of soil. Various studies proved the linear relationship between soil bacterial communities and pH value. Then, other studies showed bacterial population in the range of pH 4-8 and observed that increasing pH value and bacterial population are interrelated (Rousk *et al.* 2010).

Total Nitrogen (N): Nitrogen is a major limiting nutrient for crop production, in case absence of a source of nitrogen compound, plant need to organisms for fixed atmospheric nitrogen. from table (1) notice, increasing of nitrogen percentage in soil which was correspond to increasing of *Azotobacter* population in soil. this mean there is relationship between *Azotobacter* growth and nitrogen fixation in soil fig.(3). however this relationship was limiting with soil PH levels, although major soil PH values for soil samples which examined were situated between neutral to moderately alkaline soils, nevertheless *Azotobacter* appearing tend to growth in neutral soils more than slightly alkaline soils. whereas *Azotobacter* growth recorded fast retreat in moderately alkaline soils. table(1). (Bashan 1990) reported that, the *Azotobacter* population is low in dry and temperate zones like America and Mexico. The total nitrogen contents were suggested as the factors influencing the microbial population (Ahmed *et al*. 2008).

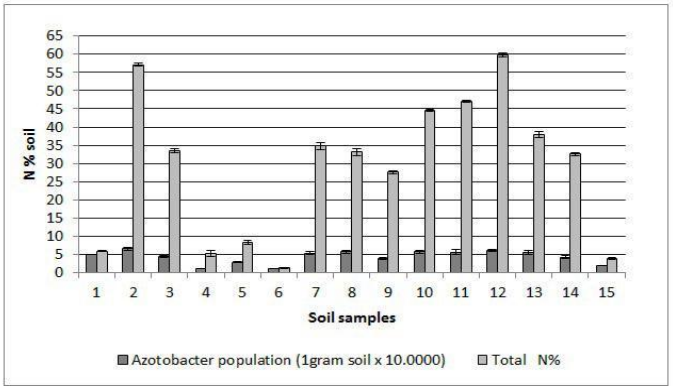


Fig (3). The relationships between total Nitrogen (N%) with *Azotobacter* population

Total phosphorus (P): In this study, the soil samples which had neutral PH such as 2,12,and 13 in table(1) appearing various values of total

phosphorus percentage in soil with *Azotobacter* population, whereas soil samples of moderately alkaline soil 4,5,6 and 15 table(1). Showing increasing in total P%. as opposite to *Azotobacter* population fig.(4). But these changes in total P% do not explain the decrease in *Azotobacter* growing in alkaline soil, because organic phosphorus decrease quickly with soil depth such as soil organic matter. Secondly the source of P in soil. in case of the source of available P in soil Ca phosphates the level of soil PH will changes from neutral to high alkaline, while in case Al and Fe phosphates are predominates P mineral in soil with PH levels below 6.5. Therefore, the value of soil PH above 8 was probably responsible for the decrease of *Azotobacter* population in soils of region of study. Some studies reported that, the native soil P is mostly unavailable to plants because its low solubility, therefore the P solubilizing bacteria and *Azotobacter* sp can play an important role in improving P bioavailability in soil, on the other side the population of rhizobacteria which includes *Azotobacter* had a different influence on phosphorus in soil (Wu *et al.* 2005). phosphorus is also a major nutrient for microorganisms and suggested to be the factors influencing the microbial population.

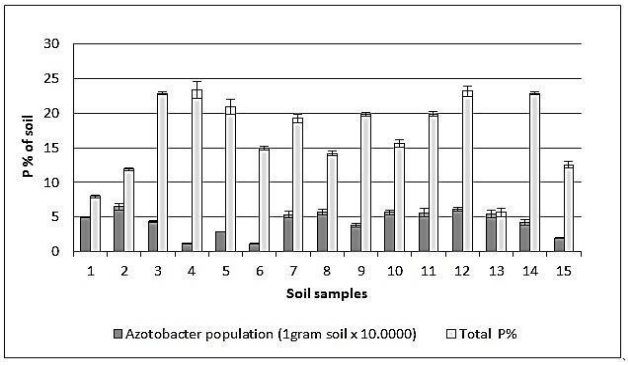


Fig (4). The relationships between total phosphorus (P%) with *Azotobacter* population

Organic Carbon (OC) : The organic carbon in soil are an importance indicator for existence soil organic matter. Through soil samples which were contain high percentage of O.C as,2,10, 12 and 14 in table (1) and fig. (5). Also, observed at same time increasing in the *Azotobacter* population and total N percentage at neutral soil 7-7.5 PH. On other hand, soil samples which were contain low O.C such as 11,9,5 and 4 do not appearing any response to *Azotobacter* population, particularly soil samples (6 ,11) which showing a clear disagreement in their contain of O.C and *Azotobacter* population. So, that mean do not there any direct relationship between O.C % and *Azotobacter* population at soils of region of study.

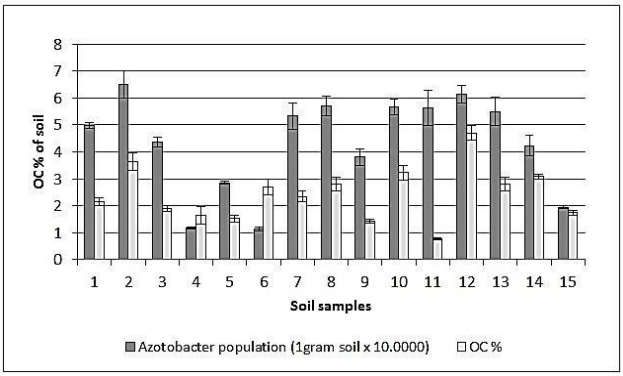


Fig (5). The relationships between Organic carbon (OC %) with *Azotobacter* population

A range of environmental factors like pH, organic carbon, total N and total P determine and influence the distribution of soil microbial population (Kennedy and Smith 1995). Organic carbon is one of the main factors influencing the number, composition and activities of microbial population(Wardle 1992). Lalfakzuala *et al*. (2008) found that gramineae influenced soil microbial number and soil respiration positively. Organic carbon affects both the chemical and physical properties of the soil (Channal *et al.* 1989). Properties influenced by organic matter include: soil structure, diversity and activity of soil organism, which might be beneficial and harmful to crop production. Soil organic matter is an accumulation of dead plant matter and animal residues (Campbell *et al.* 2000). Furthermore, The findings from this study showed that there was a Linear relationship ($p<0.01$) was observed in different soil samples for bacterial population as shown in Table (2) and significant relationship between soil pH, total N, total P and organic

carbon with microbial population, so that the number of bacterial population per gram of soil increased by increasing the compounds which, indicated, there is a significant relationship between the soil organic and mineral matters on the microbial population (Coutinho *et al*. 1999).

Table (2). Relationship with soil pH, total nitrogen, total phosphorus and organic carbon between bacterial population

Variables	Coefficient Correlation (r)
pH	0.93**
Total N	0.95**
Total P	0.90**
OC	0.75**

CONCLUSION

In conclusion, this study has shown that a significant correlation between soil pH, total nitrogen, total phosphorus and organic carbon the chemical properties of different soil samples from different soil regions of Tripoli- Libya on *Azotobacter* population which had a greater influence on it.

REFERENCES

Ahmad, F. Ahmad, I. and Khan, M. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research 163(2):173-181.

Bashan, Y. (1990). Short exposure to Azospirillum brasilense Cd inoculation enhanced proton efflux of intact wheat roots. Canadian Journal of Microbiology 36(6):419-425.

Becking, J.-H. (1981). The family Azotobacteraceae. Pages 795-817 The prokaryotes. Springer.

Becking, J. H. (1992). The family Azotobacteraceae. Pages 3144-3170 The Prokaryotes. Springer.

Benizri, E. Baudoin, E. and Guckert, A. (2001). Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Science and Technology 11(5):557-574.

Campbell, C. Zentner, R. Selles, F. Biederbeck, V. McConkey, B. Blomert, B. and Jefferson, P. (2000). Quantifying short-term effects of crop rotations on soil organic carbon in southwestern Saskatchewan. Canadian Journal of Soil Science 80(1):193-202.

Channal, H. Alagawadi, A. Bharamagoudar, T. Udupa, S. Patil P. and Mannikeri, I. (1989). *Azotobacter* population as influenced by soil properties in some soils of North Karnataka. Current science. Bangalore 58(2):70-71.

Dart, P. and Day, J. (1975). Nitrogen fixation in the field other than by nodules. Soil Microbiology. Butter Worth Sci. Publication, London.

Dobereiner, J. and Pedrosa, F. O. (1987). Nitrogen-fixing bacteria in nonleguminous crop plants. Science Tech Publishers.

Gonzalez-Lopez, J. Martinez-Toledo, M. Reina, S. and Salmeron, V. (1991). Root exudates of maize and production of auxins, gibberellins, cytokinins, amino acids and vitamins by *Azotobacter* chroococcum in chemically-defined media and dialysed-soil media. Toxicological & Environmental Chemistry 33(1-2):69-78.

Hammad, A. (1998). Evaluation of alginate-encapsulated *Azotobacter* chroococcum as a phage-resistant and an effective inoculum. Journal of Basic Microbiology 38(1):9-16.

Jensen, V. and Petersen, E. (1955). Taxonomic studies on *Azotobacter chroococcum* Beij. and *Azotobacter beijerinckii* Lipman. Royal Vet. Agric. Coll. Copenhagen Yearbook:107-128.

Kanungo, P. Ramakrishnan, B. and Rao, V. R. (1997). Placement effects of organic sources on nitrogenase activity and nitrogen-fixing bacteria in flooded rice soils. Biology and Fertility of Soils 25(2):103-108.

Kennedy, C. Rudnick, P. MacDonald, M. and Melton, T. (2005) Genus III: *Azotobacter*. In: Garrity GM, editor. Bergey's Manual of Systematic Bacteriology. The Proteobacteria, Part B, the Gammaproteobacteria. 2nd edition. Vol. 2. New York, USA: Springer. 384- 402.

Kennedy, A. C. and Smith, K. (1995). Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil 170(1):75-86.

Olsen, S. R. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department Of Agriculture; Washington.

Pandey, R. Bahl, R. and Rao, P. (1986). Growth stimulating effects of nitrogen fixing bacteria (biofertiliser) on oak seedlings. Indian Forester 112(1):75-79.

Rousk, J. Bååth, E. Brookes, P. C. Lauber, C. L. Lozupone, C. Caporaso, J. G. Knight, R. and Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4(10):1340.

Shehata, S. Saleh, S. and Junge, H. (2005). Response of sexual expression and productivity of squash plants to some biofertilizer treatments. Egypt Journal Applied Science 20(12B):680-690.

Tejera, N. Lluch, C. Martinez-Toledo, M. and Gonzalez-Lopez, J. (2005). Isolation and characterization of Azotobacter and Azospirillum strains from the sugarcane rhizosphere. Plant and Soil 270(1):223-232.

Terzaghi, B. E. (1980). Ultraviolet sensitivity and mutagenesis of Azotobacter. Microbiology 118(1):271-273.

Vikhe, P. (2014). *Azotobacter* species as a natural plant hormone synthesizer. Research Journal of Recent Sciences 3:59-63.

Walky, A. and Black, I. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid in soil analysis. 1. Experimental. Soil Sciences 79:459-465.

Wardle, D. (1992). A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67(3):321-358.

Wu, S. Cao, Z. Li, Z. Cheung, K. and Wong, M. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125(1-2):155-166.

دراسة مدى تأثير حموضة التربة pH على مستعمرات بكتيريا *Azotobacter* باستخدام الخصائص الميكروبيولوجية كمقياس حيوي في الأراضي الزراعية بمنطقة طرابلس شمال غرب ليبيا

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المستخلص : جنس *Azotobacter* لديه القدرة على تحفيز– تعزيز نمو النبات (PGPR)، ودورها في رفع ونمو وصحة النباتات. وعلاوة على ذلك، فالعديد من الأنواع الأخرى لديها القدرة على إنتاج مركبات منها المركبات المضادة لنشاط الميكروبات. في هذه الدراسة جمعت 15 عينة من منطقة الجذور لتربة مزروعة في مناطق مختلفة من طرابلس شمال غرب ليبيا لتحديد درجة تفاعل التربة pH، ونسبة النيتروجين الكلي (N)، الفوسفور(P) والكربون العضوي (OC) في التربة وأيضا لعزل بكتيريا *Azotobacter* و استخدمت البيئة الغذائية LG لعزل البكتيريا وتنقيتها على نفس البيئة لوصفها و تعريفها وقد تم تحديد جنس البكتيريا من خلال الفحص المجهري والاختبارات البيوكيميائية للعينات، وأظهرت النتائج أن البكتيريا المتحصل عليها تابعة لبكتيريا *Azotobacter* وفقا لدليل Bergey's Manual of Systematic Bacteriology (2004) وقد تم حساب أعدادها عن طريق العد للمستعمرات البكتيرية لتحديد مدي تأثير الخواص الكيميائية للترب على هذه المستعمرات. نستنتج من نتائج هذه الدراسة أن المستعمرات البكتيرية لبكتيريا *Azotobacter* تتأثر إيجابياً وسلبياً وفقا لمستويات حموضة التربة pH.

الكلمات المفتاحية: بكتيريا *Azotobacter* ، خواص التربة ، أعداد البكتيريا.

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