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SURVIVAL OF AZOSPIRILLUM BRASILENSE UNDER DIFFERENT MOISTURE CONTENT LEVELS

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Abstract:-

The survival and proliferation of free and alginate immobilized, Azospirillum brasilense in a bulk soil collected from Derna region at different moisture content levels (10%, 40% and 60 %). A reduction of cell number in the experimental units (sterile or non-sterile soil) for all the tested moisture levels was observed after 15 days after the inoculation. Nevertheless, different parameters among the level of moisture content could be observed in the reduction of the population .soil-sterilization did not affect Azospirillum survival and proliferation, and results presented in results show that Azospirillum brasilense total content was not affected by the sterility of the soil. In general, Azospirillum brasilense strain isolated from Derna soil survived well and for long period (75day) in bluk soil (without plant roots). In this study the presence of moisture in the soil as well as the soil- type of Derna which is rich in organic matter maintained Azospirillum brasilense population of a level high enough to be detected 75 days after inoculation.

Keywords:-*Azospirillum brasilense, Survival, Sterilized, Bulk soil, Derna*

INTRODUCTION

There is not enough information about PGP stay in the soil in the absence of plant (1). Even though it has been isolated *Azospirillum* successfully from the free soil (free plant) (1). Was published contradictory researches on the survival *Azospirillum*. Where in some soils; it was survival *Azospirillum* for long periods, such as the Nigerian soil that is stored in the laboratory of the United Kingdom where it was observed *Azospirillum* after 10 years (2). And when was introduced A. *lipoferum* into the soil decreased census bacteria rapidly to less than 10^2 cfu g^{-1} soil and then returned to get better remained constant at about 10^3 cfu g^{-1} soil to a period of 120 days, (3). Survival *A. brasilense* and *A.amazonense* improved to increase moisture content in the soil (4). It observed after inoculated forage crops with *A. brasilense* Cd declining population of bacteria through the disappearance of 15 days from the soil during the 25 days of the soil (5). *A. brasilense* Cd the declining numbers to less than 10^2 CFC g^{-1} during the six weeks and disappeared from the soil (6).

MATERIALS AND METHODS

Microorganisms: A. brasilense isolate has been obtained from free soil in Hai Al-salam district of Derna, Libya

Immobilization of *Azospirillum brasilense* isolate in alginate pellets:

A. brasilense isolate was immobilized by entrapment in 2% Ca-alginate. Cells encapsulated in alginate pellets were prepared by using the method applied at our laboratory (7-8-9). Briefly, 25 ml of bacteria cell suspension under aseptic condition were added to75 ml of sterile alginate solution to obtain a final concentration of 2% alginate. In some cases skim- milk (2.5%w.v) or starch (2.5%) was added as adjuvant. The mixture was vigorously stirred to allow a homogenous dissolution of alginate. Then the mixture was extruded through sterile plastic nozzle with a diameter of 1mm and the resulting drops were then projected into sterile 6 g/1 $CaCl_2$ solution forming small calcium alginate matrix beads (2mm, mean diameter) entrapping bacterial cells. The beads were maintained in $CaCl_2$ solution at room temperature for additional 1-2h to obtain regular solid beads. The $CaCl_2$ solution was pumped out, and the beads were washed twice with sterilized distilled water. Fresh beads were either used directly as fresh, or kept at 4-5˚C in sealed flacks for several days. Bacterial cells within 0.1g pellets were calculated after dissolving the pellets in phosphate buffer (pH7) solution by diluted agar plate. Survival of *Azospirillum brasilense* isolate in Derna soil under different moisture content levels.

Aliquots of 0.1ml of dense bacterial inocula (prepared by growing the bacterial strain in NB for 24 h was used to inoculate 10 g of sterilized and non-sterilized soil in glass test tubes to final concentration of 10^9CFC/g soil as free or alginate immobilized cells. The test tubes were sealed and vigorously agitated in order to distribute the inocula.

The used soil was derived from Derna region with the following characteristics: pH 8; sand 25%; Silt42% ; and clay 33%. The soil was dried, and sifted through 2 mm mesh to obtain regular soil particles. Glass test tubes of 30 ml were filled with 10 grams of the soil, and closed with a rubber stopper. The tubes were sterilized three times by autoclaving with 48 hour interval between each sterilization. Three moisture levels were tested, 10%, 40% and 60%. Moisture was adjusted with sterile distilled water after inoculation with bacterial inocula. The inoculated soils were incubated at 30°C in the dark until sampling time.

The sampling times were 15, 30, 45, 60 and 75 days after inoculation. A soil sample (10 g) was resuspended in sterile distilled water (90 ml), and a series of dilutions were prepared. Total *Azospirillum* counts was estimated using the plate count method and the selective DN medium with Congo-red (0.25 % aqueous solution), (10). The experimental units were the glass tubes with 10 g soil, sampled in triplicate for each moisture level and for each sampling time. A control test tubes without inoculation were also included.

Results

The effect of many biotic and abiotic variables on *Azospirillum* survival and proliferation in bulk soil (no roots) have been studied later (11). However, few studies have evaluated the survival of PGP bacteria *Azospirillum* under different moisture content. Results presented in Table (1-2) and Figs (1-2) show the survival and proliferation of free and alginate immobilized *A. brasilense* in a bulk soil collected from Derna region at different moisture content levels (10%, 40% and 60 %). A reduction of cell number in the experimental units (sterile or non-sterile soil) for all the tested moisture levels was observed after 15 days after the inoculation. Nevertheless, different parameters among the level of moisture content could be observed in the reduction of the population (Fig. 1 to 2). In general increased moisture content promoted cell cultureability when inocula was used as free cell in both sterile and nonsterile soil. However, the moisture content was not effective on cell total number in case of immobilized cells, and a constant high *Azospirillum* numbers were obtained tell the end of experiments (75 days). These results agree with previous observations of (12), working with *A. brasilense* strain Cd in two natural soils and in homologous artificial soil. Moreover, (8) showed that when dense population (10^7 cfu/g soil) of *Herbaspirillum* spp. was added directly to the natural soil (sand and clay), the cell concentration was reduced to less than 10^2 cfu/g soil after 50-d incubation. On the other hand, cell entrapped in an alginate matrix survived better and the wet beads released bacteria into soil with a population concentration of about 10^6cfu/g soil. Encapsulated cell survived better in clay-loamy than sandy soil. In this study the encapsulation *A. brasilense* was not affected by the soil moisture content, this could be explained on the basis that alginate matrix provides a mechanical barrier thus protect the encloused cells against the soil variable. These results are in accordance with findings of (13), they showed that alginate entrapped *Trichoderma* cells improved the production of proteolytic enzymes even in the presence of different benomy (fungicide) concentrations, and alginate immobilization provides physical protection against benomyl.

Interestingly, soil-sterilization did not affect *Azospirillum* survival and proliferation, and results presented in Table (2) show that *Azospirillum* total content was not affected by the sterility of the soil.

In general, *A. brasilense* strain isolated from Derna soil survived well and for long period (75day) in bluk soil (without plant roots). Among the mechanisms used by *Azospirillum* to overcome unfavorable conditions are: cyst formation,

flocculation, melanin production, synthesis of poly-β-hydroxybutrate (PHB), Polysaccharide synthesis and association with other microorganisms (e.g mycorrhizal fungi) (1). Indeed results of this study and laboratory studies demonstrate the importance of the physical state of the cells for their permanence in the soil after the inculcation (14). The effect of the soil moisture on survival of *Azospirillum* spp. can be of either a physical or physiological nature. Soil moisture affects the motility of the bacteria, favoring the chemotactic behavior (1). Clay content, nitrogen, organic matter and water retention capacity of the soil are positively correlated with the viability.

A. brasilense sp245 (11). (4) Indicated that soil moisture content extended the survival of *A. amazonense*. The degree of adsorption of the bacteria to soil particles is influenced by surface electrical charges, which in turn are affected by soil moisture and pH. A decrease in soil moisture or an increase in soil pH reduce the adsorption of bacteria to soil particles (1) since PGP bacterial species survive poorly in soil, in the absence of a host plant soil moisture allows the migration of the bacterial towards root exudates or more favourable niches.

In this study the presence of moisture in the soil as well as the soil- type of Derna which is rich in organic matter maintained *A. brasilense* population of a level high enough to be detected 75 days after inoculation. The more stable abiotic factors affecting bacterial survival in the soil are the soil structure and texture (15-8). In addition, amendment of the loamy sandy soil with the clay mineral bentonite substantially improved the survival of introduced rhizobi (16), possibly due to the presence of protective microhabitats created by bentonite (17).

Table (1): Survival of *Azospirllium* st. in sterilized soil under different moisture levels (No. x10^{3}cfu/g soil).
***M.C: Moisture Content**

	Days/M.C*(%)	15	30	45	60	75
Free	**10**	86	85.5	81	79	72
	40	218.5	105.5	94	115	114
	60	181	107	106	91	43.5
Immobilized	**10**	204	169	111.5	104.5	47
	40	173	122	102	101	75.5
	60	277	106	105	78	45.5

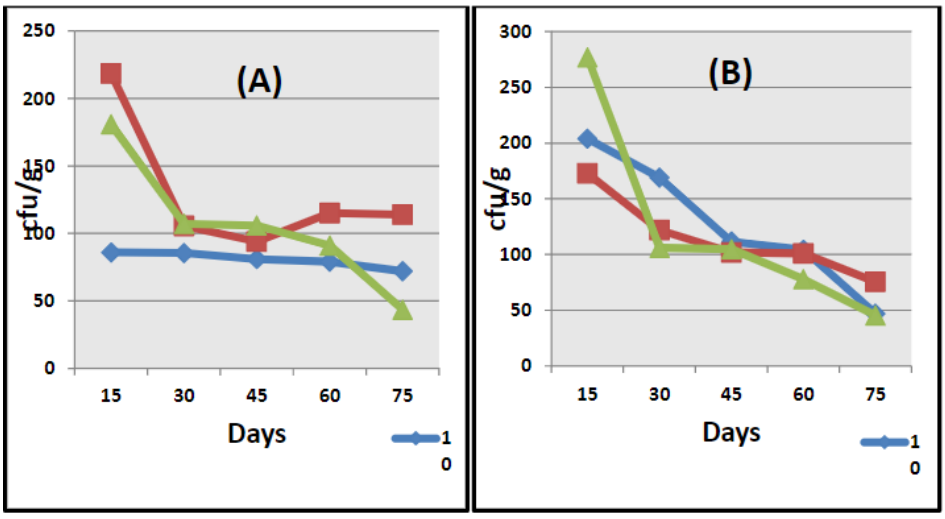

Fig. (1) : Survival of *Azospirillum* st. in sterilized soil under different moisture levels; (A) Free cells, (B) Immobilized cells

Table (2): Survival of *Azospirillum* st. Free or immobilized in nonsterilized soil under different moisture levels (No. x10^{3}cfu/g soil)

	Days/M.C*(%)	15	30	45	60	75
Free	**10**	101.5	96	88	77	53.5
	40	133.5	111	97.5	84	73.5
	60	196	119.5	106	93	82.5
Immobilized	**10**	172	117.5	100.5	101	92.5
	40	153.5	146.5	96.5	96.5	109
	60	140.5	120	100	91	62

***M.C: Moisture Content**

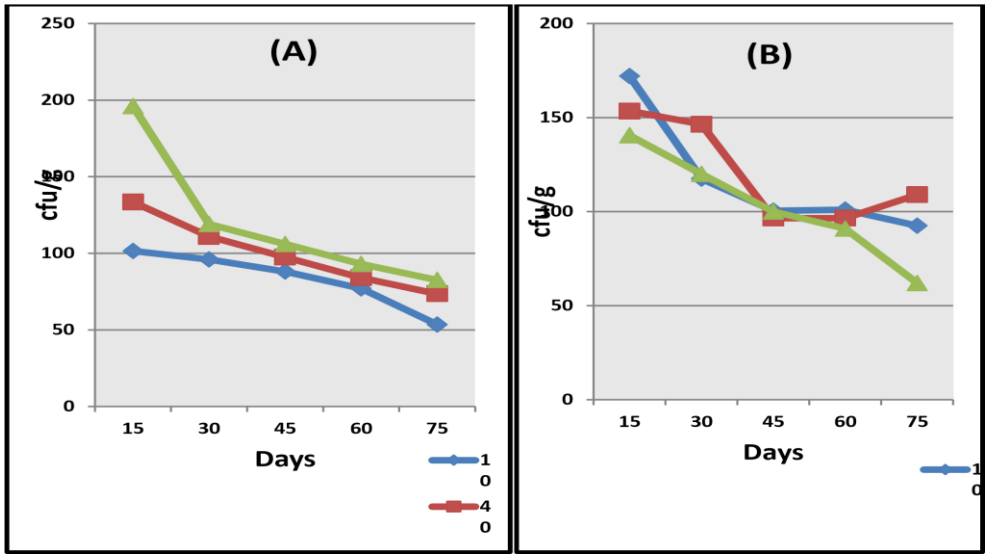

Fig. (2): Survival of *Azospirillum* st. in nonsterilized soil under different moisture levels; (A) Free cells, (B) Immobilized cells

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