# EFFECT OF RHIZOBIA ISOLATED FROM SOME ACACIAS ON GROWTH OF *ACACIA NILOTICA* UNDER SOME STRESS CONDITIONS IN NORTH AFRICA.

## 1- Characterization of Rhizobia isolated from Egyptian *Acacia* and development of a respective Rhizobial inoculant.


## ABSTRACT

Rhizobia isolates isolated from nodules of Egyptian Acacia (*A. saligna*) were purified. Selected isolates were studied comparatively with the reference strains obtained from Senegal and ARC, Egypt. Factors affecting their growth including temperature, salt and pH revealed that all selected isolates and reference strains failed to grow at 50°C, and grew heavily at 30°C, however slight or no growth was observed at 15°C. Most of selected isolates and reference strains grew in the presence of NaCl concentrations up to 0.5 M. Optimal growth was obtained at pH 7. viscosity of the tested strains as indicated by exopolysaccharide production which ranged between 0.08 to 0.19 cP. Inoculations with the tested strains increased number and dry weight of nodules/plant and nitrogenase activity. Perlite as local material gave the best result as a carrier in supporting rhizobial survival, increasing dehydrogenase activity, number and dry weight of nodules/plant, and nitrogenase activity. Perlite + vermiculite + peat (2:1:1) and vermiculite: peat (1:1) as carriers also supported inoculum but at a lesser degree.


**Keywords:** *Acacia*, *Rhizobium*, stress conditions, carrier inoculation

## INTRODUCTION

Trees of the genera *Acacia* are of considerable importance in the rural economy of many of the world's arid and semiarid areas. *Acacia* genera provide high-quality animal fodder, timber, fuel wood, charcoal, gums, and other products. Because of their association with Rhizobia, acacias fix nitrogen and therefore contribute to the improvement of soil nitrogen status **(Lal and Khanna, 1993**). In addition Acacias are used successfully for dune stabilization and reforestation **(F**agg and Stewart, **1994; Lal and Khanna, 1995; Munzbergova and Ward, 2002; Gal and Choi, 2003).**

*Rhizobium* is the most well known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. All rhizobia belong to the alpha subgroup of the Proteobacteria, based on the sequences of the gene cloning coding for small-subunit (16S) rRNA **(Maidak *et al.*, 1997).**

Recent reports support the finding that, many stress conditions e.g. salt, heat, and acid stresses affect the survival and distribution of rhizobia in soil and the rhizospheres of several plants **(Johnson *et al.*, 1981; Tate, 1995; Mashhady *et al.*, 1998; Ramos *et al.*, 1999; Zahran, 1999).**

Legume inoculation are composed of rhizobial cultures alone or rhizobial cultures inoculated into a carrier material for application to legume seeds. Therefore, the survival of rhizobia depend on the type of carrier materials such as finely ground peat, vermiculite, perlite, etc, **(Peterson and Loynachan, 1981; Kremer and Peterson, 1983; Daza *et al.*, 2000).**

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The objectives of this work were to isolate and study the characteristics of rhizobia isolated from *Acacia saligna* against reference strains in order to evaluate their efficiency for nodulation associated with *Acacia nilotica* subsp. *tomentosa*. In addition, a study was made to select suitable local carrier materials to prepare an inoculant.

## MATERIALS AND METHODS

This study was carried out at the experimental laboratory of the Microbiology Department, Soils, Water and Environmental Research Institute, Agricultural Research Center (ARC), Giza, Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, and the experimental laboratory of Natural Resource Department, Institute of African Research and Studies, Cairo University during the year 2005.

**First experimental studies:**

The objective of these experiments was to study the morphological and physiological characteristics and the nodulation capacity of one hundred and five isolates. These strains were isolated from *Acacia saligna* grown in different locations of Alexandria and Qalubia Governorates, Egypt. Four reference strains (isolated from *Acacia Senegal*, *A. tortilis* subsp. *Raddiana*, *A. laeta* and *Acacia saligna*), were obtained from Senegal and ARC, Giza, Egypt.

On 1st **June 2005**, the rhizobial nodules were collected from *Acacia* trees roots in tubes containing $CaCl_2$ as described by **Date (1982**). The isolation procedure was done according to **Somasegaran and Hoben, (1985) and Zakhia *et al*. (2004).** The morphological characteristics were examined under light microscopy to observe cell morphology using Gram-stain, and growth reaction on YEM media [yeast extract mannitol agar (YEM), containing ($g1^{-1}$): mannitol, 10; sodium glutamate, 0.5; $K_2HPO_4$, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.05; $CaCl_2$, 0.04; $FeCl_3$. 0.004; yeast extract, 1; pH 6.8; agar, 20 cultures were incubated at 35 $^oC$, under aerobic **conditions (De Lajudie *et al*., 1998; Diouf *et al.*, 2000)].**

The physiological characteristics were examined as follow:

a. Temperature tolerance: Determining the highest temperature which the isolates and reference strains tolerated by incubating cultures of rhizobia at 25, 30, 35, and 40 °C.

b. NaCl at 0.2 M tolerance by growing 1 ml of a culture of rhizobia on YEM media containing 0.2 M NaCl and inoculated at 30 °C.

c. pH tolerance: Determining the highest pH which the isolates and reference strains tolerated. Inoculated conical flasks containing 100 ml of previous media with different pH values (6, 7, 8 and 9) with 1 ml of a culture of various *Rhizobium* strains were incubated at 30 °C.

**d.** Viscosity: All isolates were tested for their culture viscosities by viscometer **(Bourn, 1982).**

- Nodulation tests in *vitro*: pods of *Acacia nilotica* subsp. *tomentosa* were collected from Orman Botanical Garden, Giza, Egypt on May. Pods were crushed to

release the seeds. The seeds were treated with concentrated sulfuric acid for 1 hour to accelerate germination **(Jackson and Peake, 1955).** Seeds were then incubated at 28 °C for germination in sterile petri dishes on a 1% agar solution for 48 hrs**. (Werner *et al.*, 1975),** and then transferred to tubes containing Jensen seedling slant agar [$CaHPO_4$, 1.0g; $K_2HPO_4$, 0.2g; $MgSO_2.7H_2O$, 0.2g; NaCl, 0.2g; $FeCl_3$, 0.1g; Water, 1.0 liter; Agar, 15.0g; Microelements (0.5% B; 0.05% Mn; 0.005% Zn; 0.005% Mo; and 0.002% Cu), 1.0 ml **(Vincent, 1970)].** For root nodulation trials (one seed for each tube and 10 plants were routinely tested with each strain). Through 10-20 days after inoculation root nodules formation was observed **(De Lajudie *et al.*, 1998).**

**Second experimental studies:**

The objective of these experiments was to study the physiological characteristics, in details, and test the nodulation capacity of two isolates and three reference strains including ASH 1, ASH 51, SWERI, ORS 1032 and ORS 1096.

On 15th June 2005, the generation time was determined each day during 3 days by Spectrophotometrically as described by **Yelton *et al*. (1983).** The physiological characteristics were carried out based on the following features:

a. Temperature tolerance: A culture of tested *Rhizobium* incubated at 15, 20, 25, 30, 35, 40, 45 and 50 °C.
b. Sodium chloride tolerance: The YEM media containing 0, 0.1, 0.15, 0.2, 0.25, 0.3, 0.5 and 0.6 M NaCl inoculated with 1 ml of a culture of tested *Rhizobium* and incubated at 30 °C.
c. pH tolerance: Conical flasks containing 100 ml of previous media with different pH values 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 were inoculated with 1 ml of a culture of tested *Rhizobium* and incubated at 30 °C.
d. Viscosity: the selected isolates and reference strains were tested, after 72 hrs incubation, for their culture viscosities by viscometer **(Bourn, 1982).**

- Nodulation tests in *vivo*: Five inoculated seeds were sown on June 15th in a 8-cm diameter plastic pots filled with sand. The physical and chemical properties of the sand are shown in Table (1). The pots were placed in a greenhouse. Each pot was inoculated with 10 ml of each isolate or reference strain to evaluate the ability of different isolates and reference strain to nodulate and form a symbotic nitrogen-fixing association with *Acacia nilotica* subsp. *tomentosa*. Number of nodules/ plant, dry weight of nodules (mg/ plant) and nitrogenase activity ($N_2$-ase) of nodules **(Hardy *et al.*, 1973; Somasegaran and Hoben, 1985)** were determined.

**Table(1):Physical and chemical characteristics of the soil used in the experiment.**

| **Physical characteristics** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Soil texture | Coarse sand (%) | Fine sand (%) | Silt (%) | Clay (%) | Field capacity (% V) | | |
| Sandy | 30.0 | 59.9 | 7.0 | 3.1 | 16.1 | | |
| **Chemical characteristics** | | | | | | | |
| pH | Organic matter (%) | $CaCO_3$ (%) | EC (dS/m)(1:25) | CEC (meq/100 g) | Available macro - nutrients (ppm) | | |
| | | | | | N | P | K |
| 7.5 | 1.11 | 0.52 | 0.61 | 5.3 | 13.9 | 1.3 | 69.0 |

**Third experimental studies:**

The objective of these experiments was to formulate a new inoculunt for *Acacia nilotica* subsp. *tomentosa*. Three materials i.e. perlite, vermiculite and peat were used to formulate different carriers. The pH and EC of different carriers are shown in Table (2).

**Table (2): Characteristics of various carrier materials.**

| Carriers | pH | EC (dS/m) |
|---|---|---|
| **Perlite** | 7.81 | 1.78 |
| **Vermiculite + peat (1:1)** | 7.67 | 0.12 |
| **Perlite + vermiculite + peat (2:1:1)** | 7.6 | 1.19 |

On 15$^{th}$ June 2005, All carriers were mixed with saturated bacteria liquid cultures (1-4 $\times 10^9$ cell ml$^{-1}$) to obtain a uniform mixture. Inoculants were sampled and viable number of bacterial cells was determined by plating the carriers of serial dilutions on Congo-red-YEM agar. Dehydrogenase activity was carried out according to **Thalman (1967).**

Seeds of *Acacia nilotica* subsp. *tomentosa* were mixed with 0.5 ml of a water solution of adhesive ( 20 % gum arabic), seeds were then inoculated with 0.5 g of inoculant. The inoculated seeds were allowed to dry at room temperature. Then five inoculated seeds were sown in a 8-cm diameter plastic pots filled with sand. The pots were placed in a greenhouse. Each pot was inoculated with 0.5g of each isolate or reference strain. Number of nodules / plant, dry weight of nodules (mg/ plant) and nitrogenase activity ($N_2$-ase) of nodules were determined as described by **Hardy *et al.* (1973) and Somasegaran and Hoben (1985).**

The layout of the experiments 2 and 3 were factorial in a randomized complete blocks design with 3 replicates. Each replicate consisted of one pot (5 seeds). The data were subjected to analysis of variance, and the means were compared using the least significant difference (L.S.D.) test at the 5% and 1% levels **(Little and Hills, 1978).** In experiment 2 with the physiological characteristics, the means for the three factors (bacterial strains and intervals period as well as temperature degree, salt concentrations or pH values) were not presented separately, because of growth absence of isolates and reference strains treated with some treatment combinations (no growth during the intervals period), which might lead to incorrect conclusion if the calculated means of the main factors were presented.

## RESULT AND DISCUSSION

**First experimental studies:**

Data presented in Table (3) revealed the morphological and physiological characteristics as well as nodulation test of different isolates and reference strains. All isolates and reference strains are short, aerobic, Gram-negative. Colonies of strains on YEM are circular, cream-coloured, semi-translucent. They often spread over an entire plate within 1-2 days, so they are considered as fast-growing isolates **(Somasegaran and Hoben 1985; Nick *et al*., 1999).**

According to the physiological characteristics, two isolates and three reference strains were selected to study the physiological characteristics in details. The selected isolates included (1) ASH 1 isolate from Alexandria isolates which gave the highest

viscosity [viscosity is an indicator of exo-polysaccharides production which are important when rhizobia infect root hairs and live as bacteroids inside nodules **(Kijne, 1992; Brewin, 1998)],** and (2) ASH 51 isolate from Qalubia as it tolerated the increase in temperature till 40 $^0$C. The three reference strains including SWERI, ORS 1032 and ORS 1096 were selected as they form nodules in the lab (*in vitro*).

**Table (3): Morphological and physiological characteristics as well as nodulation capacity of some rhizobial isolates and different strains.**

| Strain | Host plant | Geographical origin | Source or reference | Growth rate | Physiological characteristics | | | | Nodulation capacity |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Temperature tolerance | NaCl con. (0.2 M) | PH tolerance | Viscosity (cP) | |
| **New isolates No.** | | | | | | | | | |
| **ASH 1** | *Acasia saligna* | Alex., Egypt | This work | F. | 40°C | + | 9 | 0.19 | FN |
| **ASH 2** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 9 | 0.11 | AN |
| **ASH 3** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 9 | 0.12 | F. |
| **ASH 4** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 9 | 0.08 | F. |
| **ASH 5** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 9 | 0.07 | F. |
| **ASH 6** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 8 | 0.06 | F. |
| **ASH 7** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 8 | 0.08 | F. |
| **ASH 8** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 8 | 0.09 | F. |
| **ASH 9- 12** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.09 | F. |
| **ASH13 - 15** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 7 | 0.09 | F. |
| **ASH 16** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.11 | F. |
| **ASH 17** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.08 | F. |
| **ASH 18** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.05 | F. |
| **ASH 19** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.04 | F. |
| **ASH 20 - 21** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 7 | 0.07 | F. |
| **ASH 22- 25** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.10 | F. |
| **ASH 26** | *Acasia saligna* | Alex., Egypt | This work | S. | 30°C | + | 7 | 0.06 | F. |
| **ASH 27** | *Acasia saligna* | Alex., Egypt | This work | S. | 30°C | + | 7 | 0.06 | A. |
| **ASH 28** | *Acasia saligna* | Alex., Egypt | This work | S. | 35°C | + | 7 | 0.04 | A. |
| **ASH 29** | *Acasia saligna* | Alex., Egypt | This work | S. | 35°C | + | 8 | 0.04 | A. |
| **ASH 30** | *Acasia saligna* | Alex., Egypt | This work | S. | 30°C | + | 8 | 0.08 | A. |
| **ASH 31** | *Acasia saligna* | Alex., Egypt | This work | S. | 30°C | + | 8 | 0.19 | A. |
| **ASH 32** | *Acasia saligna* | Alex., Egypt | This work | S. | 30°C | + | 8 | 0.11 | A. |
| **ASH 33** | *Acasia saligna* | Alex., Egypt | This work | S. | 30°C | + | 7 | 0.12 | A. |

continued,

**Table (3): Con.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **ASH 34** | *Acasia saligna* | Alex., Egypt | This work | S. | 30°C | + | 7 | 0.08 | A. |
| **ASH 35** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.07 | A. |
| **ASH 36- 37** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.11 | A. |
| **ASH 38** | *Acasia saligna* | Alex., Egypt | This work | F. | 35°C | + | 7 | 0.11 | A. |
| **ASH 39** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.08 | A. |
| **ASH 40** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.05 | A. |
| **ASH 41- 43** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.08 | A. |
| **ASH 44- 45** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.10 | A. |
| **ASH 46** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.04 | F. |
| **ASH 47** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.07 | F. |
| **ASH 48** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.09 | F. |
| **ASH 49** | *Acasia saligna* | Alex., Egypt | This work | F. | 30°C | + | 7 | 0.12 | F. |
| **ASH 56** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.12 | F. |
| **ASH 57- 58** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.08 | F. |
| **ASH 59** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 35°C | + | 7 | 0.08 | F. |
| **ASH 60** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.08 | F. |
| **ASH 61** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.09 | F. |
| **ASH 62** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.04 | F. |
| **ASH 63- 65** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.09 | F. |
| **ASH 66** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.07 | F. |
| **ASH 67** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.10 | F. |
| **ASH 68- 71** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.11 | F. |
| **ASH 72** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.08 | F. |
| **ASH 73** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | - | 7 | 0.11 | F. |
| **ASH 74** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 35°C | - | 7 | 0.09 | F. |
| **ASH 75** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 35°C | - | 7 | 0.07 | F. |
| **ASH 76** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 35°C | - | 7 | 0.04 | A. |
| **ASH 77** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 35°C | - | 7 | 0.06 | A. |
| **ASH 78- 79** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | - | 7 | 0.09 | A. |
| **ASH 80** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | - | 7 | 0.10 | A. |
| **ASH 81** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.08 | A. |

Continued,

**Table (3): Con.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **ASH 82** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.11 | A. |
| **ASH 83-86** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.09 | A. |
| **ASH 87** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.09 | F. |
| **ASH 88** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.11 | F. |
| **ASH 89** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.09 | F. |
| **ASH 90** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.04 | F. |
| **ASH 91** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | + | 7 | 0.08 | F. |
| **ASH 92** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.05 | F. |
| **ASH 93** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.06 | F. |
| **ASH 94** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.07 | F. |
| **ASH 95** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.03 | F. |
| **ASH 96- 97** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | + | 7 | 0.06 | F. |
| **ASH 98- 99** | *Acasia saligna* | Qalubia, Egypt | This work | S. | 30°C | - | 7 | 0.05 | F. |
| **ASH 100** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | - | 7 | 0.09 | F. |
| **ASH 101** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | - | 7 | 0.07 | F. |
| **ASH 102- 105** | *Acasia saligna* | Qalubia, Egypt | This work | F. | 30°C | - | 7 | 0.04 | F. |
| **Reference strains No.** | | | | | | | | | |
| ***Rhizobium spp.* (SWERI)** | *Acacia saligna* | Monufia, Egypt | Soil,Water and Environ. Res. Inst. | F. | 40°C | + | 8 | 0.11 | F. |
| ***Mesorhizbium plurifarium* (ORS 1032)** | *Acacia senegal* | Senegal | De Lajudie *et al.* (1998) | F. | 40°C | + | 9 | 0.09 | F. |
| ***Mesorhizbium plurifarium* (ORS 1096)** | *Acacia tortilis* subsp. *raddiana* | Senegal | De Lajudie *et al*. (1998) | F. | 40°C | + | 9 | 0.19 | F. |
| ***Sinorhizbium terangae* (ORS 1009)** | *Acacia laeta* | Senegal | De Lajudie *et al*. (1994) | F. | 30°C | + | 9 | 0.04 | A. |

Abbreviation:

SWERI: Soils, water & Environment Research Institute, Agric. Res. Center, (ARC), Giza, Egypt.
ORS: ORSTOM Collection, Institute Français de Recherche Scientifique pour le Développement en Coopération, Dakar, Senegal.
ASH: Amira shawky.
F= Fast- grow S= Slow- grow
N= Gram –negative
- = No growth + = Growth
FN = Formed nodules AN = Absence of nodules

**Second experimental studies:**

**I. Physiological characterization**

**1. Temperature tolerance**

The growth of bacterial isolates and reference strains was significantly affected by different temperatures and period of growth (Table, 4). The growth of each isolate and reference strain was significantly increased as temperature increased till 30 °C, in different intervals period These results are in harmony with those obtained by **Hashem *et al*. (1998) and Zerhari *et al*. (2000)** they observed that, tropical rhizobial strains which were isolated from *Acacia* sp. can grow at 30-35°C. As temperature increased the growth was significantly decreased in all intervals period , in most cases. The significantly higher growth density of isolates and reference strains was recorded at 30 °C after incubation for 48 hrs. All isolates and reference strains were not able to grow at 50 °C.

In most cases, on different temperatures, the significantly higher growth of each isolates and reference strains was recorded after incubation period of 48 hrs.

Generally, the significantly higher growth of each isolate and reference strain was recorded on 30 °C after 48 hrs. incubation.

**2. Sodium chloride tolerance**

The growth of bacterial isolates and reference strains was significantly decreased by different salt concentrations and intervals period (Table, 4), in most cases.

Data in Table (4) revealed that the response of bacteria to different salt concentrations varied from isolate to the other. ASH 1, SWERI, and ORS 1096 survived and grew at salt concentration up to 0.5 M sodium chloride till 72 hrs incubation. These results are in agreement with the findings of **Zhang *et al*. (1991) and Zahran *et al*. (1994),** who reported that many rhizobia isolated from *Acacia* trees are capable of growing in 0.3 - 0.5 M (2-3%) NaCl.

On the other hand, ASH 51 and ORS 1032 grown on different salt concentrations did not survive with prolonged incubation period till 72 hrs. ASH 51 was not able to grow at 0.3 M NaCl.

In most cases, on different salt concentrations, the significantly higher growth of each isolate and reference strains was recorded after incubation period of 48 hrs.

**Table (4): Effect of different temperatures and salt concentrations on bacterial isolates and reference strains on growth (turbidity, nm) at different intervals period.**

| Temperature (°C) | Bacterial isolates and reference strains | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ASH 1 | | | ASH 51 | | | SWERI | | | ORS 1032 | | | ORS 1096 | | |
| | Intervals Period | | | | | | | | | | | | | | |
| | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 |
| | Growth (turbidity, nm) | | | | | | | | | | | | | | |
| 15 | 20.67 | 32.33 | 0 | 0 | 0 | 0 | 78.33 | 91 | 0 | 46.67 | 51 | 0 | 156 | 164.67 | 135 |
| 20 | 41.67 | 46.33 | 31.67 | 64.67 | 87.33 | 0 | 156 | 167.33 | 130 | 213.33 | 237.33 | 209.33 | 334 | 353 | 326 |
| 25 | 151.67 | 172.33 | 137.67 | 131.33 | 148.67 | 122.67 | 175 | 180 | 147.67 | 285.67 | 293.67 | 264 | 455.67 | 464.33 | 449.33 |
| 30 | 263.67 | 271.67 | 253.33 | 401.67 | 405.33 | 388.33 | 389.67 | 397.33 | 355.67 | 387.67 | 406 | 376 | 699.33 | 725.67 | 689.67 |
| 35 | 205.67 | 209.67 | 196.33 | 222.67 | 251.67 | 218.33 | 287.33 | 234.67 | 256.67 | 363.33 | 393.67 | 353.67 | 692.67 | 701 | 682 |
| 40 | 159 | 169.67 | 135.67 | 223.67 | 241.67 | 197.33 | 309 | 313 | 297.67 | 305.67 | 327.67 | 295.67 | 676.67 | 693.33 | 673.67 |
| 45 | 117 | 124.67 | 82.67 | 112 | 195.67 | 0 | 265 | 276.67 | 244.33 | 236.67 | 248.33 | 0 | 306.67 | 323.33 | 295.67 |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**L.S.D. Bacterial strains x intervals period x temperatures at** **0.05 = 7.95**
**0.01 = 10.22**

| Salt conc. (Molar) | Growth (turbidity, nm) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 |
| Control | 263.67 | 271.67 | 253.33 | 401.67 | 405.33 | 388.33 | 389.67 | 397.33 | 355.67 | 387.67 | 406 | 376 | 699.33 | 725.67 | 689.67 |
| 0.1 | 185.67 | 302.67 | 92.67 | 176.67 | 298.33 | 0 | 98.33 | 113.33 | 81.67 | 73.33 | 176.67 | 0 | 405.67 | 456 | 381 |
| 0.15 | 113.67 | 282.33 | 77.67 | 47.67 | 58.33 | 0 | 120.33 | 279.33 | 81.67 | 387.67 | 406 | 376 | 397.67 | 484.33 | 384.33 |
| 0.2 | 108.33 | 211.33 | 107.33 | 94.67 | 117.67 | 0 | 114.67 | 257.33 | 94.67 | 93.67 | 162.67 | 0 | 410.67 | 440.33 | 376.67 |
| 0.25 | 168.67 | 268.67 | 80.33 | 21.67 | 43.67 | 0 | 130.33 | 302.33 | 90.33 | 175 | 211 | 0 | 440 | 473 | 375 |
| 0.3 | 145.67 | 161.67 | 115.67 | 0 | 0 | 0 | 163.33 | 205.33 | 126.67 | 31 | 0 | 0 | 567.67 | 604 | 520.33 |
| 0.5 | 79.67 | 98.67 | 55.33 | 0 | 0 | 0 | 100.33 | 180 | 72.67 | 30 | 0 | 0 | 647.67 | 707.33 | 616.67 |
| 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 92.67 | 0 | 0 |

L.S.D. Bacterial strains x intervals period x salt concentrations at 0.05 = 4.00
0.01= 4.16

**3. pH tolerance**

Data in Table (5) show that the growth of bacterial isolates and reference strains was significantly affected by different pH values and intervals period. In each interval period, ASH 1 and ASH 51 could not grow at pH values of 2 and 3. The growth of different isolates and reference strains was increased with increasing pH values till 7, then it was decreased with increasing pH values till 12. There was no growth found at pH 13. Such results are in agreement with those obtained by **Zerhari *et al*. (2000)** who concluded that the growth of most rhizobial strains isolated from *Acacia* sp. was inhibited till pH 4, whereas all strains grew well at pH ranged from 7 to 9.

At each pH value, the growth of each isolate and reference strain was significantly increased with time till 48 hrs then significantly decreased at 72 hrs. incubation.

Generally, the significantly higher growth of different isolates and reference strains was recorded at pH 7 after 48 hrs. incubation.

**4. Viscosity**

As shown in Fig. (1) the culture viscosity of all isolates and reference strains of rhizobia varied from isolate to another. ASH 1 Isolate and ORS 1096 gave the highest viscosity (0.19 cP). While, ASH 51 isolate gave the lowest viscosity (0.08 cP). The average viscosity of different isolates and reference strains ranged from 0.09 to 0.11cP.

As previously mentioned, viscosity is indicating the existence of exo-polysaccharides production (a major component of the outer membrane of gram-negative bacteria such as *Rhizobium* spp) which play essential roles in the formation of the infection thread and in nodule development **(Kannenberg and Brewin, 1994; Lloret *et al*., 1998)**. Several reports have shown that these molecules are also important for the adaptation and survival of *Rhizobium* strains under severe conditions **(Soussi *et al*., 2001)**. Therefore, it can concluded that the strains which gave high viscosity are active in infection process and may give high numbers of nodules than the others strains which have lower viscosity, under stress conditions.

**Table (5): Effect of different pH on bacterial isolates and reference strains growth (turbidity, nm) at different intervals period.**

| pH | Bacterial isolates and reference strains | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ASH 1 | | | ASH 51 | | | SWERI | | | ORS 1032 | | | ORS 1096 | | |
| | Intervals Period | | | | | | | | | | | | | | |
| | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 |
| | Growth (turbidity, nm) | | | | | | | | | | | | | | |
| **2** | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 50.67 | 180.33 | 31.67 |
| **3** | 0 | 0 | 0 | 0 | 0 | 0 | 13.33 | 35.67 | 0 | 23 | 29 | 17.67 | 89 | 194.67 | 55 |
| **4** | 93.67 | 113.67 | 0 | 20.33 | 44.33 | 0 | 63.67 | 124.67 | 53.67 | 24.33 | 40 | 22 | 162.33 | 214.33 | 145 |
| **5** | 147.33 | 180 | 98.67 | 127 | 167.33 | 83.67 | 124.67 | 213.67 | 93.67 | 47 | 94.67 | 37.67 | 595.67 | 654 | 588 |
| **6** | 175.67 | 190.33 | 169.33 | 375.33 | 391.67 | 343.33 | 160.67 | 245.67 | 109.67 | 268.67 | 321 | 184 | 660 | 704.67 | 641.67 |
| **7** | 263.67 | 271.67 | 253.33 | 401.67 | 405.33 | 388.33 | 389.67 | 397.33 | 355.67 | 387.67 | 406 | 376 | 699.33 | 725.67 | 689.67 |
| **8** | 242.67 | 251.67 | 225.67 | 309.67 | 318.33 | 296 | 260 | 301.67 | 192.33 | 247 | 384.67 | 179.33 | 544.33 | 684.33 | 515.67 |
| **9** | 229.67 | 238 | 216 | 99 | 196.33 | 0 | 199.67 | 276.33 | 144 | 110.67 | 375.33 | 67.67 | 493.33 | 547.67 | 946 |
| **10** | 194.33 | 231.33 | 91.67 | 0 | 0 | 0 | 107.67 | 239.33 | 65.67 | 64 | 241 | 42.67 | 407.67 | 491 | 388.67 |
| **11** | 114.33 | 166.67 | 57.33 | 0 | 0 | 0 | 24.33 | 30 | 0 | 55.67 | 158.33 | 32.67 | 265.67 | 393 | 125.67 |
| **12** | 93.33 | 132.33 | 0 | 0 | 0 | 0 | 17.67 | 22.33 | 0 | 37.67 | 106.33 | 9.67 | 251.67 | 384.67 | 102 |
| **13** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

L.S.D. Bacterial strains x intervals period x pH at 0.05 =2.40
0.01=3.11

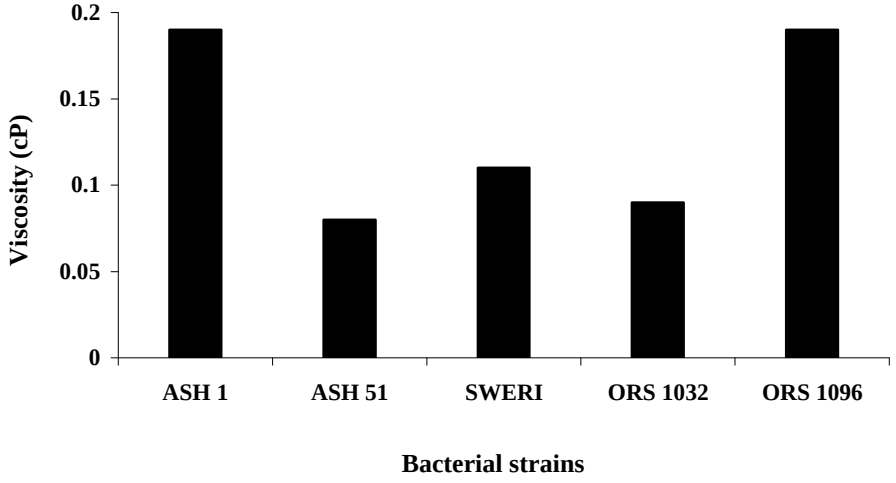


**Fig. (1) Viscosity of different isolates and refrence strains of rhizobia.**

## II. Nodulation test in *vivo*

All isolates and reference strains nodulated *Acacia nilotica* subsp. *tomentosa*, but there were differences between the strains in respect to the time required for nodule formation. As shown in Fig. (2), ASH 1 and ORS 1096 infected *Acacia nilotica* subsp. *tomentosa* faster than the others ( 60, and 70 days, respectively), followed by SWERI, ORS 1032, and ASH 51 (75, 90, and 110 days, respectively).

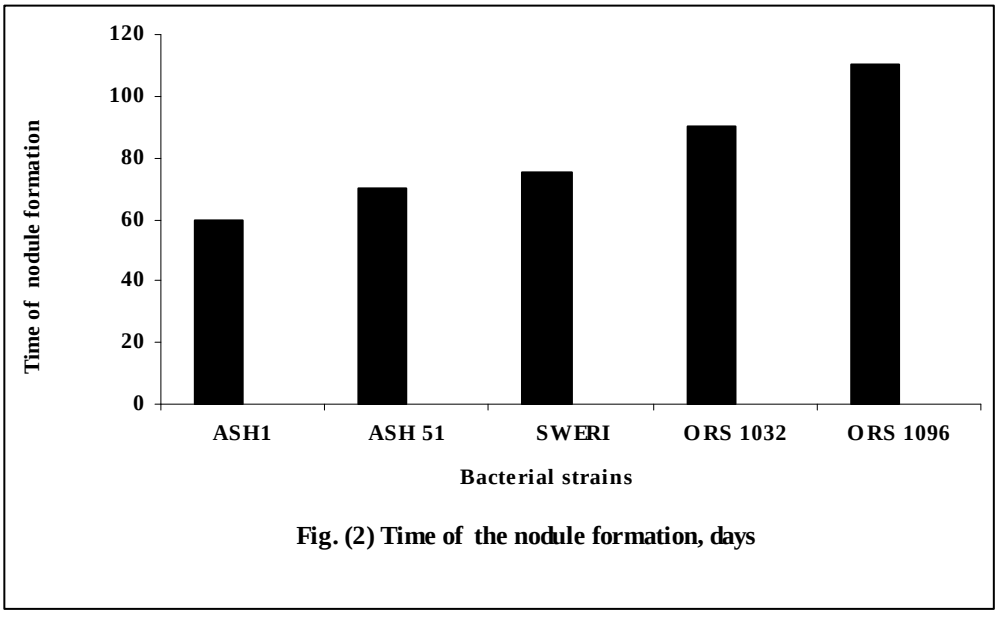


**Fig. (2) Time of the nodule formation, days**

**1-Number of nodules**

Data presented in Table (6) showed that treating *Acacia nilotica* subsp. *tomentosa* seeds with each of the tested isolates and reference strains caused a significant increase in number of nodules compared to the control. The obtained results showed that the *Rhizobium* strains were different in their ability to form nodules on the root system. ORS 1096 strain gave the highest number of nodules followed by ASH 1, ORS 1032, SWERI and ASH 1 with significant differences among them. These differences could be due to the host/ strain interactions **(Graham, 1981). Also, Abdel-Rahim *et al.* (2000**) indicated that, inoculation of *Acacia* spp. with different *Rhizobium* strains resulted in marked increases in nodule numbers than the noninoculated controls.

**Table (6): Effect of inoculation with different isolates and refrence strains on number, dry weight and nitrogenase activity (nmoles $C_2H_4$ / g dry weight nodules /hr) of *Acacia nilotica* subsp. *tomentosa* nodules in the greenhouse experiment.**

| Bacterial strains | Nodules No./ plant | Nodules dry wt (mg) / plant | Nitrogenase activity (nmoles $C_2H_4$ /ml/h) |
|---|---|---|---|
| **Control** | 15.00 | 67.00 | 16.67 |
| **ASH 1** | 98.00 | 259.00 | 254.00 |
| **ASH 51** | 40.00 | 199.00 | 168.67 |
| **SWERI** | 67.00 | 154.00 | 172.33 |
| **ORS 1032** | 72.00 | 205.00 | 168.00 |
| **ORS 1096** | 115.00 | 226.00 | 269.00 |
| **L.S.D. at** | **0.05 0.01** | **0.05 0.01** | **0.05 0.01** |
| **Bacterial strains** | **3.56 4.99** | **3.91 5.47** | **3.25 4.54** |

**2- Dry weight of nodules per plant**

Dry weight of nodules per plant was significantly affected bu inoculating seeds of *Acacia nilotica* subsp. *tomentosa* with different isolates and reference strains (Table, 6). Treating *Acacia nilotica* subsp. *tomentosa* seeds with each of the tested isolates and reference strains caused a significant increase in dry weight of nodules per plant compared to the control. ASH 1 isolate gave the heaviest dry weight of nodules per plant followed by ORS 1096, ORS 1032 strains, ASH 51 isolate and SWERI strain with significant differences among them. These results are in agreement with the findings of **Sutherland *et al.* (2000)** who concluded that inoculation of *Acacia tortilis* subsp. *spirocarpa* with the single strain of *Rhizobium* sp. gave significantly higher dry weight of nodules (ranged from 19 to 75 %) than the control. In addition, **Martin-Laurent *et al.* (2000**) found that inoculating *Acacia mangium* with *Bradyrhizobium* sp. increased dry weight of nodules compared to the control.

**3- Nitrogenase activity:**

Data presented in Table (6) showed that the effect of inoculation with isolates and reference strains of rhizobia on nitrogenase activity ($N_2$-ase) in root rhizoplane of *Acacia nilotica* subsp. *tomentosa.* Data revealed that rhizobia inoculation caused significant increases in nitrogenase activity as compared to the control. Inoculation with ORS 1096 gave the highest nitrogenase activity followed by ASH 1, SWERI, ASH 51 and ORS 1032 with significant differences among them. The nitrogenase activity may be attributed to the effect of exudation of carbon compounds that have special importance to heterotrophic the growth of nitrogen fixing microorganisms **(Omar and Ahmed, 2003). Also, Younis (2003)** found that the nitrogenase activity of *Lablab purpureus* nodules recorded the highest values when the plants were inoculated with the single strain of *Rhizobium*, compared to the un-inoculated plants.

According to the above mentioned results, it can be concluded that the selected isolates and reference strains showed different physiological characteristics under different conditions including temperature tolerance (till 45 °C), salt tolerance (till 0.5 M, NaCl), pH (till 12). Various strains also showed differences in the formation of nodules with respect to number and dry weight of nodules/ plant and nitrogenase activity in nodules.

**Third experimental studies:**

**I. Counts and dehydrogenase activity of rhizobial inoculant in different carriers in *vitro***

**1. Counts of rhizobial inoculants in different carriers**

Data illustrated in Fig. (3) showed that the perlite was more effective than other carriers in promoting rhizobial survival ($7.5 \times 10^5$ cfu / g dry carrier) during 4 months on shelf, followed by perlite + vermiculite + peat (2:1:1), and vermiculite + peat (1:1) inoculants ($7.1\times 10^5$ and $7.3 \times 10^5$ cfu /g dry carrier, respectively). This means that perlite inoculants can maintain a higher population of microorganisms than both inoculants at room temperature for 4 months. This result is in agreement with the findings of **Daza *et al.* (2000)** who found that the survived rhizobial was higher on perlite than peat-based inoculants.

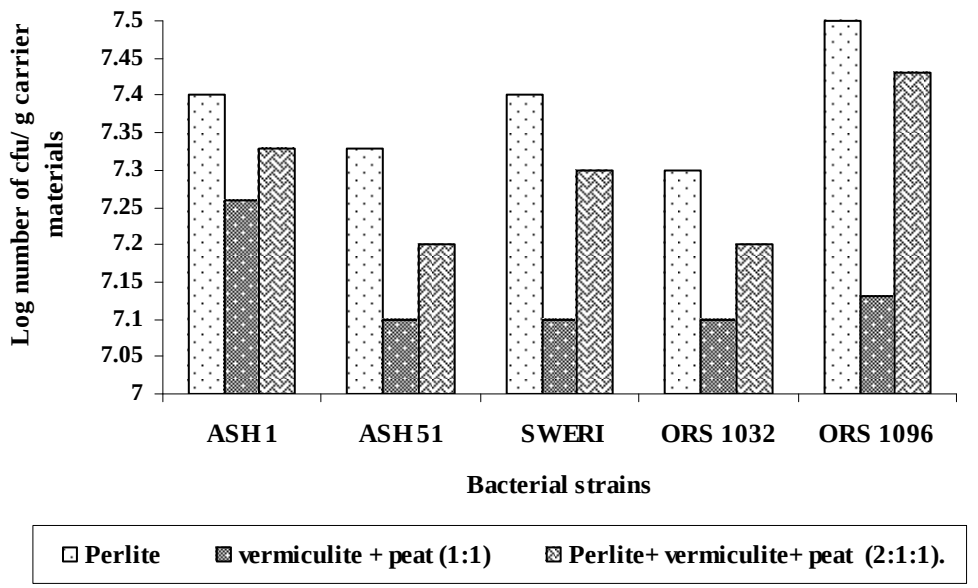


**Fig. (3) Total count of bacterial cells as (Log number ) in different carrier materials after 4 months on shelf.**

## 2. Dehydrogenase activity of rhizobial inoculant in different carriers

Data illustrated in Fig. (4) showed that the dehydrogenase activity (DHA) for rhizobial isolates and reference strains inoculant in different carriers during 4 months on shelf. Perlite-based inoculants of rhizobial isolates and reference strains showed high dehydrogenase activity (65.24 µg TPF/ g dry carrier/ day), followed by perlite + vermiculite + peat (2:1:1), and vermiculite + peat (1:1) inoculants (63.37 and 39.4 µg TPF/ g dry carrier/ day, respectively). Dehydrogenase is considered as an index for the biological activity **(Ghazal, 1980)** due to releasing carbon dioxide in the rhizosphere, which cause the formation of carbonic acid and consequently decrease the pH value. This process increases nutrient uptake and availability of the nutrient in rhizosphere and resulting in an increase in plant growth and yield as reported by **Omar and Ismail (2002).**

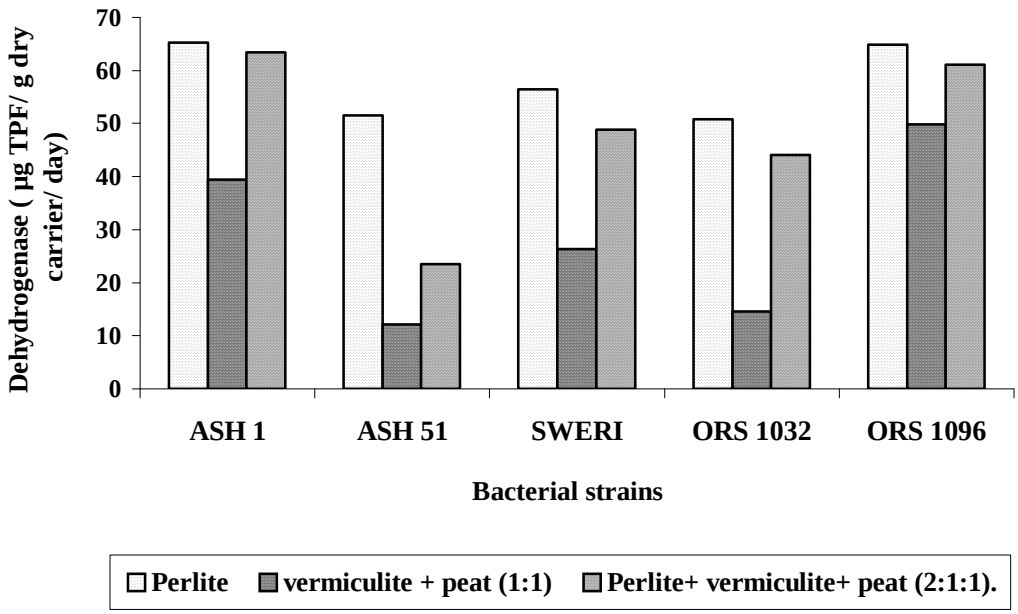


**Fig. (4) Dehydrogenase of different carrier materials after 4 months on shelf .**

## II. Number and dry weight of nodules, and nitrogenase activity ($N_2$-ase) of *Acacia nilotica* subsp. *tomentosa* nodules as affected by different carrier inoculants in *vivo.*

### 1-Number and dry weight of nodules of *Acacia nilotica* subsp. *tomentosa* nodules as affected by different carrier inoculants.

Regarding of the effect of different carrier inoculants on number and dry weight of nodules/plant, regardless of the effect of inoculation with various isolates and reference strains, data presented in Table (7) revealed that inoculation of *Acacia nilotica* subsp. *tomentosa* with perlite based-inoculant resulted in the significantly higher number and dry weight of nodules/plant followed by perlite + vermiculite + peat (2:1:1) and vermiculite + peat (1:1) with significant difference between them. These results are in conformity with those obtained by **Ronchi *et al.* (1997) and Daza *et al.* (2000),** they concluded that perlite-based inoculants increased the number and dry weight of *Phaseolus vulgaris* cv. mutin nodules more than peat- based inoculants.

Concerning the effect of inoculation with various isolates and reference strains, regardless of the effect of different carrier inoculants, data shown in Table (7) revealed that ASH 1 isolate resulted in a significantly higher number and dry weight of nodules/plant as compared to the other isolates and reference strains. On the other hand, ORS 1032 resulted in the significantly lower number and dry weight of nodules/plant as compared to the other isolates and reference strains, meanwhile the response of ASH 51, SWERI and ORS 1096 resulted in intermediate values.

The number and dry weight of nodules/plant was significantly affected by the interaction effects of various isolates and reference strains and different carrier inoculants (Table, 7). Generally using inoculant of ASH 1 in perlite gave the significantly higher number and dry weight of nodules/plant, as compared to the other combination treatments.

**Table (7): Nodules number, nodules dry weight and nitrogenase activity (nmoles $C_2H_4$ / g dry weight nodules /hr) of *Acacia nilotica* subsp. *tomentosa* affected by inoculant carrier materials.**

| Carriers | Bacterial strains | | | | | Mean |
|---|---|---|---|---|---|---|
| | ASH 1 | ASH 51 | SWERI | ORS 1032 | ORS 1096 | |
| **Nodules ( No./ plant)** | | | | | | |
| **Perlite** | 273.00 | 258.00 | 234.00 | 126.00 | 258.00 | **229.80** |
| **Vermiculite + peat (1:1)** | 130.00 | 89.00 | 87.00 | 68.00 | 105.00 | **95.80** |
| **Perlite+ vermiculite + peat (2:1:1)** | 230.00 | 199.00 | 178.00 | 84.00 | 224.00 | **183.00** |
| **Mean** | **211.00** | **182.00** | **166.33** | **92.67** | **195.67** | ---- |
| **L.S.D. at** | | **0.05** | **0.01** | | | |
| **Carrier** | | **1.87** | **2.52** | | | |
| **Bacterial strains** | | **2.41** | **3.25** | | | |
| **Carrier x Bacterial strains** | | **4.19** | **5.64** | | | |
| **Nodules dry wt. (mg)/ plant** | | | | | | |
| **Perlite** | 369.00 | 332.00 | 237.00 | 220.00 | 259.00 | **283.40** |
| **Vermiculite + peat (1:1)** | 332.00 | 259.00 | 208.00 | 208.00 | 230.00 | **247.40** |
| **Perlite+ vermiculite + peat (2:1:1)** | 355.00 | 311.00 | 209.00 | 210.00 | 243.00 | **265.60** |
| **Mean** | **352.00** | **300.67** | **218.00** | **212.67** | **244.00** | ----- |
| L.S.D. at | | 0.05 | 0.01 | | | |
| Carrier | | 1.89 | 2.55 | | | |
| Bacterial strains | | 2.44 | 3.29 | | | |
| Carrier x Bacterial strains | | 4.20 | 5.66 | | | |
| **Nitrogenase activity (nmol. $C_2H_4$ / g dry weight nodules /hr)** | | | | | | |
| **Perlite** | 289.00 | 270.00 | 186.00 | 189.00 | 273.00 | **289** |
| **Vermiculite + peat (1:1)** | 261.00 | 188.00 | 178.00 | 175.00 | 260.00 | **261** |
| **Perlite+ vermiculite + peat (2:1:1)** | 279.00 | 198.00 | 185.00 | 180.00 | 266.00 | **279** |
| **Mean** | **276.33** | **218.67** | **183.00** | **181.33** | **266.33** | ----- |
| L.S.D. at | | 0.05 | 0.01 | | | |
| Carrier | | 1.87 | 2.52 | | | |
| Bacterial strains | | 2.41 | 3.25 | | | |
| Carrier x Bacterial strains | | 4.19 | 5.65 | | | |

**2-Nitrogenase activity ($N_2$-ase) of *Acacia nilotica* subsp. *tomentosa* nodules as affected by different carrier inoculants in *vivo*.**

Concerning of the effect of different carrier inoculants on nitrogenase activity of *Acacia nilotica* subsp. *tomentosa* nodules , regardless of the effect of inoculation with various isolates and reference strains, data presented in Table (7) revealed that inoculation of *Acacia nilotica* subsp. *tomentosa* with perlite based-inoculant resulted in the significantly higher nitrogenase activity of nodules followed by perlite + vermiculite + peat (2:1:1) and vermiculite + peat (1:1) with significant difference between them.

Regarding the effect of inoculation with various isolates and reference strains, irrespect of the effect of different carrier inoculants, data shown in Table (7), revealed that ASH 1 isolate resulted in a significantly higher nitrogenase activity of nodules followed by ORS 1096, ASH 51, SWERI and ORS 1032, respectively with significant differences between them.

The nitrogenase activity of nodules was significantly affected by the interaction effects of various isolates and reference strains and different carrier inoculants (Table, 7). Using inoculant of ASH 1 in perlite gave the significantly higher nitrogenase activity, as compared to the other combination treatments.

From the above results of third experimental studies, it can be concluded that perlite inoculants can be confidently recommended in order to maintain a higher population of rhizobia than other carriers at room temperature. The maintaining higher population is an important characteristic that determines performance of an inoculant.

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# تأثير الريزوبيا المعزولة من بعض الأكاسيات على نمو الأكاسيا نيلوتيكا تحت بعض الظروف المجهدة
# فى شمال إفريقيا

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تم عزل الريزوبيا من العقد الجذرية لإحدى الأكاسيات المصرية (الأكاسيا ساليجنا) . وقد تم دراسة العزلات المختارة ومقارنتها بالسلالات المرجعية التي تم الحصول عليها من جمهورية السنغال ومركز البحوث الزراعية بمصر. لم ينجح نمو جميع العزلات والسلالات المرجعية عند درجة حرارة ٥٠ °م ، بينما كان نموها جيداً عند درجة حرارة ٣٠ °م ، أما عند درجة حرارة ١٥°م فقد كان النمو ضعيفاً أو منعدماً . كما وُجد أن معظم العزلات والسلالات المرجعية يمكنها النمو في بيئة تصل فيها نسبة كلوريد الصوديوم حتى ٠,٥ مولار ، ووجد أن أعلى نمو عند درجة حموضة ٧ . وتراوحت اللزوجة التي تعد دليلاً على إنتاج السكريدات المتعددة ما بين ٠,٠٨ – ٠,١٩ سنتيبويسيز . أدى استخدام سلالات الريزوبيا المعزولة في التاقيح البكتيري لنباتات الأكاسيا لزيادة عدد العقد الجذرية ووزنها لكل نبات ، وكذلك زيادة نشاط تثبيت الأزوت الجوي . وأدى استخدام البرليت كحامل بكتيري للحصول على أعلى نتيجة في حفظ أعداد البكتيريا وزيادة النشط التنفسي لها ، كما أدى لزيادة عدد العقد الجذرية والوزن الجاف لها وزيادة النشاط التنفسي للبكتيريا في العقد الجذرية ، يليها مخلوط البرليت + الفيرميكوليت + البيت بنسبة (١:١:٢) ، ثم الفيرميكوليت + البيت بنسبة (١:١).