RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

Environmental stress impose serious problems in the establishment of a successful symbiosis especially during the period between inocul ation of seed and germination **(Weaver and Holt, 1990).** Identification of some rhizobial strains tolerant to the different stress conditions may enhance their survival and symbiotic performance in soil.

The present investigation was carried out on 25 different strains of rhizobia represent different species to select and utilize strains of rhizobia for agronomic practices, particularly depending upon requirements as pre-conditions of the soil.

4.1. Survival of rhizobia as influenced by some stress conditions

4.1.1. Effect of NaCl salinity on survival of rhizobia

Salt stress is a major constraint limiting the production of legume crop species, particularly when the nitrogen needed for the growth of these plants is derived from symbiotic **fixation (Velagaleti and Marsh, 1989)**. Therefore, with the problems of soil salinity on the increase world wide, the identification of some rhizobial strains adapted or tolerant to salinity stress deserves high priority in the present study.

The ability of *Rhizobium meliloti*(5 strains), *Rhizobium leguminosarum* bv. *viceae* (5 strains), *Rhizobium leguminosarum* bv. *trifolii* (3 strains), *Rhizobium leguminosarum* bv. *phaseoli* (2 strains), peanut *Bradyrhizobium* sp. (5 strains) and *Bradyrhizobium japonicum* (5 strains) to tolerate increased concentrations of NaCl is presented in

Tables (3,4,5,6,7 and 8) and graphically illustrated in Figures (1) A, B, C, D, E and F respectively. Data show that all tested local and foreign strains of rhizobia were capable to tolerate 0.2% concentration of NaCl.

The number of tolerant strains generally decreased with the increased concentration of NaCl up to 50 g/L. The rhizobial strains tolerated up to 10 g/L. of NaCl were those representing *Rhizobium meliloti* (ARC 2), *Rhizobium leguminosarum* bv. *viceae* (ARC 200 F), *Rhizobium leguminosarum* bv. *trifolii* (TAL, ARC 103 and ARC 101), *Rhizobium leguminosarum* bv. *phaseoli* (ARC 302, UMR), peanut *Bradyrhizobium* sp. (618, 3339). On the other hand, three strains (3407, 110, ARC 500) of *Bradyrhizobium japonicum* can not tolerate NaCl concentration more than 0.2%, however the other two strains tested were found to survive increased NaCl concentrations up to 20 g/L. (strain 102) and 40 g/L. (strain 138).

Therefore, it may be suggested that tolerance to NaCl salinization differ according to rhizobial species and strain. In other words, there are marked variations among rhizobial species and strains in their ability to grow and survive saline conditions in YEM. Considering the different species of rhizobia under investigation, it is clear that *Rhizobium meliloti* and *Rhizobium leguminosarum* bv. *phaseoli* strains are found to be more sensitive to NaCl salinity, *Rhizobium trifolii* strains are intermediate and *Bradyrhizobium japonicum* and *Bradyrhizobium* sp. (peanut) are more tolerant to the increased NaCl salinization. These observations are in consistence with those obtained by **Singleton *et al.* (1982), Subbarao *et al.* (1990)**. On the other hand, some strains of rhizobia can grow in solutions with salinities as high as equivalent of 92% of the salinity of seawater **(Singleton *et al.*, 1982)**. This is perhaps not surprising in view of the fact

Table (3) : Effect of different concentrations of NaCl on the survival of *R. meliloti* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})				
	Concentration of NaCl (g/L)				
	Initial count	2	10	20	30
ARC 1	9.20	8.20	5.52	5.11	0
ARC 2	9.28	7.94	5.26	0	0
ARC 3	9.26	8.00	5.00	3.90	0
ARC 6	9.15	7.93	4.30	3.23	0
A2	9.26	7.98	5.08	5.26	0

Table (4) : Effect of different concentrations of NaCl on the survival of *R. leguminosarum* bv. *viceae* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of NaCl (g/L)						
	Initial count	2	10	20	30	40	50
ARC 200 F	9.62	9.47	3.60	0	0	0	0
ARC 202 F	9.58	9.69	6.26	3.68	0	0	0
ARC 206 F	9.68	9.81	6.40	3.97	0	0	0
ARC 207 F	9.78	9.58	6.58	3.91	0	0	0
ICARDA 441	8.60	8.60	6.04	4.48	3.48	3.47	0

Table (5) : Effect of different concentrations of NaCl on the survival of *R. leguminosarum* bv. *trifolii* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})			
	Concentration of NaCl (g/L)			
	Initial count	2	10	20
TAL	9.89	9.60	9.40	0
ARC 103	9.80	9.56	9.02	0
ARC 101	9.81	9.38	9.18	0

Table (6) : Effect of different concentrations of NaCl on the survival of *R. leguminosarum* bv. *phaseoli* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})			
	Concentration of NaCl (g/L)			
	Initial count	2	10	20
ARC 302	10.43	9.18	5.65	0
UMR	10.55	9.18	4.49	0

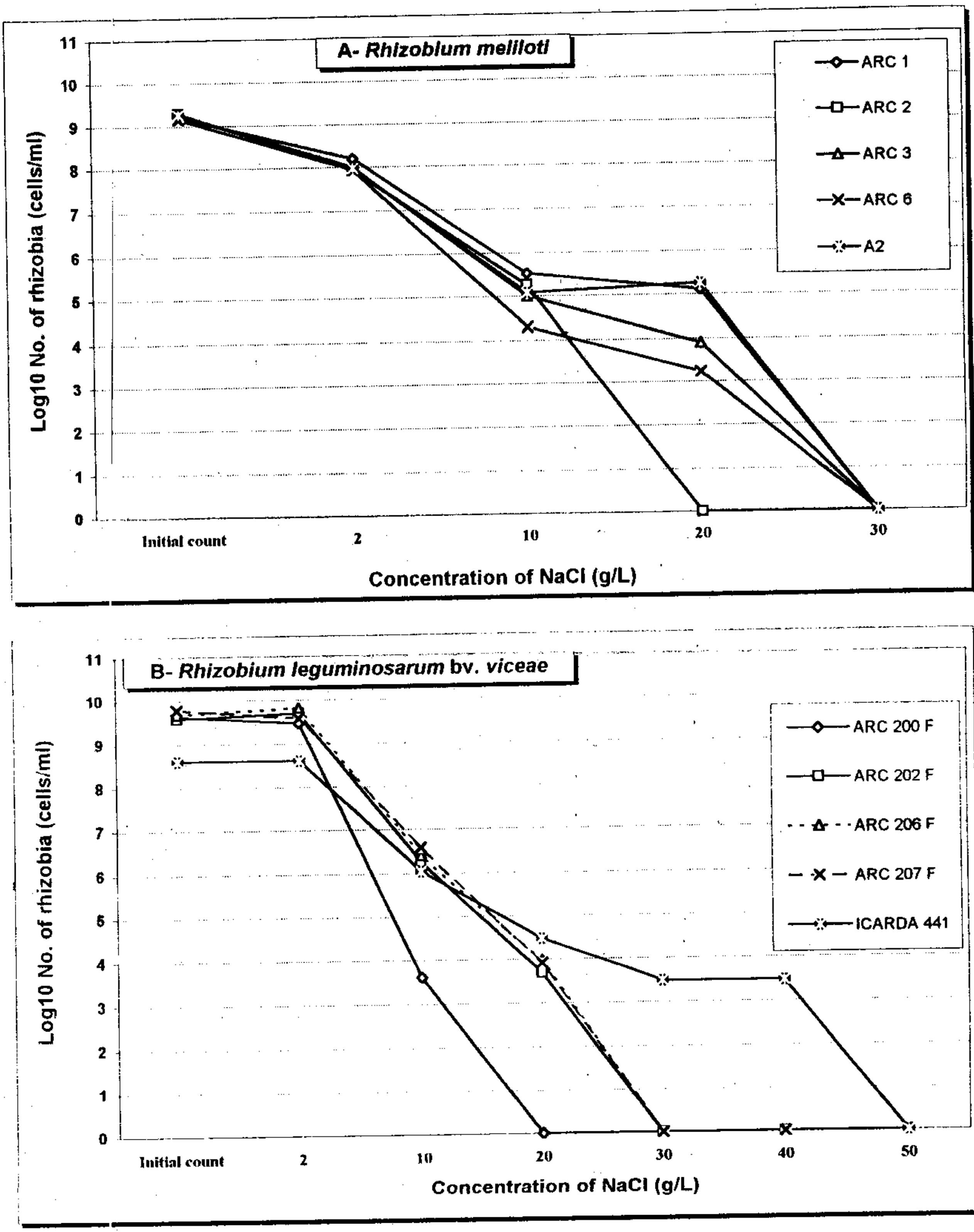
Table (7) : Effect of different concentrations of NaCl on the survival of *Bradyrhizobium* sp. (peanut) in liquid medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of NaCl g/L						
	Initial count	2	10	20	30	40	50
3456	8.41	5.90	4.56	3.95	0	0	0
618	8.46	5.02	0	0	0	0	0
619	8.23	5.00	4.23	0	0	0	0
3339	8.40	3.30	0	0	0	0	0
601	8.25	7.18	6.36	4.45	4.0	3.78	0

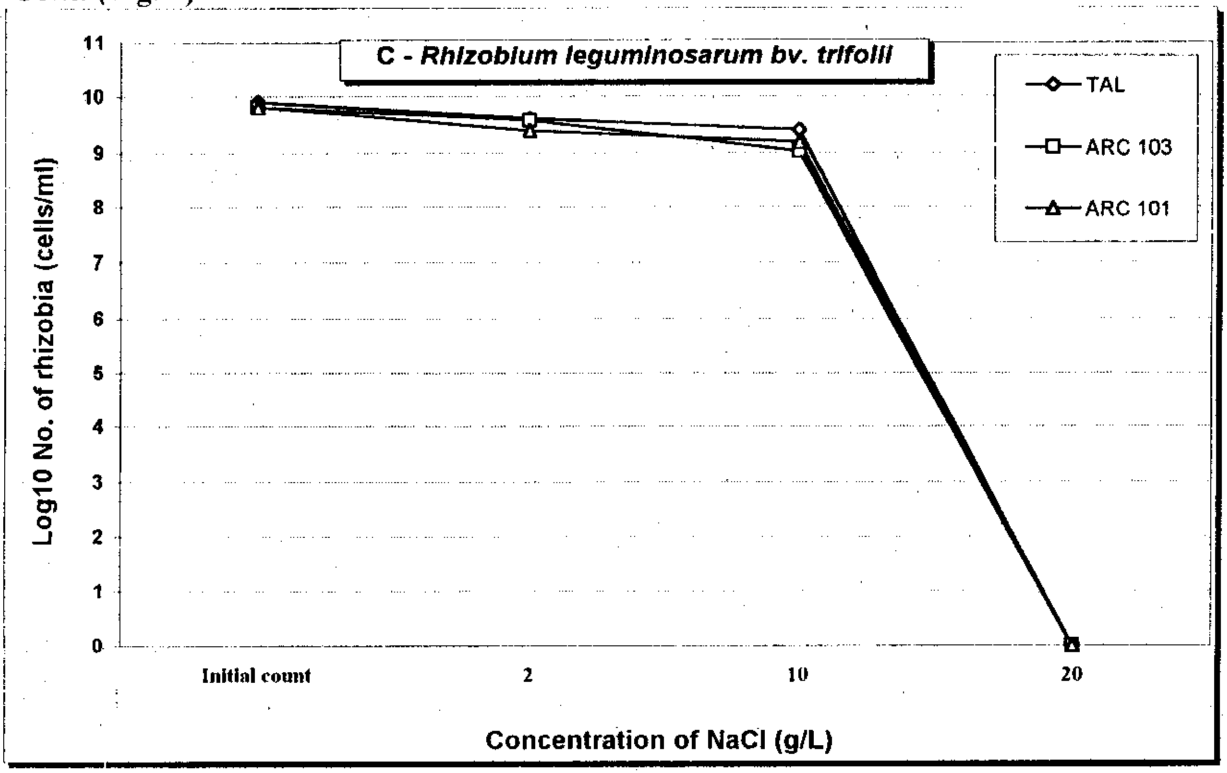
Table (8) : Effect of different concentrations of NaCl on the survival of soybean *Bradyrhizobium japonicum* in YEM medium .

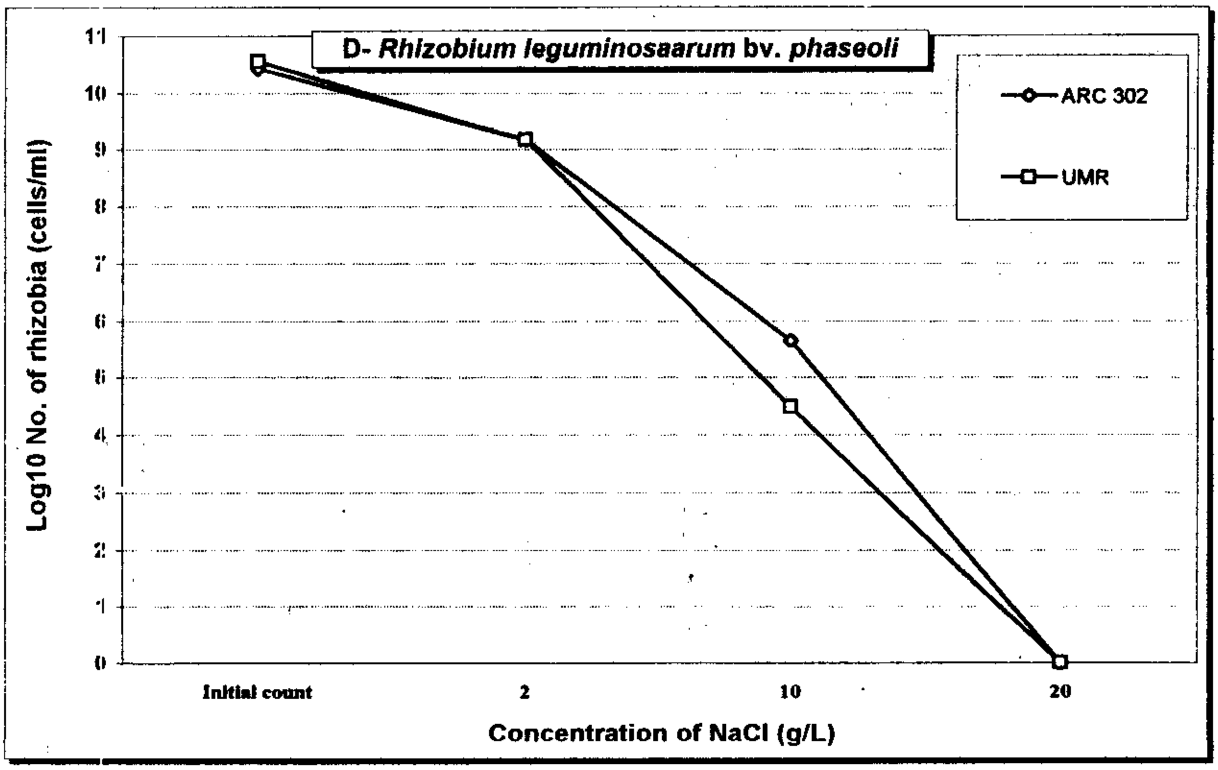
Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of NaCl (g/L)						
	Initial count	2	10	20	30	40	50
138	8.08	7.97	5.79	4.90	4.5	3.90	0
3407	8.15	8.00	0	0	0	0	0
110	8.23	8.00	0	0	0	0	0
ARC 500	8.25	7.86	0	0	0	0	0
102	8.20	7.15	4.56	3.30	0	0	0

Fig. (1) : Effect of different concentrations of NaCl on the survival of rhizobia.

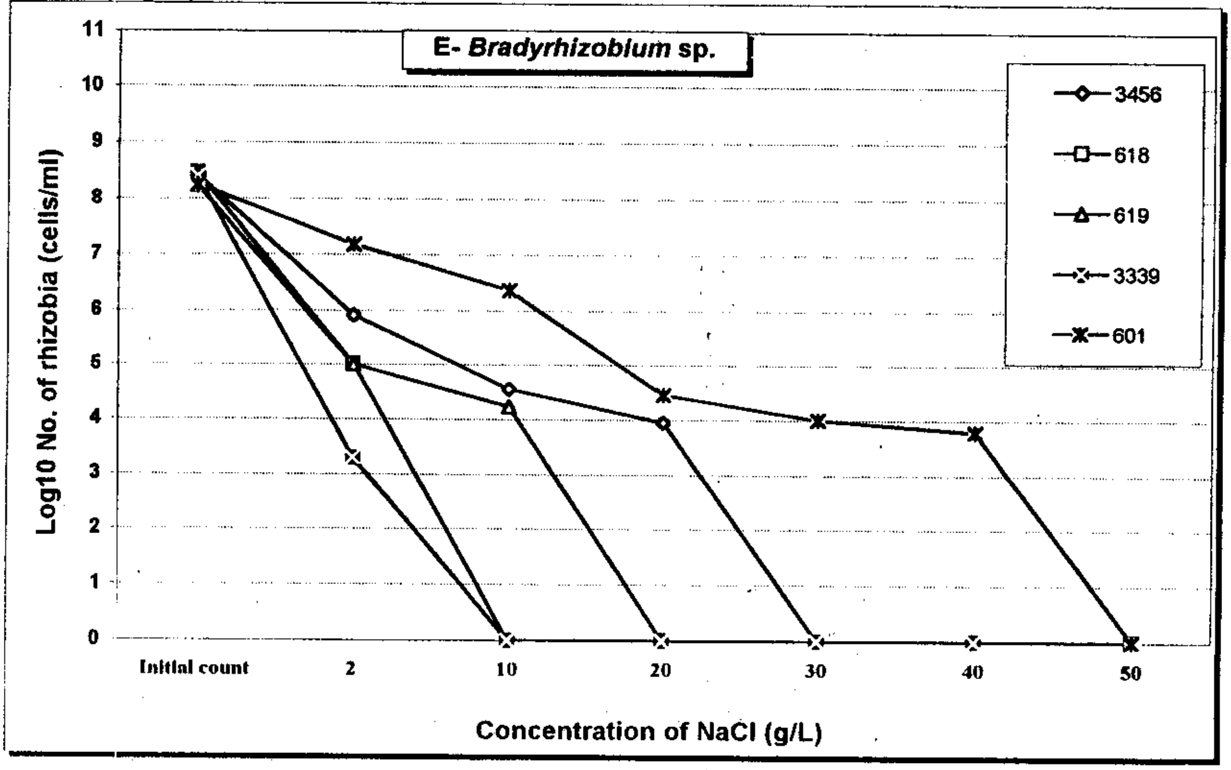


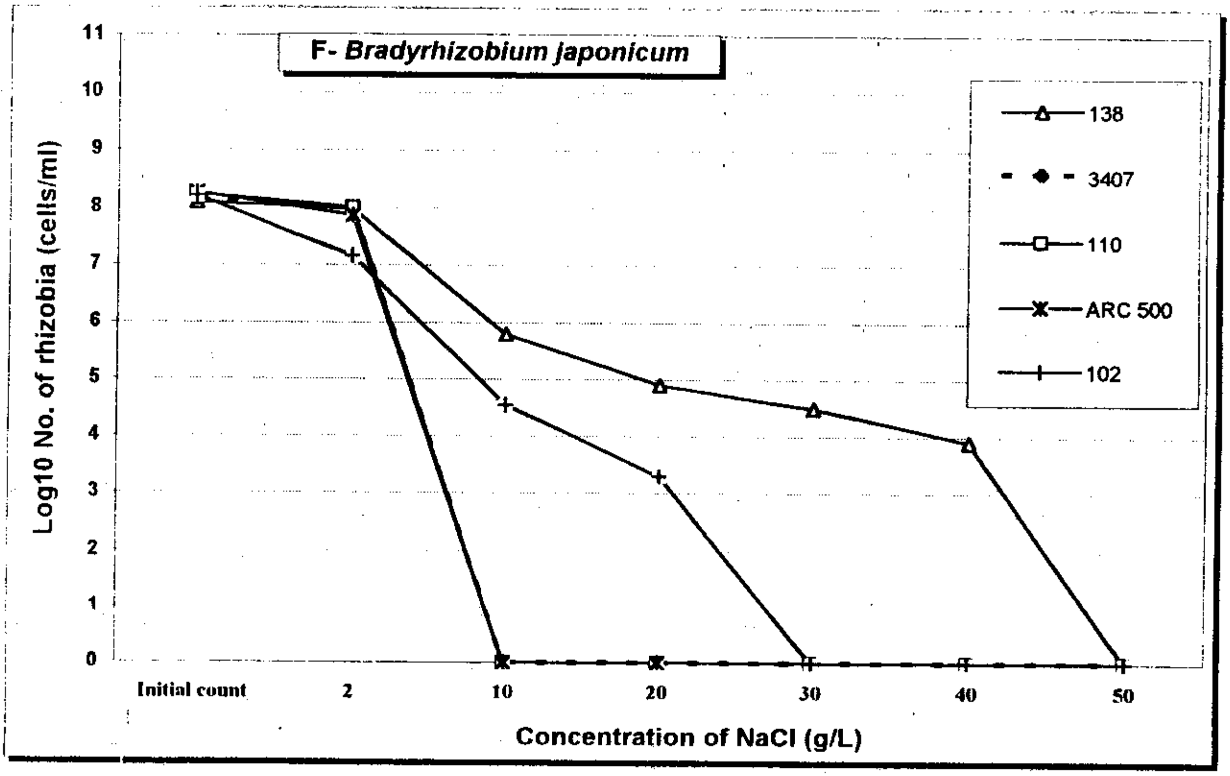
Cont. (Fig. 1)


C - Rhizobium leguminosarum bv. trifolii
TAL
ARC 103
ARC 101
Log10 No. of rhizobia (cells/ml)
11
10
9
8
7
6
5
4
3
2
1
0
Initial count
2
10
20
Concentration of NaCl (g/L)


D- Rhizobium leguminosaarum bv. phaseoli
ARC 302
UMR
Log10 No. of rhizobia (cells/ml)
11
10
9
8
7
6
5
4
3
2
1
0
Initial count
2
10
20
Concentration of NaCl (g/L)

Cont. (Fig. 1)


E- Bradyrhizobium sp.
3456
618
619
3339
601
Log10 No. of rhizobia (cells/ml)
Initial count
2
10
20
30
40
50
Concentration of NaCl (g/L)


F- Bradyrhizobium japonicum
138
3407
110
ARC 500
102
Log10 No. of rhizobia (cells/ml)
Initial count
2
10
20
30
40
50
Concentration of NaCl (g/L)

that in the symbiotic state rhizobia live within cells which have much greater solute concentrations than those generally experienced in soils **(Sprent, 1984)**.

The obtained results agree well with those obtained by **Sirry *et al.* (1980)** who found that fast growing rhizobia were more sensitive to soil salinity than slow growing-rhizobia. **Graham *et al.*** mentioned in **1992** that *Rhizobium* and *Bradyrhizobium* strains show marked variation in salt tolerance.

Nasef (1995) showed that 180 isolates of bean rhizobia were capable to tolerate 1% concentration of NaCl and some isolates were able to tolerate increased concentration of NaCl from 1.5 to 3.5 %. Also, **Swelim (1996)** identified five different groups of *Rhizobium* sp. (*Leucaena*) based on minimum inhibition concentration of NaCl which ranged from 0.75 to 4.0 % that indicated considerable variability among the rhizobial isolates.

As evident from the results there was great variance in the response of the tested strains to sodium chloride regimes. All strains were sensitive to higher concentration of NaCl (5 %). The growth of some strains was completely inhibited at 2% concentration. Some of the strains were sensitive to even 10 g/L NaCl concentration as reduction in cells number was observed at this concentration. However, rhizobia may survive in soils at much higher levels of salinity than those at which growth is restricted **(Singleton *et al.*, 1982)**.

Two *Rhizobium leguminosarum* bv. *viceae* strains ARC 200F and ICARDA 441 tolerant to 10 g/L and 40 g/L NaCl respectively were selected for further symbiotic performance study.

Table (9) : Effect of elevated temperature on the survival of *R. meliloti* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	Temperature (°C)					
	Initial count	30	35	40	45	50
ARC 1	7.70	8.18	8.18	6.70	6.70	0
ARC 2	7.81	7.51	6.48	6.30	5.90	0
ARC 3	8.38	7.34	6.59	4.45	0	0
ARC 6	7.38	8.08	6.26	2.90	0	0
A2	7.60	7.60	6.60	5.30	5.70	0

Table (10) : Effect of elevated temperature on the survival of *R. leguminosarum* bv. *viceae* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	Temperature (°C)					
	Initial count	30	35	40	45	50
ARC 200 F	8.18	9.40	7.48	6.90	4.78	0
ARC 202 F	8.34	8.00	7.60	6.60	2.48	0
ARC 206 F	8.40	8.04	6.00	0	0	0
ARC 207 F	8.08	7.95	5.78	0	0	0
ICARDA 441	7.90	7.90	6.60	2.6	0	0

Table (11): Effect of elevated temperature on the survival of *R. leguminosarum* bv. *trifolii* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	Temperature (°C)					
	28	30	35	40	45	50
TAL	8.93	8.88	6.08	5.86	4.08	0
ARC 103	8.73	8.97	4.70	0	0	0
ARC 101	8.23	8.81	5.90	3.04	0	0

Table (12): Effect of elevated temperature on the survival of *R. leguminosarum* bv. *phaseoli* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	Temperature (°C)					
	Initial count	30	35	40	45	50
ARC 302	9.50	9.18	8.27	7.90	5.6	0
UMR	9.52	9.17	8.08	5.83	4.04	0

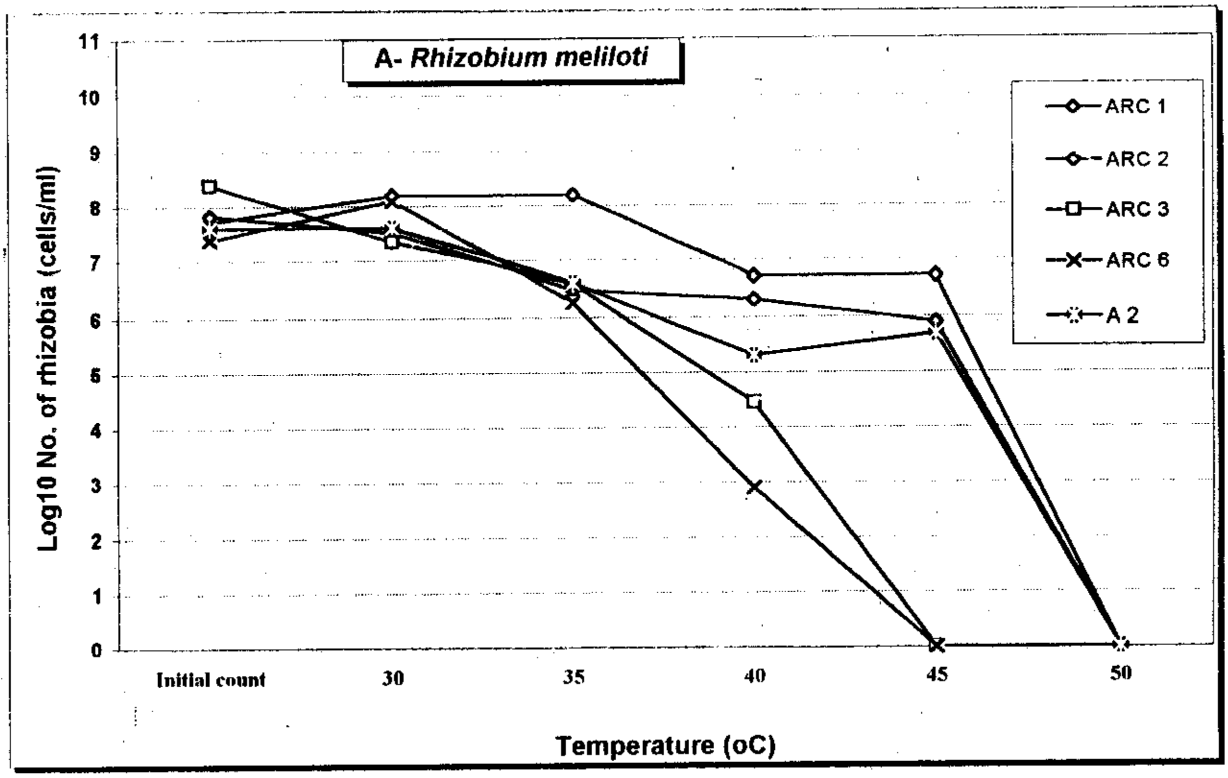
Table (13): Effect of elevated temperature on the survival of peanut *Bradyrhizobium* sp. in YEM medium .

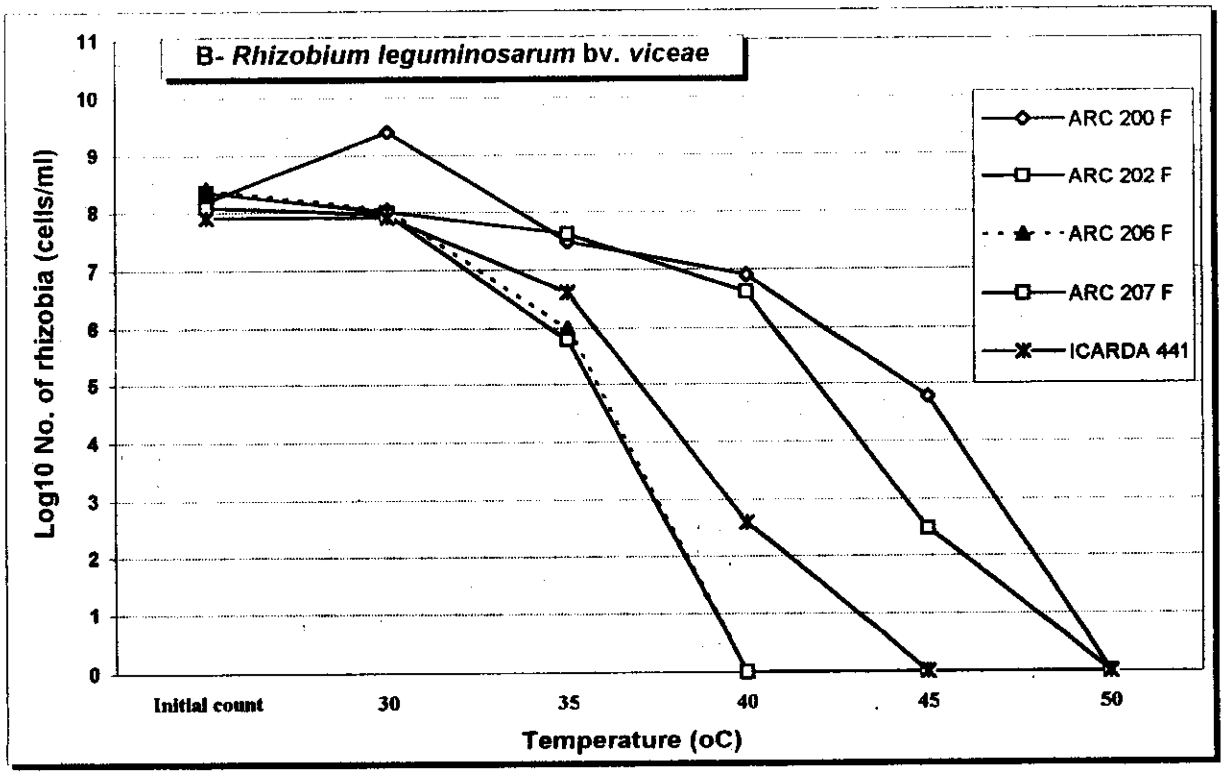
Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	Temperature (°C)					
	Initial count	30	35	40	45	50
3456	8.59	8.43	7.32	4.71	0	0
618	8.36	8.28	7.43	4.04	0	0
619	8.36	8.32	7.78	5.00	4.08	0
3339	8.49	8.96	7.88	4.23	3.90	0
601	8.00	8.00	8.40	3.78	3.30	0

Table (14): Effect of elevated temperature on the survival of soybean *Bradyrhizobium japonicum* in YEM medium .

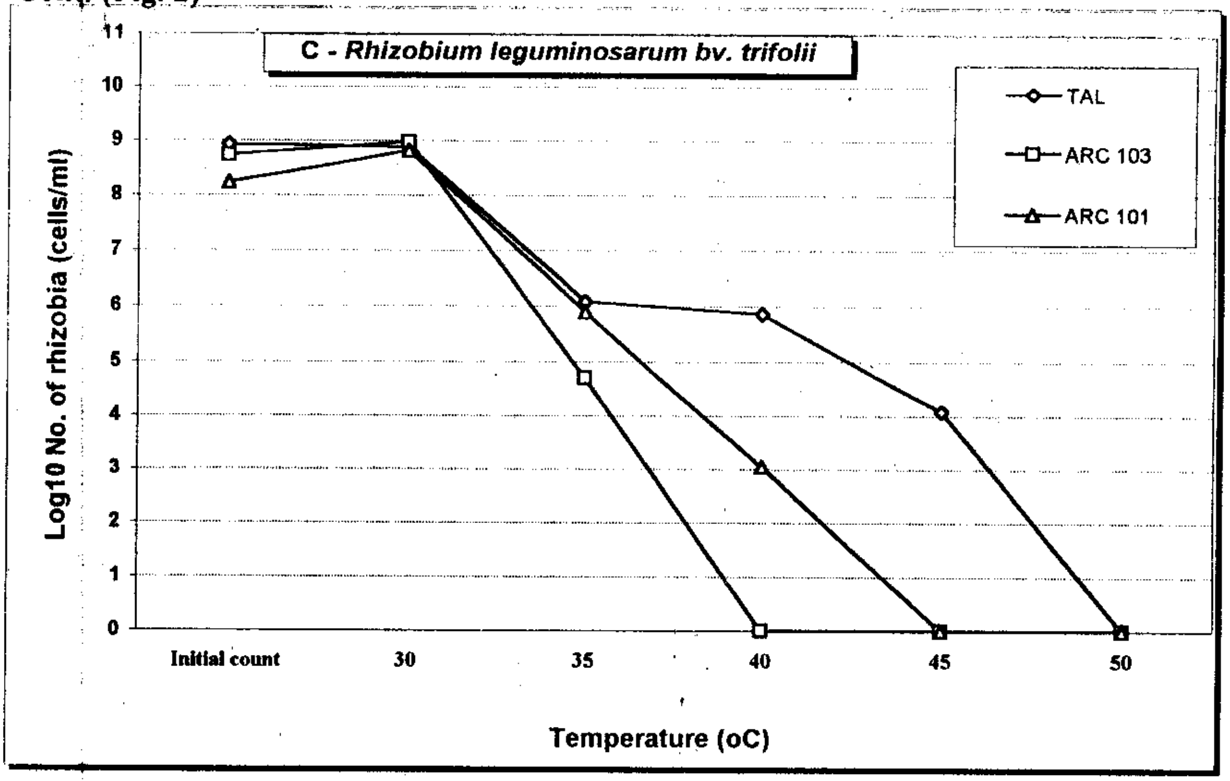
Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	Temperature (°C)					
	Initial count	30	35	40	45	50
138	9.59	9.57	8.43	6.34	4.90	0
3407	9.53	9.80	7.57	4.04	0	0
110	9.36	9.41	7.08	6.48	4.00	0
ARC 500	9.52	9.75	7.45	4.58	3.48	0
102	9.66	9.49	8.25	4.91	0	0

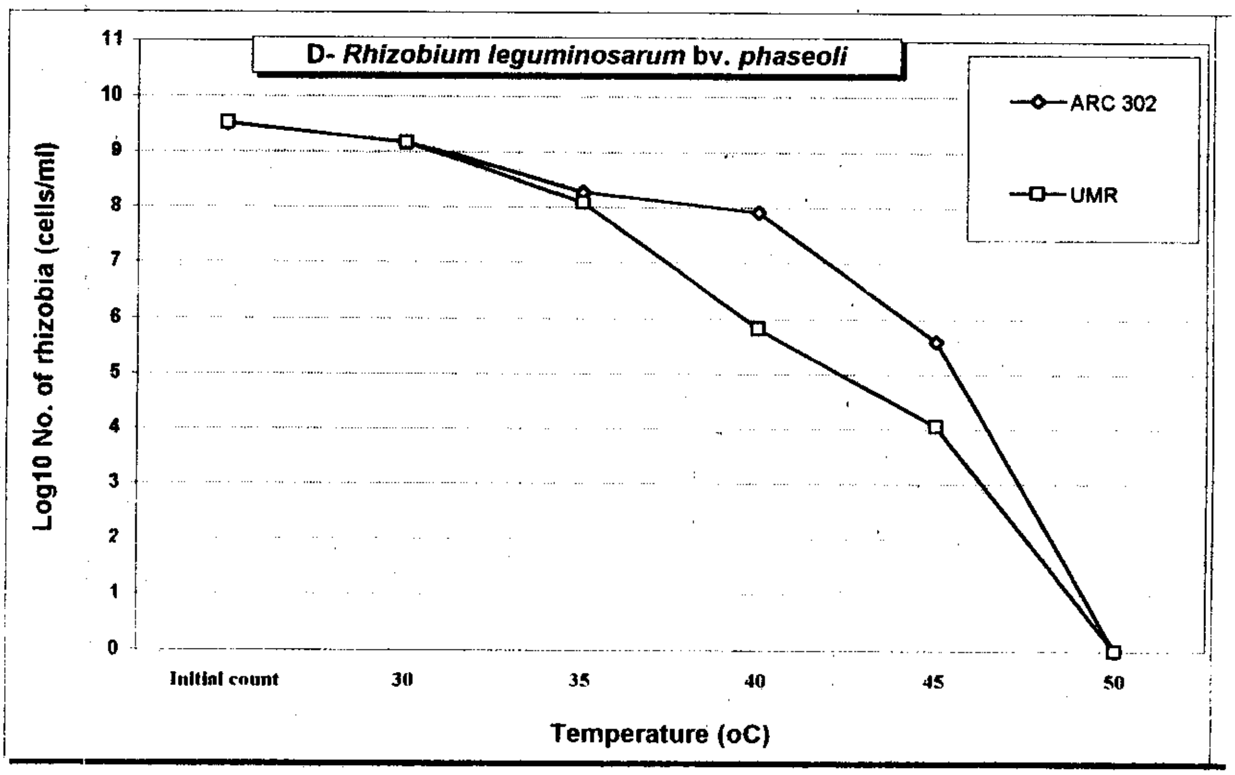
Fig. (2) : Effect of elevated temperature on the survival of rhizobia.



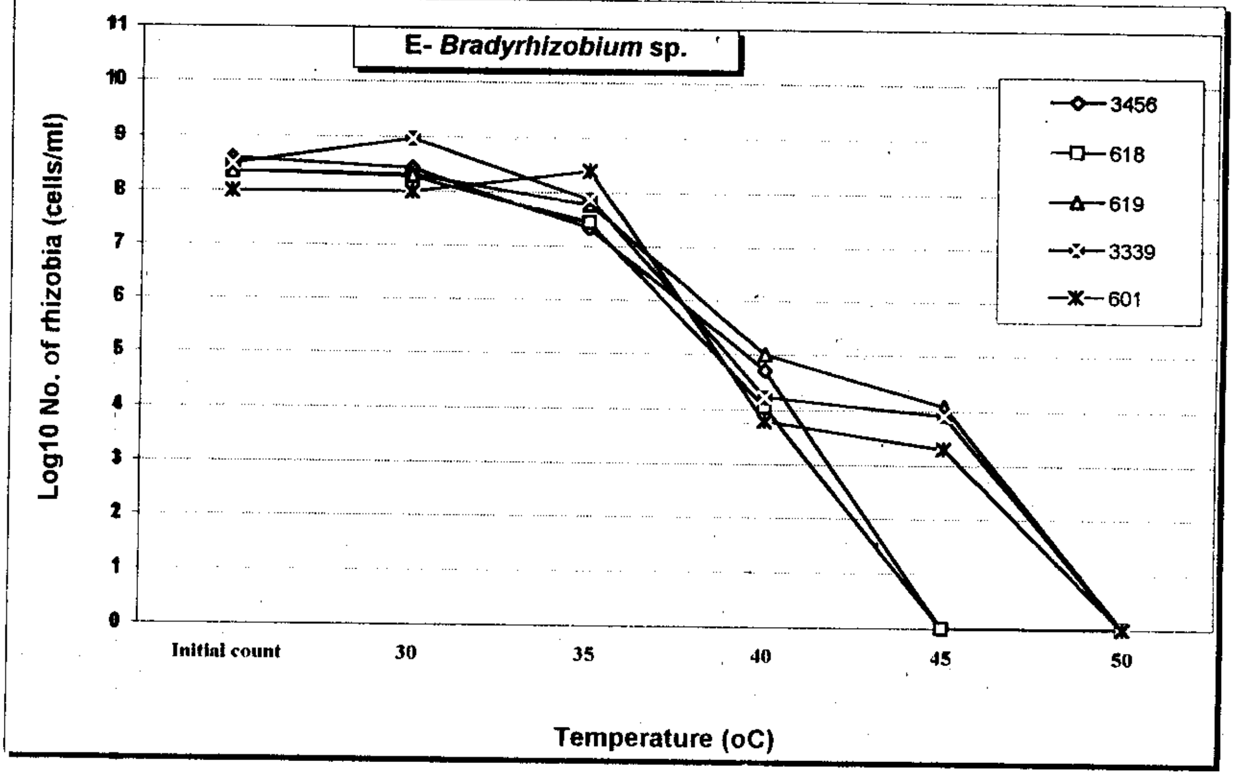


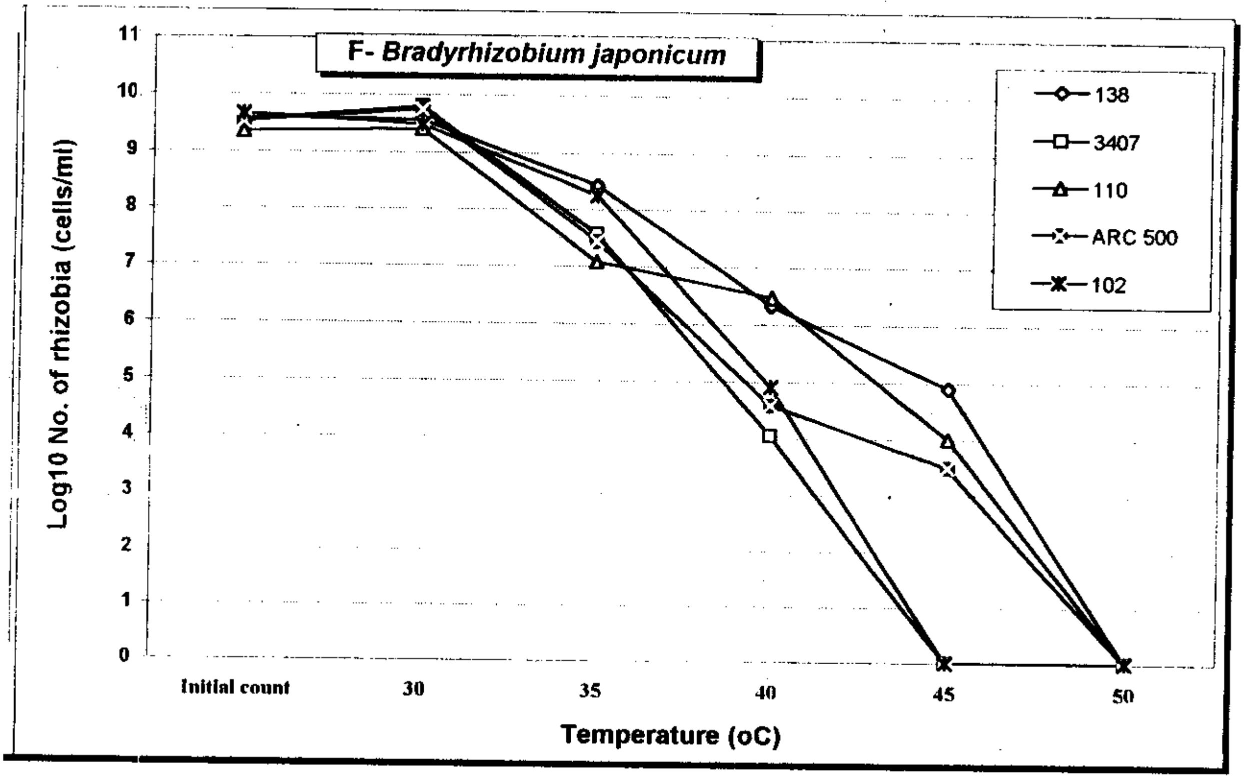
Cont. (Fig. 2)


C - Rhizobium leguminosarum bv. trifolii
TAL
ARC 103
ARC 101
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
Initial count
30
35
40
45
50
Temperature (oC)


D- Rhizobium leguminosarum bv. phaseoli
ARC 302
UMR
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
Initial count
30
35
40
45
50
Temperature (oC)

Cont. (Fig. 2)


E- Bradyrhizobium sp.
3456
618
619
3339
601
Log10 No. of rhizobia (cells/ml)
11
10
9
8
7
6
5
4
3
2
1
0
Initial count
30
35
40
45
50
Temperature (oC)


F- Bradyrhizobium japonicum
138
3407
110
ARC 500
102
Log10 No. of rhizobia (cells/ml)
11
10
9
8
7
6
5
4
3
2
1
0
Initial count
30
35
40
45
50
Temperature (oC)

strains tested high temperature - tolerant strains were selected which tolerate 45 °C. On the other hand, all strains tested were not able to grow at 50 °C.

Also, great diversity are observed among the rhizobial strains in response to increasing temperature and indicate that susceptibility to higher temperature 50 °C is common among the rhizobial species and strains tested. However, fourteen strains were found to tolerate temperature up to 45 °C such as *Rhizobium meliloti* (ARC1 , ARC2 and A2) (Table, 9), *Rhizobium leguminosarum* bv. *viceae* (ARC 200 F and ARC 202F) (Table, 10), *Rhizobium leguminosarum* bv. *trifolii* (TAL) (Table, 11), *Rhizobium leguminosarum* bv. *phaseoli* (ARC 302 and UMR) (Table 12), *Bradyrhizobium* sp. (619, 3339 and 601) (Table, 13) and *Bradyrhizobium japonicum* (138, USDA 110 and ARC 500) (Table, 14). On the other hand, eight strains showed temperature tolerance up to 40 °C, these strains belong to different species such as *Rhizobium meliloti* (ARC3 and ARC 6) , *Rhizobium leguminosarum* bv. *viceae* (ICARDA 441), *Rhizobium leguminosarum* bv. *trifolii* (ARC 101), *Bradyrhizobium* sp. (3456 and 618) and *Bradyrhizobium japonicum* (3407 and 102). Only three strains, however, are susceptible to elevated temperature more than 35 °C such as *Rhizobium leguminosarum* bv. *viceae* (ARC 206 F and ARC 207 F) and *Rhizobium leguminosarum* bv. *trifolii* (ARC103) .

These results are in harmony with those obtained by **EK-Jander** and **Fahraeus (1971)**, and **Graham (1992)** who found that for most rhizobia the temperature optimum for growth in culture is from 28 to 31 °C. On the other hand, **Habish** and **Khairi (1970)** observed that rhizobial strains from *Acacia* sp. attained optimum growth at 30-35 °C.

However, the optimum temperature for *Rhizobium meliloti* is 35 °C **(Allen and Allen, 1950)** and 90 % of cowpea strains obtained from the hot, dry environment grew well at 40 °C **(Eaglesham and Ayanaba, 1984)**.

The ability to grow at 42 °C was previously considered to be rare among rhizobial strains, even those of tropical origin **(Eaglesham *et al.*, 1987)**. To the contrary, in this study, it was found that fourteen and eight out of twenty five strains were tolerant to 45 and 40 °C respectively. This finding agrees well with the results of **Somasegran *et al.* (1984)** and **Hafeez *et al.* (1991)**. Also, **Munevar** and **Wollum (1981 a)** are in agreement with the obtained results and showed that characteristic temperatures varied among rhizobial strains, i.e., optimum temperature (27.4 to 35.2 °C) , maximum permissive temperature (29.8 to 38.0 °C) and maximum survival temperature (33.7 to 48.7 °C).

Generally, *Rhizobium* strains or species vary greatly in their susceptibility to high temperatures. *Rhizobium meliloti* survives better than *Rhizobium trifolii* on seed lying for periods in hot-dry soil **(Brockwell and Whally, 1970)**, *Rhizobium lupini* and *Rhizobium japonicum* are less susceptiple than *Rhizobium trifolii* when in dry soil at elevated temperatures **(Chatel** and **Parker, 1973b; Marshall, 1964)**. Hence, it should be possible to obtain or devise rhizobia able to survive, grow, or fix nitrogen under conditions where elevated temperatures are of importance.

Strain adaptation of high temperature (45 °C) has also been reported by **Wilkins (1967)**, **Hartel** and **Alexander (1984)**, and **Karanja** and **Wood (1988 b)**. In addition six strains out of 18 different strains of peanut rhizobia were classified as high temperature tolerant by

Elsaeid ***et al.*** **(1990)**. **Baldani** and **Weaver (1992)** suggested an involvement of some plasmids in *Rhizobium leguminosarum* bv. *trifolii* that mediating the response to the stress for resistance to heat stress.

Pronounced capability of tolerating the incubation temperature of 60 °C was recorded by **Nasef (1995)** for some local bean rhizobia and *Rhizobium tropici* . Also, nine out of thirteen isolates of rhizobia nodulating *Leucaena* were also reported resistant to 42 °C **(Swelim, 1996)**.

Four rhizobial strains representing *Bradyrhizobium* sp. (peanut) (3456 T 40 °C and 619 T 45 °C) and *Bradyrhizobium japonicum* (3407 T 40 °C and 138 T 45 °C) were selected for capacity of infective and effectiveness studies against the original strains.

4.1.3. Effect of mineral nitrogen levels on surivial of rhizobia

The rhizobial inocula must not only be effective in its symbiotic performance in controlled environments but also be highly competitive under the prevailing soil conditions, for instance in soil rich in nitrogen due to mineralization (Martensson et al., 1989). Therefore, the present study aimed to select suitable rhizobial strains tolerant to high nitrogen levels and investigating whether different N levels applied to the soil influence strain symbiotic performance.

Twenty-five strains represent the different species of rhizobia were screened for mineral nitrogen (KNO_3) tolerance at 100, 200, 300, 400, 500 and 600 ppm ; i.e.; 14, 28, 42, 56, 70 and 84 kg N per feddan, respectively in liquid medium.

Data in Tables 15-20 and Figures (3) A, B, C, D, E and F represent the effect of increased concentrations of mineral nitrogen (KNO_3) on survival and growth (log10 /ml) of the twenty five strains under investigation.

Results show that all strains tested were able to grow and survive under stress of 200 ppm KNO_3. However, strains representing *Rhizobium leguminosarum* bv. *viceae*, bv. *trifolii*, bv. *phaseoli* and *Bradyrhizobium* sp. (peanut) were able to tolerate up to 300 ppm KNO_3 in liquid medium. On the other hand, all strains representing *Rhizobium meliloti* were able to tolerate up to 400 ppm KNO_3 and two strains (ARC 6 and A2) were superior to the other tested strains in surviving up to 500 ppm KNO_3 .

In the present study it was clear that increasing KNO_3 concentration up to 400 ppm was found to have inhibitory effect on the growth and survival of the two strains representing *Rhizobium leguminosarum* bv. *phaseoli* (ARC 203 and UMR). Under the above mentioned conditions (400 ppm KNO_3) one out of five strains representing *Rhizobium leguminosarum* bv. *viceae*, two out of three strains representing *Rhizobium leguminosarum* bv. *trifolii* (ARC 103 and ARC 101) and three out of five strains representing *Bradyrhizobium* sp. (peanut) (618, 619 and 601) were not able to survive or grow. Some strains were found to tolerate higher concentrations of KNO_3 (500 ppm) such as ICARDA 441, TAL, 3456 and 3339, 3407 and ARC 102 which represent *Rhizobium leguminosarum* bv. *viceae*, *Rhizobium leguminosarum* bv. *trifolii*, *Bradyrhizobium* sp. and *Bradyrhizobium japonicum* , respectively.

Table (15) : Effect of different concentrations of KNO_3 on the survival of *R. meliloti* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of KNO_3 (ppm)						
	Initial count	100	200	300	400	500	600
ARC 1	8.20	8.92	8.00	6.07	6.26	0	0
ARC 2	8.28	8.20	6.97	6.00	5.86	0	0
ARC 3	8.08	8.00	7.11	7.04	6.95	0	0
ARC 6	8.15	8.00	8.04	6.94	6.83	5.70	0
A2	8.08	8.04	8.11	6.08	5.93	3.00	0

Table (16) : Effect of different concentrations of KNO_3 on the survival of *R. leguminosarum* bv. *viceae* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of KNO_3 (ppm)						
	Iinitial count	100	200	300	400	500	600
ARC 200 F	8.60	8.56	6.28	4.40	0	0	0
ARC 202 F	8.81	8.68	7.30	5.58	3.70	0	0
ARC 206 F	8.55	8.34	7.26	4.78	3.95	0	0
ARC 207 F	8.20	8.00	7.08	4.11	3.84	0	0
ICARDA 441	8.68	8.04	7.48	7.46	5.20	4.8	0

Table (17) : Effect of different concentrations of KNO_3 on the survival of clover *R. leguminosarum* bv. *trifolii* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of KNO_3 (ppm)						
	Initial count	100	200	300	400	500	600
TAL	8.78	8.00	5.78	5.60	3.78	3.78	0
ARC 103	8.90	5.08	3.90	3.08	0	0	0
ARC 101	8.88	7.90	4.04	1.70	0	0	0

Table (18) : Effect of different concentrations of KNO_3 on the survival of *R. leguminosarum* bv. *phaseoli* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})				
	Concentration of KNO_3 (ppm)				
	Iinitial count	100	200	300	400
ARC 302	8.28	9.18	9.30	8.15	0
UMR	7.70	7.52	6.08	4.00	0

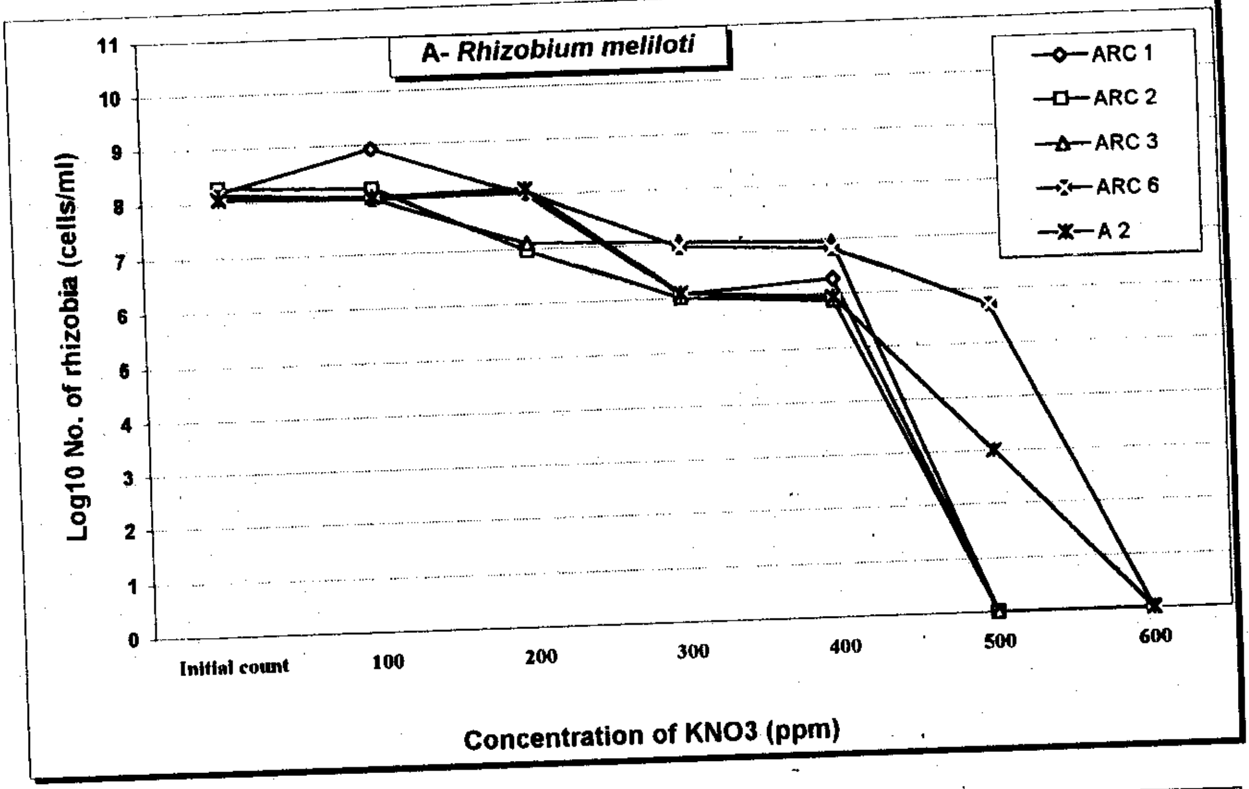
Table (19) : Effect of different concentrations of KNO_3 on the survival of *Bradyrhizobium* sp. (peanut) in YEM medium .

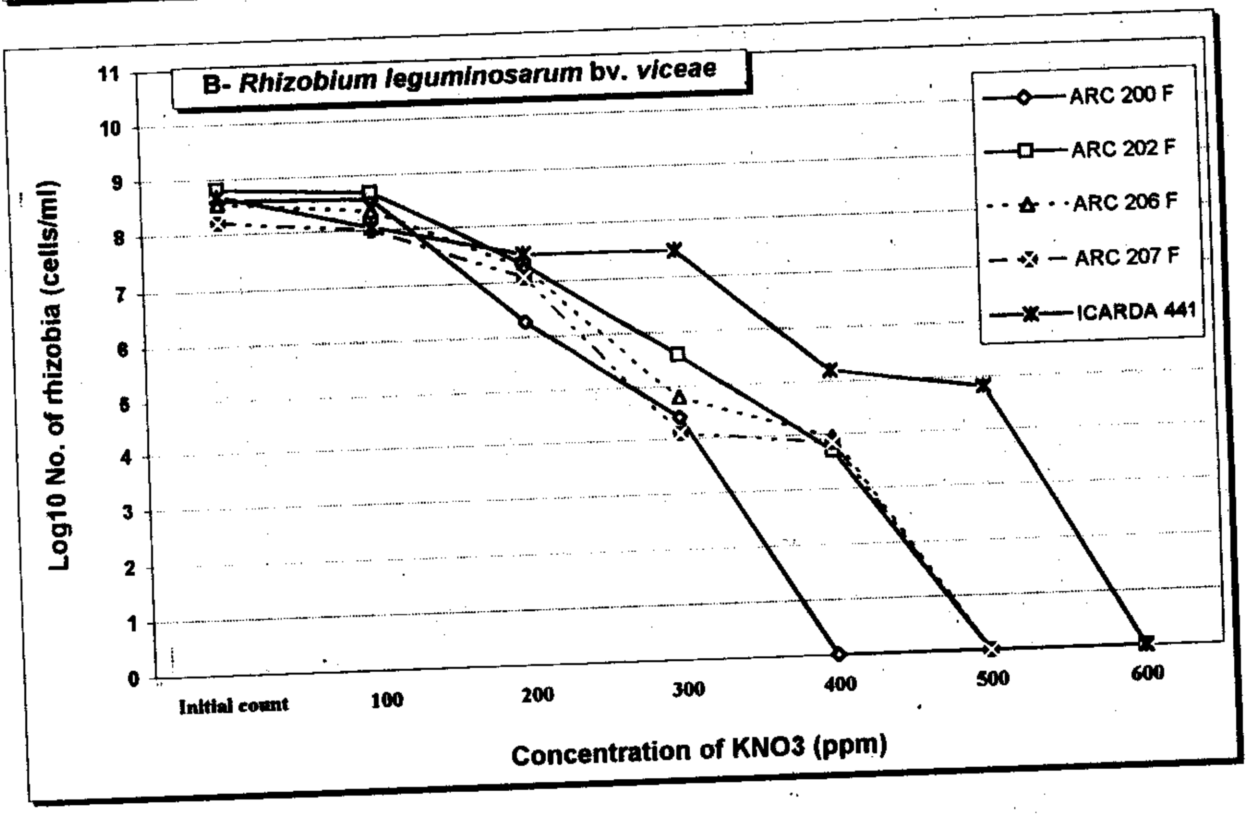
Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of KNO_3 (ppm)						
	Initial count	100	200	300	400	500	600
3456	8.49	8.45	7.30	5.32	3.46	4.45	0
618	8.40	8.30	6.00	4.88	0	0	0
619	8.26	8.18	5.88	4.54	0	0	0
3339	8.28	6.98	7.08	5.04	3.08	4.04	0
601	8.25	6.00	5.70	3.60	0	0	0

Table (20) : Effect of different concentrations of KNO_3 on the survival of soybean *Bradyrhizobium japonicum* in YEM medium .

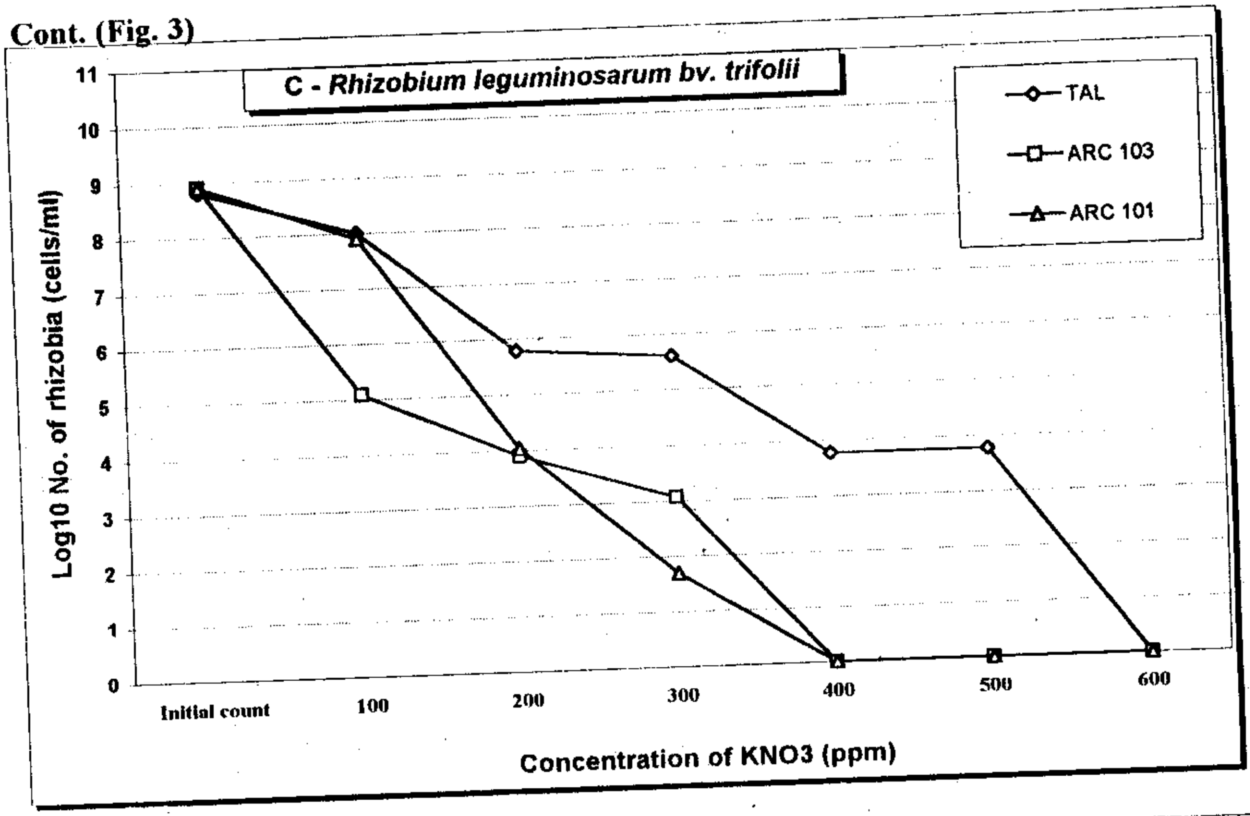
Rhizobial strains	Log number of rhizobia (cells ml^{-1})						
	Concentration of KNO_3 (ppm)						
	Initial count	100	200	300	400	500	600
138	8.08	8.08	5.04	0	0	0	0
3407	8.15	9.95	8.26	7.15	5.15	5.04	0
110	8.23	8.20	4.96	0	0	0	0
ARC 500	8.26	8.14	4.94	0	0	0	0
102	8.20	8.98	8.04	8.08	7.11	4.76	0

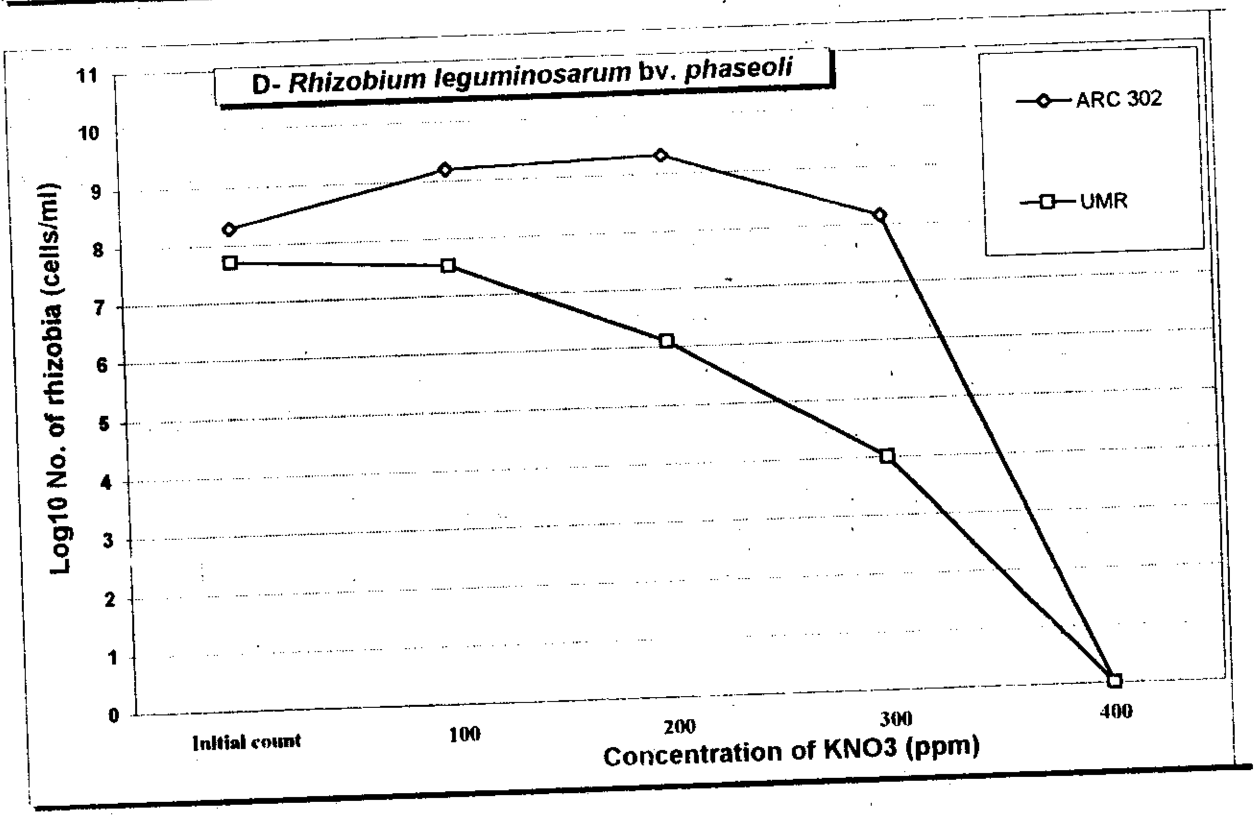
Fig. (3) : Effect of different concentrations of KNO3 on the survival of rhizobia.



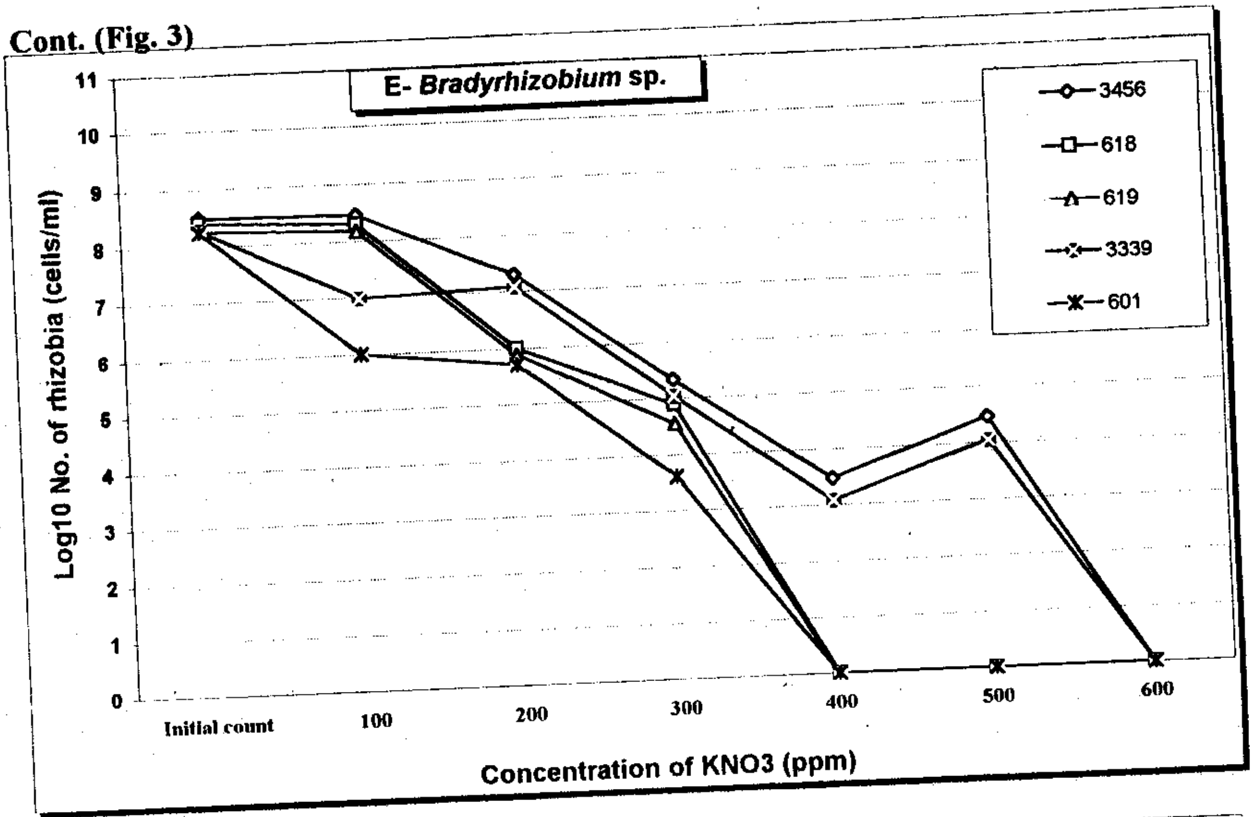


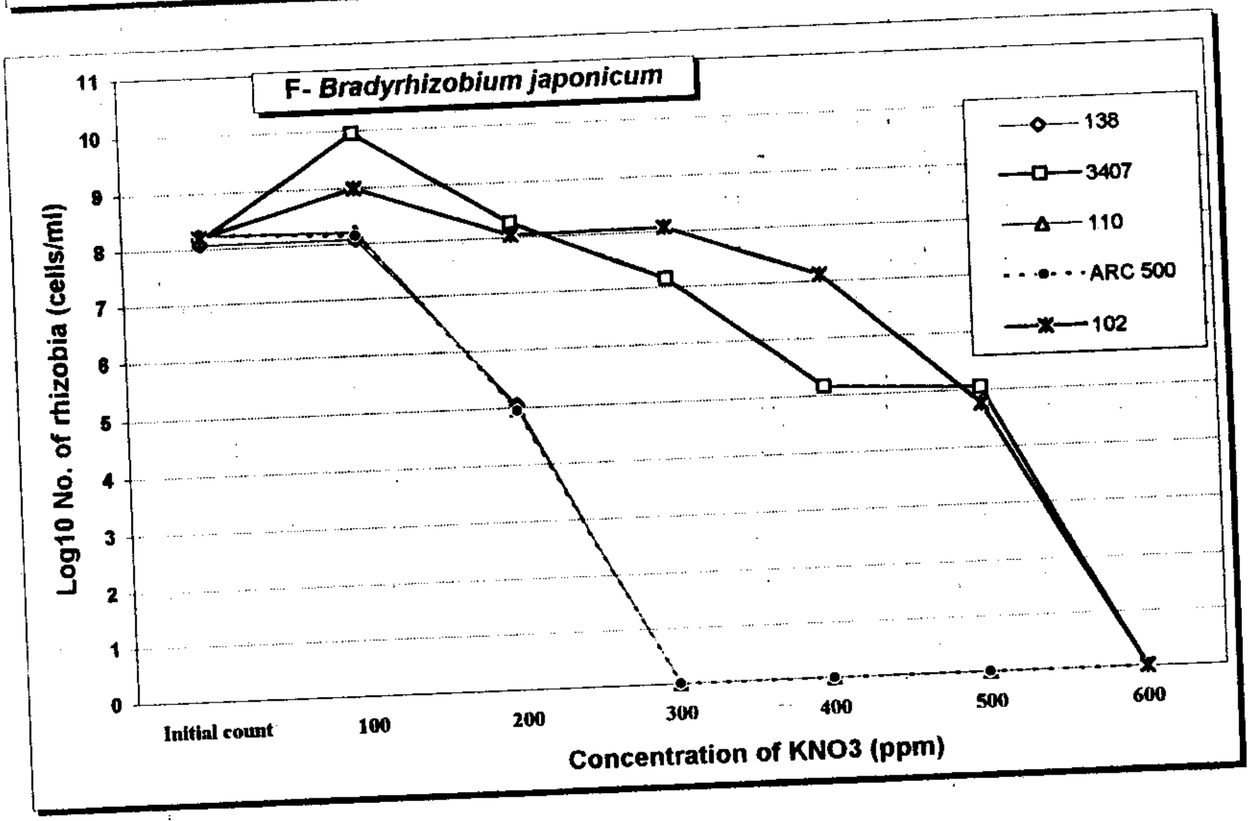
Cont. (Fig. 3)


C - Rhizobium leguminosarum bv. trifolii
TAL
ARC 103
ARC 101
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
Initial count
100
200
300
400
500
600
Concentration of KNO3 (ppm)


D- Rhizobium leguminosarum bv. phaseoli
ARC 302
UMR
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
Initial count
100
200
300
400
Concentration of KNO3 (ppm)

Cont. (Fig. 3)


E- Bradyrhizobium sp.
3456
618
619
3339
601
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
Initial count
100
200
300
400
500
600
Concentration of KNO3 (ppm)


F- Bradyrhizobium japonicum
138
3407
110
ARC 500
102
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
Initial count
100
200
300
400
500
600
Concentration of KNO3 (ppm)

Regarding *Bradyrhizobium japonicum* strains tested data showed that survival under stress of increased levels of mineral nitrogen (Table,20) classified the strains into two groups. Three strains (138, 110 and ARC 500) were very sensitive to higher concentrations of KNO_3 more than 200 ppm, whereas two strains (3407 and 102) were able to tolerate up to 500 ppm. The same pattern of mineral nitrogen tolerance was also observed for *Bradyrhizobium* sp. (peanut) strains tested except that grouping of the strains observed was at KNO_3 concentrations more than 300 ppm.

With increasing the dose of mineral nitrogen up to 600 ppm (KNO_3), no growth was observed for any of the tested strains. Again, higher mineral nitrogen concentrations more than 500 ppm were totally inhibitor for the growth or survival of all rhizobial strains under investigation.

Variation between rhizobial strains in their capacity to tolerate different levels of KNO_3 is in agreement with **Koermendy and Eaglesham (1984)**. They showed differences between strains of *Rhizobium japonicum* to survive in the presence of nitrate. **Maier and Brill (1978)** also isolated mutant of *Rhizobium japonicum* which showed a greater tolerance to combined N compared to the parent strain. The sensitivity of nitrogen fixation to combined nitrogen was dependent upon both the rhizobial strain and host-*Rhizobium* association. Therefore, it is advisable to describe a certain bacterium as being fully effective / ineffective or sensitive / tolerant to combined nitrogen, except in relation to a particular host genotype.

In the present study two rhizobial strains nodulating peanut 619 and 3456 tolerant to 300 and 500 ppm N respectively, were selected for competence study against the original sensetive ones. Two strains of *Bradyrhizobium japonicum* (138 T 200 ppm N and 3407 T 500 ppm N) were also selected for symbiotic performance evaluation as compared to sensitive original ones.

4.1.4. Effect of acidity and alkalinity on survival of rhizobia

Acid soils constraint agriculture production on perhaps 25% of the world's cropland **(Munns, 1986)**. pH can be the major factor limiting the number of microorganisms in the soil **(Borockwell *et al.*, 1991)**.

In the present study, the growth and survival of the different rhizobial species and some strains within each species were tested against lower and higher pH values.

Data in Tables 21-26 and Figures (4 A,B,C,D,E and F) showing the survival and growth of the rhizobial strains against variable pH values in YEM medium revealed that different rhizobial strains varied widely in their growth tolerance to pH. On the other hand, the pH value most favourable for growth was at pH 7.0, but growth was also possible outside of this value. There was a corresponding decrease in growth of all strains tested with the increase or decrease in pH outside 7.0.

Strains of *Rhizobium leguminosarum* bv. *phaseoli* ARC 302 and *Bradyrhizobium* sp. 618, 3339 and 601 were more tolerant to lower pH values up to 5. The growth rate ranged from 72-99% as compared with growth at pH 7. For the other tested strains, survival number (log10) diminished by decreasing the pH of the growth medium from 7.0 to 6.0.

Table (21) : Effect of different pH values on the survival of *R. meliloti* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	pH values					
	5	6	7	8	9	10
ARC 1	4.20	7.95	8.20	4.15	4.04	0
ARC 2	5.83	7.84	8.28	4.30	4.00	0
ARC 3	4.90	8.20	8.08	4.30	3.20	0
ARC 6	4.11	8.26	8.14	4.90	3.23	3.62
A2	4.26	8.26	8.26	4.30	4.00	3.54

able (22) : Effect of different pH values on the survival of *R. leguminosarum* bv. *viceae* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	pH values					
	5	6	7	8	9	10
ARC 200 F	3.97	4.07	8.51	0	0	0
ARC 202 F	3.81	5.80	8.34	6.86	3.26	0
ARC 206 F	4.00	5.28	7.40	0	0	0
ARC 207 F	4.08	5.15	8.08	0	0	0
ICARDA 441	4.37	7.72	7.90	6.28	4.56	0

Table (23) : Effect of different pH values on the survival of *R. leguminosarum* bv. *trifolii* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	pH values					
	5	6	7	8	9	10
TAL	3.56	8.89	8.93	2.11	0	0
ARC 103	3.30	8.80	8.87	6.60	4.20	2.28
ARC 101	5.00	6.37	8.85	6.26	3.85	0

Table (24) : Effect of different pH values on the survival of *R. leguminosarum* bv. *phaseoli* in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	pH values					
	5	6	7	8	9	10
ARC 302	9.28	9.32	9.36	6.04	3.91	0
UMR	5.32	6.11	8.28	7.28	6.00	1.2

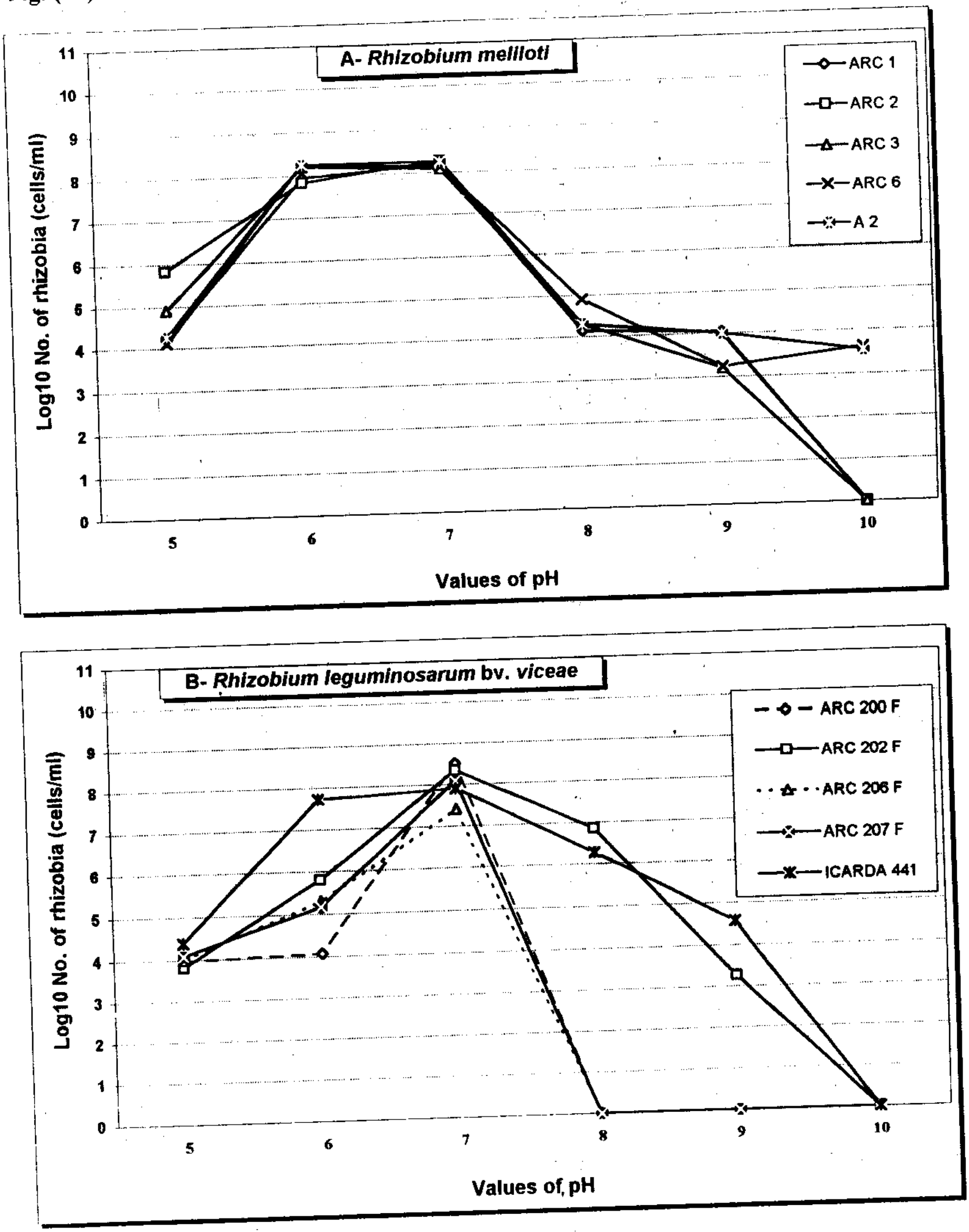
Table (25) : Effect of different pH values on the survival of peanut *Bradyrhizobium* sp. in YEM medium .

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	pH values					
	5	6	7	8	9	10
3456	4.28	7.48	8.61	3.90	4.04	0
618	6.32	6.46	8.46	6.32	4.30	3.30
619	0	5.16	8.26	4.08	0	0
3339	6.04	8.30	8.40	6.70	4.26	0
601	7.07	8.15	8.26	3.92	4.81	0

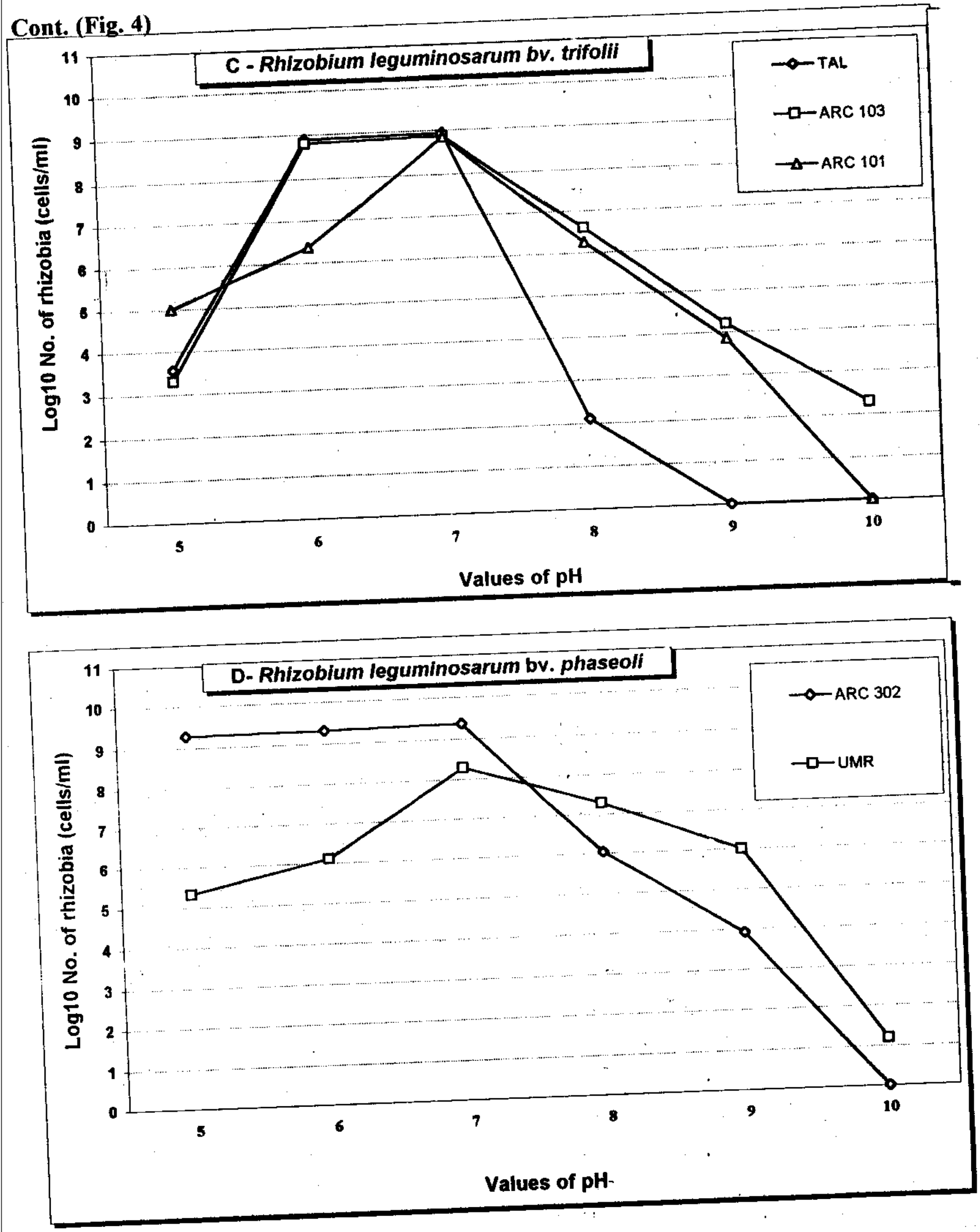
Table (26) : Effect of different pH values on the survival of soybean *Bradyrhizobium japonicum* in YEM medium.

Rhizobial strains	Log number of rhizobia (cells ml^{-1})					
	pH values					
	5	6	7	8	9	10
138	0	4.00	8.08	7.06	5.04	4.08
3407	4.89	5.89	8.15	6.67	5.83	3.95
110	4.94	5.18	8.21	5.52	5.34	0
ARC 500	4.38	5.53	8.26	3.75	0	0
102	0	4.08	8.20	7.24	5.05	0

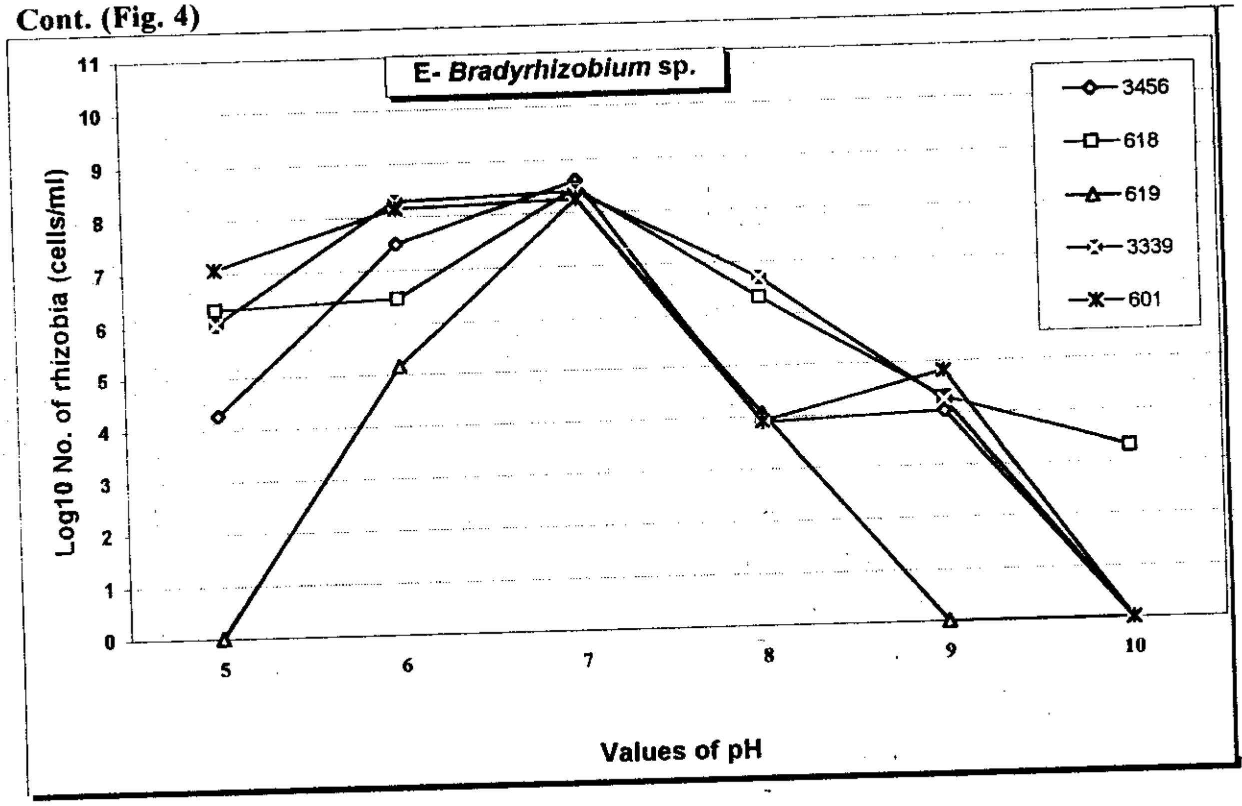
Fig. (4) : Effect of different pH values on the survival of rhizobia.

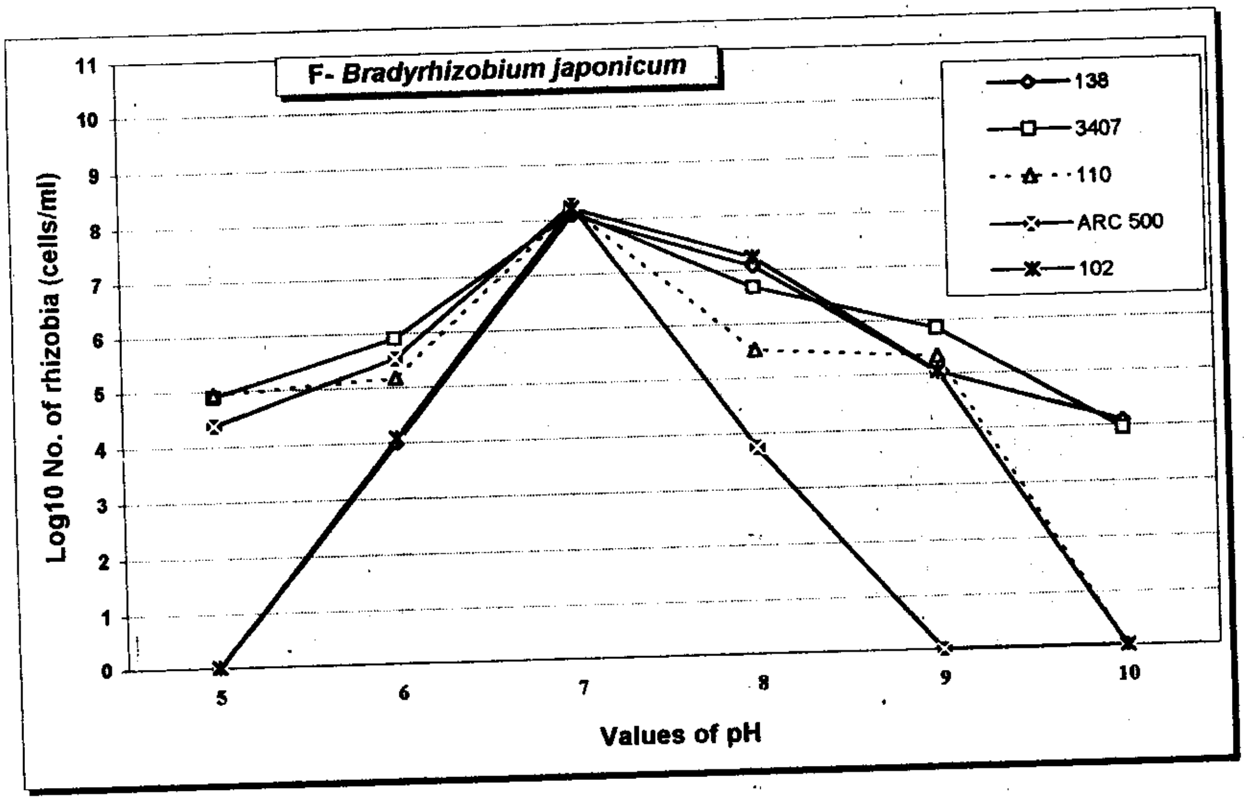


Cont. (Fig. 4)


C - Rhizobium leguminosarum bv. trifolii
TAL
ARC 103
ARC 101
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
5
6
7
8
9
10
Values of pH
D- Rhizobium leguminosarum bv. phaseoli
ARC 302
UMR
Log10 No. of rhizobia (cells/ml)
0
1
2
3
4
5
6
7
8
9
10
11
5
6
7
8
9
10
Values of pH

Cont. (Fig. 4)


E- Bradyrhizobium sp.
Log10 No. of rhizobia (cells/ml)
Values of pH
3456
618
619
3339
601
0
1
2
3
4
5
6
7
8
9
10
11
5
6
7
8
9
10


F- Bradyrhizobium japonicum
Log10 No. of rhizobia (cells/ml)
Values of pH
138
3407
110
ARC 500
102
0
1
2
3
4
5
6
7
8
9
10
11
5
6
7
8
9
10

Further, the growth rate was slowed more by the decrease in pH level up to 5.0 .

The growth rate of the three strains *Bradyrhizobium* sp. 619, *Bradyrhizobium japonicum* 138 and *Bradyrhizobium japonicum* 102 decreased markedly at pH 6.0. The growth rate percentage recorded were 62.4, 49.5 and 49.8 respectively, as compared with growth at pH 7.0. On the other hand, with decreasing pH value to 5.0, the growth of the above mentioned strains was totally inhibited which indicated its higher sensitivity to lower pH (pH 5.0).

Tolerance to pH 8.0 was clear for all tested species except three *Rhizobium leguminosarum* bv. viceae strains (ARC 200F, ARC 206F and ARC 207F) were very sensitive (Table 22) and were not able to grow with increasing pH values more than 7.0. On the other hand, tolerance to pH 9.0 varied among species and strains tested. All strains tested representing *Rhizobium meliloti* and *Rhizobium leguminosarum* bv. *phaseoli* tolerated pH 9.0 as indicated in Tables 21 and 24 respectively. Also, the same pattern of tolerance was scored for two out of three strains (*Rhizobium leguminosarum* bv. *trifolii*) Table (23), four out of five strains (*Bradyrhizobium japonicum*) (Table, 26).

Regarding higher pH values tested only seven out of twenty-five rhizobial strains were able to tolerate pH values up to 10.0. These strains representing *Rhizobium meliloti* (ARC 6 and A2), *Rhizobium leguminosarum* bv. *trifolii* (ARC 103), *Rhizobium leguminosarum* bv. *phaseoli* (UMR), *Bradyrhizobium* sp. (618) and *Bradyrhizobium japonicum* (138 and 3407).

Strains with higher tolerance to hydrogen ion concentration have been identified in most rhizobia species **(Cooper, 1982; Graham *et al*., 1982; Lowendorf and Alexander, 1983; Vargas and Graham, 1988; Richardson and Simpson, 1989)**, and in several cases performed better under acid-soil conditions in the field **(Graham *et al*., 1982; Howieson *et al*., 1988)**.

Also, the present observations are in accordance with that reported by **Nassef (1995)**, who found that the 180 isolates of bean rhizobia tested were capable to grow at pH 7. When the pH of the medium was decreased to 6.5, 5.5, 4.5 and 3.5, the number of isolates tolerated those conditions were 160, 102, 20 and 8 respectively.

Also, the obtained results are in harmony with those obtained by **Swelim (1996)**, who found that tolerance of sixteen isolates of rhizobia nodulating *Leucaena* varied among the isolates; 31% grew at pH 4.5, 46% grew at pH 5.0 and all grew at pH 5.5 and 10.0 .

The ability of *Rhizobium* strains to tolerate low pH was correlated with extracellular polysaccharide production **(Cunningham and Munns, 1984)** attributed to production of an arginine deaminase **(Casiano-Colon and Marquis, 1988; and Marquis *et al*., 1987)**, metabolic changes leading to reduce acid lading **(Terraccino *et al*., 1988; Rossi and Arst, 1990)** or maintaining higher cytoplasmic pH than acid sensitive strains **(O'Hara *et al*., 1989)**.

In the present study attempts to identify acid or/and alkaline tolerant strains of rhizobia by screening for rhizobial growth in acidified or alkaline YEM medium have been successful. The symbiotic performance of the tolerant strains to pH 5, pH 10 and pH 11 were investigated with peanut and soybean.

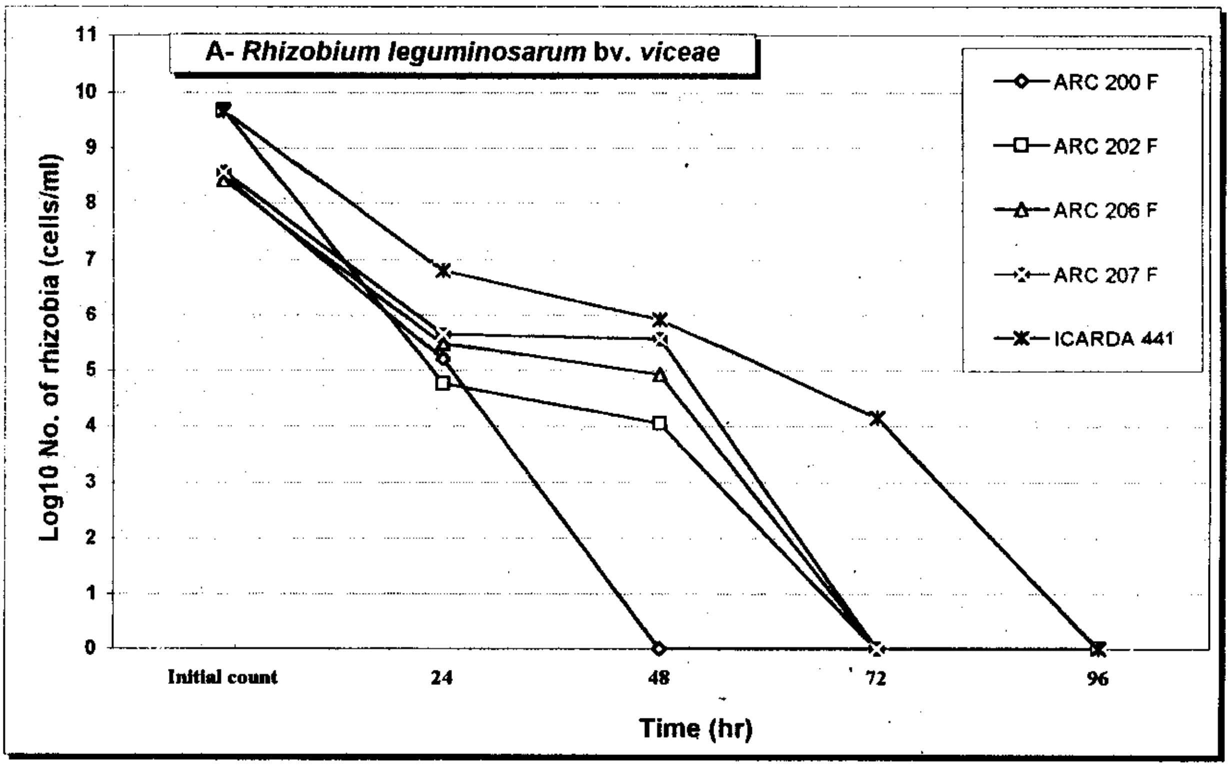
4.1.5. Effect of desiccation on survival of rhizobia :

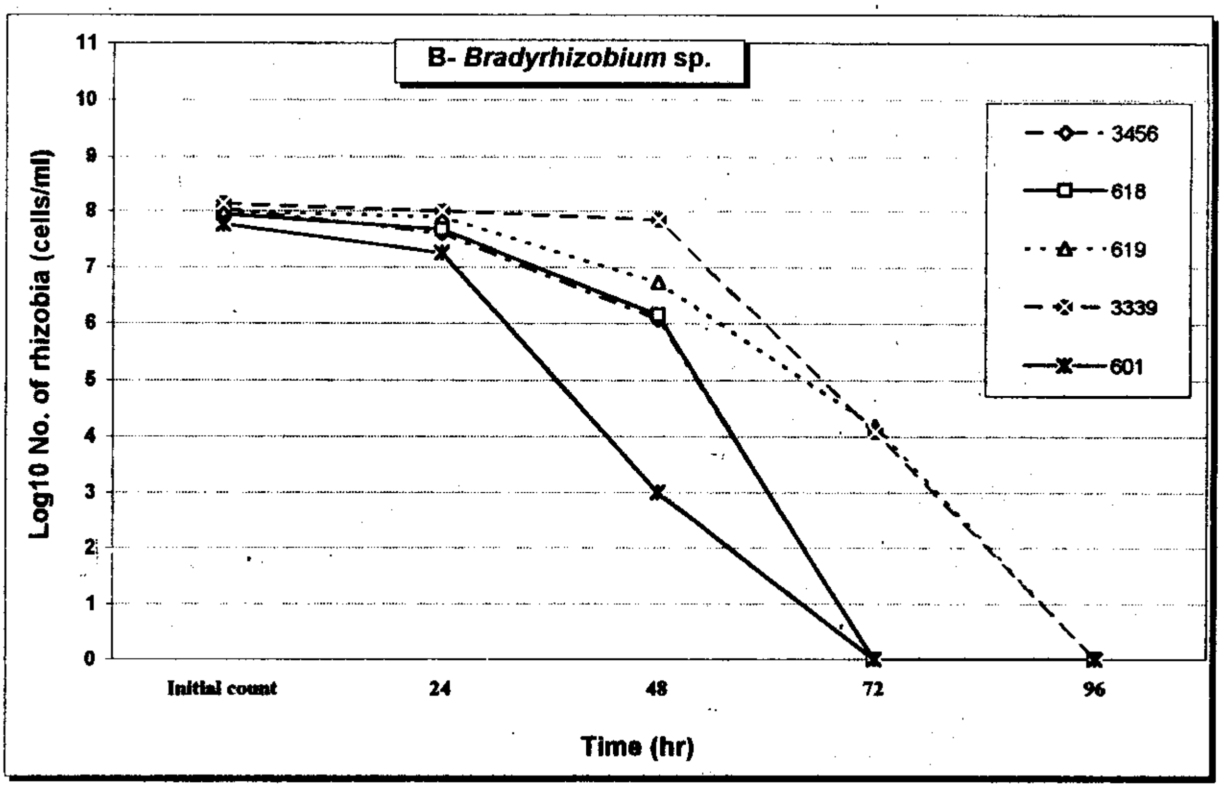
Nearly all areas of the world are subject to rainfree periods of sufficient duration to result in extensive drying of soil. The rhizobia that must survive in these soils in order to bring about nitrogen fixation in the succeeding crop may thereby become too few in number to cause extensive nodulation. Since root nodule bacteria do not produce endospores **(Graham *et al.*, 1963)**, they are susceptible to the detrimental effects of desiccation on inoculated seed and in natural soil. Therefore, in the present study, selection of some rhizobial strains that are better able to survive desiccation conditions and not seriously affected by the drying of soil was carried out.

Representative strains of *Rhizobium leguminosarum* bv. *viceae* and *Bradyrhizobium* sp. 5 strains each, were chosen for study. Tables 27 and 28 and Figures (5) A and B show the effect of desiccation for 24-96 hr. on growth and survival of the rhizobial strains nodulating faba bean and peanut respectively. Drying stress was imposed on the legume seeds coated with the rhizobial strains as peat-based inoculant kept in $CaCl_2$ desiccator for 96 hr. Survival of rhizobia was followed by determination of the viable number (log10) per seed at intervals of 24hr.

The growth rate of the rhizobial strains generally declined by increasing the duration of desiccation. No strains from either two species tested were able to survive desiccation up to 96 hr. However, one strain (ICARDA 441) of *Rhizobium leguminosarum* bv. *viceae* and two strains (619 and 3339) of *Bradyrhizobium* sp. retained viability after 72 hr. of

Fig. (5) : Effect of desiccation on the survival of rhizobia





The nodulation and N_2-fixation capability of the obtained strains under different levels of water stress were investigated.

4.2. Symbiotic performace of stress-tolerant rhizobial strains

A compatible symbiosis, under field conditions, is established only with the strains of rhizobia that are able to survive in the soil under stress of edaphic factors, such as salinity, temperature , high levels of nitrogen, pH and moisture **(Graham, 1992)**. Therefore, in the present study rhizobial strains representing the different species of rhizobia and tolerant to the different soil factors were selected and their competence with their specific hosts were evaluated.

4.2.1. The symbiotic performance of *Rhizobium leguminosarum* bv. *viceae* tolerant to different concentrations of NaCl with faba bean under soil salinity stress

Saline soils are formed under hot-arid conditions due to an accumulation of salts in the top soil and can form naturally or as the result of poorly-managed irrigation **(Nortcliff, 1988)**.

In the present study, data showed that strains of rhizobia are able to survive at high salinities. On the other hand, legumes are generally much more sensitive to salinity as compared to rhizobia **(Bhardwaj, 1975; Singleton *et al.*, 1982)**. Therefore, influence of salinity on the different aspects of symbiosis was investigated in the present study.

The soil was salinized with NaCl (0.1 up to 1.5%) before potting. Data in Table 29 and Figures 6A, B, C and D showed that inoculation, generally improved nodulation status of faba bean cv. Giza 204 plants as compared to uninoculated ones under the different levels of soil NaCl.

In addition, **Chao** and **Alexander (1982)** reported that prolonged drought or even a short period of drying is a major stress on *Rhizobium*. Also, with some strains of bacteria that infect *Lotus corniculatus*, the decline in soil kept dry for several months may be four logarithmic orders of magnitude **(Foulds, 1971)**. The conditions under which water is lost from the soil affect the extent of the decline **(Bushby** and **Marshall, 1977 b)**.

The results illustrate that the number of rhizobial cells/seed under desiccation stress were declined drastically as duration increased. Comparison of survival in drying conditions have indicated that *Bradyrhizobium* strains are more tolerant to desiccation than strains of *Rhizobium* over short periods. These results agree well with those of **Bushby** and **Marshall (1977)**. In contrast, other workers **(Mahler** and **Wollum, 1981)** found no simple relationship between the desiccation tolerance of fast-or slow growing rhizobia but they found specific strains of each survived in much greater numbers than others. Strains which survive under greater water stress are those which retain less water within the cells **(Bushby** and **Marshall, 1977 a; Al-Rashidi *et al.*, 1982)**.

The observations that nearly half of the viable cells of some strains of *Rhizobium* and *Bradyrhizobium* are not killed under stress of drying after 48 and 72 hr respectively, whereas 100% of the cells of other strains die under identical circumstances after 96 hr are in consistence with those obtained by **Osa-Afiana** and **Alexander (1982)**. In the present study, it appears that exposure to desiccation can be applied to obtain rhizobial strains (*Rhizobium leguminosarum* bv. *viceae*, ICARDA 441) not seriously affected under stress of desiccation or drying conditions.

drying stress. The viability rate recorded was 42.9, 52.3 and 50.1 % for the above mentioned strains respectively.

Comparing the viability responses after 24hr *Rhizobium leguminosarum* bv. *viceae* strains showed consistently lower growth rate than *Bradyrhizobium* sp. strains with a decrease in water potential imposed by desiccation. The viability rate was between 49.1 - 70.4 and between 93.5-98.7 for *Rhizobium leguminosarum* bv. *viceae* and *Bradyrhizobium* sp. respectively, as compared to initial count. On the other hand, after 48 hr of desiccation exposure, all strain tested were able to tolerate drying except one strain represent *Rhizobium leguminosarum* bv. *viceae* (ARC 200F) which showed no growth. Although strains from either species were able to survive drying upto 48 and 72 hr, the viable counts (log 10) were gradually decreased.

Generally, data indicate that the slow-growing strains have a greater ability to survive the effect of desiccation than the fast growing strains. These results are in agreement with those of **Marshall (1964)** and **Bushby** and **Marshall (1977 a)**. The survival of rhizobial strains on desiccation was not influenced by the degree of extracellular polysaccharide production in strains of *Rhizobium trifolii* (**Bushby** and **Marshall, 1977 a**).

The above mentioned findings indicate the importance of drying as a factor affects the viability and survival of the rhizobial strains in free living state. These observations are in consistence with **Pena-Cabriales** and **Alexander (1979)** with many strains of all species of *Rhizobium*, exposure of the soil to drying reduces the viable population between 50-99% or more, and several cycles of soil wetting followed by drying reduce the population still further .

Table (29) : Symbiotic performance of *Rhizobium leguminosarum* bv. *viceae* tolerant to different concentrations of NaCl with faba bean (75 DAP).

Treatments NaCl %	Nodulation status No./Plant	Nodulation status D.W. (mg/plant)	Shoots D.W. (g/plant)	Nitrogen content (mg/plant)
Uninoculated				
0.0	15	678.0	8.96	146
0.1	18	714.4	7.33	111
0.2	17	422.4	6.47	91
0.4	7	173.0	5.26	71
0.6	9	133.1	4.76	56
0.8	3	79.6	2.88	13
1.0	3	40.1	2.74	5
1.5	0	0.0	1.99	3
ARC 200 F				
0.0	78	1334.1	10.31	169
0.1	64	956.8	8.33	154
0.2	41	449.4	7.16	142
0.4	20	149.8	4.97	94
0.6	22	105.5	4.44	77
0.8	6	25.7	2.66	8
1.0	0	0.0	1.43	2
1.5	0	0.0	1.11	2
ICARDA 441				
0.0	86	1592.8	10.38	199
0.1	74	1314.8	9.13	191
0.2	50	747.6	8.86	176
0.4	40	505.0	7.63	133
0.6	24	171.8	6.29	102
0.8	21	141.4	4.02	76
1.0	13	45.7	3.56	22
1.5	5	39.3	2.77	8
L.S.D.				
Inoc. 0.05	4.879	63.77	0.4088	7.005
0.01	6.508	85.07	0.5453	9.345
Salts 0.05	7.967	104.10	0.6680	11.440
0.01	10.630	138.90	0.8910	15.260
I. x S. 0.05	13.800	180.40	1.1560	19.810
0.01	18.410	240.60	1.5420	26.430

Nodulation, growth and nitrogen fixation capability of faba bean plants grown on salinized soil and inoculated with NaCl tolerant *Rhizobium leguminosarum* bv. *viceae* are present in Table (29) and illustrated by Fig. (6 A,B,C and E) .

The number of noduels of the uninoculated plants varied between 18 to 0 / plant under NaCl soil stress of 0.1% - 1.5% respectively. The number of nodules among the inocuolated plants with salt tolerant strains, however, varied between 64 to 0 nodules / plant with ARC 200F and 74 to 0 nodules / plant with ICARDA 441 under the same rate of soil salinity stress. Data also, showed that inoculation with strains tolerant to high concentration of NaCl (ARC 200F and ICARDA 441) produced significant increases in plant dry matter and nitrogen contents under stress of NaCl salinized soil up to 0.6% as compared to uninoculated plants. Strain ICARDA 441 was superior in producing the higher number and dry weight of nodules, plant dry weight accumulation and nitrogen content. The above mentioned parameters decreased with increasing the soil NaCl. The decrease was very clear in uninoculated plants in which nodules resulted from the native rhizobia. For strain ARC 200F (tolerant to NaCl up to 1%) was able to induce nodules on faba bean plants under stress of salinized soil (0.8% NaCl), however, strain ICARDA 441 (tolerant to NaCl up to 4%) was infective up to 1.5% NaCl salinized soil and induced 5 nodules/plant. On the other hand, as salinity increased from 0.6 to 1.5% NaCl , faba bean plants inoculated with ARC 200F showed a reduction in nodulation (6-0 nodules/plant). However, under the same rate of soil salinity, faba bean plants inoculated with ICARDA 441 the number of nodules formed ranged from 21-5 nodules/plant. Uninoculated plants under stress of more than 0.4% NaCl were severly depressed regarding the nodules number (9-0 / plant) and dry weight (133.1 - 0 mg/plant).

At high soil salinity (0.6% NaCl) which greatly depressed plant growth, there was good nodulation of 22 and 24 nodules/plant due to inoculation with ARC 200F and ICARDA 441 respectively. These observations indicate that the rhizobia (*Rhizobium leguminosarum* bv. *viceae*) can tolerate higher levels of soil salinity than the host plant and reflect the importance of selecting varieties or cultivars of leguminous crops capable of withstanding salinity rather than attempting to introduce salt-tolerant rhizobial strains into soil. Levels of salinity that inhibit the symbiosis between legumes and rhizobia different from those that inhibit the growth of the individual symbionts **(Lauter *et al.*, 1981)**. Generally, legumes are more sensitive to osmotic stress than their microsymbionts **(Singleton** and **Bohlool, 1983)**. In contrast, rhizobia can survive in extremely high levels of salt, both in culture **(Hamdi** and **Al Tai, 1981; El-Sheikh** and **Wood, 1989 a,b)** and in the soil **(Bhardwaj, 1975)**.

Generally, increasing soil salinity resulted in a reduction of nodules number, nodules dry weight, growth and nitrogen content of faba bean plants. However, inoculation with NaCl tolerant strains (ARC 200F and ICARDA 441) resulted in increasing the above mentioned parameters under the high levels of salinity from 0.6 up to 0.8 NaCl %. **Lauter *et al.* (1981)** observed similar effects of salinity on nodulation and N_2 fixation by *Glycine max* and *Cicer arietinum* .

Naturally adapted to salt stress of strains of *Rhizobium leguminosarum* bv. *viceae* **(Abdel-Wahab *et al.*, 1993)** and

Bradyrhizobium japonicum (**Abdel-Wahab *et al.*, 1995**) were superior in nodulation and accumulation of higher amounts of atmospheric nitrogen as compared to sensitive or induced ones under soil salinity stress.

Significant differences were noticed in shoots dry weight and nitrogen content between NaCl stressed and non-stressed plants. However, stressed plants inoculated with salt tolerant *Rhizobium leguminosarum* bv. *viceae* strains showed higher shoots dry weight and nitrogen content as compared to uninoculated ones, this finding agree with **Swelim (1996)** who suggested that salt tolerant *Rhizobium* strains, compared to salt sensitive ones, can increase the ability of host plant to grow under saline conditions.

4.2.2. Nodulation and growth of peanut due to inoculation with *Bradyrhizobium* strains tolerant to high temperatures

Growth and nodulation status of peanut cv. Giza 5 grown in sandy soil as affected by inoculation with *Bradyrhizobium* sp. tolerant to high temperatures (3456 T 40 °C and 619 T 45 °C) were evaluated in a pot experiment.

Nodulation and growth parameters of the peanut plants are presented in Table (30) and illustrated in Figures (7A, B, C and D) .

Peanut roots beared variable numbers of nodules depending upon inoculation or uninoculation and the bacterial strain applied. Generally, inoculation resulted in the formation of nodules on the root system of the plants as compared to uninoculated ones. Inoculation with *Bradyrhizobium* sp. strains (peanut) tolerant to higher temperature 40 and 50 °C significantly increased the number of nodules/plant. At 45

Table (30) : Symbiotic performance of *Bradyrhizobium* sp. tolerant to different degrees of temperature with peanut .

Treatments		Nodulation status No./Plant		Nodulation status D.W. (mg/plant)		Shoots D.W. (g/plant)		Nitrogen content (mg/plant)	
	DAP	45	75	45	75	45	75	45	75
Uninoculated		0	0	0	0	4.9	6.4	90	102
3456		47	85	180	523	5.9	10.1	104	299
3456 T 40 °C		69	95	260	743	7.3	19.9	290	564
619		57	99	150	503	4.6	11.6	89	181
619 T 45 °C		69	106	267	680	8.5	18.6	258	433
L.S.D.	0.05	10.59	15.72	78.18	295.7	1.044	1.988	66.81	85.8
	0.01	19.29	39.26	111.2	320.6	1.484	2.828	95.02	164.2

Table (31) : Symbiotic performance of *Bradyrhizobium japonicum* tolerant to different degrees of temperature with soybean .

Treatments		Nodulation status No./Plant		Nodulation status D.W. (mg/plant)		Shoots D.W. (g/plant)		Nitrogen content (mg/plant)	
	DAP	45	75	45	75	45	75	45	75
Uninoculated		0	0	0	0	2.4	6.7	30	171
3407		105	165	378	1750	4.7	11.5	95	223
3407 T 40 °C		99	158	478	2683	5.7	13.5	140	568
138		109	102	282	1651	3.5	10.8	47	238
138 T 45 °C		115	124	603	2437	5.6	11.0	116	433
L.S.D.	0.05	10.34	33.96	122.6	520.4	0.638	1.774	18.11	63.99
	0.01	14.71	47.60	174.3	740.2	0.907	2.524	25.76	90.73

Fig. (7): Symbiotic performance of *Bradyrhizobium* sp. tolerant to different degrees of temperature with peanut .

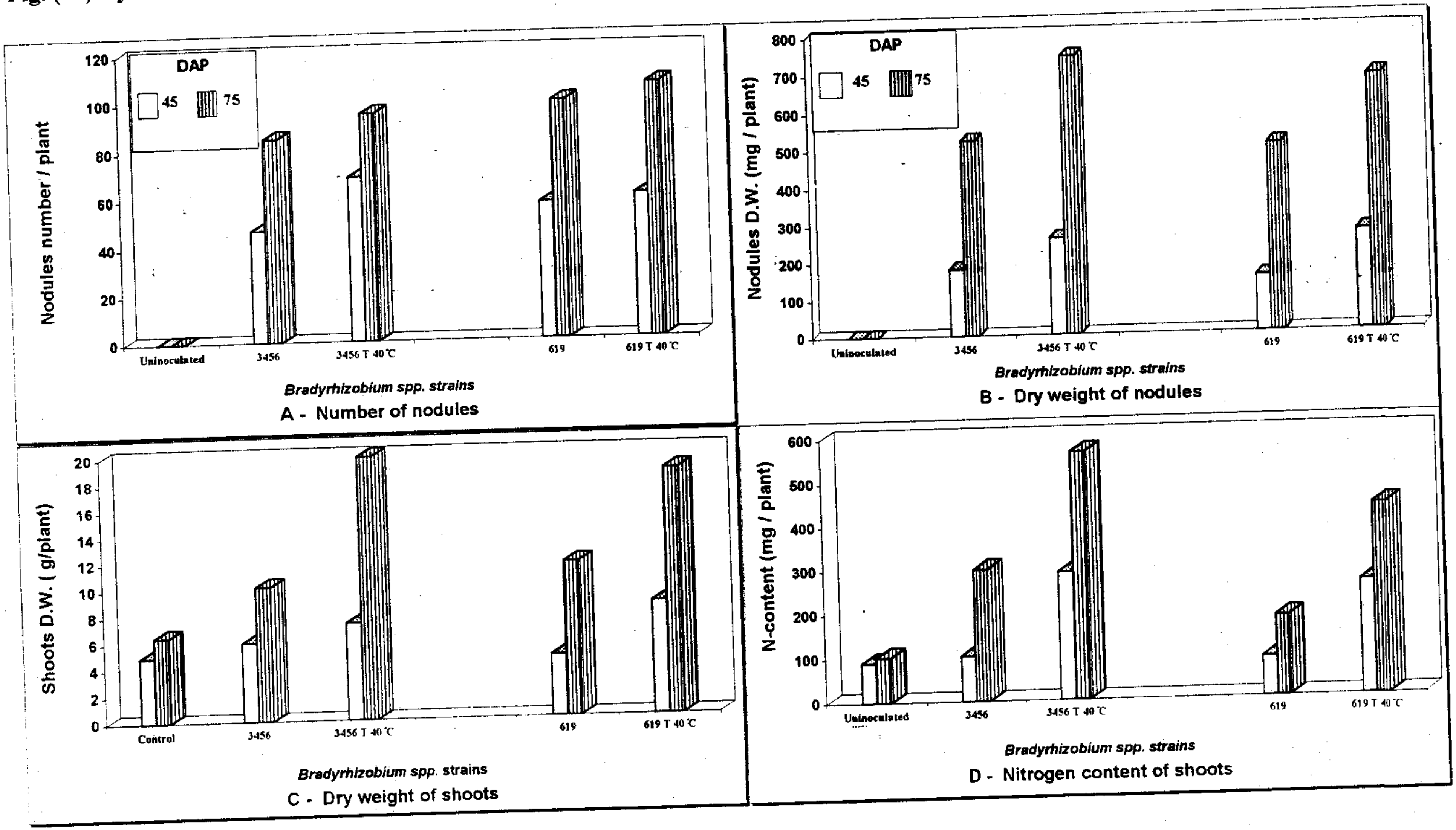
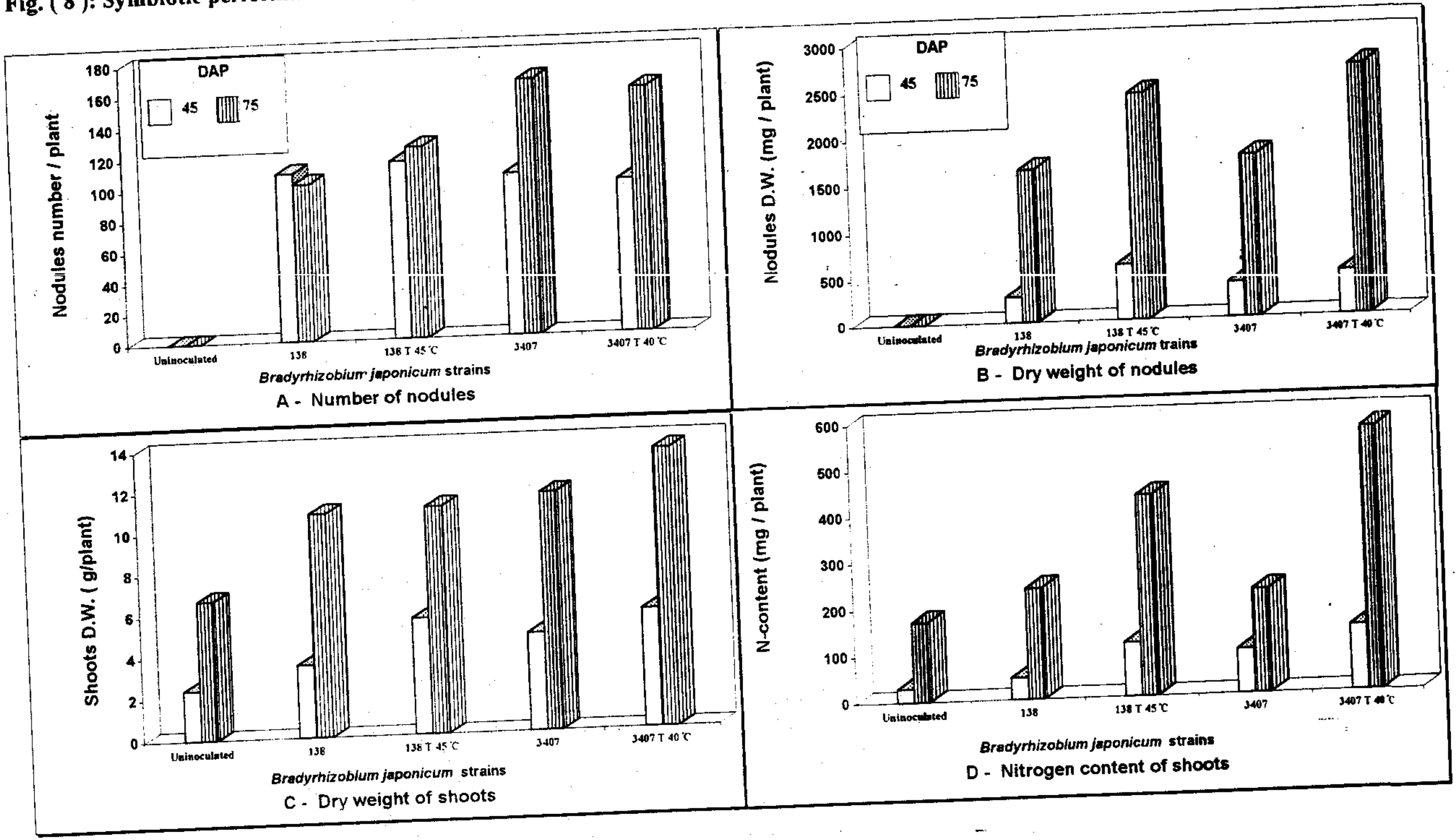


Fig. (8): Symbiotic performance of *Bradyrhizobium japonicum* tolerant to different degrees of temperature with soybean .



DAP, 69 nodules/plant were formed. The corresponding numbers for 75-days old plants were 95 (3456 T 40 °C) and 106 (619 T 45 °C) nodules/plant as compared to inoculated plants with original sensitive ones (3456 and 619), the number of nodules formed ranged from 85 to 99 nodules/plant.

Significant variations in nodules dry weight were recorded among the different rhizobial strains applied. In respect to plants inoculated with high temperature tolerant strains, the nodules formed were higher in dry weights (680-743 mg/plant). On the other hand, inoculation with original sensitive ones resulted in lower dry weight (503-523 mg/plant). *Bradyrhizobium* sp. (3456 T 40 °C) was superior among the four strains tested in producing higher nodules weight of 743 mg/plant.

Regarding shoots biomass of peanut plants as affected by inoculation with strains tolerant to high temperatures , data in Table (30) and illustrated in Figure (7 C) show significant variation at early growth period (45 DAP). Thereafter, differences became more pronounced after 75 DAP.The influence of inoculation with 3456 T 40°C and 619 T 45 °C was promotive and produced the highest dry matter yield of 19.9 and 18.6 g/plant respectively, as compared to 10.1 and 11.6 g/plant for sensitive original strains. Highly significant differences in shoot nitrogen content were observed between experimental treatments (Table, 30 and Figure 7D). The lowest nitrogen quantities (90-102 mg/plant) were recorded in shoot tissues of uninoculated peanut plants. However, inoculation with tolerant rhizobial strains to high temperature seemed to have the superior influence in increasing the nitrogen content of shoots tissues by 82.7-189.9% (45 DAP) and 88.6 - 139.2% (75 DAP) over inoculated plants with sensitive original strains.

4.2.2.1. Nodulation and growth of soybean as affected by inoculation with *Bradyrhizobium japonicum* strains tolerant to high temperatures

Response of soybean cv. Crawford to inoculation with two strains *Bradyrhizobium japonicum* strains tolerant to high temperature (138 T 45 °C and 3407 T 40 °C) was evaluated in sandy soil. Nodulation status and growth parameters of the plants are presented in Table (31) and illustrated in Figures (8A, B, C and D).

Uninoculated soybean plants showed no nodules formation. No significant difference were obtained in nodules number of soybean plants inoculated with original and high temperature tolerant *Bradyrhizobium japonicum* strains where averages recorded were 105 - 109 and 99 - 115 nodules/plant respectively (45 DAP). The same trend was noticed with older plants (75 DAP).

At the plant age of 75 days an increase of 53.3 and 47.6% in nodules dry weight (Table, 31 and Figure, 8B) were attributed to inoculation with 3407 T 40 °C and 138 T 45 °C respectively, over inoculation with original sensitive ones (3407 and 138).

The rate of increases in plant dry matter yield was different according to rhizobial strain character as tolerant or sensitive to high temperature. Percentage increases in biomass of 17.4 and 1.8 were recorded for 3407 T 40 °C and 138 T 45 °C respectively, over inoculated plants with original sensitive *Bradyrhizobium japonicum* rhizobial strains tested (3407 and 138). It is clear that tolerance to high temperature of strain 3407 was positively correlated and superior in promoting soybean nodulation status, infectivity and nodules growth, compared to the other strain tested (138 T 45 °C).

The above mentioned finding was clear also for nitrogen content of soybean plants inoculated with 3407 T 40 °C . Percentage increase of 154.7 was recorded over inoculated plants with 3407. In contrast, percentage increase due to inoculation with 138 T 45 °C was 81.9% over inoculated plants with the original strain 138 .

It is of rather interest to mention that inoculation of peanut or soybean plants, as summer legumes, with high temperature (40 and 45°C) tolerant rhizobial strains resulted in significantly increasing the nodulation status, growth and nitrogen content of the plants. Also, the symbiotic performance of the high temperature tolerant strains was positively correlated to their abilities to grow and survive at high temperatures in liquid culture and the strain genotype. The present findings are in accordance with those obtained by **Munevar** and **Wollum (1982 b)** who reported that high - temperature tolerant rhizobial strains nodulated better and fix more N_2 when exposed to high temperatures as compared to strains considered to be temperature neutral or intolerant. In another study **(Elsaeid *et al.*, 1990)**, high - temperature tolerant strains of *Bradyrhizobium japonicum* survived longer and in greater numbers in soils exposed to a continuous temperature of 32 °C than high temperature - intolerant strains exposed to similar conditions. A positive correlation has been shown between the ability of strains of *Rhizobium* sp. (*Leucaena*) to nodulate and fix nitrogen at constant high temperature and their ability to grow when cultured on artificial media at high temperatures **(Swelim, 1996)**.

Soil temperature is an important environmental variable that affects general biological activity . Nodulation and N_2-fixation are observed under a wide range of temperatures with an optimum between

20 and 30°C. Elevated temperatures affect nodule initiation and develpment in temperate legumes, whereas in tropical legumes it is mainly N_2-fixation efficiency that is affected **(Toro, 1996)**. Also, temperature changes affect the competitive ability of *Rhizobium* strains, and there are specific temperature - sensitive legume- *Rhizobium* combinatios. This is the case in *Rhizobium leguminosarum* bv. *trifolii* TAL that forms nodules with *Trifolium subterraneum* cv. *Woogenellup* in the laboratory above 25 °C but not below 22 °C , although it nodulates other cultivars at low temperatures **(Gibson, 1968)**.

4.2.3. Competence of *Bradyrhizobium* sp strain (peanut) tolerant to mineral nitrogen under stress of soil nitrogen

Legume nodulation decrease in the presence of high levels of soil inorganic nitrogen **(Hardarson *et al.*, 1984 b)**. The degree of inhibition depends largely on the host plant, rhizobial strain, plant age, rate and form of combined nitrogen applied **(Pate and Alkins, 1963)**.

Nitrogen, especially nitrate, reduce root hair infection, available infection sites on the root **(Munns, 1977)** and lectin binding **(Dazzo *et al.*, 1985)**.

Nodulation status and growth of peanut cv. Giza 5 as affected by inoculation with *Bradyrhizobium* strains tolerant to mineral nitrogen (619 T 300ppm N and 3456 T 500ppm N) under stress of increasing amounts of soil nitrogen (20, 50 and 75kg N/fed.) were evaluated in a pot experiment.

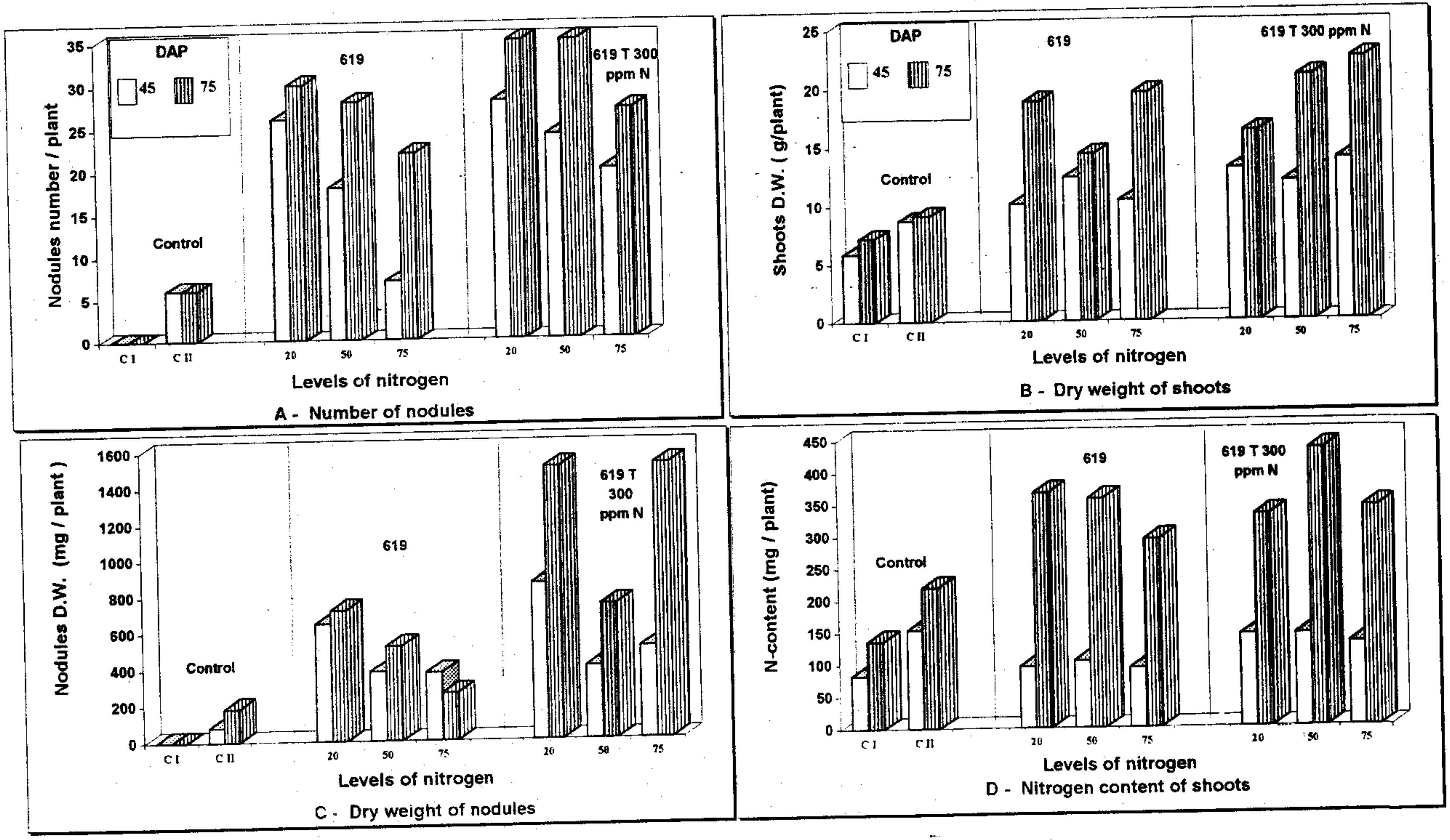
Nodulation and growth parameters of the peanut plants grown in sandy soil are presented in Table (32) and illustrated in Figures (9 A, B, C and D). Peanut roots beared variable numbers of nodules depending

Table (32) : Symbiotic performance of *Bradyrhizobium* sp. tolerant to different concentrations of nitrogen with peanut .

Treatments	Nodulation status				Shoots D.W. (g/plant)		Nitrogen content (mg/plant)	
	No./Plant		D.W. (mg/plant)					
	DAP							
	45	75	45	75	45	75	45	75
C I *	0	0	0	0	5.9	7.2	83	136
C II **	6	6	80	183	8.7	9.1	153	219
619								
+ 20 kg N/fed	26	30	646	720	10.1	18.9	96	367
+ 50 kg N/fed	18	28	380	520	12.4	14.4	105	357
+ 75 kg N/fed	7	22	372	260	10.4	19.6	93	293
619 T 300 ppm N								
+ 20 kg N/fed	28	35	860	1507	13.1	16.3	143	331
+ 50 kg N/fed	24	35	400	740	12.0	21.0	144	434
+ 75 kg N/fed	20	27	504	1520	13.9	22.5	130	342
3456								
+ 20 kg N/fed	19	33	486	640	11.8	12.0	155	121
+ 50 kg N/fed	17	25	400	500	12.1	13.8	244	178
+ 75 kg N/fed	10	23	301	160	14.5	17.2	438	240
3456 T 500 ppm N								
+ 20 kg N/fed	42	41	552	980	12.2	16.1	220	385
+ 50 kg N/fed	31	36	420	480	12.7	18.6	346	235
+ 75 kg N/fed	27	31	226	340	14.5	19.1	249	303
L.S.D.								
N 0.05	16.03	7.12	226.26	168.4	2.64	1.55	48.93	54.29
0.01	21.61	9.95	303.52	225.9	3.54	2.09	65.64	72.82
S 0.05	8.010	3.69	112.58	83.79	1.31	0.77	24.35	27.01
0.01	10.80	4.98	151.76	112.95	1.77	1.04	32.82	36.41
N x S 0.05	22.67	10.44	318.42	236.99	3.71	2.19	68.86	76.4
0.01	30.56	14.07	429.24	319.47	5.00	2.95	95.83	102.99

* Uninoculated unfertilized
** Uninoculated + 20 kg N/fed.

Fig. (9): Symbiotic performance of *Bradyrhizobium* sp. tolerant to mineral nitrogen (619 T 300 ppm N) with peanut under strss of soil nitrogen .



upon nitrogen level applied and rhizobial strains. Uninoculated peanut plants produced the lowest nodules numbers of 0 and 6 nodules/plant in case of unfertilized and fertilized with 20kg N/fed respectively at 45 and 75 DAP. Irrespective of the plant age and rhizobial strains, nodule numbers decreased with increasing the level of nitrogen in the soil being lower (7- 27 nodules/plant) at 75kg N/fed than 20kg N/fed (19 - 41 nodules plant).

On the other hand, peanut plants inoculated with nitrogen tolerant rhizobial strains formed higher number of nodules ranging from 20-27 nodules/plant under nitrogen fertilized soil with 75kg N/fed compared to the sensitive orginal strains (7-10 nodules/plant) at 45 DAP. The corresponding values at 75 DAP were 27-31 nodules/plant and 22-23 nodules/plant in the same order.

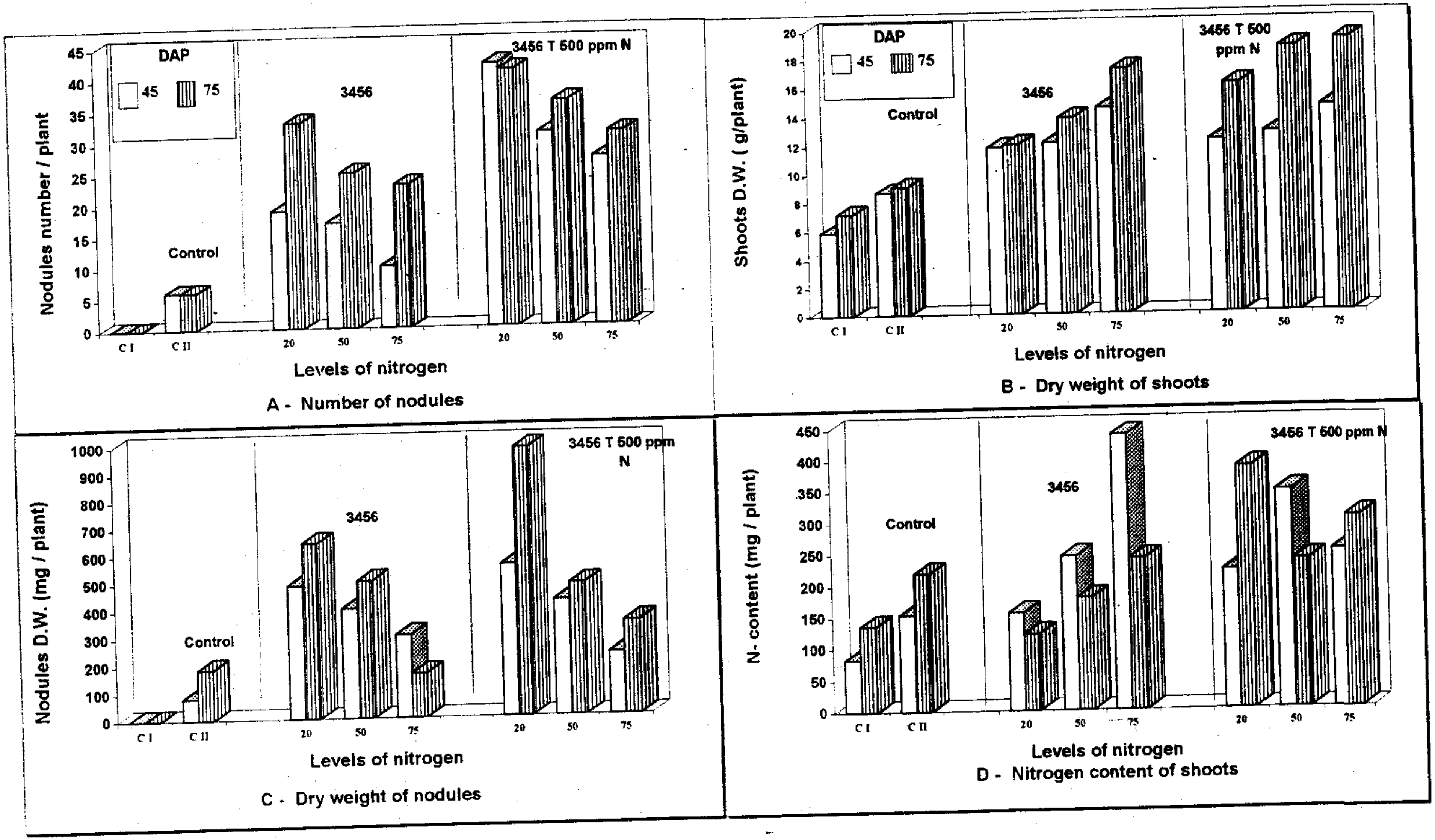
Considerable variations in noduels dry weight were found among the different treatments (Table, 32 and Figure 9 B). It could be noticed that nodules dry weight drastically decreased by application of higher doses of nitrogen (75kg N/fed) at the two periods of plant sampling. This finding was clear for inoculation with original *Bradyrhizobium* sp. peanut strains and their derivatives nitrogen- tolerant ones. On the other hand, irrespective of rhizobial strain, the development and growth of nodules formed were generally increased at nitrogen fertilizer doses of 20 and 50 kg N/fed with increasing the plant age from 45-75 DAP. At 75kg N/fed, the case was different where inoculation with nitrogen tolerant rhizobial strains (619 T300 ppm N) increased the dry weight of nodules from 504 to 1520 mg/plant at 45 and 75 DAP respectively. The corresponding values for 3456 T 500 ppm N were 226 to 340 at 45 and 75 DAP respectively. In contrast, inoculation with original rhizobial strains under stress of 75 kg N/fed negatively affected the growth and development of the nodules reached -30.1 and -46.8% for 619 and 3456, respectively, lower at 75 than 45 DAP.

Inoculation, in general, increased the shoots biomass of the peanut plants as compared to uninoculated ones. However, little fluctuations of shoots dry weight (Table 32 and Fig 9 C) in majority of inoculated treatments were recorded at plants age of 45 DAP. In contrast, at 75 DAP, inoculated plants with nitrogen tolerant rhizobial strains showed pronounced significantly differences as compared to inoculation with sensitive original ones. Percentage increases induced by 619 T300 ppm N were 45.8 and 18.4 under stress of 50 and 75 kg N/fed respectively. The corresponding percentages increases were 34.8 and 11.0 for 3456 T500ppm N over 3456 at 50 and 75kg N/fed respectively. It is clear that competence of rhizobial strains tolerant to nitrogen is more pronounced under stress of 50kg N/fed than higher dose of 75kg N/fed. This finding is also observed for shoots nitrogen content, percentage increases recorded were 30.0 and 16.7 for 619 T300 ppm N over 619 under stress of 50 and 75kg N/fed respectively. Also, inoculated plants with 3456 T500 ppm N increased the shoot nitrogen content by 32.0 and 15.4% over 3456 with the application of 50 and 75kg N/fed, respectively.

4.2.3.1. Symbiotic performance of *Bradyrhizobium japonicum* tolerant to mineral nitrogen under stress of N-fertilization :

Response of soybean cv. Crawford to inoculation with *Bradyrhizobium japonicum* strains 3407 and 138 and their nitrogen tolerant derevatives 3407 T 500 ppm N and 138 T 200 ppm N respectively was evaluated under different doses of nitrogen fertilization (20, 50 and 75kg N/fed) in sandy soil.

Fig. (10): Symbiotic performance of *Bradyrhizobium* sp. tolerant to mineral nitrogen (3456 T500 ppm N) with peanut under stress of soil nitrogen .



Nodulation and growth parameters of soybean plants as affected by inoculation with mineral nitrogen tolerant *Bradyrhizobium japonicum* strains are presented in Table (33) and illustrated in Figures (11 and 12). As mentioned for peanut plants, inoculation of soybean plants resulted in the highest increases of nodules number (Table, 33 and Figures 11 A and 12A). This finding was clear for inoculation with *Bradyrhizobium japonicum* strains tolerant to nitrogen or original ones.

In general, the two selective strains tolerant to nitrogen (3407 T 500ppm N and 138 T 200ppm N) showed different infectivity competence as compared to their original ones. Table 33 and Figure 11A demonstrate the higher infectivity (94-125 nodules/plant) of 3407 T500 ppm N at all levels of nitrogen applied than the original one (25-81 nodules/plant) . However, little fluctuation of no significance in number of nodules between 138 (66-125 nodules/plant) and 138 T 200 ppm N (80 - 123 nodules/plant) under stress of all doses of nitrogen (Table 33 and Figure 12 A).

High nitrogen doses 75 kg N/fed reduced nodules numbers/plant, this as reported by **Norris and Date (1976)** illustrates the adverse effects of nitrogen on nodulation. The reduction of nodulation may be due to the ready source of nitrogen in the soil, which makes the nodules ineffective in fixing atmospheric nitrogen. Combined nitrogen had its main effect in increasing plant weight. However, nodules weight/numbers per plant decreased by combined nitrogen **(Buttery *et al.*, 1990).**

The same trend was also observed regarding nodules dry weight and dry weight of plant shoot. In the majority of cases <u>*Bradyrhizobium*</u> *japonicum* strains 3407 T500 ppm N was superior in producing higher dry weight of nodules and shoot biomass as compared to 3407 at the two periods of sampling. In contrast, there was no significant differences between 138 and 138 T200 ppm N regarding the above mentioned parameters (Table 33 and Figures 12 B and C).

Regarding accumulation of nitrogen in plant shoots (Table 33 and Figures 11D and 12D), higher significant quantities of nitrogen in shoot tissues of soybean plants inoculated with 3407 T 500ppm N than recorded when inoculation with 3407. This finding was clear under stress of all doses of nitrogen (20, 50 and 75 N/fed). In contrast, the influence of inoculation with 138 T200 ppm N on soybean shoots nitrogen content was rather unpromising (unpromotive) as compared to 138 original one.

These results indicate that competence of the rhizobial strain tolerant to higher doses of nitrogen may depend upon the rhizobial species, strain, dose of nitrogen and the legume host .

The inhibition of nitrogen fixation by combined nitrogen prevents optimal exploitation of both pathways of legume nitrogen nutrition, i.e., atmospheric N_2 fixation and soil nitrogen assimilation. This inhibition results from complex events occurring at different stages of nodule development **(Streeter, 1988)** and depends upon many factors: plant genotype, rhizobial strain, form and concentration of combined N, and period of nitrogen supply (**Harper** and **Cooper, 1971; Wych** and **Rains, 1979)**.

The mechanism by which NO_3^- inhibits nodule function is not yet completely understood **(Serraj *et al.*, 1992)**. However, several hypotheses have been reviewed by **Drevon *et al.* (1988 a)** and **Streeter (1988)**.

Table (33) : Symbiotic performance of *Bradyrhizobium japonicum* tolerant to different concentrations of nitrogen with soybean .

Treatments	Nodulation status				Shoots D.W. (g/plant)		Nitrogen content (mg/plant)	
	No./Plant		D.W. (mg/plant)					
	DAP							
	45	75	45	75	45	75	45	75
C I *	0	0	0	0	2.1	5.5	12	80
C II **	3	7	70	60	3.3	6.7	19	135
3407								
+ 20	81	66	458	1070	2.8	12.3	126	457
+ 50	56	61	420	1300	4.5	14.1	243	224
+ 75	37	25	332	840	6.2	17.0	370	187
3407 T 500 ppm N								
+ 20	112	125	567	1100	3.3	16.2	127	781
+ 50	102	97	480	1320	4.3	17.5	142	635
+ 75	109	94	476	1500	5.3	18.5	176	675
138								
+ 20	85	110	607	1270	3.2	11.9	182	464
+ 50	66	125	438	1120	4.4	14.3	204	384
+ 75	74	104	436	1150	5.3	16.3	197	311
138T 200 ppm N								
+ 20	94	111	608	890	3.5	10.4	138	201
+ 50	85	123	545	1010	4.0	13.5	181	284
+ 75	80	114	510	910	4.5	15.6	233	251
L.S.D.								
N 0.05	13.77	19.95	111.24	232.26	0.423	0.794	21.802	59.40
N 0.01	18.75	26.86	149.77	312.70	0.570	1.069	29.353	79.97
S 0.05	6.88	9.97	55.62	116.13	0.212	0.397	10.901	29.70
S 0.01	9.27	13.43	74.89	156.35	0.285	0.534	14.680	39.99
N x S 0.05	19.48	28.21	157.32	328.46	0.599	1.123	30.830	84.00
N x S 0.01	26.22	37.98	211.81	422.22	0.806	1.512	41.510	113.10

* Uninoculated unfertilized

** Uninoculated + 20 kg N/fed.

Fig. (11): Symbiotic performance of *Bradyrhizobium japonicum* tolerant to mineral nitrogen (138 T 200 ppm N) with soybean under stress of soil nitrogen .

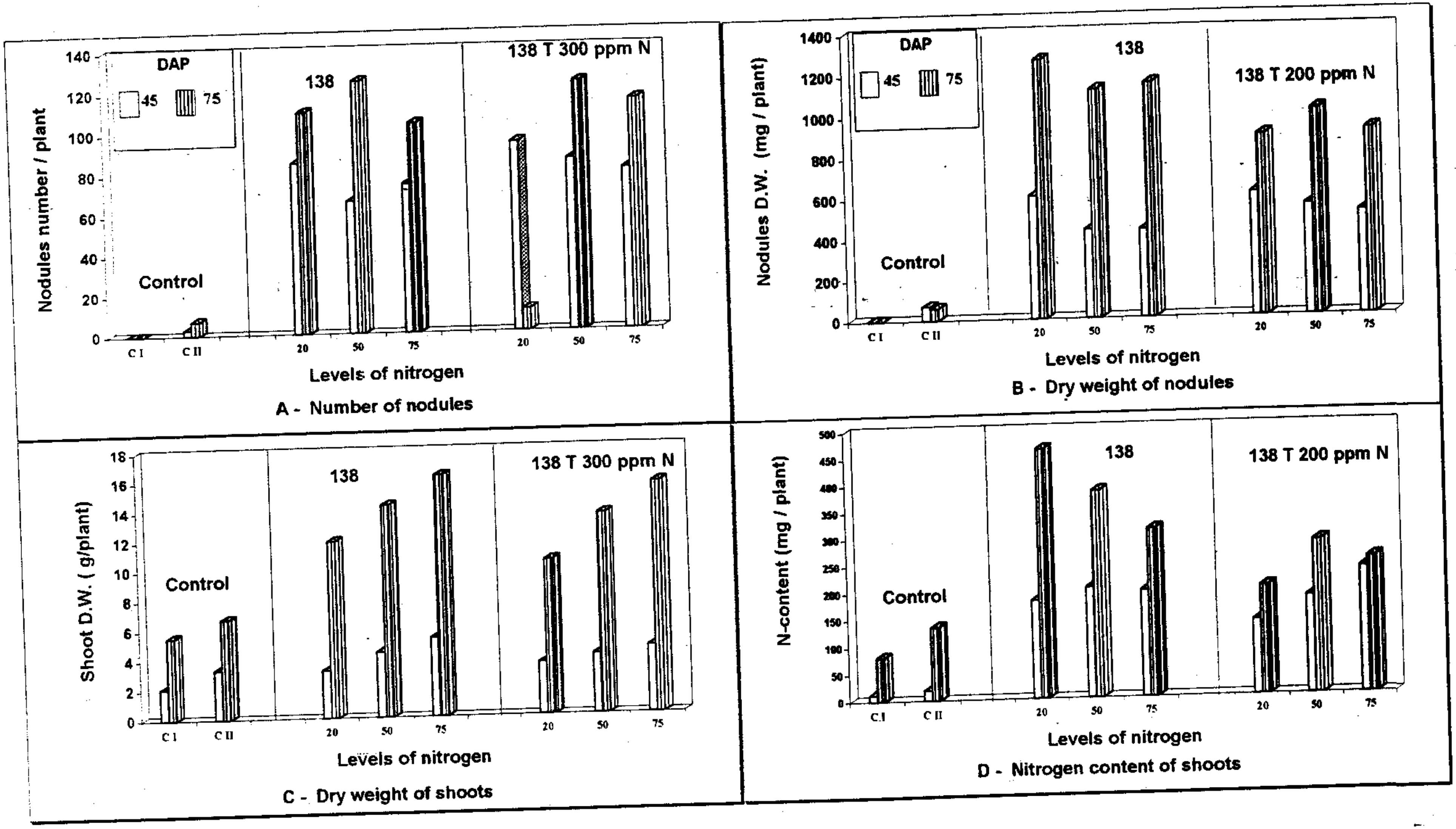
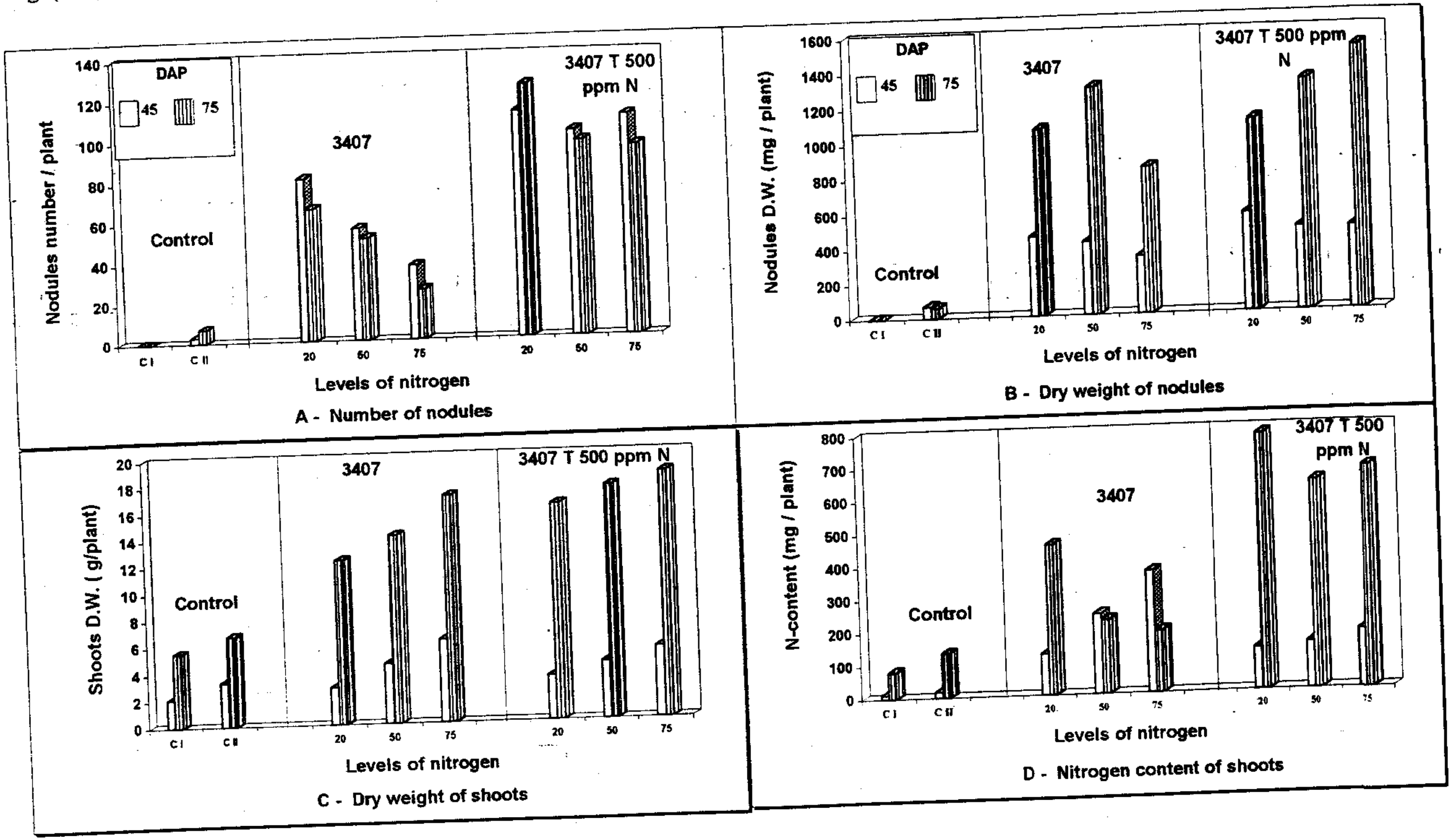


Fig. (12): Symbiotic performance of *Bradyrhizobium japonicum* tolerant to mineral nitrogen (3407 T 500 ppm N) with soybean.



A - Number of nodules

B - Dry weight of nodules

C - Dry weight of shoots

D - Nitrogen content of shoots

Variation in the sensitively of N_2 fixation to combined nitrogen occurs between and within legume-species **(Carroll *et al.*, 1985)**. The symbiosis of faba beans- *Rhizobium leguminosarum* was tolerant to high levels of combined N **(Harper** and **Gibson, 1984)** whereas soybean - *Bradyrhizobium japonicum* symbioses were rather sensitive **(Ruschel *et al.*, 1979)**. Variation between rhizobial strains has also been observed. **Koermendy** and **Eaglesham (1984)** showed differences between strains of *R. japonicum* in their capacity to nodulate in the presence of nitrate. **Maier** and **Brill (1978)** also isolated mutant strains of *R. japonicum* which showed a greater tolerance to combined N compared to the parent strains.

It is inadvisable to describe a certain rhizobial strain as being fully effective / ineffective or sensitive / tolerant to combined nitrogen, except in relation to a particular host genotype. These is scope for obtaining promising host - *Rhizobium* associations tolerant to combined nitrogen through exploiting the interactive effects between the host - genotypes and strains of *Rhizobium* **(senaratine *et al.*, 1987)**.

4.2.4. Symbiotic performance of three different rhizobial strains nodulating peanut and soybean and their derivatives tolerant to low and high pH values

One *B. japonicum* sp. (618, peanut) rhizobial strain tolerant to acidity and alkalinity (618 T pH 5 and 618 T pH 10) and two *B. japonicum* (soybean) strains 3407 (tolerant to pH 5 and pH 10) and 138 (tolerant to pH 11) were selected for infectiveness and effectiveness investigation with peanut and soybean plants.

Uninoculated peanut and soybean plants did not produce any root nodules which may be due to the absence of the native specific rhizobia in the sandy soil under investigation . However, inoculation with original or tolerant to lower or higher pH values generally leads to induction of higher number of nodules/plant which increased by increasing the plant age (Tables, 34 and 35 and Figures 13A and 14A). On the other hand, tolernace of the rhizobial strains to higher pH values (10 and 11) was parallel to the highest number of nodules induced for the two intervals of plant sampling 45 and 75 DAP. The corresponding number of nodules recorded were 44, 92 and 84 for 618 T pH 10, 3407 T pH 11 and 138 T pH 11 at 45 DAP respectively, then the number of nodules increased to 91, 174 and 123 in the same order at 75 DAP. The dry weight of nodules was found to have the same trend. In other words tolerance of rhizobial strains tested to higher pH values was positively correlated to higher nodules dry weight. This finding is recorded for peanut and soybean rhizobial strains tested .

Irrespective of sampling date, rhizobial inoculation promoted peanut and soybean growth compared to uninoculated plants. However, inoculation with alkaline (pH 10-11) tolerant *Bradyrhizobium* sp. (618 T pH 10) and *Bradyrhizobium japonicum* (3407 T pH 11 and 138 T pH 11) was superior in increasing peanut and soybean growth compared to original and acidity tolerant strains. Percentage of increases of 12.3, 37.1 and 27.9 were recorded for inoculation with 618 T pH 10 (peanut), 3407 T pH 11 (soybean) and 138 T pH 11 (soybean) respectively over uninoculated plants with original rhizobial strains (Tables, 34 and 35, Figures, 13 C and 14 C). In contrast, tolerance of the two rhizobial strains (618 T pH 5, peanut and 3407 T pH 5, soybean) to acidity (pH 5) was correlated with lower dry weight of shoots (15.28 and 7.6 g/plant) as

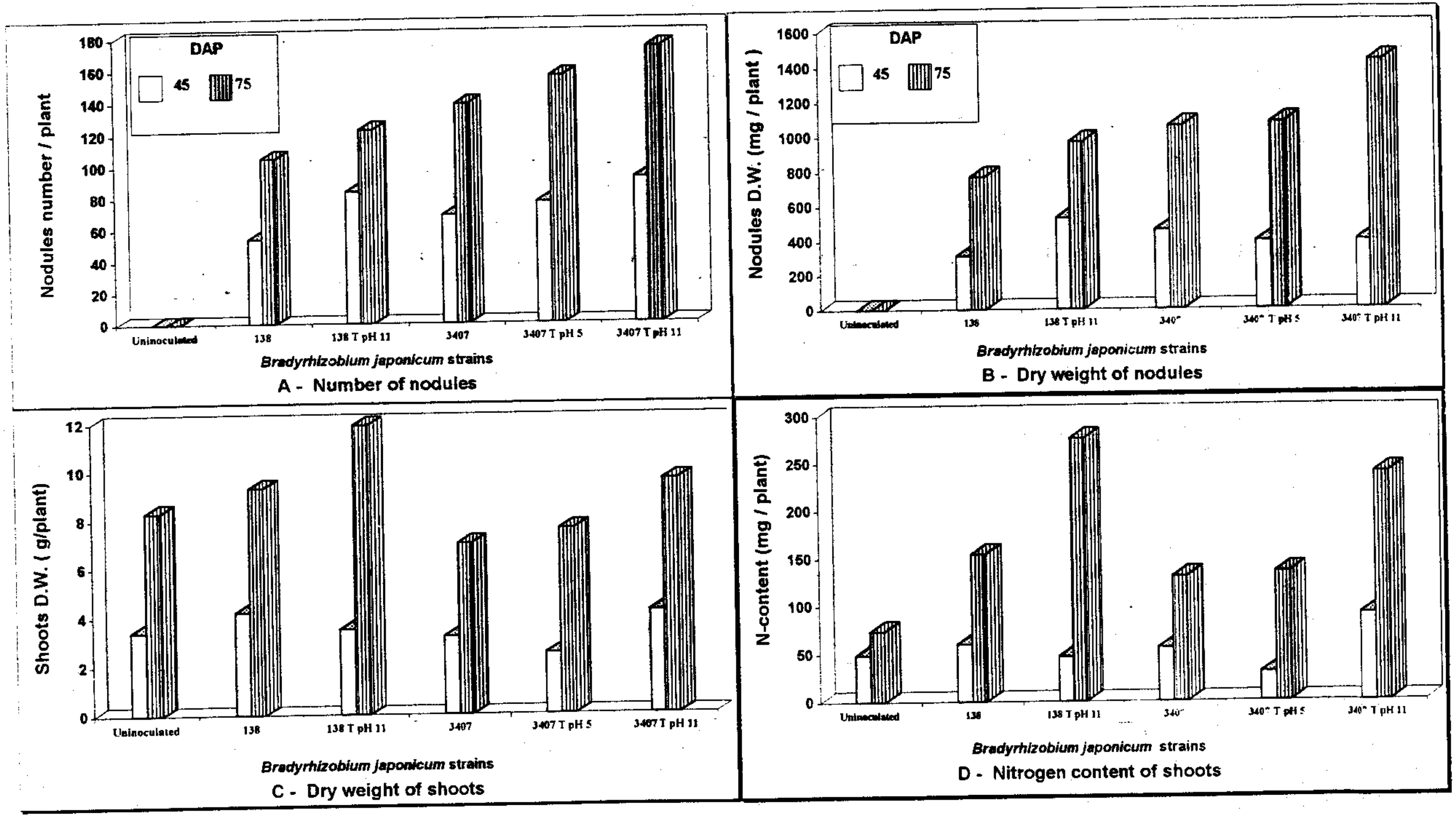
Table (34) : Symbiotic performance of *Bradyrhizobium* sp. (peanut) tolerant to different pH values with peanut .

Treatments	Nodulation status No./Plant		Nodulation status D.W. (mg/plant)		Shoots D.W. (g/plant)		Nitrogen content (mg/plant)	
	DAP 45	75	45	75	45	75	45	75
Uninoculated	0	0	0	0	7.02	11.35	127	190
618	27	80	326	340	13.3	18.07	94	225
618 T pH 5	25	51	246	320	12.2	15.28	88	247
618 T pH 10	44	91	413	640	14.6	20.30	189	396
L.S.D. 0.05	5.829	10.07	47.5	97.8	2.151	4.865	11.42	50.0
L.S.D. 0.01	14.30	29.20	65.2	187.8	4.584	11.44	89.37	125.5

Table (35) : Symbiotic performance of *Bradyrhizobium japonicum* tolerant to different pH values with soybean .

Treatments	Nodulation status No./Plant		Nodulation status D.W. (mg/plant)		Shoots D.W. (g/plant)		Nitrogen content (mg/plant)	
	DAP 45	75	45	75	45	75	45	75
Uninoculated	0	0	0	0	3.4	8.3	49	74
3407	69	139	460	1060	3.2	7.0	56	130
3407 T pH 5	77	156	394	1080	2.5	7.6	30	135
3407 T pH 11	92	174	394	1430	4.2	9.6	91	239
138	54	105	312	770	4.2	9.3	60	154
138 T pH 11	84	123	532	970	3.5	11.9	47	276
L.S.D. 0.05	6.69	22.95	32.2	59.8	0.918	1.570	10.78	28.54
L.S.D. 0.01	23.32	46.20	85.3	104.4	1.286	2.201	29.14	59.03

Fig. (14): Symbiotic performance of *Bradyrhizobium japonicum* tolerant to different pH values with soybean.



compared to original and alkaline - tolerant ones (20.3 and 9.6 g/plant) respectively at 75 DAP.

The higher nitrogen content of peanut (Table, 34 and Figure 13D) and soybean (Table, 35 and Figure, 14D) plants inoculated with alkaline - tolerante strains confirm their effectiveness superiority compared to original and acid - tolerant ones. Alkaline-tolerant rhizobial strains 618 T pH 10, 3407 T pH 11 and 138 T pH 11 resulted in the accumulation of 76.0, 83.8 and 79.2 % more nitrogen compared to original sensitive ones, respectively at 75 DAP. The corresponding percentage recorded over acid tolerant strains 618 T pH 5 and 3407 T pH 5) being 60.3 and 77.0 respectively.

This finding is in harmony with **Van Schreven (1972)**, who suggested that prolonged exposure to acidity can result in selection for strains having reduced efficiency in symbiosis. In contrast, this was not evident by **Vargas and Graham (1988)**, who found that acid-pH tolerant rhizobial strains (*Rhizobium leguminosarum* bv. *phaseoli*) were highly effective in N_2 fixation.

4.2.5. Competence of desiccation tolerant *Rhizobium leguminosarum* bv. *viceae* strains with faba bean under drought stress :

In the present study two *Rhizobium leguminosarum* bv. *viceae* ARC 200F and ICARDA 441 were identified as low and high desiccation tolerant strains, respectively. The symbiotic performance of the two obtained rhizobial strains with faba bean under soil drought stress of 45 and 30 % water holding capacity (WHC) compared to an optimum supply of water (around 60-75% WHC) was dtermined 75DAP.

Nodulation status , shoots dry weight and nitrogen content of faba bean plants cv. Giza 402 as influenced by inoculation with desiccation tolerant rhizobial strains under different WHC% (75, 60, 45 and 30) were evaluated.

Results in Table (34) and illustrated by Figure 15A show that uninoculated faba bean plants formed a considerable number of nodules being 45 nodules/plant at 75% WHC. However, by decreasing the WHC to 60, 45 and 30% adversely affect the number of nodules formed being 34, 23 and 7 nodules/plant respectively. On the other hand, inoculated plants with desiccation tolerant strain ICARDA 441 show similar trend as uninoculated plants that the number of nodulaes / plant formed decreased by decreasing the soil WHC. However, the highest number of nodules/plant recorded ranged between 135-30 nodules/plant. Inoculated plants with ARC 200F showed lower average number of nodules being 114-13 nodules / plant. These results therefore suggest that the native rhizobia nodulating faba bean was not able to tolerate lower WHC of 30% . Under water stress of 30% WHC, number of nodules of 7, 13 and 30 / plant was recorded for uninoculated, inoculated with ARC200F and ICARDA 441 respectively. The corresponding values recorded for nodules dry weight were 41.8, 64.3 and 160.6 mg/plant in the same order.

These results may suggest that the native rhizobia nodulating faba bean showed lower infectivity under drought stress of 30% WHC, however under the same condition low desiccation tolerant one (ARC 200F) recorded medium infectivity. The highest nodules numbers and dry weight were induced on faba bean plants inoculated with ICARDA 441 which recorded more desiccation tolerance up to 72 hrs (Table, 36 and

Figure, 15 A and B). These findings indicate that there was a positive correlation between rhizobial high infectivity and high tolerance of desiccation under soil drought stress.

By maturity (75 DAP), the drought stress of 30% WHC had considerably reduced shoot dry matter, this finding was pronounced for uninoculated plants and inoculated ones with ARC 200F. The shoot dry weight values recorded were 4.72 and 5.88 g/plant in the same order mentioned above . The largest estimates of shoots dry weight were for inoculation with ICARDA 441 being 9.7 and 8.18 g/plant at 45 and 30% WHC respectively. The same trend was also observed for shoots nitrogen content of 95.1, 106.7 and 132.5 mg/plant for uninoculated, inoculated with ARC 200F and inoculated plants with ICARDA 441 respectively at 30% WHC. Under uninoculated treatments it was noticed that shoot dry matter was not significantly affected by decreasing soil WHC of 75, 60, 45 and 30%, the estimated shoots-dry matter were 5.03, 5.22, 5.02 and 4.72 g/plant respectiely. These results therefore suggest that the host plant was reasonably successful in adapting the reduced WHC% .

Symbiotic nitrogen fixation in most species is sensitive both to excess water and to drought. **Sprent (1972 a)** reported that nitrogen fixation (acetylene reduction) in field-grown *V. faba* was maximum at soil moistures around field capacity .

Busse and Bottomely (1989) indicated the importance of soil-WHC% to the success of microsymbiont both in the free-living state and during the establishment of a symbiosis. The ability of rhizobia to survive at low water potential in soil has been established by many studies **(Al-Rashidi *et al.*, 1982; Fuhrmann *et al.*, 1986).**

An optimum supply of water, around 60-75% of the water-holding capacity, is considered essential for maximum plant growth **(Mary *et al.*, 1986).**

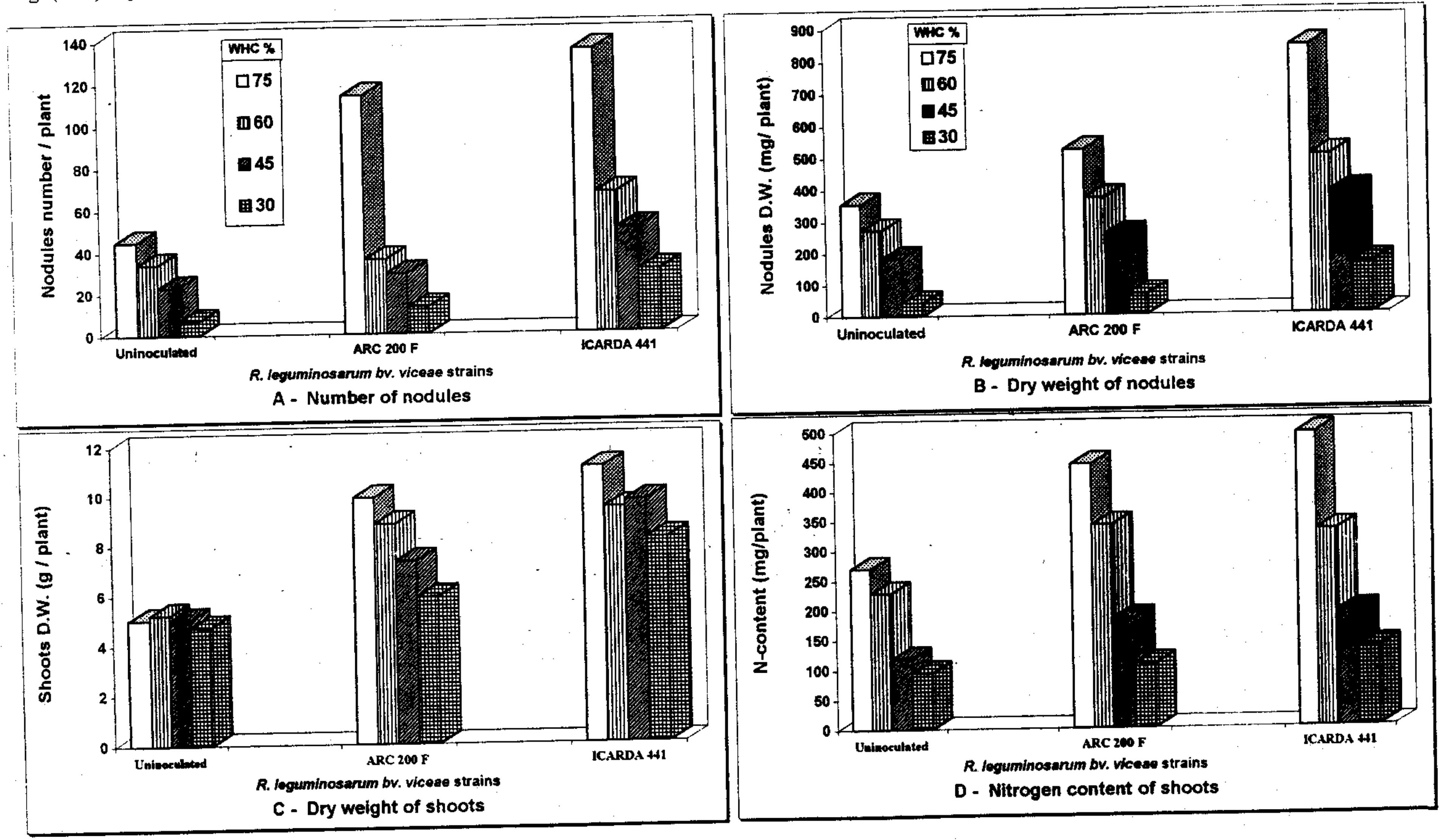
Dry soils inhibit normal root hair formation and, hence, infection by *Rhizobium* . On the other hand, nodule development initiated under normal moisture conditions is set back by later dry conditions **(Worral** and **Roughley, 1976).**

Table (36) : Symbiotic performance of *Rhizobium leguminosarum* bv. *viceae* tolerant to desiccation with faba bean under water stress (75 DAP) .

Treatments	W.H.C* %	Nodulation status		Shoots D.W. (g/plant)	Nitrogen content (mg/plant)
		No./Plant	D.W. (mg/plant)		
Uninoculated					
	75	45	354.7	5.03	269.6
	60	34	271.6	5.22	228.3
	45	23	182.6	5.02	118.5
	30	7	41.8	4.72	95.1
ARC 200 F					
	75	114	522.3	9.89	444.2
	60	36	370.9	8.83	340.7
	45	29	253.5	7.33	183.3
	30	13	64.3	5.88	106.7
ICARDA 441					
	75	135	841.8	11.07	494.5
	60	67	499.9	9.42	329.9
	45	50	385.4	9.70	192.6
	30	30	160.6	8.18	132.5
L.S.D.					
Inoc.	0.05	18.46	110.8	1.252	22.23
	0.01	28.57	150.1	1.697	30.12
W.H.C.	0.05	32.86	127.9	1.446	25.66
	0.01	44.53	173.3	1.959	34.78
I. x W.	0.05	56.92	221.5	2.504	44.45
	0.01	77.13	300.2	3.393	60.24

* W.H.C. Water holding capacity

Fig. (15): Symbiotic performance of *Rhizobium leguminosarum* bv. *viceae* tolerant to desiccation with faba bean under water stress.



Conculding Remarks :

1- Variation within the rhizobial strains, against the different stress conditions, is well recognized and can be used to select valuable strains for the preparation of rhizobial inoculants selectively better under certain environmental stress conditions. On the other hand, selection of the rhizobial strains, has been carried out under conditions, supposed to be optimal for the symbiosis and showed highley effective under green house conditions.

2- The most pronounced rhizobial strains tested tolerated more than one stress factor (Table, 37) were as follows:

a- *R. meliloti* A2 which showed tolerance to high temperature (45°C) , high level of N (500 ppm) and alkaline pH.

b- *Rhizobium leguminosarum* bv. *viceae* ICARDA 441 which showed tolerance to NaCl salinity of 40 g/L high level of nitrogen (500ppm) and desiccation up to 72 hr.

c- *Rhizobium leguminosarum* bv. *trifolii* TAL showed tolerance to high temperature (45 °C) and high level of nitrogen (500 ppm).

d- *Rhizobium leguminosarum* bv. *phaseoli* ARC 302 was tolerant to high temperature (45 °C) and acidic pH 5. Also strain UMR showed tolerance to high temperature of 45 °C and alkaline pH 10.

e- *Bradyrhizobium* sp. (peanut) strain 601 showed tolerance to high salinity, high temperature and acidic pH 5. In addition, strain 619 was tolerant to high temperature and desiccation. Strain 3339 was superior in tolerating high temperature, high nitrogen and desiccation.

f- Only one *Bradyrhizobium japonicum* strain 138 was found to be tolerant to three different stress factors such as high salinity, high temperature and alkalinity pH. However, strain 3407 showed tolerance to high level of nitrogen and alkaline pH.

3- Generally, it is possible to select rhizobial strains which give relatively better symbiotic association under certain environmental stress conditions.

4- It may be assumed that the results obtained can be used as a guide to select special rhizobial strains (*Rhizobium* and *Bradyrhizobium*) to be tolerant for certain environmental stress conditions and to predict symbiotic performance of the selected strains as rhizobial inoculant.

5- Almost nothing is known about the effect of two or more stress factors on the survival and symbiosis.

6- In some cases, conditions favourable for survival, nodule formation and effectiveness of the rhizobial strain may not be so for the host growth. Therefore, plant breeders are well aware of the possibilities to select plants adapted to certain environments to maximize the symbiotic performance under different stress conditions.

Table (37): The rhizobial strains tolerant to more than one of stress conditions tested.

Stress conditions	*R. meliloti*	*R. leguminosarum* bv. *viceae*	*R. leguminosarum* bv. *trifolii*	*R. leguminosarum* bv. *phaseoli*	*m* sp. (peanut)	*B. japonicum*
Salinity (40 g/L) NaCl	None	ICARDA 441	None	None	601	138
Temperature (45 °C)	ARC 1 ARC 2 A 2	ARC 200 F ARC 202 F	TAL	ARC 302 UMR	619 3339 601	138 110 ARC 500
Nitrogen (500ppm)	ARC 6 A 2	ICARDA 441	TAL	None	3456 3339	3407 102
Alkaline pH	ARC 6 A 2	None	ARC 103	UNR	618	138 3407
Acidic pH	None	None	None	ARC 302	601	None
Desiccation (72 hr.)	Not tested	ICARDA 441	Not tested	Not tested	619 3339	Not tested