RESULTS

1. Screening the Symbiotic Effectiveness of Different Rhizobial Strains/ Isolates with Different Peanut Cultivars

To investigate the most promising peanut nodulated rhizobia, the effect of different bradyrhizobial strains/ isolates on different peanut cultivars was evaluated under greenhouse conditions. The pot experiments involved two *Bradyrhizobium* strains (USDA 3456 and USDA 3339), four local isolates (ARC 601, ARC 617, isolate 1 and isolate 2) and six peanut cultivars (*Early bunch*, *Gregory*, *NC*, *Giza 5*, *Giza 6* and *Ismailia 1*)

1.1. Evaluation the Symbiotic Effectiveness of Different Rhizobial Strains/Isolates with Peanut Cultivar, *Giza5*

Screening the effect of different rhizobial strains/isolates compared to un-inoculated control on growth, nodulation, nitrogen content of peanut, *Giza5* plants was carried out in pot experiments (Table 7 and 8). All tested rhizobia displayed a prolific nodulation patterns with peanut plants (Table 7). *Bradyrhizobium* strain USDA 3456 gave the highest number of nodules by a dry weight of 182.8 mg/plant. However, the un-inoculated control did not form any nodules. The tested rhizobia increased the dry weight of plants shoot and root compared to un-inoculated control. *Bradyrhizobium* strain USDA 3456 and local isolate 2 displayed the highest dry weight of roots by 2.56 and 2.48 g/plant, respectively compared to un-inoculated control by 0.56 g/plant (Table 8). In the same context, *Bradyrhizobium* strain USDA 3456, local isolate 2, ARC 601 and 1 gave the highest dry weight of shoots by 6.09, 5.77, 5.74 and 5.72 g/plant, respectively compared to the un-inoculated control by 4.19 g/plant (Table 8). In respect to shoot nitrogen content, *Bradyrhizobium* strain USDA 3456 exhibited the highest nitrogen content in shoot by 137.56 mg/plant compared to the un-inoculated control by 80.64 mg/plant (Table 8)

Table (7): Effect of different rhizobial strains/ isolates on nodulation status of peanut cultivar, *Giza5*

Treatment	Nodulation Status	
	No. of Nodules*/plant	Dry Wt. of Nodules* (mg/plant)
Control (Un-inoculated)	0.00 E	0.00 E
USDA 3339	58.00 C	135.83 C
USDA 3456	81.67 A	182.83 A
ARC 617	46.67 D	122.92 D
ARC 601	68.00 B	145.42 C
Isolate 1	73.67 AB	161.20 B
Isolate 2	74.33 AB	162.77 B
LSD at 0.05	8.42	10.95

*Means followed by the same letter are not significantly different at 5% level

Table (8): Effect of different rhizobial strains/ isolates on growth parameters and shoot nitrogen content of peanut cultivar, *Giza5*

Treatment	Dry Wt. of Root* (g/ plant)	Dry Wt. of Shoot* (g/ plant)	Shoot Nitrogen Content* (mg/ plant)
Control (Un-inoculated)	0.56 D	4.19 C	80.64 D
USDA 3339	1.37 C	5.36 B	111.55 C
USDA 3456	2.56 A	6.09 A	137.56 A
ARC 617	1.32 C	4.39 C	85.24 D
ARC 601	1.46 C	5.74 AB	123.44 B
Isolate 1	2.13 B	5.72 AB	123.28 B
Isolate 2	2.48 A	5.77 A	128.08 B
LSD at 0.05	0.20	0.40	8.55

*Means followed by the same letter are not significantly different at 5% level

1.2. Evaluation the Symbiotic Effectiveness of Different Rhizobial Strains/Isolates with Peanut Cultivar, *Giza6*

The effect of different rhizobial strains/isolates compared to un-inoculated control on growth, nodulation, nitrogen content of peanut, *Giza6* plants was evaluated through pot experiments (Table 9 and 10). All tested rhizobia strains/isolates exhibited a different nodulation patterns with peanut plants (Table 9). *Bradyrhizobium* USDA 3456 strain gave the highest number of nodules (54 nodules/ plant) by a total dry weight of 168.03 mg/plant. On the other hand, the un-inoculated control did not form any nodules. The tested rhizobia strains/isolates resulted in significant improvement of plant growth parameters and shoot nitrogen content in comparison with the un-inoculated control. *Bradyrhizobium* USDA 3456, USDA 3339 strains and local isolate 2 gave the highest dry weight of plant roots by 1.24, 1.18 and 1.17 g/plant, respectively compared to the un-inoculated control which gave 0.55 g/plant (Table 10). *Bradyrhizobium* USDA 3456 strain and local isolate 2 displayed the highest dry weight of shoots by 4.30 and 4.10 g/plant, respectively compared to the un-inoculated control by 2.68 g/plant (Table 10). Concerning shoot nitrogen content, it is clear from Table (10) that *Bradyrhizobium* USDA 3456 strain exhibited the highest nitrogen content in shoot by 104.34 mg/plant compared to the un-inoculated control by 52.23 mg/plant.

Table (9): Effect of different rhizobial strains/ isolates on nodulation status of peanut cultivar, *Giza6*

Treatment	Nodulation Status			
	No. of Nodules*/plant		Dry Wt. of Nodules* (mg/plant)	
Control (Un-inoculated)	0.000	E	0.000	F
USDA 3339	45.33	B	136.78	C
USDA 3456	54.00	A	168.03	A
ARC 617	35.33	D	112.33	E
ARC 601	40.67	C	123.12	D
Isolate 1	38.67	CD	121.77	D
Isolate 2	46.33	B	154.58	B
LSD at 0.05	3.82		7.34	

*Means followed by the same letter are not significantly different at 5% level

Table (10): Effect of different rhizobial strains/ isolates on growth parameters and shoot nitrogen content of peanut cultivar, *Giza6*

Treatment	Dry Wt. of Root* (g/ plant)		Dry Wt. of Shoot* (g/ plant)		Shoot Nitrogen Content* (mg/ plant)	
Control (Un-inoculated)	0.55	D	2.68	D	52.23	E
USDA 3339	1.18	AB	3.71	B	83.24	C
USDA 3456	1.24	A	4.30	A	104.34	A
ARC 617	1.02	C	3.30	C	70.42	D
ARC 601	1.15	B	3.61	B	77.28	C
Isolate 1	1.12	B	3.60	BC	78.05	C
Isolate 2	1.17	AB	4.10	A	92.18	B
LSD at 0.05	0.08		0.31		6.19	

*Means followed by the same letter are not significantly different at 5% level

1.3. Evaluation the Symbiotic Effectiveness of Different Rhizobial Strains/Isolates with Peanut Cultivar, *Ismailia 1*

The response of peanut, *Ismailia*1 plants to different rhizobial strains/isolates compared to un-inoculated control was studied in pot experiments. Growth, nodulation and shoot nitrogen content of peanut plants were determined (Table 11 and 12). Different nodulation patterns were distinct by rhizobial strains/ isolates (Table 11). *Bradyrhizobium* USDA 3456, USDA 3339 strains and local isolate 1 gave the highest number of nodules by 40.0, 36.0 and 36.0 nodules/plant, respectively. However, the un-inoculated control did not form any nodules. *Bradyrhizobium* USDA 3456 strain gave the highest dry weight of nodules by 252.50 mg/plant (Table 11). All tested rhizobial strains/ isolates increased dry weight of plant root and shoot compared to un-inoculated control. Biomass of peanut plants has been determined among all rhizobial strains/ isolates treatments compared to the un-inoculated control (Table 12). *Bradyrhizobium* USDA 3456 strain exhibited the highest dry weight of root by 1.53 g/plant compared to the un-inoculated control by 0.40 g/plant. Similarly, *Bradyrhizobium* USDA 3456 strain gave the highest dry weight of shoot by 6.76 g/plant compared to the un-inoculated control which gave 4.66 g/plant. The effect of USDA 3456 treatment on peanut plants compared to un-inoculated control is clearly appeared in Fig (2). Regarding to nitrogen content of shoot, all rhizobial strains/ isolates significantly improved shoot nitrogen content. *Bradyrhizobium* USDA 3456 strain showed the highest shoot nitrogen content by 163.59 mg/plant in comparison with the un-inoculated control by 97.25 mg/plant (Table 12).

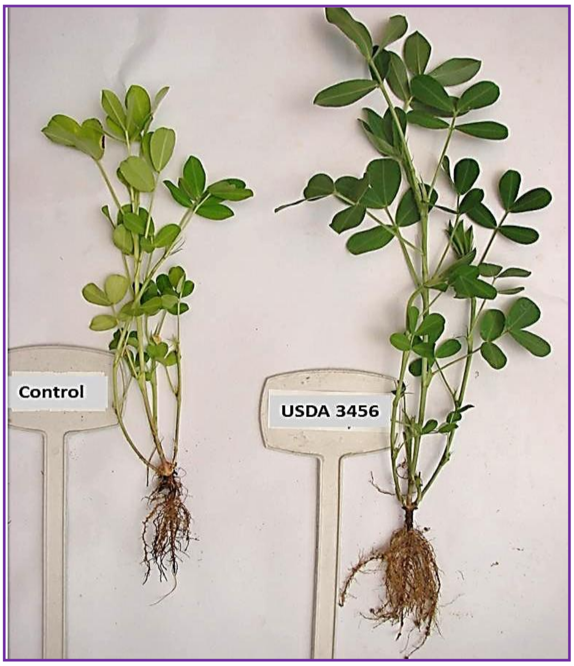


Fig (2): Effect of rhizobial inoculant (USDA 3456) on peanut plants cultivar, *Ismailia 1* compared to un-inoculated control

Table (11): Effect of different rhizobial strains/ isolates on nodulation status of peanut cultivar, *Ismailia 1*

Treatment	Nodulation Status			
	No. of Nodules*/plant		Dry Wt. of Nodules* (mg/plant)	
Control (Un-inoculated)	0.00	E	0.00	F
USDA 3339	36.00	AB	196.27	B
USDA 3456	40.00	A	252.50	A
ARC 617	33.33	BC	158.00	C
ARC 601	28.00	D	75.02	E
Isolate 1	36.00	AB	153.53	C
Isolate 2	30.67	CD	93.97	D
LSD at 0.05	4.28		17.74	

*Means followed by the same letter are not significantly different at 5% level

Table (12): Effect of different rhizobial strains/ isolates on growth parameters and shoot nitrogen content of peanut cultivar, *Ismailia 1*

Treatment	Dry Wt. of Root* (g/ plant)		Dry Wt. of Shoot* (g/ plant)		Shoot Nitrogen Content* (mg/ plant)	
Control (Un-inoculated)	0.40	F	4.66	D	97.25	D
USDA 3339	1.42	B	5.52	C	129.12	C
USDA 3456	1.53	A	6.76	A	163.59	A
ARC 617	1.33	CD	5.40	C	123.02	C
ARC 601	1.21	E	4.35	D	94.69	D
Isolate 1	1.38	BC	6.26	B	144.20	B
Isolate 2	1.30	D	5.38	C	120.76	C
LSD at 0.05	0.08		0.39		10.07	

*Means followed by the same letter are not significantly different at 5% level

1.4. Evaluation the Symbiotic Effectiveness of Different Rhizobial Strains/Isolates with Peanut Cultivar, *Gregory*

The symbiotic effectiveness of bradyrhizobial strains/ isolates with peanut variety, *Gregory* was investigated under greenhouse conditions. Un-inoculated control was included. Nodulation status, growth parameters and shoot nitrogen content of peanut, *Gregory* plants are clearly shown in Tables (13 and 14). All tested rhizobial strains/ isolates significantly enhanced nodulation status of plants compared with the un-inoculated control. *Bradyrhizobium* USDA 3456 and USDA 3339 strains gave the highest number of nodules by 35.33 and 32.67 nodules/ plant, respectively with highest dry weight by 235.33 and 201.33 mg/ plant, respectively. In contrast, the un-inoculated control did not form any nodules (Table 13). Dry weight of plant roots and shoots were affected by different bradyrhizobial strains/ isolates treatments. *Bradyrhizobium* USDA 3456 strain gave the highest dry weight of plant root and shoot by 1.52 and 6.23 g/plant, respectively compared to the un-inoculated control by 0.82 and 4.54 g/plant, respectively (Table 14). In the same context, *Bradyrhizobium* USDA 3456 strain gave the highest shoot nitrogen content by 148.25 mg/plant compared to the un-inoculated control which gave 78.17 mg/plant (Table 14).

Table (13): Effect of different rhizobial strains/ isolates on nodulation status of peanut cultivar, *Gregory*

Treatment	Nodulation Status			
	No. of Nodules*/plant		Dry Wt. of Nodules* (mg/plant)	
Control (Un-inoculated)	0.00	F	0.00	E
USDA 3339	32.67	AB	201.33	B
USDA 3456	35.33	A	235.33	A
ARC 617	28.67	BC	190.00	B
ARC 601	15.33	E	133.33	D
Isolate 1	21.67	D	143.00	CD
Isolate 2	25.67	CD	153.22	C
LSD at 0.05	4.85		12.26	

*Means followed by the same letter are not significantly different at 5% level

Table (14): Effect of different rhizobial strains/ isolates on growth parameters and shoot nitrogen content of peanut cultivar, *Gregory*

Treatment	Dry Wt. of Root* (g/ plant)		Dry Wt. of Shoot* (g/ plant)		Shoot Nitrogen Content* (mg/ plant)	
Control (Un-inoculated)	0.82	E	4.54	D	78.17	E
USDA 3339	1.42	B	5.62	B	121.59	B
USDA 3456	1.52	A	6.23	A	148.25	A
ARC 617	1.40	BC	5.63	B	119.30	B
ARC 601	1.27	D	4.91	C	95.68	D
Isolate 1	1.35	C	5.77	B	108.41	C
Isolate 2	1.38	BC	5.48	B	117.02	C
LSD at 0.05	0.07		0.30		10.28	

*Means followed by the same letter are not significantly different at 5% level

1.5. Evaluation the Symbiotic Effectiveness of Different Rhizobial Strains/Isolates with Peanut Cultivar, *NC*

Studying the effect of different rhizobial strains/isolates compared to the un-inoculated control on growth, nodulation and nitrogen content of peanut, *NC* plants was carried out in pot experiments (Table 15 and 16). Growth parameters, nodulation status, and shoot nitrogen content of peanut *NC* plants were positively affected by all tested bradyrhizobial strains/ isolates compared to the un-inoculated control. *Bradyrhizobium* USDA 3456 strain gave the highest number of nodules by 45.67 nodules/ plant with highest dry weight by 192.17 mg/ plant. On the other hand the un-inoculated control did not form any nodules (Table 15). Furthermore, *Bradyrhizobium* USDA 3456 strain gave the highest dry weight of plant root and shoot by 2.27 and 8.63 g/ plant, respectively compared to the un-inoculated control which gave 1.06 and 5.63 g/ plant, respectively (Table 16). Concerning shoot nitrogen content, it is clear from Table (16) that *Bradyrhizobium* USDA 3456 strain exhibited the highest nitrogen content in shoot by 200.27 mg/plant compared to the un-inoculated control which gave 95.92 mg/plant.

Table (15): Effect of different rhizobial strains/ isolates on nodulation status of peanut cultivar, *NC*

Treatment	Nodulation Status	
	No. of Nodules*/plant	Dry Wt. of Nodules* (mg/plant)
Control (Un-inoculated)	0.00 F	0.00 E
USDA 3339	34.67 B	136.00 B
USDA 3456	45.67 A	192.17 A
ARC 617	23.00 CD	124.33 C
ARC 601	19.33 DE	123.00 C
Isolate 1	26.33 C	134.78 B
Isolate 2	17.67 E	104.89 D
LSD at 0.05	4.26	8.82

*Means followed by the same letter are not significantly different at 5% level

Table (16): Effect of different rhizobial strains/ isolates on growth parameters and shoot nitrogen content of peanut cultivar, *NC*

Treatment	Dry Wt. of Root* (g/ plant)	Dry Wt. of Shoot* (g/ plant)	Shoot Nitrogen Content* (mg/ plant)
Control (Un-inoculated)	1.06 E	5.63 D	95.92 E
USDA 3339	2.17 B	7.43 B	169.69 B
USDA 3456	2.27 A	8.63 A	200.27 A
ARC 617	2.15 B	6.80 C	145.72 C
ARC 601	1.96 C	6.48 C	133.50 D
Isolate 1	2.20 B	7.73 B	168.74 B
Isolate 2	1.86 D	6.51 C	128.48 D
LSD at 0.05	0.07	0.52	11.22

*Means followed by the same letter are not significantly different at 5% level

1.6. Evaluation the Symbiotic Effectiveness of Different Rhizobial Strains/Isolates with Peanut Cultivar, *Early Bunch*

The effect of different rhizobial strains/isolates compared to un-inoculated control on growth, nodulation and shoot nitrogen content of peanut, *Early bunch* plants was observed in pot experiments (Table 17 and 18). All tested rhizobial strains/ isolates exhibited a different nodulation patterns with peanut plants (Table 17). *Bradyrhizobium* USDA 3456 strain, gave the highest number of nodules by 37.00 nodules/ plant with the highest dry weight by 196.10 mg/ plant. However, the un-inoculated control did not form any nodules. Dry weight of plant roots and shoots significantly increased due to different rhizobial treatments compared to un-inoculated control. Data in Table (18) revealed that *Bradyrhizobium* USDA 3456 and USDA 3339 strains exhibited the highest dry weight of plant roots by 1.44 and 1.37 g/ plant, respectively compared with the un-inoculated control which gave 0.99 g/plant. Regarding to dry weight of plant shoots, *Bradyrhizobium* USDA 3456 strain gave the highest dry weight of plant shoot by 7.18 g/plant compared to the un-inoculated control which gave 4.43 g/plant (Table 18). The effect of rhizobial treatment (USDA 3456) on peanut plants compared to un-inoculated control is clearly shown in Fig (3). Shoot nitrogen content was positively affected by different rhizobial treatments in comparison with the un-inoculated control. *Bradyrhizobium* USDA 3456 strain showed the highest nitrogen content of peanut plant shoots by 172.90 mg/plant compared to un-inoculated control which gave 77.57 mg/plant (Table 18).

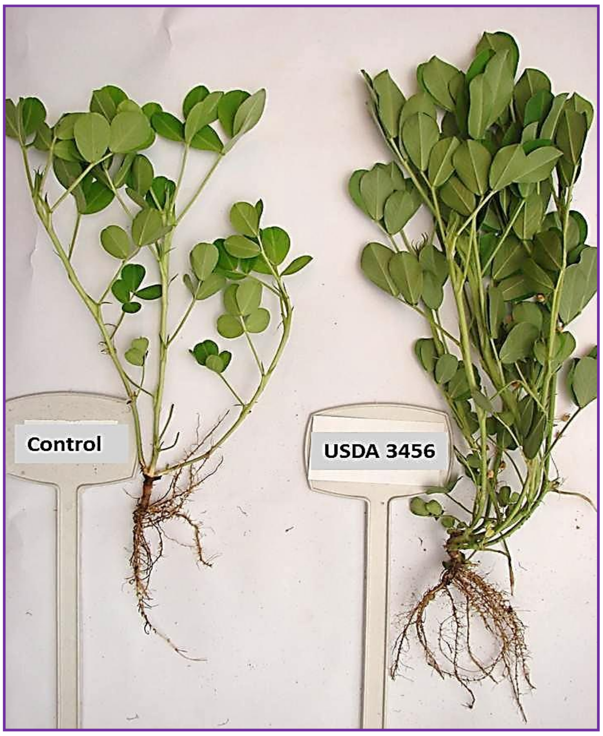


Fig (3): Effect of rhizobial inoculant (USDA 3456) on peanut plants cultivar, *Early bunch* compared to the un-inoculated control

Table (17): Effect of different rhizobial strains/ isolates on nodulation status of peanut cultivar, *Early bunch*

Treatment	Nodulation Status	
	No. of Nodules*/plant	Dry Wt. of Nodules* (mg/plant)
Control (Un-inoculated)	0.00 F	0.00 F
USDA 3339	31.00 B	187.50 B
USDA 3456	37.00 A	196.10 A
ARC 617	19.67 D	129.08 DE
ARC 601	26.67 BC	153.00 C
Isolate 1	13.67 E	125.90 E
Isolate 2	24.00 CD	135.56 D
LSD at 0.05	4.71	6.49

*Means followed by the same letter are not significantly different at 5% level

Table (18): Effect of different rhizobial strains/ isolates on growth parameters and shoot nitrogen content of peanut cultivar, *Early bunch*

Treatment	Dry Wt. of Root* (g/ plant)	Dry Wt. of Shoot* (g/ plant)	Shoot Nitrogen Content* (mg/ plant)
Control (Un-inoculated)	0.99 D	4.43 D	77.57 D
USDA 3339	1.37 A	6.36 B	149.35 B
USDA 3456	1.44 A	7.18 A	172.90 A
ARC 617	1.15 C	6.42 B	139.60 B
ARC 601	1.24 B	6.34 B	145.65 B
Isolate 1	1.14 C	5.82 C	125.52 C
Isolate 2	1.22 BC	6.61 B	148.72 B
LSD at 0.05	0.08	0.47	12.17

*Means followed by the same letter are not significantly different at 5% level

2. Screening the Antagonistic Activity of Rhizobacterial Isolates Against Fungal Growth

To investigate the most effective rhizobacterial isolates against peanut fungal pathogens, five isolates of *Serratia marcescens* sp. (SER4, EG10, GT, BF9 and BEF91) and three isolates of *Pseudomonas* sp. (*P. putida* , *P. aeruginosa* and *P. fluorescens*) were tested for their antagonistic activity *in-vitro* by assaying their ability to inhibit the mycelial growth of *Aspergillus flavus* on PDA media (Fig. 4). Only those isolates that produced a clear zone of inhibition or caused lysis of fungal mycelium were considered effective. *Aspergillus flavus* inoculated PDA plates were used as a negative control (Fig. 4A). The results demonstrated that GT isolate of *Serratia marcescens* sp. exhibited the highest antagonistic effect against *Aspergillus flavus* (Fig. 4B); it had the largest inhibition zone. On the contrary, other tested isolates of *Serratia marcescens* sp. were sensitive to fungal growth and did not form any inhibition zone (Fig. 4C). In the same context, *Pseudomonas putida* sp. exhibited the highly antagonistic effect against *Aspergillus flavus*; it had the largest inhibition zones (Fig. 4D), compared to other tested isolates of *Pseudomonas* sp. which did not show any antagonistic effect against fungal growth (Fig. 4E).

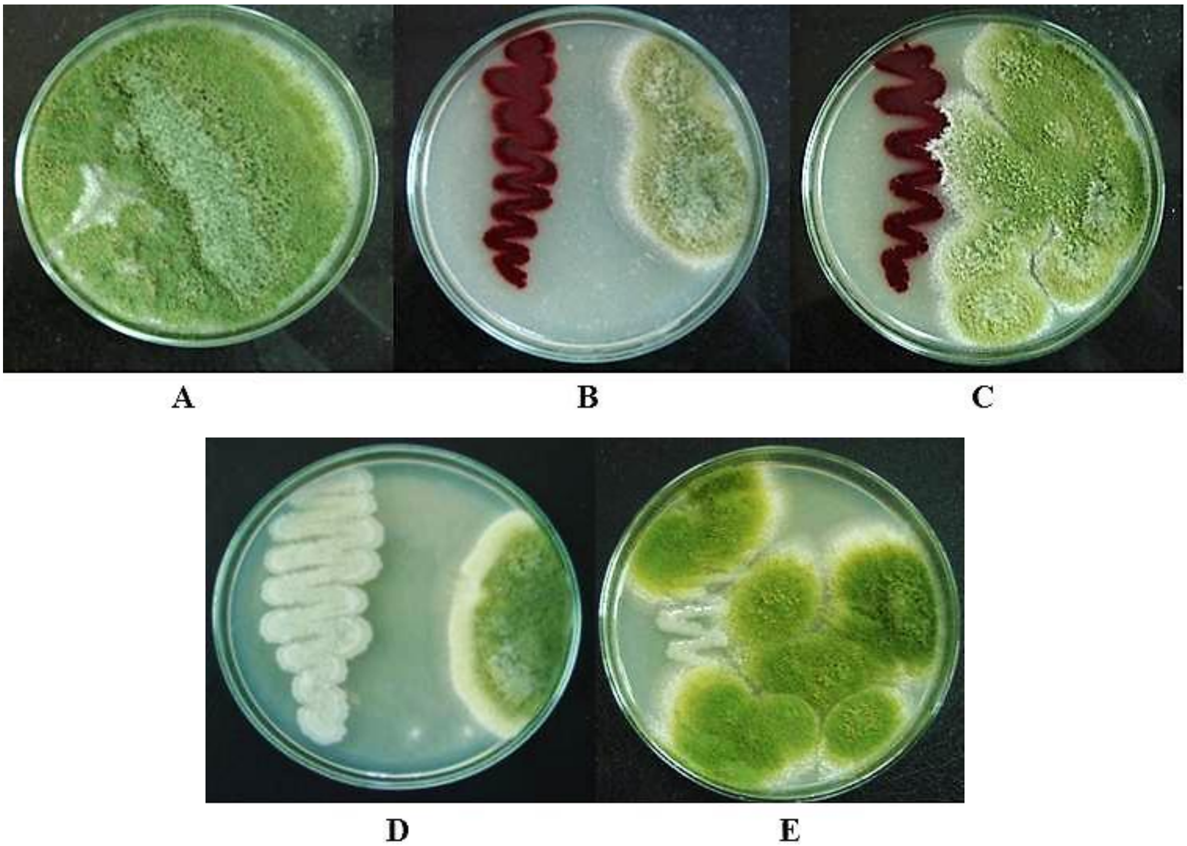


Fig (4): The antagonistic effect of rhizobacterial isolates on *Aspergillus flavus* growth compared to negative control

A: *Aspergillus flavus* (negative control)

B: Antagonistic effect of *Serratia marcescens* sp., GT against *A. flavus*

C: negative effect of other isolates of *Serratia marcescens* sp. on *A. flavus*

D: Antagonistic effect of *Pseudomonas putida* against *A. flavus*

E: negative effect of other isolates of *Pseudomonas* sp. on *A. flavus*

3. Bacterial Conjugation

Based on the symbiotic effectiveness and antagonistic activity of all tested bacterial strains/ isolates, bacterial conjugation process was carried out using the most effective bradyrhizobia, USDA 3456 reference strain as a recipient and the most antagonistic rhizobacteria, *Serratia marcescens* GT as well as *Pseudomonas putida* sp. isolate as donors. This conjugation aimed to obtain new rhizobial isolates that have dual functions: symbiotic effectiveness with peanut and antagonistic activity against peanut fungal pathogens.

3.1. Antibiotic Marking for Bacterial Strains/ Isolates

The three bacterial strains/ isolates used in this experiment were genetically marked using four antibiotics (ampicillin, chloramphenicol, neomycinsulphate and streptomycin). The results in Tables (19 and 20) demonstrated that Ampicillin was much more effective to inhibit the growth of all bacterial strains/ isolates in comparison with the other used antibiotics. On the other hand, all bacterial strains/ isolates were resistant to neomycinsulphate. Bradyrhizobial USDA 3456 strain was sensitive to streptomycin by inhibition zone of 2.0 cm diameter (Fig. 5A) and resistant to chloramphenicol (Fig. 5B). On the contrary, *Serratia* sp. (GT) was resistant to streptomycin (Fig. 6A) and sensitive to chloramphenicol by inhibition zone of 1.3 cm diameter (Fig. 6B). Similarly, *P. putida* was resistant to streptomycin (Fig. 7A) and sensitive to chloramphenicol by inhibition zone of 2.3 cm diameter (Fig. 7B). Therefore, the two antibiotics, streptomycin and chloramphenicol were selected as antibiotic markers and were supplemented to media on which conjugation was carried out.

Table (19): Diameter (cm) of inhibition zones according to bacterial strains/isolates response to different antibiotics*

Bacterial strains/ isolates	Inhibition zones (cm)			
	Chloramphenicol	**Ampicillin**	**Streptomycin**	**Neomycinsulphate**
USDA 3456	**0.0**	**1.2**	**2.0**	**0.0**
***P. putida* sp.**	**2.3**	**1.3**	**0.0**	**0.0**
***Serratia* sp.(GT)**	**1.3**	**1.5**	**0.0**	**0.0**

*All tested antibiotics were used in a concentration of 100µg/ml.

Table (20): Susceptibility of bacterial isolates/ strains against tested antibiotics

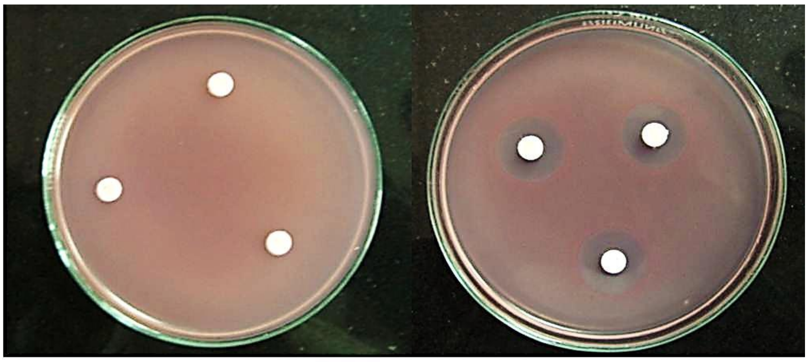
Antibiotics	Bacterial strains/ isolates		
	USDA 3456	***Serratia* sp. (GT)**	***P. putida***
Chloramphenicol	+	-	-
Ampicillin	-	-	-
Streptomycin	-	+	+
Neomycinsulphate	+	+	+

+/ - means resistant and sensitive to antibiotics respectively.

A B

Fig (5): Effect of streptomycine and chloroamphenicol on *Bradyrhizobium* USDA 3456

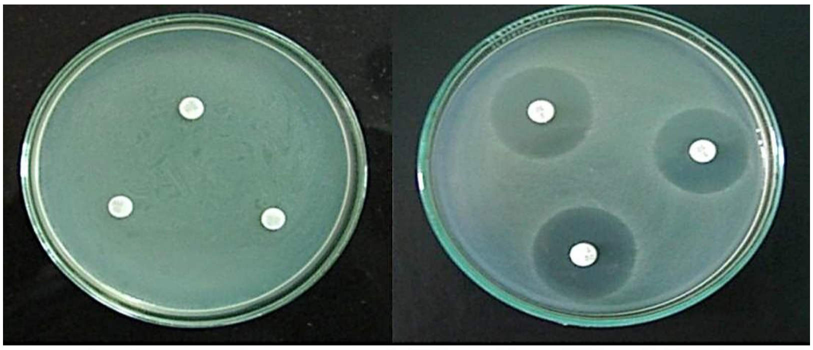
A: Sensitivity of *Bradyrhizobium* USDA 3456 to streptomycine
B: Resistance of *Bradyrhizobium* USDA 3456 to chloroamphenicol



A B

Fig (6): Effect of streptomycine and chloroamphenicol on *S. marcescens* GT

A: Resistance of *S. marcescens* GT to streptomycine
B: Sensitivity of *S. marcescens* GT to chloroamphenicol



A B

Fig (7): Effect of streptomycine and chloroamphenicol on *P. putida* sp.

A: Resistance of *P. putida* sp. to streptomycine
B: Sensitivity of *P. putida* sp. to chloroamphenicol

4. Evaluation of New *Rhizobium* Transconjugants

4.1. Evaluation the Symbiotic Effectiveness of New *Rhizobium* Transconjugants

The two *Rhizobium* transconjugants (DiM 71 and DiM 73) were tested for their symbiotic effectiveness compared to their parent *Bradyrhizobium* strain USDA 3456 under greenhouse conditions. The pot experiments involved the parent *Bradyrhizobium* strain, USDA 3456 and the transconjugants DiM 71 (which resulted from diparental mating between USDA 3456 and *Serratia marcescens* isolate GT) and DiM 73 (which resulted from diparental mating between USDA 3456 and *Pseudomonas putida).* The pot experiments conducted with three peanut cultivars (*Giza 6, Gregory* and *Early bunch*).

4.1.1. Evaluation the Symbiotic Effectiveness of New *Rhizobium* Transconjugants with Peanut Cultivar, *Giza6*

The effect of new rhizobial transconjugants compared to the parent strain USDA 3456 on nodulation, growth parameters and shoot nitrogen content of peanut plants (*Giza6* cultivar) was carried out in pot experiments (Table 21 and 22). The un-inoculated control was included. All rhizobial treatments significantly improved the nodulation status of peanut plants compared to un-inoculated control. DiM 71 and DiM 73 transconjugants gave 42.33 and 43.67 nodules/ plant, respectively by dry weight 212.10 and 226.25 mg /plant, respectively compared to parent strain USDA 3456 which gave 49 nodules/ plant by dry weight 236.09 mg/ plant. On the other hand, the un-inoculated control did not form any nodules (Table 21). In respect to growth parameters and nitrogen content of peanut plants, there was no significant difference between the tested rhizobial transconjugants and the parent *Bradyrhizobium* USDA 3456. Dry weight of root and shoot of peanut plants were positively affected by the three *Rhizobium* treatment compared to the un-inoculated control (Table 22). DiM 73 gave the highest dry weight of root by 1.55 g/ plant followed by DiM 71 and USDA 3456 by 1.13 and 1.07 g/ plant, respectively compared to un-inoculated control which gave 0.57 g/ plant (Table 22). DiM 71 and DiM 73 gave 6.26 and 6.22 g/ plant, respectively compared to the parent strain USDA 3456 by 6.27 g/ plant. Regarding the shoot nitrogen content, DiM 71 and DiM 73 gave 107.10 and 102.76 mg/ plant compared to USDA 3456 by 116.62 mg/ plant (Table 22).

Table (21): Effect of parent *Bradyrhizobium* strain (USDA 3456) and new transconjugants (DiM 71 and DiM 73) on nodulation status of peanut cultivar, *Giza 6.*

Treatment	Nodulation Status	
	No. of Nodules*/plant	Dry Wt. of Nodules* (mg/plant)
Control (Un-inoculated)	0.00 B	0.00 C
USDA 3456	49.00 A	236.09 A
DiM 71	42.33 A	212.10 A
DiM 73	43.67 A	226.25 A
LSD at 0.05	8.23	54.10

*Means followed by the same letter are not significantly different at 5% level

Table (22): Effect of parent *Bradyrhizobium* strain (USDA 3456) and new transconjugants (DiM 71 and DiM 73) on growth parameters and shoot nitrogen content of peanut cultivar, *Giza6*

Treatment	Dry Wt. of Root* (g/ plant)	Dry Wt. of Shoot* (g/ plant)	Shoot nitrogen Content* (mg/ plant)
Control (Un-inoculated)	0.57 B	2.76 B	40.54 B
USDA 3456	1.07 AB	6.27 A	116.62 A
DiM 71	1.13 AB	6.26 A	107.10 A
DiM 73	1.55 A	6.22 A	102.76 A
LSD at 0.05	0.88	1.68	29.22

*Means followed by the same letter are not significantly different at 5% level

4.1.2. Evaluation of Symbiotic Effectiveness of New *Rhizobium* Transconjugants with Peanut Cultivar, *Gregory*.

The response of peanut plants (*Gregory* cultivar) to DiM 71 and DiM 73 transconjugants compared to parent *Bradyrhizobium* USDA 3456 strain was studied under greenhouse conditions. Nodules number and dry weights, as well as shoot biomass and total nitrogen content were determined (Tables 23 and 24). DiM 71 and DiM 73 gave 49.00 and 43.33 nodules/ plant by total dry weight 179.73 and 174.75 mg/ plant, respectively compared to USDA 3456 which gave 48.67 nodules/ plant by dry weight 168.10 mg/ plant. On the contrast, the un-inoculated control did not form any nodules. The effect of new transconjugants on peanut plants compared to the parent USDA 3456 strain and the un-inoculated control is clearly appeared in Fig. (8). Results in Table (24) revealed that there were no significant difference between parent strain USDA 3456 and new transconjugants regarding to peanut growth parameters and shoot N-content. DiM 71 and DiM 73 showed root dry weight by 1.99 and 1.71 g/plant, respectively compared to USDA 3456 by 1.42 g/ plant. In the same context, DiM 71 and DiM 73 displayed shoot dry weight by 6.79 and 6.94 g/ plant, respectively compared to strain USDA 3456 which gave 7.29 g/ plant. Concerning shoot nitrogen content, DiM 71 and DiM 73 gave 119.89 and 127.15 mg/ plant compared to strain USDA 3456 by 145.57 mg/ plant (Table 24).

Fig (8): Effect of DiM 71 and DiM 73 transconjugants on peanut, *Gregory* plants compared to *Bradyrhizobium* USDA 3456 and the un-inoculated control

Table (23): Effect of parent *Bradyrhizobium* strain (USDA 3456) and new transconjugants (DiM 71 and DiM 73) on nodulation status of peanut cultivar, *Gregory*.

Treatment	Nodulation Status			
	No. of Nodules*/plant		Dry Wt. of Nodules* (mg/plant)	
Control (Un-inoculated)	0.00	B	0.00	B
USDA 3456	48.67	A	168.10	A
DiM 71	49.00	A	197.73	A
DiM 73	43.33	A	174.75	A
LSD at 0.05	8.47		74.38	

*Means followed by the same letter are not significantly different at 5% level

Table (24): Effect of parent *Bradyrhizobium* strain (USDA 3456) and new transconjugants (DiM 71 and DiM 73) on growth parameters and shoot nitrogen content of peanut cultivar, *Gregory*.

Treatment	Dry Wt. of Root* (g/ plant)		Dry Wt. of Shoot* (g/ plant)		Shoot nitrogen Content* (mg/ plant)	
Control (Un-inoculated)	0.60	B	3.23	B	48.64	B
USDA 3456	1.42	AB	7.29	A	145.57	A
DiM 71	1.99	A	6.79	A	119.89	A
DiM 73	1.71	A	6.94	A	127.15	A
LSD at 0.05	1.10		2.02		44.21	

*Means followed by the same letter are not significantly different at 5% level

4.1.3. Evaluation of Symbiotic Effectiveness of New *Rhizobium* Transconjugants with Peanut Cultivar, *Early Bunch.*

The new rhizobial transconjugants, DiM 71 and DiM 73 compared to the parent *Bradyrhizobium* USDA 3456 strain were tested for their symbiotic effectiveness with peanut cultivar, *Early bunch* under greenhouse conditions. All tested treatments enhanced the nodulation patterns of peanut plants compared to the un-inoculated control (Table 25). DiM 71 and DiM 73 gave 51.67 and 49.00 nodules/ plant by total dry weight 174.60 and 162.87 mg/ plant, respectively compared to parent strain (USDA 3456) which gave 52.00 nodules/ plant by total dry weight 192.73 mg/ plant. In respect to root and shoot dry weight as well as shoot N-content of peanut plants, there was no statistical difference between DiM 71 and DiM 73 and USDA 3456 strain (Table 26). DiM 71 gave the highest dry weight of root by 2.28 g/ plant followed by parent USDA 3456 strain and DiM 73 which gave 1.95 and 1.88 g/ plant, respectively (Table 26). Similarly, DiM 73 displayed the highest dry weight of shoot by 7.67 g/ plant followed by parent strain USDA 3456 and DIM 71 by 6.83 and 6.48 g/ plant, respectively. Regarding to shoot nitrogen content, DiM 73 and DiM 71 increased nitrogen content of shoot as compared by parent strain USDA 3456. They gave 134.52 and 130.33 mg/ plant respectively compared to strain USDA 3456 which gave 110.20 mg/ plant (Table 26).

Table (25): Effect of parent *Bradyrhizobium* strain (USDA 3456) and new transconjugants (DiM 71 and DiM 73) on nodulation status of peanut cultivar, *Early bunch.*

Treatment	Nodulation Status			
	No. of Nodules*/plant		Dry Wt. of Nodules* (mg/plant)	
Control (Un-inoculated)	0.00	B	0.00	B
USDA 3456	52.00	A	192.73	A
DiM 71	51.67	A	174.60	A
DiM 73	49.00	A	162.87	A
LSD at 0.05	14.90		89.02	

*Means followed by the same letter are not significantly different at 5% level

Table (26): Effect of parent *Bradyrhizobium* strain (USDA 3456) and new transconjugants (DiM 71 and DiM 73) on growth parameters and shoot nitrogen content of peanut cultivar, *Early bunch.*

Treatment	Dry Wt. of Root* (g/ plant)		Dry Wt. of Shoot* (g/ plant)		Shoot nitrogen Content* (mg/ plant)	
Control (Un-inoculated)	0.80	B	3.75	B	52.88	B
USDA 3456	1.95	A	6.83	A	110.20	AB
DiM 71	2.28	A	6.48	AB	130.33	A
DiM 73	1.88	A	7.67	A	134.52	A
LSD at 0.05	1.67		2.78		64.84	

*Means followed by the same letter are not significantly different at 5% level

4.2. Evaluation of Antagonistic Activity of New *Rhizobium* Transconjugants

To confirm that, the antagonistic activity trait has been transferred from the parent rhizobacteria (*S. marcescens* sp. GT and *P. putida* sp.) to the obtained transconjugants. DiM 71 and DiM 73 were tested for their antagonistic activity against phytopathogenic fungi *Aspergillus flavus in-vitro* (Fig. 9). Results revealed that the tested *Rhizobium* transconjugants, DIM 71 and DIM 73 exhibited highly antagonistic effect against peanut fungal pathogen (*A. flavus*) (Figs. 9B and 9C, respectively) compared to the negative control (Fig. 9A).

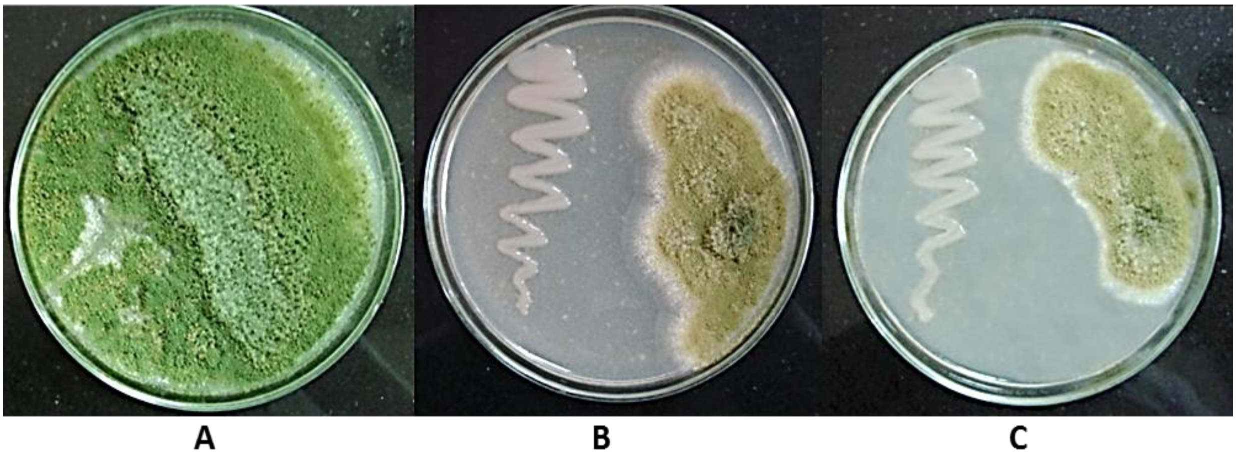


Fig (9): The antagonistic effect of new *Rhizobium* transconjugants on *Aspergillus flavus* growth compared to negative control

A: *Aspergillus flavus* (negative control)

B: Antagonistic effect of DiM 71 against *A. flavus*

C: Antagonistic effect of DiM 73 against *A. flavus*

5. Proteomic Analysis

5.1. Protein Analysis of Peanut Nodulated Rhizobial Strains/ Isolates

The total protein contents of rhizobial isolates/ strains (isolate 1, isolate 2, ARC 601, ARC 617, *Bradyrhizobium* USDA 3339 and *Bradyrhizobium* USDA 3456) were separated by using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Fig. 10). Bioinformatics analysis of rhizobial protein profiles was carried out using Phoretix 1D software (TotalLab, UK). The electrophoretic analysis clarified that most of separated protein bands are dominant in all tested peanut rhizobia (Fig. 10). More than 25 protein bands could be resolved ranged in the size from 24 to 138 KDa. SDS protein profile of isolate 1 and ARC 601, 617 and USDA 3456 showed high genetic similarity and were clustered in one group at 80% level of similarity (Fig11). This group was characterized by discriminating protein bands with approximately molecular weight of 76.6 KDa and 55.0 KDa (Fig. 10). On the other hand, isolate 2 and USDA 3339 showed high genetic resemblance and were ranked in one group at 73% level of similarity (Fig. 11). This group was characterized by distinguishing protein band with approximately molecular weight of 52.4 KDa (Fig. 10).

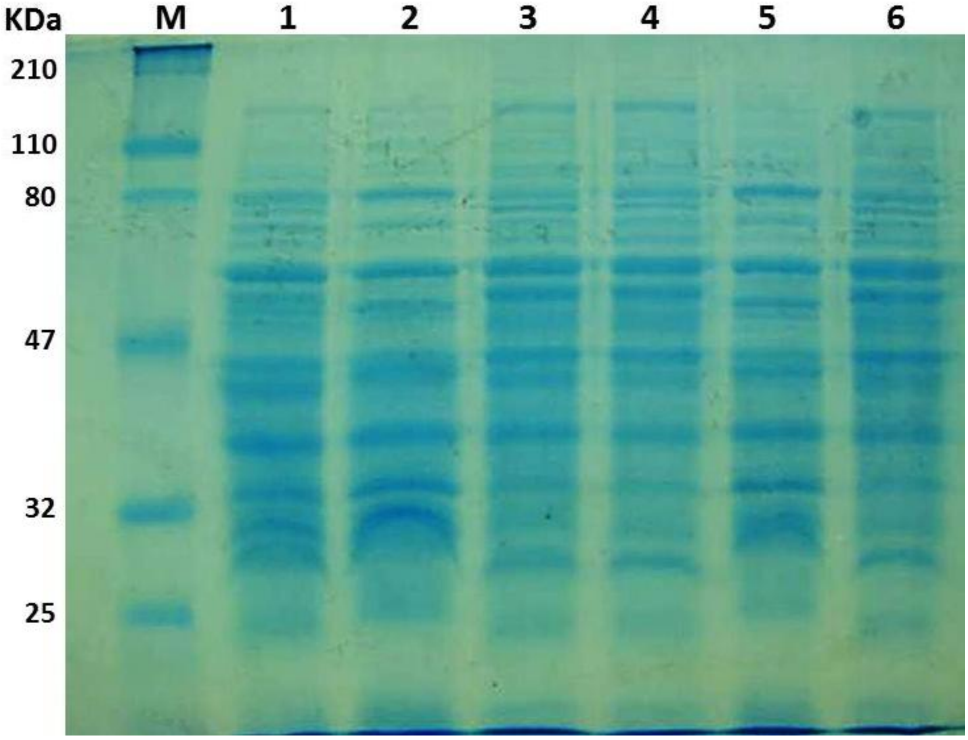


Fig (10): SDS gel electrophoresis of total cellular protein of tested bradyrhizobial isolates/strains
M: Pierce® protein marker
1-6: Isolate 1, Isolate 2, ARC 601, ARC 617, USDA 3339 and USDA 3456

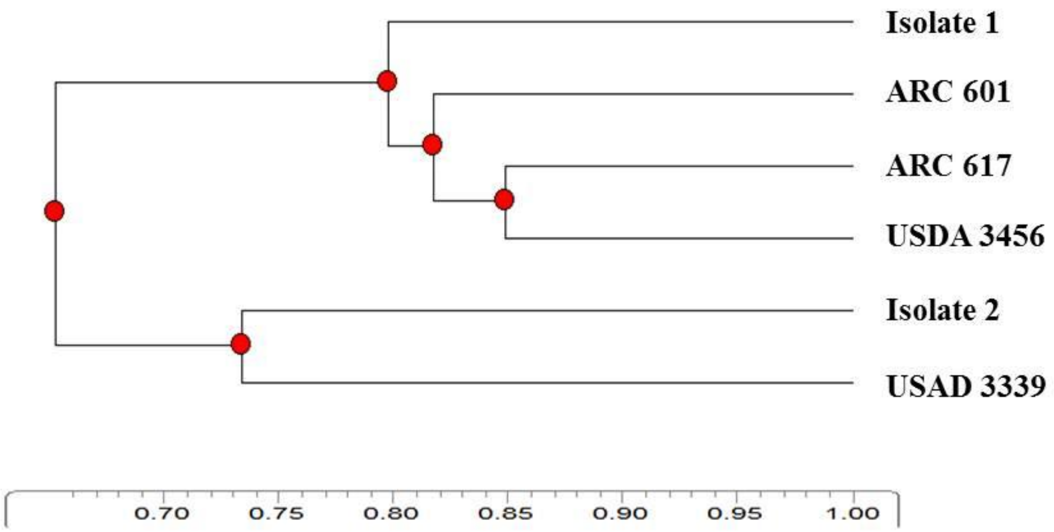


Fig (11): Dendrogram showing the similarity among the electrophoretic protein patterns (SDS-PAGE) of the tested rhizobial isolates/strains

5.2. Protein Analysis of Parent Bacterial Isolates/ Strains and New Transconjuagnts

SDS-PAGE was used as a molecular marker to monitor the conjugation process at higher level between the donor "parent" bacterial isolates/ strains (*Bradyrhizobium* USDA 3456 and isolates of *S. marcescens* GT and *P. putida*) and the obtained transconjugants, DiM 71 (USDA 3456 + *Serratia marcescens*) and DiM 73 (USDA 3456 + *P. putida*) (Fig.12). The protein analysis of *S. marcescens* GT exhibited the presence of discriminating protein bands with high molecular weight of approximately 70.0, 83.3 and 96.0 KDa in addition to, low molecular weight proteins of approximately 34.7 and 37.1 KDa, respectively (Fig. 12). Similarly, the existence of distinctive protein bands was found in *P. putida* sp. isolate with high molecular weight of approximately 71.4, 76.2 and 84.5 KDa as well as low molecular weight proteins of approximately 35.2 and 38.0 KDa, respectively (Fig.12). Also, the presence of distinct protein bands was clearly appeared in *Bradyrhizobium* USDA 3456 at approximately 32.9, 55.0 and 63.0 KDa (Fig. 12).

The SDS protein profiles of the new transconjugants, DiM 71 and DiM 73 clarified the transfer and expression of different proteins from their related donors. In respect to DiM 71, the electrophoretic analysis showed the presence of major proteins of both *Bradyrhizobium* USDA 3456 (approximately 32.9, 55.0 and 63.0 KDa) and *S. marcescens* GT (approximately 34.7, 37.1, 70.0, 83.3 and 96.0). Likewise, SDS-PAGE analysis of

DiM 73 displayed expression of the distinctive proteins of *Bradyrhizobium* USDA 3456 (approximately 32.9, 55.0 and 63.0 KDa) and *P. putida* (approximately 71.4, 76.2 and 84.5 KDa). However, some *P. putida* related proteins (approximately 35.2 and 38.0 KDa) did not clearly express in the obtained DiM 73 transconjugant.

The genetic relationship among the parent bacteria (*Bradyrhizobium* USDA 3456 and isolates of *S. marcescens* GT and *P. putida*) and the obtained transconjugants, DiM 71 and DiM 73 was assessed using bioinformatics analysis, Phoretix 1D software (Fig. 13). The obtained dendrogram showed high genetic resemblance among the DiM 71 and DiM 73 transconjugants and USDA 3456, which were clustered in one group at 53% level of similarity (Fig. 13). The genetic similarity of DiM73 with USDA 3456 was higher than appeared between DiM 71 and USDA 3456 by 89 and 55 % level of similarity, respectively. On the other hand, a high genetic relationship was detected between *S. marcescens* GT and *P. putida* sp. which were grouped in one lineage at 47% level of similarity (Fig. 13).

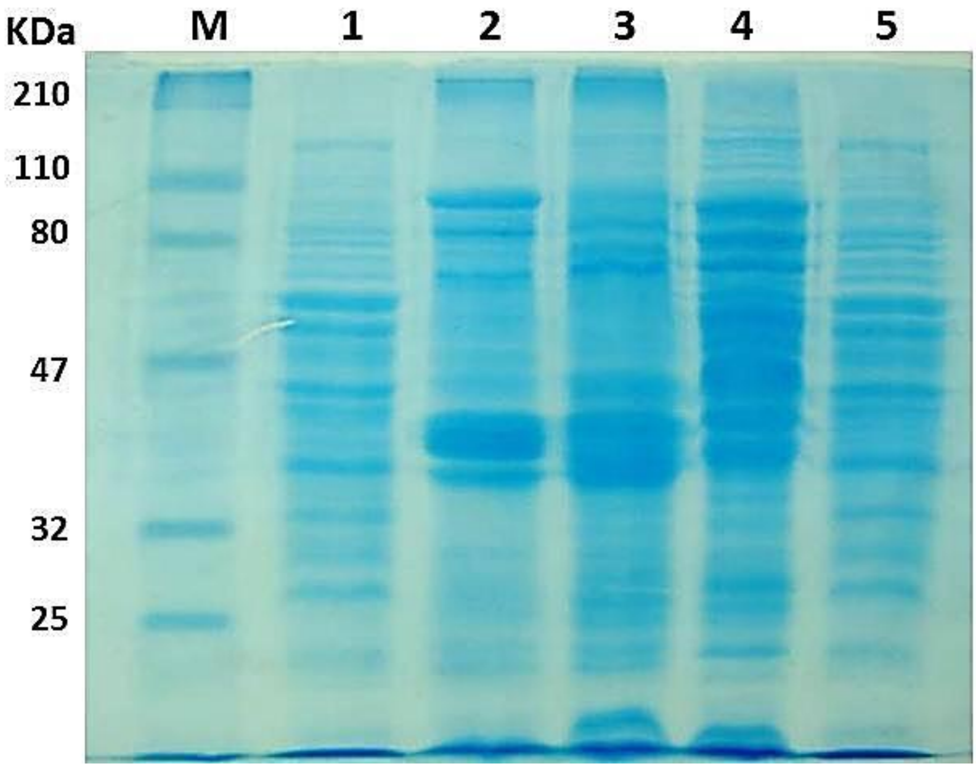


Fig (12): SDS gel electrophoresis of total cellular protein of the donor bacterial isolates/strains and new transconjugants

M: Pierce® protein marker

1-5: USDA 3456, *S. marcescens* GT and *P. putida* sp., DiM 71 and DiM 73

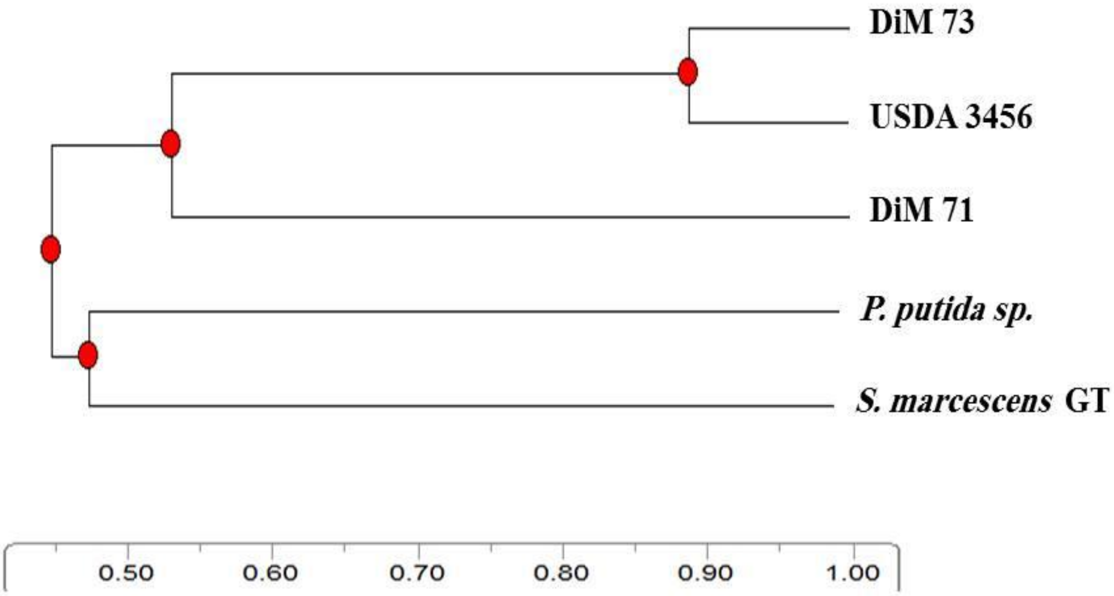


Fig (13): Dendrogram showing the similarity among the electrophoretic protein patterns (SDS-PAGE) of the donor bacterial isolates/strains and new transconjugants