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Evaluation of Native Rhizobia Strains on the Growth and Yield Attributes of Rainfed Chickpea

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Abstract: This research aims to explore the effect of inoculation with nitrogen-fixing bacteria and with or without chemical nitrogen fertilizer on the growth and productivity of chickpea under field conditions. The experiment was carried out in split-plot design with three replicates at Hout Research Station of Agricultural Scientific Research Center in As-Suwayda governorate. Nitrogen fertilizer treatments (N 0%, N 100% of recommended dose) were according to the ministry of agriculture recommendation; the bacterial treatments were ten (eight treatments were inoculation with different chickpea bacterial isolates and a ninth one was inoculation with mixture of eight isolates and the tenth was uninoculated control treatment). The results showed that the isolates R1, R4 and R8 exceeded all others in terms of productivity (t ha^{-1}), weight of dry matter, weight of dry nodules per plant (g $plant^{-1}$), plant height (cm) and seeds number per plant. As for the interaction between bacterial inoculation and chemical nitrogen fertilization, the treatments R1 N 100% and R4 N 100% exceeded all others in terms of all the studied parameters of growth and productivity. Rhizobia inoculation and chemical nitrogen fertilization for the treatments R1 N 100% increased the weight of dry nodules per plant by 228.66% compared to non-inoculated plants. Results indicated that application of suitable amounts of N fertilizer can be beneficial to improve nodulation, growth, and final yield of inoculated chickpea plants. Based on results, it can be concluded that the two strains R1 and R4 can be promising to increase the growth and productivity of chickpea plants.

Key words: Rainfed chickpea, nitrogen fixing bacteria, nitrogen fertilization, bio-fertilizer, rhizobia, productivity.

Low soil fertility is one of the major problems that limits economically successful agricultural production worldwide. Depletion of nutrients from the soil is a specific problem in the areas where grain legumes, one of the most important economic resources for small landowners, are heavily cultivated (Baset and Shamsuddin, 2010). Studies showed that annual average value of nutrients loss is 22 kg nitrogen ha^{-1} (N ha^{-1}), 25 kg phosphorus ha^{-1} and 15 kg potassium ha^{-1}. Therefore, the addition of fertilizers has become an urgent need to correct low soil fertility and provide the nutrients needed for optimal crop growth (Elsheikh *et al*., 2005). Chickpea requires about 13-41 kg N ha^{-1} for growth and development (Siddiqi and Mahmood, 2001). As a legume plant, it also has a significant impact on improving soil fertility and growth of succeeding field crops. This is achieved by entering into a symbiotic (mutually beneficial) relationship with nodulating bacteria, where the plant provides bacteria with photosynthates and the bacteria supply the plant with fixed nitrogen (Peoples *et al*., 1995; Andrews *et al*., 2017; Sprent *et al*., 2017; Vanlauwe *et al*., 2019). It is therefore not necessary to add nitrogen to chickpea crop because it meets 60-80% of its requirement from the atmospheric nitrogen (Siddiqi and Mahmood, 2001; Kucuk and Kıvanc, 2008). Legumes use their seed/root exudate to control plant and microbial growth in soil, and subsequently promote germination and growth of specific species. Though legumes and N_2-fixing diazotrophs, through their N_2 fixation ability puts them at an advantage over non-fixing systems in occupying various ecological niches (Dakora, 2004).

Biological nitrogen fixation (BNF) is carried out by a specific group of prokaryotes, which use an enzyme called nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_4+); i.e. a form that can be easily absorbed by the plant (Franche *et al*.,

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Table 1. Physical, chemical and fertility characteristics of site soils

Depth cm	pH	EC dS m^{-1}	OM %	$CaCO_3$ %	N ppm	P_2O_5 ppm	K_2O ppm	SAND %	SILT %	CLAY %
0-15	7.74	0.24	0.90	1.00	9.43	8.6	450	12	22	60
15-30	6.77	0.25	0.90	0.00	9.63	8.0	400	10	21	68
30-45	7.99	0.18	0.79	0.00	6.88	0.5	80	14	21	65
45-60	8.00	0.13	0.65	1.00	7.10	1.0	80	22	18	60

2009). Bio-fertilization through atmospheric nitrogen-fixing bacteria is a natural method of increasing soil nitrogen content, meeting crop nutrient requirements, and thus sustaining agricultural systems (Papastylianou and Danso, 1991; Rhinhart *et al.*, 2003). Innoculation of plants with rhizobia increases root nodule number, nitrogen uptake, growth and weight of 100 seeds, yield of grain (70-72%) and protein content of chickpea seeds (Rudresh *et al.*, 2005; Sogut, 2006; Erman *et al.*, 2011).

However recommendation of a biological fertilizer also depends on ability to fix atmospheric nitrogen within a wide range of environmental conditions, competition of other strains, reproduction in the environment, survival in the carrier, survival when incorporated into seed coated materials, formation of root nodules and fixation of atmospheric nitrogen in the presence of soil nitrogen, confronting inappropriate environmental factors, especially physical ones such as drought, high temperature, freezing and finally genetic stability (Keyser *et al.*, 1993). Thus, it is necessary to inoculate with strains that have high ability to fix atmospheric nitrogen (more efficient) and are acclimatized to local environmental conditions. Thus, the present research aims in identifying promising nitrogen fixers, in relation to applied nitrogen fertilizers for enhancing food-security of the citizen by increasing expansion in legumes cultivation, which leads to increase in the productivity and economic profitibilty of these crops.

So, the objective of the present research is to evaluate efficiency of local rhizobia bacteria in chickpea in Syria (at many locations in As-Suwayda governorate) with respect to their competence in root nodule formation and nitrogen fixation in comparison to the existing soil micro-organisms so as to improve growth and productivity of chickpea crop.

Materials and Methods

Study area

A field experiment was conducted in Hout Research Station of the Agricultural Scientific Research Center at As-Suwayda governorate, Syria, which is located 30 km south of As-Suwayda city center (32°42′45″N 36°34′00″E). Fertility characteristics of soil were determined. Mineral nitrogen, total phosphorus and total potassium were determined (FAO, 2007). Samples were taken from three profiles of soil (one in each replicates) and from four depths as shown in Table 1.

Two treatments of nitrogen fertilizer are:

N 0%: without fertilizer

N 100%: Treatment using (20 kg N ha^{-1}) of chemical nitrogen fertilizer (urea) and ten treatments of bacterial inoculation were as follows: eight treatments using chickpea bacterial isolates such as: R1, R2, R3, R4, R5, R6, R7, R8, R9 (treatment with mixture of the R1 to R8) and R10 (untreated control).

Characterization tests: To define rhizobia isolates, the following essential tests were performed: Gram test (Suslow *et al.*, 1982), Catalase test (Goszczynska *et al.*, 2000), Lactose metabolism (De oliveira *et al.*, 2007), growth test on YMA (yeast manitol agar; medium mannitol 1%, agar 1.5%, yeast 0.1%, di-potassium phosphate 0.08%, sodium chlorine 0.01%, calcium carbonate 0.1%, aqueous magnesium water 0.02%), oxidase test, gelatin decomposition by Frasier method, and test of sugary oxidation, sporulation, starch degradation, respiration, dye, and accumulation of poly-B-hydroxybutyrate granules (Abu Ghurra, 1997).

The experiment was designed in split-plot design, statistical analysis was performed by taking the mean of three replicates and the data was analyzed using MSTAT-C. Treatment effects were assessed based on the Duncan test at a significant level of 0.05.

Preparation of bacterial suspension

Pure bacterial isolates from (Rhizobia) bacteria, used in this research, were isolated and characterized in plant disease laboratory (at the Faculty of Agriculture, Damascus University). First, these isolates were grown on petri dishes containing yeast mannitol agar and incubated at 28°C for 48 hours. Then, 200 ml of LP liquid medium (Peptone: 7 g L^{-1}, yeast: 7 g L^{-1}) was sterilized within flasks and left to cool. Next, jugs containing the sterile LP liquid were inoculated with 1 ml of bacterial suspensions which were prepared from dishes containing the isolates, and incubated at 28°C with speed shaking of 100 r min^{-1} for 48 hours (Kantar *et al.*, 2003).

Cultivation conditions

Soil was ploughed well before planting and nitrogen fertilizers were added at the rate of 20 kg N ha^{-1} in the form of urea in two equal batches (i.e. each batch of 10 kg N ha^{-1}) at the planting and flowering stage. Phosphorus and potassium fertilizers were not added due to their high content in soil. Chickpea seeds were planted on 10.03.2018 for the first season and on 11.03.2019 for the second season, after soaking in bacterial suspension with $1*10^6$ CFU ml^{-1} for two hours. Seeds were planted in experimental plots of 3 m length and 2 m width and in 5 lines in each experimental plot. Planting distances were 40 cm between lines and 20 cm between plants, with three seeds per groove. Later they were thinned at a four-leaf phase and one plant was left in each groove.

Growth and yield parameters

The following parameters were recorded: plants height (cm), seeds average number per plant (seed $plant^{-1}$), average weight of plant dry matter (g $plant^{-1}$), average weight of dry nodules (g $plant^{-1}$), plant productivity calculation (t ha^{-1}) [Average weight of grain per square meter was calculated and converted to t ha^{-1} (FAO, 2007)].

Results and Discussion

Definition of isolated rhizobia isolates

Laboratory analysis that included biological and phenotypic tests for bacterial colonies besides microscopy, are presented in Table 2. These results showed that the eight isolates are similar in terms of the studied parameters: single-cell, bacilli-shaped, dimensions less than 2 microns, accumulation of poly-B-hydroxybutyrate granules and colonies bacterial growth on the medium of mannitol yeast has a full-edged convex circular shape with creamy-color and negative Gram stain. This corresponds to the morphological traits of rhizobia (Holt *et al.*, 1994). Also, all isolates are positive for catalase, negative oxidase activity and are able to use some sugars like

Table 2. Vital characterizing qualities of the used isolates

Isolates name	Growth on YMA	Colony shape	Colony color	Cell shape	Gram	Catalase	Oxidase	Gelatin disintegration	Starch disintegration	Lactose metabolism	Oxidase sugar group*	Glucose metabolism	Respiration
R1 R2 R3 R4 R5 R6 R7 R8	Positive	full-edged convex circular shape	creamy-colored	bacilli-shaped	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Air oxydation

Table 3. Mean effect of rhizobia inoculation on chickpea growth and productivity during consecutive agriculture seasons (mean over both nitrogen levels)

Treatment	Production (t ha^{-1})	Weight of dry matter (g $plant^{-1}$)	Plant height (cm)	No. of seeds per plant (seed $plant^{-1}$)	Weight of dry nodules (g $plant^{-1}$)
R1	1.51 A	15.16 A	42.25 A	97.50 A	0.402 A
R4	1.36 B	12.49 B	40.00 B	80.83 B	0.323 B
R8	1.34 B	12.48 B	39.75 B	77.67 B	0.305 C
R9	1.26 C	11.15 C	38.63 C	64.00 C	0.268 D
R3	1.16 D	10.43 D	37.67 D	59.83 D	0.275 D
R5	1.12 E	9.25 E	36.62 E	49.33 E	0.243 E
R7	1.12 E	9.07 F	36.58 E	48.00 E	0.238 EF
R2	1.08 F	9.01 F	36.18 E	46.17 E	0.227 F
R6	0.93 G	6.88 G	34.40 F	39.00 F	0.192 G
R10	0.83 H	5.99 H	32.75 G	32.00 G	0.167 H
LSD	0.017	0.174	0.634	3.744	0.012

Values followed by different letter in column are significantly different w.r.t. the parameter.

xylose, maltose, fructose, galactose, sucrose and mannitol as a carbon source, and these results are consistent with the characteristics of rhizobia mentioned by Deora *et al*., 2010; Fatima *et al*., 2008; Kanika *et al*., 2010;Teng *et al*., 2015.

Additionally, they were able to metabolize glucose but were not able to metabolize lactose and this is consistent with the results of Oliveira *et al*., 1997. Further, all isolates were non-sporadic, reflected aerobic oxidation, gelatin decomposition and were unable to utilize starch and these responses were consistent with that of Rosenberg *et al*., 2014.

Effect of atmospheric nitrogen fixation by bacteria on plant productivity (t ha^{-1})

Grain yield was significantly affected by inoculation with rhizobia bacteria and plants inoculated with R1 surpassed significantly all other plants that were treated with other isolates (Table 3); noticeably the average grain yield resulting from this isolate was (1.51 t ha^{-1}), followed by isolates R4 and R8, and lowest value of grain yield was in non-inoculated plants (0.83 t ha^{-1}). Increase in production due to rhizobia inoculation has also been reported in legumes from other parts of world (Singh, 1977; Sinha and Ramakrishna, 1996).

Fertilizer treatment N 100% exceeded the fertilizer treatment N 0% with an increase of 26.21% (Table 4).

This has also been reported by (Kalaghatagi *et al*., 2000; Das *et al*., 2000; Leary *et al*., 2008; Vora *et al*., 2019.

By comparing control treatments (without and with nitrogen) treatment C N 100% significantly exceeded C N 0% with an increase of 28.57%. The results in Table 5 also showed that highest value of productivity average was in R1 N 100% (1.63 t ha^{-1}), followed by R4 N 100% (1.54 t ha^{-1}), R8 N 100% (147 t ha^{-1}) and M N 100% (1.45 t ha^{-1}) in the sequence mentioned. Lowest value of mean yields was in the treatment C N 0% that was 0.7 t ha^{-1}. This corroborates with the results of earlier studies on various other crops (Karadavut and Ozdemir, 2001; Fatima *et al*., 2008; Öğütçü *et al*., 2010; Poole *et al*., 2018; Mathenge *et al*., 2019).

Since at low levels of nitrogen fertilizer, a positive catalytic effect in the fixation process has been reported, so adding a small amount of mineral nitrogen (20-25 kg ha^{-1}) to the soil

Table 4. Mean effect of mineral nitrogen fertilization on chickpea growth and productivity (mean over all isolate treatments)

Chemical fertilization treatment	Production (t ha^{-1})	Weight of dry matter (g $plant^{-1}$)	Plant height (cm)	No. of seeds per plant (seed $plant^{-1}$)	Weight of dry nodules (g $plant^{-1}$)
N 100%	1.3	12.24	39.34	73.97	0.309
N 0%	1.03	8.14	35.62	44.9	0.219
% increase	26.21	50.37	10.44	64.74	41.10

Table 5. Interaction effects of rhizobia inoculation and mineral nitrogen fertilization on chickpea growth and productivity (as mean of two consecutive agriculture seasons)

Treatment	Production (t ha^{-1})	Weight of dry matter (g plant^{-1})	Plant's height (cm)	No. of seeds per plant (seed plant^{-1})	Weight of dry nodules (g plant^{-1})
R1 N 100%	1.63 A	17.06A	43.83 A	127.00 A	0.493 A
R1 N 0%	1.39 D	13.25 F	40.67 DE	68.00 F	0.310 F
R4 N 100%	1.54 B	15.64 B	43.00 A	110.00 B	0.403 B
R4 N 0%	1.21 H	9.95 J	37.50 GH	55.33 GH	0.267 I
R8 N 100%	1.47 C	15.00 C	42.00 B	100.00 C	0.360 C
R8 N 0%	1.17 I	9.33 K	37.00 HI	51.67 HI	0.250 J
R9 N 100%	1.45 C	14.46 D	41.67 BC	85.33 D	0.343 D
R9 N 0%	1.13 J	8.47 L	36.27 IJ	48.33 IJ	0.243 K
R3 N 100%	1.40 D	13.83 E	41.00 CD	79.67 E	0.323 E
R3 N 0%	0.98 L	7.19 N	35.00 KL	41.67 KL	0.227 L
R5 N 100%	1.35 E	12.31 G	39.87 E	60.33 G	0.300 G
R5 N 0%	0.91 M	6.85 O	34.33 LM	38.67 LM	0.210 N
R7 N 100%	1.30 F	11.64 H	38.90 F	57.33 G	0.280 H
R7 N 0%	0.86 N	6.39 P	33.67 MN	34.33 MN	0.207 N
R2 N 100%	1.27 G	10.82 I	38.17 FG	57.00 G	0.217 M
R2 N 0%	0.82 O	5.94 Q	33.00 NO	33.00 N	0.193 O
R6 N 100%	1.04 K	7.82 M	35.80 JK	45.00 JK	0.183 P
R6 N 0%	0.80 O	5.82 Q	32.50OP	32.00 N	0.153 R
R10 N 100%	0.90 M	6.28 P	33.50 MN	34.00 MN	0.15 R
R10 N 0%	0.70 P	5.70 Q	32.00 P	30.00 N	0.15 R
LSD	0.023	0.246	0.896	5.294	0.005

Significance of interaction between bacterial inoculation and chemical nitrogenous fertilizers denoted by different following letters.

contributes to the formation of a sufficiently flat leafy surface which in turn facilitates and provides photosynthesis products which are necessary for root nodules so as to perform the fixation process from atmospheric nitrogen. Other researchers also reported same results with respect to the effects of N application (McKenzie and Hill, 1995; Walley *et al*., 2005; Achakzai and Bangulzai, 2006; Amany, 2007; Caliskan *et al*., 2008; Salvagiotti *et al*., 2008) and rhizobium inoculation (Singh, 1977; Shrivastava *et al*., 2000; Rudresh *et al*., 2005; Malik *et al*., 2006; Chemininggwa and Vessey, 2006; Albayrak *et al*., 2006; Togay *et al*., 2008) ultimately resulting in improved grain yield in different legume crops.

Effect of atmospheric nitrogen fixation by bacteria on dry matter weight

Weight of dry matter was significantly affected by bacterial inoculation. Results depicted in Table 3 shows that the highest value of dry matter weight averages was in plants treated with R1 isolation (15.16 g plant^{-1}), followed by isolates R4 and R8, and lowest value of dry matter weight was in non-inoculated plants (5.99 g plant^{-1}); this corroborates with Kimiti and Odee, 2010; Gao *et al*., 2019. Also, chemical nitrogen fertilization treatment N 100% was superior to that of the treatment N 0% reflecting an increase of 50.37% (Table 4).

As for the interaction between bacterial inoculation and chemical nitrogen fertilization, the weight of dry matter was significantly affected by bacterial inoculation and chemical nitrogen fertilization. Highest mean weight of dry matter was found in treatments R1 N 100% (17.06 g plant^{-1}) followed by R4 N 100% (15.64 g plant^{-1}), while lowest value of dry matter weight was in control C N 0% (5.7 g plant^{-1}).

Results in Table 5 are consistent with studies of (Ali *et al*., 2004; Amany, 2007; Caliskan *et al*., 2008; Öğütçü *et al*., 2010). Poole *et al*., 2018 also reported that the fixed amount of N_2 is an important factor that directly affects the growth of plant biomass. Nitrogen is known to be an essential nutrient for plant growth and development (Werner and Newton, 2005; Sogut, 2006; Salvagiotti *et al*., 2008) and it is involved in vital plant functions such as photosynthesis, DNA synthesis, protein formation, respiration, and N_2 fixation (Werner and Newton, 2005; Caliskan *et al*., 2008). Parameters of growth such as leaf area index (LAI), biomass, and leaf photosynthesis significantly decreased because of unsatisfactory N availability (Malik *et al*., 2006; Cheminingwa and Vessey, 2006; Caliskan *et al*., 2008). Results obtained from this study indicated that application of N fertilization have positive effects on chickpea yield and its attributes. Addition of N increases the production of total dry matter in plants (McKenzie and Hill, 1995; Salvagiotti *et al*., 2008; Caliskan *et al*., 2008), which can improve plant potential to gain more plant height and produce more branches, pods and seeds that ultimately resulted in high grain and biological yields. Nitrogen fertilization increases the total dry matter for a number of reasons: (i) Nitrogen can increase LAI in plants (McKenzie and Hill, 1995; Malik *et al*., 2006; Caliskan *et al*., 2008). Increase in LAI subsequently increases interception of solar radiation by plant that consequently results in more biomass accumulation (Caliskan *et al*., 2008), (ii) Nitrogen can increase the photosynthesis rate in plants which can be attributed to increase in amount of chlorophyll pigments, because N is one of the main components of chlorophyll (Werner and Newton, 2005; Caliskan *et al*., 2008). Thus, plants are able to positively influence yield components that result in more grain yield. Inoculation of legumes with rhizobia, for the purpose of enhancing N_2 fixation and yield in legume crops, is considered possibly as an oldest and most common method of voluntarily-releasing-microorganism into the environment (Werner and Newton, 2005; Cheminingwa and Vessey, 2006). The influence of rhizospheric bacteria on legume growth promotion has been documented by many researchers (Shrivastava *et al*., 2000; Rudresh *et al*., 2005; Malik *et al*., 2006; Cheminingwa and Vessey, 2006; Albayrak *et al*., 2006; Togay *et al*., 2008). The observed benefits on chickpea by rhizobium inoculation seem to be due to the limited supply by N to the crop (Cheminingwa and Vessey, 2006; Togay *et al*., 2008). Moreover, growth promoting substances (phytohormones like auxin) were also reported to be produced by these organisms. Phytohormones are known to play a key role in plant-growth regulation through promotion of seed germination, root elongation, and stimulation of leaf expansion. In addition, root development and proliferation of plants in response to rhizobia activities enhances water and nutrient uptake (Werner and Newton, 2005). While (Sogut, 2006). Further, as symbiotic N is already in the organic reduced form and it is hence more readily available for plant metabolism. In contrast, in the absence of symbiotic N, plants must spend a lot of energy to take up nitrates and reduce them to the level of NH_3. Consequently, inoculation resulted in more dry matter accumulation compared to only N fertilization.

Effect of nitrogen fixation bacteria on plant height

Height of plant was significantly improved by bacterial inoculation (Table 3). The R1 isolate was the best with respect to height (42.25 cm), followed by isolates R4 and R8. The non-inoculated plants (treatment C) recorded lowest plant height (32.75 cm) and this corresponds to the observations of Rao and Dass, 1989 where they observed that inoculation with nitrogen fixing bacteria enhanced height and dry weight of plants as compared to the uninoculated control plants. The chemical fertilization treatment equivalent to N 100% was superior to N 0% (without chemical fertilization) with plant height (Table 4). With regard to the interaction between bacterial inoculation and chemical nitrogen fertilization, Table 5 shows highest value of plant in treatment R1 N 100% (43.83 cm), followed by treatment R4 N 100% with a height of 43 cm, while lowest value of control treatment C N 0% was with a plant height of 32 cm. Similar observations have also been reported in the studies of Rudresh *et al*., 2005; Amany, 2007; Caliskan *et al*., 2008; Namvar *et al*., 2011 and Liu *et al*., 2018 wherein plant height increased with application of N fertilization and rhizobia inoculation.

Effect of nitrogen fixation bacteria on seed number per plant

Seed number per plant was significantly affected by bacterial inoculation, where isolation R1 recorded highest value for seeds number in the plant (97.5 seeds plant^{-1}) with a significant increase of 204.69% compared to non-inoculated control, followed by isolates R4 and R8 (Table 3). Chemical fertilization treatment N 100% out performed the treatment N 0% (without chemical fertilization) with respect to seeds number (Table 4). As for the interaction between bacterial inoculation and chemical nitrogen fertilization, seeds number in the plant was significantly affected by bacteria inoculation along with chemical nitrogen fertilization, where R1 N 100% was considerably superior to the other treatments in terms of seeds number (127 seeds plant^{-1}) followed by treatment R4 N 100% with 110 seeds plant^{-1} (Table 5). These findings correspond to those observed by Walley *et al*., 2005; Amany, 2007 who reported that number of pods and grains per plant was affected statistically with rhizobia inoculation in chickpea. These researchers noted that this trait increased from 11.50 pods per plant in non-inoculated plants to 12.35 pods per plant in inoculated plants. Application of 75 kg urea ha^{-1} in inoculated chickpea plants increased grain number per plant by 40.82% compared to the control (Namvar *et al*., 2011). Maximum effect on grains number per plant at 75 kg urea ha^{-1} rather than other fertilizer levels, may be due to more effectiveness of rhizobia inoculation at this level compared to other levels of N applied. Previous studies also justified the positive effects of N application and rhizobia inoculation on number of grains per plant (Togay *et al*., 2008).

Effect of atmospheric nitrogen fixation by bacteria on the weight of dry nodules per plant

The highest value of the average weight of dry nodules on plants was observed in the isolate R1 (0.402 g plant^{-1}), followed by isolate R4 (0.323 g plant^{-1}) and the lowest value of the average weight of dry nodules was with non-inoculated plants (0.167 g plant^{-1}) (Table 3). This corresponds to the earlier observations of Sinha and Ramakrishna, 1996.

It was noted that the fertilizer treatment N 100% was superior to fertilizer treatment N 0%, with an increase of 41.10% (Table 4).

Thus, weight of nodules on the total root was significantly influenced by both bacterial inoculation and chemical nitrogen fertilization. Highest mean weight of dry nodules on plants was in treatment R1 N 100% and that was 0.493 g plant^{-1}. The plants in the treatment (C N 0%) and (C N 100%) gave the lowest mean weight of dry nodules at 0.15 g plant^{-1}. These results corroborate with those of Kurdali, 1996; Begum *et al*., 2001; Adgo and Schulze, 2002; Rudresh *et al*., 2005; Stancheva *et al*., 2006; Ogutcu *et al*., 2008; Poole *et al*., 2018 and Liu *et al*., 2018.

It is evident from the literature that the presence of high content of N in soil can inhibit the nodulation and symbiotic N fixation in legumes (Clayton *et al*., 2004; Walley *et al*., 2005; Ogutcu *et al*., 2008). A negative exponential relationship was observed between N fertilizer rate and N_2 fixation when N was applied in the top 0-20 cm of soil or on the soil surface (Salvagiotti *et al*., 2008). Further, mineral nitrogen fertilization reduces the bacterial efficiency in nodule formation and fixation of atmospheric nitrogen. Mineral nitrogen is one of the factors that inhibits the fixation of atmospheric nitrogen during the different phases of coexistence, including root capillary injury, growth and development of nodules and the effectiveness of nitrogenase enzyme activity (Kurdali, 2001). Rawsthorne *et al*., 1985 considered the effects of supplemental nitrate on chickpea and reported that nitrate at 0.71 and 1.43 mM stimulated early nodulation and nodule growth, but higher concentrations of nitrate (2.86 mM) decreased nodulation and symbiotic N_2 assimilation significantly. Biological N fixation begins around 2-5 weeks after planting (Werner and Newton, 2005), and therefore N uptake from biological N fixation is negligible in early growth stages. Thus, application of a small amount of N at planting is beneficial to improve early growth and yield of legumes in most cases (Walley *et al*., 2005; Werner and Newton, 2005; Caliskan *et al*., 2008). Our study also indicated that application of N at low dose (i.e., 20 kg urea ha^{-1}) had positive effects not only on yield and its components, but also on nodulation of chickpea. In the present study weight of nodules per plant showed no statistically significant difference in non-inoculated chickpea plants. This work was in field conditions that are not sterile soil, i.e. the possibility of presence of rhizobia bacteria in the soil exists. So it is possible to form root nodules. However, in actual study it does not exist because soil was poor with original strains of rhizobia; where the weight of the dry nodules in non-inoculated chickpea plants (control) was 0.167 g plant^{-1} (Table 3). This may be due to lack of native rhizobia that inoculates chickpea in the experimental field. Ideally, inoculation is required in the absence of compatible resident rhizobia or where the resident rhizobia population density is very low, or where the resident rhizobia is less infective and effective for N_2 fixation than alternative (inoculant) strains (Cheminingwa and Vessey 2006; Herrmann *et al*., 2014). Soils lacking in rhizobia are found in areas where either indigenous related legumes are absent or where levels of pH, osmotic stress, high temperature and heavy metals are at detrimental levels for rhizobia populations (Werner and Newton, 2005; Cheminingwa and Vessey, 2006).

Conclusion

Isolates R1, R4 and R8 outperformed all other bacterial isolates and non-inoculated control on its effect on yield (t ha^{-1}), dry matter weight, plant height (cm), seeds number per plant and nodulation. The treatment R1 N 100% exceeded the rest of the treatments for all studied growth and productivity indicators followed by treatment R4 N 100%. These results are consistent with other studies (Karadavut and Ozdemir, 2001; Fatima *et al*., 2008). The results pointed out that N fertilization (20 kg urea ha^{-1}) can be beneficial to improve N nutrition of inoculated chickpea and stimulate BNF and this is consistent with Roy *et al*., 2012. The study also emphasized that rhizobia inoculation is also required to enhance the nodulation and BNF in the experimental area.

So, this research suggests using isolated bacteria to inoculate chickpea seeds and to convert these isolates into commercial inoculators for widespread use in order to establish an economic project to produce effective bacterial inoculation that will provide good profits. Institution should also develop the project and conduct similar studies on isolates from different regions in Syria to boost legume cultivation for long term goal of nutritional security.

References

Abu Ghurra, M. 1997. *Bacterial Plant Diseases* (theoretical and practical). Damascus: Damascus University Publications: 350-359.

Achakzai, A.K.K. and Bangulzai, M.I. 2006. Effect of various levels of nitrogen fertilizer on the yield and yield attributes of pea (*Pisum sativum* L.) cultivars. *Pakistan Journal of Botany* 2: 331-340.

Adgo, E. and Schulze, J. 2002. Nitrogen fixation and assimilation efficiency in Ethiopian and German pea varieties. *Plant and Soil* 239: 291-299.

Albayrak, S., Sevimay, C.S. and Tongel, O. 2006. Effect of inoculation with rhizobium on seed yield and yield components of common vetch (*Vicia sativa* L.). *Turkish Journal of Agriculture and Forestry* 30: 31-37.

Ali, H., Khan, M.A. and Randhawa, Sh. A. 2004. Interactive effect of seed inoculation and phosphorus application on growth and yield of chickpea (*Cicer arietinum* L.). *International Journal of Agriculture and Biology* 6: 110-112.

Andrews, M. and Andrews, M.E. 2017. Specificity in legume-rhizobia symbioses. *International Journal of Molecular Sciences* 18: 705.

Amany, A.B. 2007. Effect of plant density and urea foliar application on yield and yield components of chickpea (*Cicer arietinum* L.). *Research Journal of Agriculture and Biological Sciences* 3: 220-223.

Baset Mia, M.A. and Shamsuddin, Z.H. 2010. *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production. *African Journal of Biotechnology* 9: 6001-6009.

Begum, A.A., Leibovitch, S., Migner, P. and Zhang, F. 2001. Inoculation of pea (*Pisum sativum* L.) by *Rhizobium leguminosarum* bv. *viceae* preincubated with naringenin and hesperetin or application of naringenin and hesperetin directly into soil increased pea nodulation under short season conditions. *Plant and Soil* 237: 71-80.

Caliskan, S., Ozkaya, I., Caliskan, M.E. and Arslan, M. 2008. The effect of nitrogen and iron fertilization on growth, yield and fertilizer use efficiency of soybean in Mediterranean type soil. *Field Crops Research* 108: 126-132.

Cheminingwa, G.N. and Vessey, J.K. 2006. The abundance and efficacy of *Rhizobium leguminosarum* bv. *viciae* in cultivated soils of eastern Canadian prairie. *Soil Biology and Biochemistry* 38: 294-302.

Clayton, G.W., Rice, W.A., Lupwayi, N.Z., Johnston, A.M., Lafond, G.P., Grant, C.A. and Walley, F. 2004. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N_2 fixation and nitrogen partitioning. *Canadian Journal of Plant Science* 84: 79-88.

Dakora, F.D. 2004. Effects of symbiotic legumes and rhizobia on plant and microbial biodiversity in Natural and Agricultural Ecosystems. *Annals of Arid Zone* 43(3&4): 377-390.

Das, A., Jakasaniya, M.S., Patel, K.H. and Patel, P.T. 2000. Effect of graded levels of nitrogen and phosphorus on yield and nutrients uptake by hybrid fodder sorghum under rainfed situation. *Annals of Arid Zone* 39(2): 163-168.

De Oliveira, A.N., De Oliveira, L.A., Andrade, J.S. and Chagas, J.A.F. 2007. Rhizobia amylase production using various starchy substances as carbon substrates. *Brazilian Journal of Microbiology* 38: 208-216.

Deora, G.S. and Singhal, K. 2010. Isolation, biochemical characterization and preparation of bio-fertilizers using rhizobium strains for commercial use. *Bioscience Biotechnology Research Communications* 3: 132-136.

Elsheikh, M.A., EL-Tilib, A.M.A. and ELSheikh, E.A.E. 2005. A Note on the effect of phosphate rock, triple superphosphate, Bradyrhizobium and their combination on the available soil phosphorus in shambat clay soil. *University of Khartoum Journal of Agricultural Sciences* 13: 488-493.

Erman, M., Demir, S., Ocak, E., Tufenkci, S., Oğuz, F. and Akköprü, A. 2011. Effects of rhizobium, arbuscular mycorrhiza and whey applications on some properties in chickpea (*Cicer arietinum* L.) under irrigated and rainfed conditions 1-yield, yield components, nodulation and AMF colonization. *Field Crops Research* 122: 14-24.

FAO 2007. Methods of analysis for soils of arid and semi-arid regions. *Food and Agriculture Organization*. Rome, Italy. pp. 64-77.

Fatima, Z., Bano, A., Sial, R. and Aslam, M. 2008. Response of chickpea to plant growth regulators on nitrogen fixation and yield. *Pakistan Journal of Botany* 40: 2005-2013.

Franche, C., Lindstrom, K. and Elmerich, C. 2009. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and Soil* 32: 35-59.

Gao, Fang-Lei Che, Xiu-Xia Yu, Fei-Hai and Li, J.M. 2019. Cascading effects of nitrogen, rhizobia and parasitism via a host plant. *Flora* 251: 62-67.

Goszczynska, T., Serfontein, J.J. and Serfontein, S. 2000. Introduction to practical phytobacteriology (A manual for phytobacteriology), first edition. Safrient-loop of *BioNet-International*. ISBN: 0-620-25487-4: 83.

Herrmann, L., Chotte, J.L., Thuita, M. and Lesueur, D. 2014. Effects of cropping systems, maize residues application and N fertilization on promiscuous soybean yields and diversity of native rhizobia in Central Kenya. *Pedobiologia-Journal of Soil Ecology* 57: 75-85.

Holt, J.G., Kreig, N.R., Sneath, P.H.A., Staley, J.T. and Williams. S.T. 1994. Berge's Manual of determinative bacteriology. 9th Ed., Williams and Wilknis, Baltimore, USA: 40-169.

Kalaghatagi, S.B., Jirali, D.I., Wali, S.Y. and Nagod, M.S. 2000. Response of foxtail millet (*Setaria italica*) to nitrogen and phosphorus under rainfed conditions of northern dry zone of Karnataka. *Annals of Arid Zone* 39(2): 169-171.

Kanika, M., Dogra, T. and Nain, L. 2010. Biochemical and molecular characterization of Mesorhizobiumciceri Containing acdS Gene. *Journal of Plant Biochemistry & Biotechnology India* 19: 107-110.

Kantar, F., Elkoca, E., Ögütçü, H. and Algur, Ö. 2003. Chickpea yields in relation to rhizobium inoculation from wild chickpea at high altitudes. *Journal of Agronomy and Crop Science* 189: 291-297.

Karadavut, U. and Ozdemir, S. 2001. Effect of rhizobium inoculation and nitrogen application on yield and yield characters of chickpea. *Anadolu* 11: 14-22.

Keyser, H.H., Somasegaran, P. and Bohlool, B.B. 1993. Rhizobia ecology and technology. In *Soil Microbial Ecology, Application in Agricultural and Environmental Management Blain Metting* (Ed. F. Blaine Metting), pp. 205-226. Marcel Dekker, Inc., New York.

Kimiti, J.M. and Odee, D.W. 2010. Integrated soil fertility management enhances population and effectiveness of indigenous cowpea rhizobia in semi-arid eastern Kenya. *Applied Soil Ecology* 45: 304-309.

Kucuk, C. and Kıvanc, M. 2008. Preliminary characterization of rhizobium strains isolated from chickpea nodules. *African Journal of Biotechnology* 7: 772-775.

Kurdali, F. 1996. Nitrogen and phosphorus assimilation, mobilization and partitioning in Rain-fex Chickpea (*Cicer arietinum* L.). *Field Crop Research* 47: 81-92.

Kurdali, F. 2001. *Biological Stabilization of Atmospheric Nitrogen*. Publications of the Syrian Atomic Energy Commission, Damascus, 124 p.

Leary, G.J.O., Joshi, N.L. and Van Oosterom, E.J. 2008. A Simulation study of the response of plant-type and nitrogen fertilization on the grain yield of pearl millet. *Annals of Arid Zone* 47(2): 121-137.

Liu, A., Contador, C.A., Fan, K. and Lam, H. 2018. Interaction and regulation of carbon, nitrogen, and phosphorus metabolisms in root nodules of legumes. *Frontiers in Plant Science* 18: 1860-1876.

Malik, M.A., Cheema, M.A. and Khan, H.Z. 2006. Growth and yield response of soybean (*Glycine max* L.) to seed inoculation and varying phosphorus levels. *Journal of Agricultural Research* 44: 47-53.

Mathenge, C., Thuita, M., Masso, C., Gweyi-Onyango, J. and Vanlauwe, B. 2019. Variability of soybean response to rhizobia inoculant, vermicompost, and a legume-specific fertilizer blend in Siaya County of Kenya. *Soil & Tillage Research* 194: 104290.

McKenzie, B.A. and Hill, G.D. 1995. Growth and yield of two chickpea (*Cicer arietinum* L.) varieties in

Canterbury, New Zealand. *New Zealand Journal of Crop and Horticultural Science* 23: 467-474.

Namvar, A., SeyedSharifi, R. and Khandan, T. 2011. Growth analysis and yield of chickpea (*Cicer arietinum* L.) in relation to organic and in organic nitrogen fertilization. *Ekologija* 57: 97-108.

Ogutcu, H., Algur, O.F., Elkoca, E. and Kantar, F. 2008. The determination of symbiotic effectiveness of rhizobium strains isolated from wild chickpea collected from high altitudes in Erzurum. *Turkish Journal of Agriculture and Forestry* 32: 241-248.

Öğütçü, H., Kasimoğlua, C. and Elkoca, E. 2010. Effects of rhizobium strains isolated from wild chickpeas on the growth and symbiotic performance of chickpeas (*Cicer arietinum* L.) under salt stress. *Turkish Journal of Agriculture and Forestry* 34: 361-371.

Oliveira, A., Ferreira, E.M. and Pampulha, M.E. 1997. Nitrogen fixation, nodulation and yield of clover plants co-inoculated with root-colonizing bacteria. *Symbioses* 23: 35-42.

Papastylianou, I. and Danso, S.K.A. 1991. Nitrogen fixation and transfer in vetch and vetch-oats mixture. *Soil Biology and Biochemistry* 23(5): 447-452.

Peoples, M.B., Herridge, D.F. and Ladha, J.K. 1995. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production. *Plant and Soil* 174: 3-28.

Poole, P., Ramachandran, V. and Terpolilli, J. 2018. Rhizobia: From saprophytes to endosymbionts. *Nature Reviews Microbiology* 16: 291-303.

Rao, A.V. and Dass, H.C. 1989. Growth of fruit plants as influenced by nitrogen fixing bacteria. *Annals of Arid Zone* 28(1-2): 143-147.

Rawsthorne, S., Hadley, P., Summerfield, R.J. and Roberts, E.H. 1985. Effect of supplemental nitrate and thermal regime on the nitrogen nutrient of chickpea (*Cicer aretinum* L.). *Plant and Soil* 83: 279-293.

Rhinhart, K., Petrie, S., Blake, N., Jacobson, E., Correa, R., Coppock, L. and Hulick, D. 2003. *Growing Chickpea in Eastern Oregon*. Oregon Deptt. of Agric: 1-24.

Rosenberg, E., Delong, E.F., Lory, S., Stackebrandt, E. and Thompson, F. 2014. *The Prokaryotes*. 4th Ed., Springer-Verlag, Berlin, pp. 315-328.

Roy, S., Saxena, P. and Bano, R. 2012. Soil biota build-up under organic and inorganic fertilization in semi-arid central India. *Annals of Arid Zone* 51(2): 91-98.

Rudresh, D.L., Shivaprakash, M.K. and Prasad, R.D. 2005. Effect of combined application of rhizobium, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). *Applied Soil Ecology* 28: 139-146.

Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A. and Dobermann, A. 2008. Nitrogen uptake, fixation and response to N in soybeans: A review. *Field Crops Research* 108: 1-13.

Shrivastava, U.K., Rajput, R.L. and Dwivedi. M.L. 2000. Response of soybean-mustard cropping system to sulfur and biofertilizers on farmer's field. *Legume Research* 23: 277-278.

Siddiqi, Z.A. and Mahmood, I. 2001. Effects of rhizobacteria and root symbionts on the reproduction of *Meloidogyne javanica* and growth of chickpea. *Bioresource Technology* 79: 41-45.

Singh, S. 1977. Effect of rhizobia inoculation on nodulation and yield of moong [*Vigna radiata* (L) Wilczek]. *Annals of Arid Zone* 16(1): 61-66.

Sinha, K. and Ramakrishna, K. 1996. Variation among promising genotypes of fenugreek (*Trigonella foenungraecum* Linn.) for symbiotic nitrogen fixation. *Annals of Arid Zone* 35(4): 331-334.

Sogut, T. 2006. Rhizobium inoculation improves yield and nitrogen accumulation in soybean (*Glycine max*) cultivars better than fertilizer. *New Zealand Journal of Crop and Horticultural Science* 34: 115-120.

Sprent, J.I., Ardley, J. and James, E.K. 2017. Biogeography of nodulated legumes and their nitrogen-fixing symbionts. *New Phytol* 215: 40-56.

Stancheva, I., Geneva, M., Zehirov, G., Tsvestkova, G., Hristozkova, M. and Georgiev, G. 2006. Effects of combined inoculation of pea plants with arbuscular mycorrizal fungi and rhizobium on nodule formation and nitrogen-fixing activity. *General and Applied Plant Physiology* (special issue): 61-66.

Suslow, T.V., Schroth, M.N. and Isaka, M. 1982. Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology* 72(7): 917-918.

Teng, Y., Wang, X., Li, L., Li, Z. and Luo, Y. 2015. Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. *Frontiers in Plant Science* 6:32.

Togay, N., Togay, Y., Cimrin, K.M. and Turan, M. 2008. Effect of rhizobium inoculation, sulfur and phosphorus application on yield, yield components and nutrient uptake in chick pea (*Cicer aretinum* L.). *African Journal of Biotechnology* 7: 776-782.

Vanlauwe, B., Hungria, M., Kanampiu, F. and Giller, K.E. 2019. The role of legumes in the sustainable intensification of African small holder agriculture: Lessons learnt and challenges for the future. *Agriculture, Ecosystems and Environment* 284, 106583.

Vora, V.D., Hirpara, D.S., Vekariya, P.D., Vala, F.G. and Modhvadiya, V.L. 2019. Effect of nutrient management on yield of Bt. cotton under dry farming conditions in north Saurashtra agro-climatic zone. *Annals of Arid Zone* 58(1&2): 61-63.

Walley, F.L., Boahen, S.K., Hnatowich, G. and Stevenson, C. 2005. Nitrogen and phosphorus fertility management for desi and kabuli chickpea. *Canadian Journal of Plant Science* 85: 73-79.

Werner, D. and Newton, W.E. 2005. Nitrogen Fixation in Agriculture, Forestry, Ecology and Environment (New York: Springer): 5-13.

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