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Influences of Mineral Fertilization with NPK, Inoculation and Methods of Inoculation on Seedling Growth of Two Woody Legume Trees

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Abstract: In order to investigation the effect of method of inoculations, type as well as source of bacteria strains on growth response of two woody tree species *Acacia saligna* and *Leucaena leucocephala*, the present study was carried out at the nursery of the Department of Forestry and Wood Technology at Experimental Station, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. The main objective of the study is to evaluate growth, ability to inoculation with different strains in presence of NPK fertilizers as well as method of inoculations. The mineral fertilizer included treatments *viz.,* application PK, NPK and inoculation with Rhizobium as well as control (without NPK and Rhizobium). Inoculation was used as soil and seed inoculations. The results indicated that the seedlings of *A. saligna* and *L. leucocephala* inoculation with Rhizobium in combination with mineral fertilizers were significantly affected on the growth and nodulation of seedlings. The soil inoculation surpassed seed inoculation. The native Rhizobium strains were highly significant effect than the foreign Rhizobium strain, NGR234. *Leucaena leucocephala* was more responded to inoculate with Rhizobium strains in presence of mineral fertilizer treatments as compared to *Acacia saligna.* Concurrent use of fertilization and inoculation in the nursery may provide opportunity to produce larger seedlings that have high potential to reduce the time needed to achieve canopy closure and thereby help achieve restoration objectives.

Key words: Inoculation methods · NPK fertilization · Rhizobium strains · Woody legume trees

INTRODUCTION

Nitrogen is an essential element needed by plants and soil, with low level of nitrogen the plant growth become limit. Chemical fertilizer has different effects with different agrochemical soil properties. Ammonium sulfate as example impairs the agrochemical properties of soils and with long term use became ineffective [1]. Nitrogen fixing leguminous trees is frequently used to rehabilitate degraded lands in different parts of the world but in arid and semi-arid zone it is important to find root nodules on plant with the capacity to fix nitrogen symbiotically with appropriate species of Rhizobium. The formation of nitrogen-fixing nodules on roots is a feature share by many plant legume species. These nodules are the result of a symbiotic association between the legumes and soil bacteria called Rhizobia [2-4]. Rhizobia are able to take atmospheric nitrogen and convert it into a form that plants can use it and able to establish symbiosis with a wide range of legumes, such as trees, shrubs, herbs and aquatic legumes [5-9]. Work on the *Rhizobiurn-legume* symbiosis has been carried out, but less attention has been given to the symbionts of woody legumes such as the *Acacia* species. *Acacia* species can facilitate understory plants by nitrogen fixation [10, 11]. The growth of understory plants will develop plant community as a result of modifying soil physical and chemical properties beneath canopy [12]. Inoculation of

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legume seed is an efficient way to introduce effective Rhizobium to soil and to rhizosphere of legumes [13]. The efficacy of inoculation varies depending the number of viable Rhizobium available to infection the legume roots. Although, several studies reported that yield increased up to 25% when used the inoculation, however applying high quantities of inoculants is technically difficult and uneconomical. Alternatively, higher number of viable rhizobia on seed and substantial demand for commercially pre inoculated legume seed. Seed and soil inoculation techniques have advantages and disadvantages. So selection of a method depends on the, seed size and availability of equipment. Chemical fertilizers are an important component for agriculture now [14]. However, concerning about the environmental consequences of mineral nitrogen use emphasize the need to develop new technologies for production that are sustainable in both economically and ecologically [15]. The NPK fertilizer gave the soil equal amount of nitrogen, phosphorus and potassium and this will enable us to evaluate equal application of nutrient and their functions to legume trees or shrubs. The mineral fertilizer may affect the adaptation persistence, ability to dominate and flourish best in legume production [16]. The present study was carried out to evaluate the effect of NPK fertilization and Rhizobium inoculation methods on seedlings growth of two woody legume trees and to compare the ability of local and foreign Rhizobium strains under different treatments of fertilization on seedlings properties growth.

MATERIALS AND METHODS

The present study was carried out at the nursery of the Forestry and Wood Technology Department at Experimental Station of Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University, Abies region, Alexandria, Egypt during two successive seasons (2009-2010) and (2010/2011). The aim of this study were to evaluate growth response to inoculation methods and ability of three isolated root nodules bacteria namely (VFR, MSR and NGR234) and two woody tree species namely *Leucaena leucocephala* and *Acacia saligna* to NPK fertilization.

Tree Species: Two woody legume trees namely *Acacia saligna* (Labill.) H. L. Wendl and *Leucaena leucocephala* (Lam.) De Wit growing in Egypt were used in this study. Seeds were obtained from the Department of Forestry and Wood Technology, Agricultural Research Center at Antoniadis, Alexandria, Egypt during 2009 and 2010.

Table 1: Physical and chemical characteristics of soil used in the study.

Parameter	Value
Practical size distribution	
Sand %	77.50
Silt %	14.25
Clay %	8.25
Soil texture	Sandy loam
pH	7.45
EC(mmhos/cm)	1.60
Soluble cations (meq/l)	
Ca^{++}	7.20
Mg^{++}	4.50
Na^{+}	3.27
K^{+}	0.50
Soluble anions (meq/l)	
CO_3^{--}	1.00
HCO_3^{-}	8.00
Cl^{-}	5.30
SO_4^{--}	4.10
Available N (mg/kg)	29.40
Available P (mg/kg)	7.30

Bacterial Species: Two Rhizobia strains were isolated from *Vicia faba* and *Medicago sativa* roots as a local species and one strain NGR234 was obtained from Faculty of Sciences, Genève University, Switzerland as a foreign strain were used in the present study.

Soil: Sterilized sandy loam soil was used. It was chosen from the Nursery of the Experimental Station of Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University, Alexandria. The chemical and physical characteristics of the soil are presented in Table 1.

Mineral Fertilizers and Inoculation Methods: Three common commercial chemical fertilizers were used in this study. The fertilizers were; Ammonium nitrate (33%N) as a source of nitrogen 0.3g/plant, superphosphate (15% P_2O_5) as a source of phosphorus 0.33g/plant and potassium sulfate (50% K_2O) as a source of potassium 0.1g/plant. Two treatments of fertilization were used, the first treatment was PK 1:1 and the other was NPK 1:1:1. The doses of fertilizers treatments were divided in two equal portions the first was added on June and the second one was added on July in the two successive seasons. Two inoculation methods were used to infect the tree seedlings; seed inoculation (mixture method) (by mixed 15ml of bacteria, sugar solution and activated charcoal with seeds during germination in March) and soil inoculation (injection method), the soil per plant was injection with 15ml of Rhizobium strains and this methods were added to three months old seedlings in May.

Experimental Procedure

Isolation of Root-nodule Bacteria: Seedlings of *Vicia faba* and *Medicago sativa* were removed from the soil in the field and the roots were gently shaken free of the soil. Healthy root-nodules of selected plants were cleared from adherent soil particles by washing with tap water carefully. Nodules were excised from the roots and surface sterilized by 0.1% HgCl solution for 4-5 minutes and then washed several times with distilled water. The nodules were immersed for 3 minutes in ethyl alcohol (95%) and rinsed several times with distilled water. With a sterile glass rod, individual nodules were crushed in one ml of sterile water in a sterile Petri dish. A loop of each nodule suspension, prepared was streaked into yeast extract mannitol agar (YEM). The plates were incubated at 28°C for 3-7 days. The strain NGR234 was tested and grown at trypton with antibiotic chlorophenicol at the same temperature for 3-7 days [17].

Testing the Isolated Strains for Purity: The colony morphology of the isolated root-nodules bacteria and NGR234 were examined for purity by testing each isolate for gram stain reaction, characteristic growth in yeast extract mannitol agar (YEM), trypton yeast (TY), yeast extract mannitol agar + bromothymol blue (YEM+BTB), yeast extract mannitol agar + Congo red (YEM+CR), trypton yeast + bromothymol blue (TY+BTB) and trypton yeast + Congo red (TY+CR). After an incubation of 3 to 7 days at 28°C, individual colonies were characterized based on their size, color, shape, transparency, borders and gram stain reaction [17].

Seedlings Production and Infection with Rhizobia: Seeds of the two legume trees; *Acacia saligna and Leucaena leucocephala* were soaked in hot water for 48 hrs to prevent the inhibitory effect of seed coats on germination. Seeds of *A. saligna and L. leucocephala* germinated in a mixture of sand and clay at 2:1 (v/v) in the green house, maintained at day/ night temperatures of approximately 20-25°C. Seeds were sown in plastic bag 15 cm in diameter containing 1 kg of a mixture of sand and clay at 2: 1 (v/v) moistened with water. Seeds were watered manually with tap-water. Seedlings growing in the plastic bags and were inoculated with 15ml of Rhizobium strains (approximately 10^8 bacterial cells ml/plant) grown in trypton yeast and yeast extract mannitol broth incubated for 4 days with shaking at 28°C (NGR234, VFR strain, MSR strain). Two methods of infection were used to inoculate the three months old tree seedlings in May. The seedlings were placed in greenhouse between March and the first week of April 2011. The daily maximum temperature ranged 23-27°C and the minimum 19-20°C. After 4 weeks from the inoculation with the strains, the seedlings of the trees were harvested and checked for root nodule formation. The number of nodules per plant, the fresh and dry weight of nodules and fresh and dry weight of root and shoots were determined (g). The treatments used in the study included, control treatment; inoculated with local Rhizobium only by using injection method and/ or mixture method; inoculated with foreign Rhizobium only by using injection method and/ or mixture method; inoculated with local Rhizobium with PK1:1 by using injection method and/ or mixture method; inoculated with foreign Rhizobium with PK1:1 by using injection method and/ or mixture method; inoculated with local Rhizobium with NPK 1:1:1 by using injection method and/ or mixture method and inoculated with foreign Rhizobium with NPK 1:1:1 by using injection method and/ or mixture method.

The Experimental Design: The split-split plot in randomized complete block design (RCBD) was used, where the main plot was devoted for tree species and N, P and K treatments in sub plots and Rhizobium species in the sub-sub plots with three replicates for each treatment. Statistical analysis was done according to Snedecor and Cochran [18] by using ANOVA, F-test and L.S.D procedures available within the MSTAT-C software package (version 2.0 1998) and SAS software package (version 9.13 2008).

RESULTS AND DISCUSSION

Characteristics of Studied Rhizobium Isolates

Isolates Purity Testing: Two root noduling bacteria were isolated from the root of *Vicia faba* and *Medicago sativa.* In addition to pure culture of Rhizobium strain: NGR234 obtained from Faculty of Sciences, University of Genève, Switzerland was used to test their purity and characteristics. Table 2 indicates that, all isolates had the

Table 2: Growth of Rhizobia isolates on yeast mannitol agar (YAM) containing bromothylmol blue and Congo red.

		Acid Production	
Strain	Source of Strain	BTB	CR
NGR234	Geneva	++	--
VFR	Egypt	++	++
MSR	Egypt	++	--

Rhizobium isolated from *Vicia faba* (VFR); Rhizobium isolated from *Medicago sativa* (MSR) and *Rhizobium* (NGR234). BTB: Bromothymol blue; CR: Congo red ; (++): Acid production

Table 3: Mean values of growth parameters of tree species under the fertilization treatments during the two seasons.

	Growth parameters									
	Seedling height (cm)		Stem diameter (cm)		Leaves dry weight (g)		Stem dry weight (g)		Root dry weight (g)	
Tree species	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Leucaena leucocephala	14.22	12.15	0.10	0.12	12.79	11.54	23.80	18.90	11.58	9.32
Acacia saligna	10.14	9.21	0.07	0.07	7.30	5.19	13.42	11.28	8.43	6.68
LSD 0.05	3.37	1.09	0.02	0.03	5.29	1.16	2.62	2.71	0.65	0.76

Table 4: Mean values of growth parameters under fertilization treatments during the two seasons

	Growth parameters									
	Seedling height (cm)		Stem diameter (cm)		Leaves dry weight (g)		Stem dry weight (g)		Root dry weight (g)	
Fertilization Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	6.85	5.65	0.05	0.05	1.26	1.12	2.79	2.71	1.18	0.86
Rhizobium	8.26	7.31	0.07	0.07	6.20	6.28	9.01	7.31	7.0	5.40
PK1:1+ Rhizobium	17.30	16.42	0.10	0.15	17.74	14.15	31.97	25.34	17.19	14.08
NPK 1:1:1+Rhizobium	16.33	13.33	0.11	0.10	14.48	11.91	30.67	25.01	14.66	11.65
LSD 0.05	2.30	1.30	0.02	0.04	1.94	1.58	4.17	3.47	1.65	1.74

same colony morphology and produced high, mucous transparent to creamy colored colonies on yeast extract mannitol agar (YEM) and trypton yeast agar plates after 3 and 7 days of incubation at 28°C. Also, all tested Rhizobium isolates grew on YEM agar containing bromothymol blue indicator and all of them produced an acid reaction changed the color of the medium. Also, all tested isolates grew on YAM agar containing Congo red indicator, only *Vicia faba Rhizobium* (VFR) isolated from root nodules of *Vicia faba* changed the color of the medium.

Growth Parameters

Seedling Height: The mean average of the seedlings height at the two seasons showed that, *Leucaena leucocephala* recorded the higher seedling height than *Acacia saligna* with average 14.22 and 12.15 cm and 10.14 and 9.21cm, respectively (Table 3).The most effective fertilizer was PK at 1:1 with Rhizobium (17.30 and 16.42 cm) followed by NPK 1:1:1 with Rhizobium (16.33 and 13.33 cm), while the control gave the lower seedling height with average 6.85 and 5.56 cm (Table 4). The results of inoculation methods with Rhizobium isolates under the fertilization treatments on seedling height showed that, the inoculation using the soil inoculation method was more effective than seed inoculation method. The most effective inoculation methods with Rhizobium isolates was *Vicia faba* Rhizobium (soil inoculation) VFRI with averages (17.31 and 16.48 cm) followed by *Medicago sativa* Rhizobium (soil inoculation) MSRI. The averages were 13.22 and 11.63 cm, respectively, while the lowest one was NGR234 (seed inoculation) NGRM with averages 8.51 and 7.70 cm, respectively (Table 5). The results from the two seasons indicated that, *Leucaena leucocephala* was more responded to fertilization treatment and inoculation methods with Rhizobium isolates than *Acacia saligna*. The PK 1:1 was the most effective fertilizer treatment compared with the other fertilizer treatments used in the study. The soil inoculation method was the most effective method that increased the seedling height compared with seed inoculation method. Therefore, the present findings are in conformity with the results obtained by Bekere and Hailemariam [19] and Ahmadi and Chaichi [20]. The inoculation and chemical nitrogen fertilizer application caused more nutrients concentration and growth plant increased. The fertilizer rate was significantly affected every measured response variable. Fertilized seedlings had significantly larger height [21, 22]. The local Rhizobium isolates were more effective on growth parameters and height than the foreign strain. This result was in harmony with results obtained by Shetta [23], Pueppke and Broughton [24] and Swelim *et al*. [25]. Rhizobium NGR234 was unable to compete the native isolates, however the reduction of seedlings parameters noticed with strain NGR234 might be due to weak symbiosis compared with local Rhizobium isolates used in this study, but are in contradiction with results obtained by Sánchez *et al*. [26], who indicated that native Rhizobium population were not effective enough to obtain a significant increase in shoot height. Also, the fertilized treatments showed less nodulation than the control, but are in contradiction with results obtained by

Table 5: Mean values of growth parameters of inoculation method under the fertilization treatments during the two seasons

Inoculation Method with Rhizobium	Growth parameters: Seedling height (cm) 1st season	Seedling height (cm) 2nd season	Stem diameter (cm) 1st season	Stem diameter (cm) 2nd season	Leaves dry weight (g) 1st season	Leaves dry weight (g) 2nd season	Stem dry weight (g) 1st season	Stem dry weight (g) 2nd season	Root dry weight (g) 1st season	Root dry weight (g) 2nd season
VFRI	17.31	16.48	0.10	0.16	13.52	11.06	22.71	19.17	12.45	9.90
VFRM	12.87	9.96	0.08	0.08	9.80	8.27	21.51	16.35	10.88	9.15
MSRI	13.22	11.63	0.10	0.09	12.31	10.13	21.89	16.37	11.32	9.08
MSRM	11.22	9.56	0.06	0.09	8.68	7.74	16.48	14.32	9.42	7.74
NGRI	10.01	8.75	0.09	0.07	8.84	6.98	16.33	13.43	8.29	6.47
NGRM	8.51	7.70	0.08	0.07	7.12	6.03	12.76	10.99	7.76	5.92
LSD 0.05	2.64	2.13	0.02	0.04	2.93	1.74	3.62	2.67	1.68	1.28

Vicia faba Rhizobium (soil inoculation) = VFRI
Vicia faba Rhizobium (seed inoculation) = VFRM
Medicago sativa Rhizobium (soil inoculation) = MSRI
Medicago sativa Rhizobium (seed inoculation) = MSRM
NGR234 (soil inoculation) = NGRI
NGR234 (seed inoculation) = NGRM

Ahmed *et al*. [27], Hoque and Haq [28] and Khalil *et al*. [29], who found that the inoculation of seed with Rhizobium significantly increased plant height in many legume plants.

Stem Diameter: The analysis of variance of the stem diameter was varied between the two seasons. Highly significant difference was found between tree species and fertilization treatments, while no significant difference was found between inoculation methods with Rhizobium isolates and all interactions treatments in the first season, while the interaction was not significant at the second season. The mean average of stem diameter indicated that seedlings of *Leucaena leucocephala* had the bigger diameter (0.10 and 0.12 cm) than *Acacia saligna* (0.07 and 0.10 cm), respectively (Table 3). The fertilizer treatments showed that the NPK 1:1:1 with Rhizobium had the widest stem diameter with averages 0.11 and 0.10 cm, followed by PK 1:1 with Rhizobium (0.10 and 0.15 cm) and Rhizobium only (0.07 cm in both seasons), while lowest value was found in the control (0.05 cm) at the two seasons (Table 4). The effect of inoculation methods with Rhizobium isolates under the fertilization treatments on seedlings diameter indicated that the seedlings diameter was bigger with soil inoculation. The inoculation with the VFRI gave the highest diameter averaged (0.10 and 0.16 cm), followed by MSRI (0.10 and0.09 cm) and Rhizobium NGRI (0.09 and 0.07 cm), respectively. On the other hand, inoculation the seeds with seed inoculation method had the lowest diameters averaged compared with the soil inoculation methods (Table 5). The results of stem diameter in the two seasons indicated that, the fertilizer treatments were varied in the two seasons where as the NPK 1:1:1 associated with Rhizobium gave the biggest stem diameter in the first season, while the PK1:1had the bigger stem diameter in the second season. The fertilizer rate affected significantly root diameters of *Acacia kae* [22]. Moreover, the stem diameter was increased with inoculation by Rhizobium isolates using the soil inoculation method compared to seed inoculation. The inoculation efficiency was varied depending on several factors that affected on number of viable rhizobia available for infection root legume [13]. Inoculation with Rhizobium strain increased the root length, root hair and root diameter. This result is in line with the findings of Deaker *et al*. [13] and Molla *et al*. [30], but was in contradiction with results obtained by Philpotts [31]. The seed inoculated plants exhibited significantly greater root and shoot as compared to control plants. The inoculating seed only was less effective as indication of bacterial survival started decreasing soon after seed inoculation [27. 32]. The inoculated with indigenous strain of Rhizobium enhance the growth parameters [33], this result was in harmony with the results obtained from our study. The local Rhizobium isolates were more effective on growth parameters and height than the foreign strain.

Leaves Dry Weight: The same trend was found in leaves dry weight, the analysis of variance at the two studied seasons were varied. *Leucaena leucocephala* recorded the higher dry weight of leaves 12.79 and 11.54g than *Acacia saligna* 7.30 and 5.19g (Table 3). The fertilization with PK1:1associated with Rhizobium gave the highest leaves dry weight compared with NPK 1:1:1 with Rhizobium, followed by Rhizobium only and the control.

The mean averages were (17.74 and 14.15g), (14.48 and 11.91g), (6.20 and 6.28g) and (1.26 and 1.12g), respectively (Table 4). The effect inoculation method on leaves dry weight showed that soil inoculation (injection method) was more effective than the other inoculation methods. The inoculation with Rhizobium of VFRI and MSRI gave the highest dry weight of leaves with averages 13.52 and 12.31g, respectively in the first season and 11.06 and 10.13g in the second season, followed by inoculation with NGRI (8.84 and 6.98g), respectively at the two seasons. Also the same trend was found in the seed inoculation method, the VFRM had the highest leave dry weight compared with the MSRM and NGRM (Table 5). The soil inoculation method was not effective with inoculation with NPK 1:1:1. The inoculation with Rhizobium only came in the third rank after PK1:1and NPK 1:1:1 associated with Rhizobium.

Stem Dry Weight: The results indicated highly significant differences were found between tree species, fertilization treatments and inoculation methods with Rhizobium isolates as well as their interactions in the first season. While, no significant differences at the interaction between tree species, inoculation methods with Rhizobium isolates and fertilization treatments and inoculation methods in the second season. *Leucaena leucocephala* was more responded to fertilization treatments and Rhizobium isolates than *Acacia saligna.* Also, the fertilization treatment PK1:1associated with Rhizobium gave the highest stem dry weight compared with other fertilization treatments, as well as the inoculation with injection method compared with mixture method. The mean average of the seedlings stem dry weight showed that, *Leucaena leucocephala* recorded the higher stem dry weight than *Acacia saligna.* The averages were 23.80g, 18.90g, 13.42g and 11.28g, respectively (Table 3). Also the fertilization with PK1:1associated with Rhizobium gave the highest shoots dry weight compared with NPK 1:1:1 with Rhizobium, the mean averages were 31.97 and 30.67g, respectively in the first season and 25.34 and 25.01g in the second season, respectively (Table 4). The effect inoculation method on stem dry weight showed that the soil inoculation method was more effective than the seed inoculation method (Table 5). The inoculation with Rhizobium of VFRI and MSRI gave the highest dry weight of stem 22.71 and 19.17g and 21.89 and 16.37g, respectively followed by inoculation with NGRI 16.33g in the first season and 13.33g in the second season, also the same trend was found in the seed inoculation method (Table 5).

Roots Dry Weight: Highly significant differences between tree species, fertilization treatments, inoculation methods with Rhizobium isolates were found at root dry weight during the two seasons. On the other hand, there were no significant differences in their interactions in the second season. *Leucaena leucocephala* had a higher root dry weight (11.58g and 9.32g), followed by *Acacia saligna* (8.43g and 6.68g), respectively as shown in (Table 3). Also, the fertilization with PK1:1associated with Rhizobium gave the same trend compared with NPK 1:1:1 associated with Rhizobium. The averages were 17.19 and 14.08 while were 14.64 g and 11.65 g, respectively at the two seasons (Table 4). The soil inoculation (injection method) gave a higher roots dry weight than seed inoculation (mixture method) at the two studied seasons. The averages of inoculation with VFRI and MSRI were higher 12.45g and 11.32g in the first season and 9.90g and 9.08g, in the second season, respectively compared with NGRI. Also, the same results were found with the seed inoculation method, the inoculation with VFRM gave the highest root dry weight than the other isolates (Table 5). On the other hand the results obtained from the study were in harmony with results obtained by Bekere and Hailemariam [19], who indicated that seed and soil inoculations and fertilizer with phosphorus increased the shoots dry matter. Ali *et al.* [34] found that P fertilizer along with Rhizobium inoculation increased the dry weight of roots and shoots of mung bean.

Nodules Dry Weight: The results of nodules dry weight revealed that highly significant differences were found between tree species, fertilization treatments, inoculation methods with Rhizobium isolates and their interactions. However, *L. leucocephala* had the highest nodules dry weight (0.26g and 0.23g) than *Acacia saligna* (0.11g and 0.10g) (Fig.1A). The same result was found at the fertilization with PK1:1associated with Rhizobium gave the highest nodules dry weight compared with Rhizobium only and NPK 1:1:1 associated with Rhizobium, the mean averages were 0.46, 0.23 and 0.06g, at first season and 0.38, 0.20 and 0.09g, respectively (Fig. 1B).

The inoculation method showed that the soil inoculation method was more effective than the seed inoculation method. The inoculation with Rhizobium of VFRI and MSRI gave the highest dry weight of nodules. The averages were 0.40 and 0.28g and 0.32 and 0.24g, respectively followed by inoculation with NGRI 0.18 and 0.13g, respectively. The same trend was found at the seed inoculation method. The inoculation with Rhizobium of VFRM and MSRM gave the highest dry weight of nodules followed by inoculation with NGRM (Figure 1C).

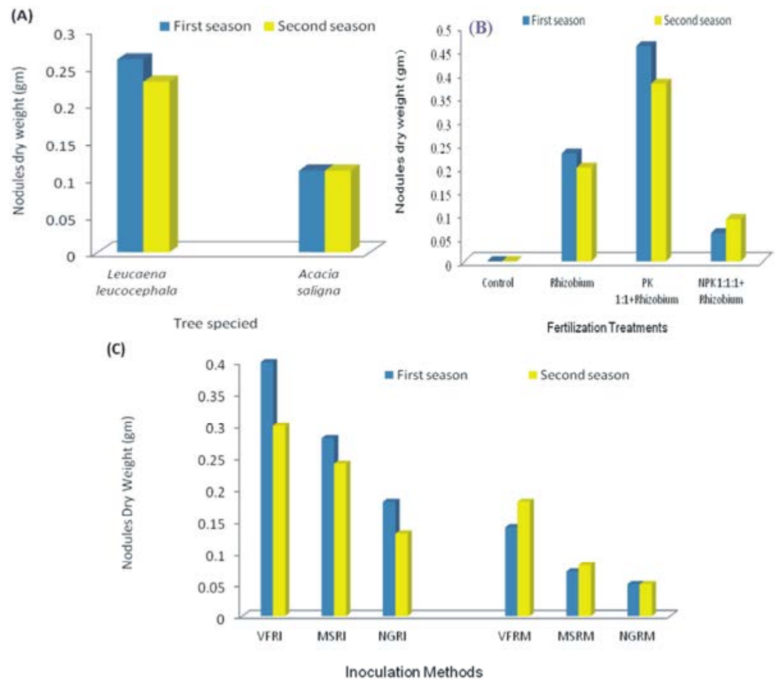

Fig. 1: Effect of fertilization treatments and inoculation methods on nodules dry weight during the study

Nodules Number: *Leucaena leucocephala* showed more responded to fertilization treatments and Rhizobium isolates than *Acacia saligna*. Also, the fertilization treatment PK1:1associated with Rhizobium gave the highest nodules number compared with other fertilization treatments, as well as the inoculation with soil inoculation method (injection method) compared with seed inoculation method (mixture method) at the two seasons. The mean average of the seedlings nodules number showed that, *L. leucocephala* recorded the higher nodules number than *A. saligna.* The averages were 23.78 and 18.88 nodule/plant in the first season and 22.18 and 12.15 nodule/plant, respectively (Fig.2A). For fertilization, the fertilization with PK1:1associated with Rhizobium gave the highest nodules number compared with Rhizobium only and NPK 1:1:1 associated with Rhizobium. The mean averages were 43.03, 33.75 and 9.25 nodule/plant in the first season as well as 32.75, 26.25 and 9.67 nodules/plant, respectively (Fig. 2B).The effect of inoculation methods on nodules number showed that soil inoculation method was more effective than the seed inoculation method. The inoculation with Rhizobium of VFRI and MSRI gave the highest nodules number 34.71and 29.42 nodule/plant for VFRI and 31.83 and 26.50 nodules/ plant, for MSRI respectively followed by inoculation with NGRI 31.54 and 16.79 nodules/plant at the two seasons. The seed inoculation with Rhizobium of VFRM, MSRM and NGRM were varied during the two seasons (Fig.2C). The results obtained from the present study indicated that, *Leucaena leucocephala* had the higher number of nodules per seedlings and nodules dry weight in the two seasons than the *A. saligna.* The PK1:1associated with the local Rhizobium was the higher effective on number of nodules and dry weight of nodules during the two seasons as compared to NPK 1:1:1 or Rhizobium only. The soil inoculation was the most effective on root nodules and dry weight of nodules than the seed inoculation. The results obtained are in agreement with the pervious findings of Bekere and Hailemariam [19], who indicated that fertilization with P and K without N increased the nodules number and nodules dry weight as well as the total nitrogen at shoots. The applied N fertilizer significantly and linearly reduced the nodules number and fresh and dry weight of nodules. Achakzai [35], found that legume group fertilized with NPK resulted in a decrease in nodulation, number of nodules. Hatam, [36], Jamro *et al.* [37] and Vessey [38] demonstrated that no need to apply nitrogen fertilizer to legumes (pea) when it inoculated with good quality of inoculants. The inoculation methods did not influence any of the growth parameters or nodulation formation, this result attributed to presence of numerous in effective indigenous rhizo-competitions between Rhizobium strains in the soil was a common phenomenon as the introduced inoculum strains compete with indigeneous Rhizobium [22]. The fertilizer treatments affected nodules formation, number of nodules and dry

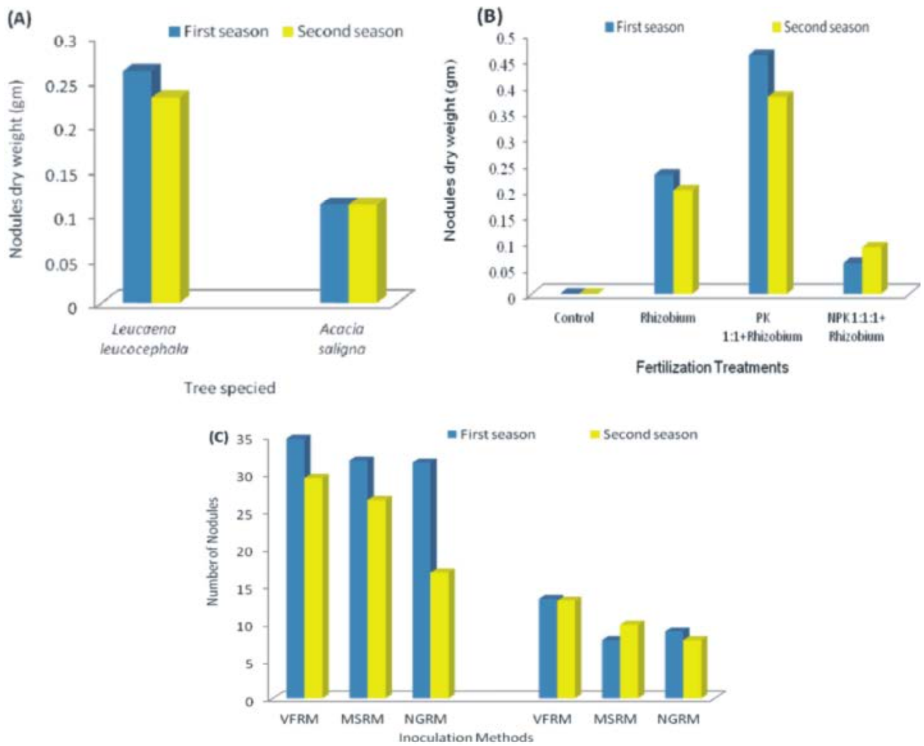

Fig. 2: Effect of fertilization treatments and inoculation methods on number of nodules during the study.

weight of nodules. This result is in line with the findings of Korenkov [1], who found that the legume ability to nodulate was affected by the NPK treatments at various levels. Also who indicated that fertilizer application at various rates affected nodulation and plant height.

Javaid [14], Ali *et al*. [34] and Hoshiyar *et al*. [39] indicated that phosphorus application along with Rhizobium inoculation increased the dry weight of nodules significantly. The nodules number was significantly enhanced by inoculation with Rhizobium isolates and NPK. The results obtained are disagreement with results obtained by Umamaheswari *et al*. [40], who indicated that number of nodules was not significantly influenced by different fertilizer treatments. The study indicated also that the soil inoculation was the most effective method to infect the root of *leucaena* and *Acacia* seedlings. The results of the present study were in contradiction with results obtained by Ahmed *et al*. [27] and Hoque and Haq [28]. The inoculation of seed with Rhizobium significantly increased the nodulation and plant height. Also they found that seed inoculation and nitrogen fertilizer significant increased the number of nodules, fresh and dry weight of nodules as well as the biological yield. Seed, soil and seed+ soil inoculations increased the plant parameters and shoot dry matter [19].

CONCLUSIONS

On the basis of this study, it is concluded that inoculation of soil with Rhizobium in combination with mineral fertilizers significantly affected the growth and nodulation as compared to seed inoculation method. Soil inoculation with PK1:1performed better than other treatments and produced the maximum growth and root nodules. The native Rhizobium isolates showed highly effective on tree legume seedlings as compared to foreign strain NGR234, which effect may be due to the competition between introduced strain and native isolates for nodules. A high competitiveness of inoculums isolates in comparison with native Rhizobium isolates is as important as effectiveness of symbiotic nitrogen fixation. The Rhizobium NGR234 was unable to complete the native isolates. *Leucaena leucocephala* was more responded to inoculate with Rhizobium isolates under the fertilizer treatments compared to *Acacia saligna.* Concurrent use of fertilization and inoculation in the nursery may provide opportunity to produce larger seedlings that have high potential to reduce the time needed to achieve canopy closure and thereby help achieve restoration objectives. It is recommended however to inoculate leguminous seedlings of timber trees with appropriate isolates of Rhizobium and use soil inoculation with a line of phosphorus, potassium without nitrogen fertilization to exaggerate its growth in the framework of afforestation and reforestation programs.

REFERENCES

1. Korenkov, D.A., 1976. Nitrogen Fertilizer Agrokhimiya Azotnykh Udobrenii. Nauka Publishers. Moscow, pp: 39.
2. Allen, O.N. and E. K. Allen, 1981. The Leguminosae: A source Book of Characteristics. Use and Nodulation, University of Wisconsin Press, Wisconsin, pp: 812.
3. Barker, D.G., S. Bianchi, F. Blondon, Y. Dattee, G. Duc, S. Essad, P. Flament, P. Gallusci, G. Genier, P. Guy, X. Muel, J. Tourneur, J. Denarie and T. Huguet,1990. *Medicago truncatula*, a model plant for studying the molecular genetics of the *Rhizobium* legume symbiosis. Plant Molecular Biology Reporter, 8(1): 40-49.
4. Mylona, P., K. Pawlowski and T. Bisseling, 1995. Symbiotic nitrogen-fixation. Plant Cell, 7(7): 869-885.
5. Khbaya, B., M. Neyra, P. Normand, K. Zerhari and A. Filali-Maltouf, 1998. Genetic diversity and phylogeny of rhizobia that nodulate *Acacia* spp. in Morocco assessed by analysis of rRNA genes. Applied and Environmental Microbiology, 64(12): 4912-4917.
6. Donate-Correa, J., M. Leon-Barrios, M. Hernandez, R. Perez-Galdona and M. del Arco-Aguilar, 2007. Different *Mesorhizobium* species sharing the same symbiotic genes nodulate the shrub legume *Anagyris latifolia*. Systematic and Applied Microbiology, 30(8): 615-623.
7. Giongo, A., A. Ambrosini, L. K. Vargas, J.R.J. Freire, M.H. Bodanese-Zanettini and L.M.P. Passaglia, 2008. Evaluation of genetic diversity of *bradyrhizobia* strains nodulating soybean *Glycine max* (L.) Merrill isolated from South Brazilian fields. Applied Soil Ecology, 38(3): 261-269.
8. Odee, D.W., K. Haukka, S.G. McInroy, J.I. Sprent, J.M. Sutherland and J.P.W. Young, 2002. Genetic and symbiotic characterization of rhizobia isolated from tree and herbaceous legumes grown in soils from ecologically diverse sites in Kenya. Soil Biology and Biochemistry, 34: 801-811.
9. McInroy, S.G., M. Andrews and J.I. Sprent, 1988. Growth, nodulation and nitrate reeducates activity in the aquatic legume *Neptunia plena* (L.) Benth at different external nitrate concentrations. Aquatic Botany, 30(4): 379-387.
10. Ludwig, F., T.E. Dawson, H. de Kroon, F. Berendse and H.H.T. Prins, 2005. Hydraulic life in *Acacia tortilis* trees on East African savanna. Oecologia, 134(3): 293-300.
11. Yang, L., N. Liu, H. Ren and J. Wang, 2009. Facilitation by two exotic *Acacia*: *Acacia auriculiformis* and *Acacia mangium* as nurse plants in South China. Forest Ecology and Management, 257(8): 1786-1793.
12. Armas, C. and F.I. Pugnaire, 2005. Plant interactions govern population dynamics in a semi-arid plant community. Journal of Ecology, 93: 978-989.
13. Deaker, R.J. Roughley and I.R. Kennedy, 2004. Legume seed inoculation technology: A review. Soil Biology & Biochemistry, 36: 1275-1288.
14. Javaid, A., 2009. Growth, nodulation and yield of black gram [*Vigna mungo* (L.) Hepper] as influenced by biofertilizers and soil amendments. African Journal of Biotechnology, 8(21): 5711-5717.
15. Khaliq, A., M.K. Abbasi and T. Hussain, 2006. Effect of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. Bioresource Technology, 97: 967- 972.
16. Ngwu, O.E., 2007. Effect of NPK 15:15:15 on physicochemical properties, nodulation and plant height of *Arachis hypogea* and *Voandzeia subterranea* using indigenous *Rhizobium*. Continental J. Agronomy, 1: 11-17.
17. Vincent, J. M., 1970. A manual for the Practical Study of Root-nodule Bacteria. Blackwell Scientific, Oxford.
18. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Ed. - Iowa State Univ. Press, Ames, Iowa.
19. Bekere, W. and A. Hailemariam, 2012. Influences of inoculation methods and phosphorus levels on nitrogen fixation attributes and yield of soybean (*Glycine max* L.) at Haru, western Ethiopia. American Journal of Plant Nutrition and Fertilization Technology, 2(2): 45-55.

20. Ahmadi, A.H.A. and M.R. Chaichi, 2007. Nitrogen fertilizing systems and harvest: Frequency effects on percent hard seedless and hard seed breakdown trend in annual medic (*Medicago scutellata* Var. Robinson). World Journal of Agricultural Sciences, 3(5): 597-601.
21. Villar-Salvador, P., F. Valladares, S. Domı´nguez-Lerena, B. Ruiz-Dı´ez, M. Ferna´ndez-Pascual, A. Delgado and J.L. Pen˜uelas, 2008. Functional traits related to seedling performance in the Mediterranean leguminous shrub *Retama sphaerocarpa*: Insights from a provenance, fertilization and rhizobial inoculation study. Environ. Exp. Bot., 64: 145-154.
22. Dumroese, R.K., D.F. Jacobs and A.S. Davis, 2009. Inoculating *Acacia koa* with *Brady Rhizobium* and applying fertilizers in the nursery: Effects on nodule formation and seedling growth. Hortscience, 44(2): 443-446.
23. Shetta, N.D., 2010. Bio-Role of *Acacia karroo* in nitrogen fixation at different locations of North-West Egypt region. American-Eurasian J. Agric. & Environ. Sci., 7(4): 471-477.
24. Pueppke, S.G. and W.J. Broughton, 1999. *Rhizobium sp*. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges. The American Phytopathological, 12(4): 293-318.
25. Swelim, D.M., F.M. Hashem, L.D. Kuykendall, N.I. Hegazi and S.M. Abel-Wahab, 1997. Host specificity and phenotypic diversity of *Rhizobium* strains nodulating *Leucaena*, *Acacia* and *Sesbania* in Egypt. Biology and Fertility of Soils, 25: 224-232.
26. Sánchez, R.C.L., B. Eichler-Löbermann, E.G. Padilla and E. Schnug, 2007. Response of *Leucaena leucocephala* cv. Peru to *Rhizobium* inoculation under salt stress. Landbauforschung, 4(57): 307-311.
27. Ahmed, Z.I., M.S. Anjum and C.A. Rauf, 2006. Effect of *Rhizobium* inoculation on growth and nodule formation of green gram. International Journal of Agriculture & Biology, 8(2): 235-237.
28. Hoque, M.M. and M.F. Haq, 1994. Rhizobial inoculation and fertilization of lentil in Bangladesh. Lens Newsletter, 21: 29-30.
29. Khalil, S.K., M.S. Afridi and M. Iqbal, 1989. Response of monoculture and intercropped maize and mungbean to various levels of NPK. Pakistan Journal of Agricultural Research, 10: 23-9.
30. Molla, A.H., Z.H. Shamsuddin, M.S. Halimi, M. Morziah and A.B. Puteh, 2001. Potential for enhancement of root growth and nodulation of soybean co- inoculated with *Azospirillum* and *Brady Rhizobium* in laboratory systems. Soil Biology and Biochemistry, 33(4-5): 457-463.
31. Philpotts, H., 1977. Effect of inoculation method on *Rhizobium* survival and plant nodulation under adverse conditions. Australian Journal of Experimental Agriculture and Animal Husbandry, 17(85): 308-315.
32. Ahmed, Z.I., M. Ansar, M. Tariq and M.S. Anjum, 2008. Effect of different *Rhizobium* inoculation methods on performance of lentil in Peshawar region. International Journal of Agriculture & Biology, 10(1): 81-84.
33. Shaheen, A. and Rahmatullah, 1996. Growth and nodulation of groundnut inoculation with *Rhizobium* strains based on different carriers. Pakistan Journal of Soil Science, 12: 3-4.
34. Ali, A., A. Amjad, J. Akhtar and M. Yaseen, 2010. Effects of phosphorus in combination with Rhizobium inoculation on growth and yield parameters of mungbean. Crop & Environment, 1(1): 53-56.
35. Achakzai, A.K.K., 2007. Effect of various levels of nitrogen fertilizer on nodulation of pea cultivars. Pak. J. Bot., 39(5): 1673-1680.
36. Hatam, M., 1977. Effect of various sources of nitrogen and added organic matter on nodulation, nodule activity and nitrogen content in soybean. Journal of Science and Technology, 1(1): 31-35.
37. Jamro, G.H., G.H. Memon and K.A. Ibupota, 1990. Effect of combined nitrogen and row spacing on nodulation and grain yield of soybean. Journal of Agriculture, 6(2): 107-112.
38. Vessey, J.K., 2002. Improvements to the Inoculation of Pea and Lentil Crops in Southern Manitoba, ARDI (Agric-Food Research & Development Initiative), Project Results. Manitoba Agriculture, Food and Rural Initiatives, Manitoba, Canada.
39. Hoshiyar, S., P.S. Pathore, A. L. Mali and H. Singh, 1994. Influence of phosphorus and inoculation on nutrient uptake, recovery and response of applied P on green gram (*Phaseolus radiata*). Indian Journal of Agronomy, 39: 316-118.
40. Umamaheswari, P., Y. Padmalatha and M. Murali Rao, 2001. Evaluation of different sources of bio-fertilizers in association with inorganic and organic manures in groundnut. Agricultural Science Digest, 21(4): 250-252.