



Ability of *Rhizobium leguminosarum* inoculum to improve fava beans (*Vicia faba*) growth and produce some hydrolytic enzyme

Hutaf A. A. Alsalmim*, Azhar abood, Lamees M.R. Abbas

Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Biological experiment was performed (with Complete Random Design (CRD)) by using sterile and non-sterile soil, to investigate *Rhizobium leguminosarum* isolate ability to enhance fava beans (*Vicia faba*) plant. The experiment includes: control group (C), fertilizer treatment as group 1 (F), and *Rhizobium leguminosarum* inoculum treatments as group 2 (R). Fava beans seeds were planted in pots filled with 5 Kg soil (sterile and non-sterile), and after seven weeks of germination, length and weight of plant vegetative part and plant root were measured. The ability of *Rhizobium leguminosarum* isolate to produce hydrolytic enzymes (chitinase, pectinase, protease, and lipase) was studied. The results show that the isolate was able to produce chitinase, pectinase, protease, and lipase enzymes. Furthermore, the addition of *Rhizobium leguminosarum* inoculum to fava beans plant increased the length and dry weight of plant vegetative part (20.3% and 51.5% respectively) and also increased the length and dry weight of plant root (49.28% and 56.2% respectively) as compared with control group.

Keywords: *Rhizobium leguminosarum*, hydrolytic enzyme, fava beans, (*Vicia faba*), biofertilizer.

قدرة بكتريا *Rhizobium leguminosarum* على إنتاج بعض الانزيمات الحالة وتعزيز نمو نبات الباقلاء (*Vicia faba*)

هتاف عبدالملك احمد السالم* ، ازهار عبود، لميس محمد رياض

قسم التقنيات الإحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

نفذت تجربة بايولوجية (بالتصميم العشوائي التام (CRD)) باستعمال تربة معقمة وغير معقمة للتحرري عن قدرة العزلة *Rhizobium leguminosarum* في تعزيز نمو نبات الباقلاء (*Vicia faba*). شملت التجربة على المعاملات الاتية : مجموعة السيطرة (C) ، والمجموعة الاولى المعاملة بسماذ ال NPK (F)، والمجموعة الثانية المعاملة بلقاح بكتريا *Rhizobium leguminosarum* (R). زرعت بذور الباقلاء في الاصص الحاوية على 5 كغم من التربة (المعقمة وغير المعقمة) ، وبعد مرور سبعة اسابيع من الانبات تم قياس اطوال واوزان الجزء الخضري والجزء الجذري للنبات . كما اختبرت قدرة العزلة *Rhizobium leguminosarum* في إنتاج بعض الانزيمات الحالة (الكابتينيز والبكتينيز والبروتيز واللايبيز).

أظهرت النتائج أن العزلة كانت قادرة على إنتاج انزيمات الكابتينيز والبكتينيز والبروتيز واللايبيز . كذلك لوحظ ان إضافة لقاح بكتريا الرايزوبيا (*Rhizobium leguminosarum*) إلى نبات الباقلاء أدى إلى زيادة في طول ووزن الجزء الخضري الجاف للنبات (بنسبة 20.3% و 51.5% على التوالي) وكذلك زيادة في طول

*Email: hutafalsalmim67@gmail.com

وزن الجزء الجذري الجاف للنبات (بنسبة 49.28% و 56.2% على التوالي) مقارنة مع مجموعة السيطرة
(C).

Introduction

The Rhizobia are special bacteria that can live in the soil or in nodules formed on the roots of legumes. The root-nodule symbiosis provides the most efficient source of biologically fixed ammonia fertilizer for agricultural crops [1]. Biological nitrogen fixation can be stimulated by applying the correct Rhizobia to their legume crops, inoculation. Rhizobium inoculation has been reported to increase the biomass of plant and seed production [2, 3] suggest that non-nodulating strains may be important competitors at the root-soil interface and that their capacity to attenuate this symbiosis should be considered in efforts to use rhizobia as biofertilizers. *Rhizobium* spp. increased plant protection by cellulase, protease, lipase and β -1,3 glucanase production [4]. Also they have ability to produce amylase, cellulase and hemicellulase enzymes [5, 6]. Hydrolytic enzyme breaks down protein, lipids, nucleic acids, carbohydrate and fat molecules into their simplest units. The presence of hydrolytic enzymes in plant infected microorganisms are widespread in nature.

This study was aimed to assess *Rhizobium leguminosarum* inoculum as biofertilizer by investigate its ability to produce hydrolytic enzymes and improve length and weight of fava beans plant shoot and root.

Materials and methods

Rhizobium inoculum

The *Rhizobium leguminosarum* isolate used in this study was provided by Department of Biotechnology /College of Science/ University of Baghdad.

The isolate was reactivated in yeast mannitol agar, (15 gm/ L agar, 10gm/ L mannitol, 0.5 gm/ L K_2HPO_4 , 0.2 gm/L $MgSO_4 \cdot 7H_2O$, 0.1 gm/ L NaCl, and 1 gm/ L yeast extract, in 1000 ml distilled water at pH 6.8-7.0) incubated at 30 °C for 3 days [7].

Determination *Rhizobium* ability to produce hydrolytic enzymes

Chitin and Pectin medium containing plates were inoculated with *Rhizobium leguminosarum* isolate and incubated at 30 °C for 3-7 days. Clear zone formation indicate positive result for chitinase and pectinase enzymes respectively [8].

Protease production investigated on milk agar, after inoculation with the bacterial isolate and incubation at 30 °C for 24h, by the formation of a clear zone [9].

Mineral salt olive oil agar was inoculated with the isolate and incubated at 30 °C for 3-7 days, to investigate the production of lipase. Positive result appear as bacterial growth and reduction of medium oil [10].

Biological experiment

Soil preparation

Soil obtained from Baghdad University fields Table-1 was divided into two groups. The first was autoclaved three times at 121 °C for 1 hour each time [11], the second was left without sterility.

Inoculum preparation

Preparation of inoculum was achieved by growing of *Rhizobium leguminosarum* isolate in 100 ml of yeast mannitol broth culture for three days at 30 °C. The optical density of bacterial culture at 600 nm was 0.86.

Plant seeds preparation

Seeds of fava beans plant, which provided by Ebaa agriculture research center / Iraq, were prepared by surface sterilization using 2% $HgCl_2$ and 95% Ethanol for 2 min, and then washed well with distilled water [12].

Biological experiment

Biological experiment performed with Complete Random Design (CRD). The experiment includes three groups: control group includes soil without addition (C), NPK fertilizer addition group (100 Kg.hect.⁻¹) (F), and *Rhizobium leguminosarum* inoculum addition group (R), which performed with sterile and non-sterile soil and with three replicates. Sterile and non-sterile soil was distributed in 18 sterile (5 Kg) pots (9 for each). Seeds, treated with inoculum, were soaked with 100ml of *Rhizobium leguminosarum* fresh culture and mixed with sterilized Arabic gum. Seeds (treated and non-treated with inoculum) were cultured, pots were arranged randomly inside a plastic house and irrigated with

tap water. After seven weeks from seeds germination, length of each plant vegetative part and plant root were measured. Plant matters were oven dried at 60°C, until weight stability. Then plant vegetative part and roots were weighted.

Table 1-The properties of soil used in the biological experiment.

properties	Soil content
Electric conductivity (EC)(ds.m ⁻¹)	1.8
pH	7.3
Cation exchange capacity(CEC) (cmol.Kg ⁻¹)	18.6
Organic matter (gm.Kg ⁻¹)	1.4
Nitrogen (mg.Kg ⁻¹)	2.21
Phosphor (mg.Kg ⁻¹)	14.53
Calcium carbonate (gm.Kg ⁻¹)	235
Sand (gm.Kg ⁻¹)	233
Silt (gm.Kg ⁻¹)	273
Clay (gm.Kg ⁻¹)	494
Soil texture	Sandy loam

Results and Discussion

Production of hydrolytic enzymes by *Rhizobium leguminosarum*

The results showed that *Rhizobium leguminosarum* isolate was able to produce chitinase, pectinase, protease and lipase enzymes. The hydrolysis zone formation on chitin, pectin and milk agar indicate chitinase, pectinase and protease production respectively [9, 8]. The growth in mineral salt olive oil agar and reduced the oil prove lipase enzyme production [10, 6]found That *Rhizobium* species isolated from fenugreek roots have the potential to produce amylase and cellulose enzymes. Hydrolytic enzymes production by some rhizospheric bacterial community indicate its ability to promoting plant growth [13].

Effect of *Rhizobium leguminosarum* inoculum

Height of the vegetative plant part

The *Rhizobium leguminosarum* inoculum addition caused an increase in height of the vegetative part of fava beans plants comparing with control groups in sterile and non-sterile soil (Figure-1). Fertilizer groups show no significant increase from *Rhizobium leguminosarum* inoculum groups but their increase were significant from control groups. The absence of soil normal flora may cause height decreases of plants vegetative part in sterile soil comparing with non-sterile soil. The trajectory of the co-evolutionary interactions between rhizobia and legumes is differentiated across different environments [14].

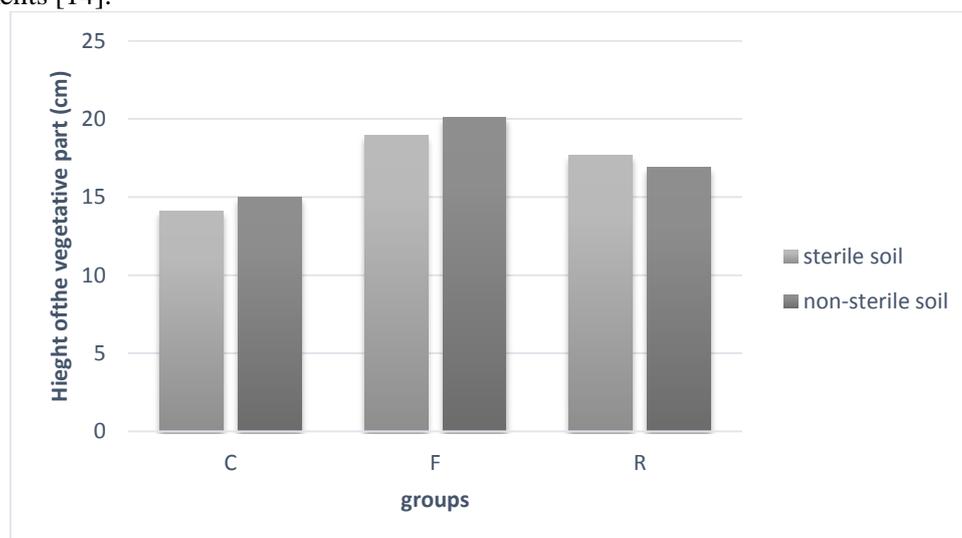


Figure 1-The height mean of fava beans vegetative part (cm) for control (C), Fertilizer (F), and *Rhizobium leguminosarum* inoculum (R) groups. (LSD_{0.05} value = 4.25)

Dry weight of the vegetative plant part

Plants vegetative part in fertilizer groups presented the best weight which differ significantly from other groups Figure-2. *Rhizobium leguminosarum* inoculum groups recorded an increase in the weight of plants comparing with control groups in sterile and non-sterile soil. The increasing in plant growth via *Rhizobium* inoculum due to soil nutrient enrichment through nitrogen fixation, siderophore production, phosphate solubilization and phytohormones production [4].

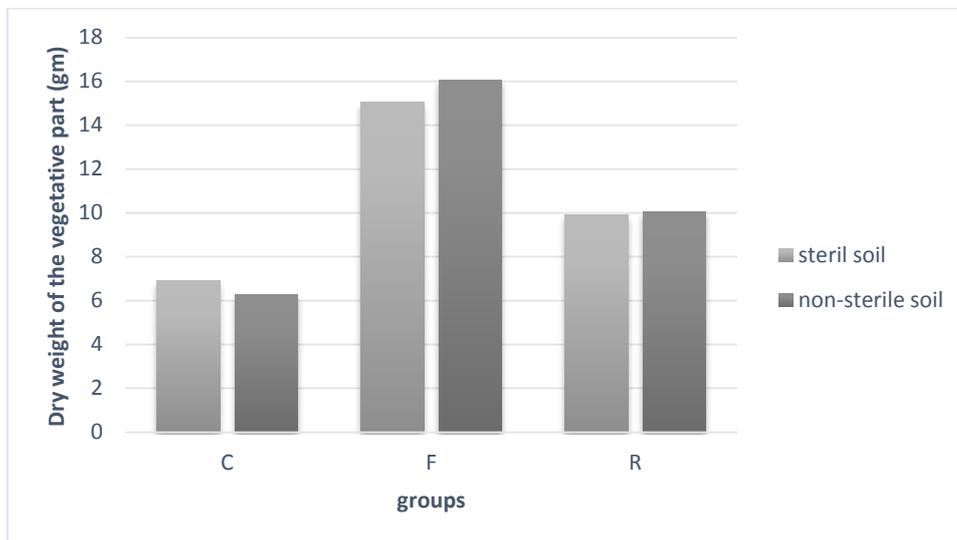


Figure 2-The dry weight mean of fava beans vegetative part (gm) for control (C), Fertilizer (F), and *Rhizobium leguminosarum* inoculum (R) groups. (LSD_{0.05} value = 4.48)

Length of the roots

The results in Figure-3 showed that addition of *Rhizobium leguminosarum* inoculum increase the length of plant root in contrast with control group, while fertilizer addition exhibit highest length of plant root. The roots length in non-sterile soil are higher than in sterile soil (15.36 and 17.64 cm respectively), but the different are not significant.

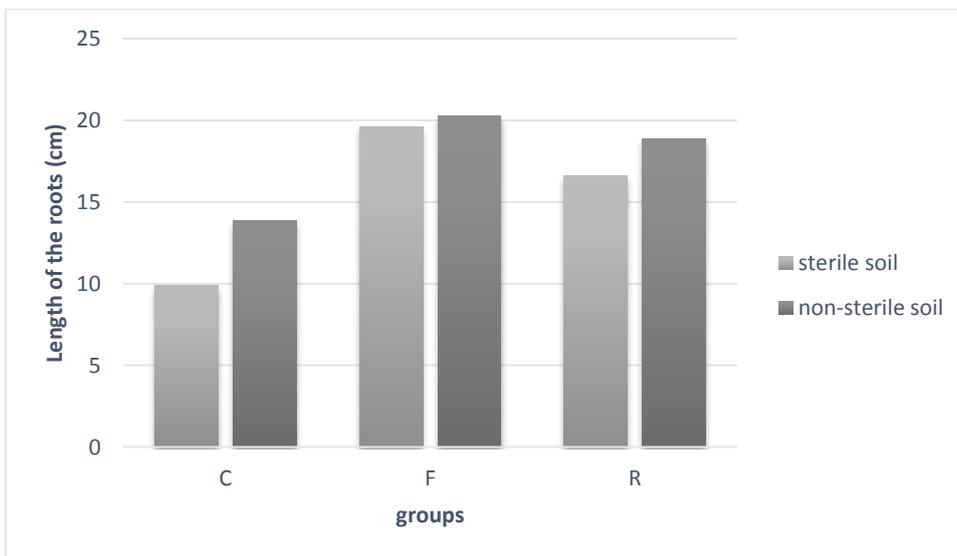


Figure 3-The length mean of fava beans roots (cm) for control (C), Fertilizer (F), and *Rhizobium leguminosarum* inoculum (R) groups. (LSD_{0.05} value = 9.53)

Dry weight of the roots

The results in Figure-4 showed that *Rhizobium leguminosarum* inoculum addition enhance the roots weight, especially in non-sterile soil. The inoculum groups show no significant differences from fertilizer groups. Roots weight in none-sterile soil are improved better than in sterile soil (the averages

are 9.06 and 6.94 respectively). Plant growth promoting rhizobia increased plant growth through enhancing soil nutrient [4] and biocontrol agent properties [15,16] found that the *Rhizobium leguminosarum* strain PEPV16 was able to promote the growth of lettuce and carrots by increasing the dry matter of shoots and roots, as well as the uptake of N and P in the edible parts of both plant species. These data confirmed the suitability of *Rhizobium* as biofertilizer for nonlegumes.

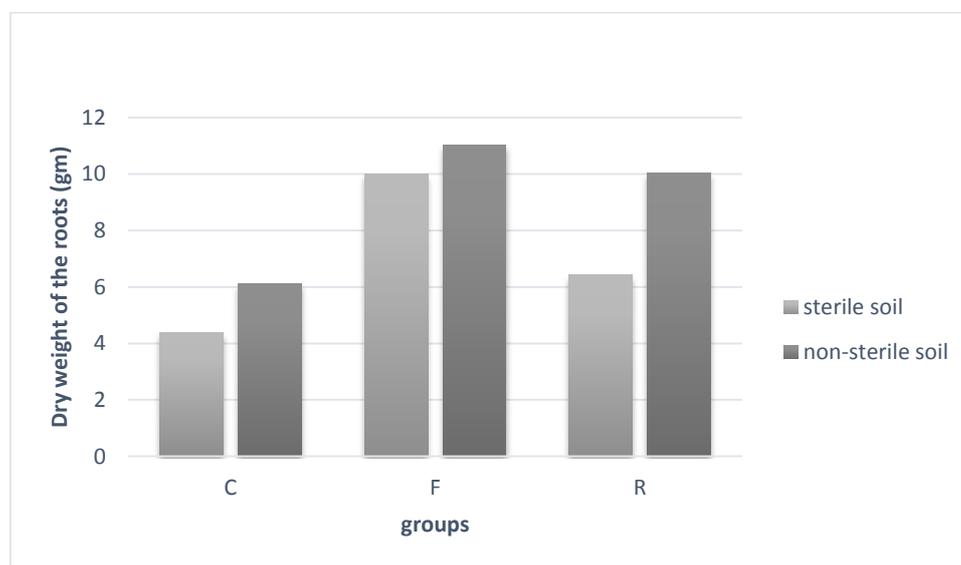


Figure 4-The dry weight mean of fava beans roots (gm) for control (C), Fertilizer (F), and *Rhizobium leguminosarum* inoculum (R) groups. (LSD_{0.05} value = 3.52)

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