Tikrit Journal for Agricultural Sciences (2022) 22 (4):73 -80
https://doi.org/10.25130/tjas.22.4.10

IRAQI Academic Scientific Journals
المجلات الأكاديمية العلمية العراقية
ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

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Evaluation of effect of the organic fertilizer prepared from Azolla primaeva and inoculation with Rhizobium bacteria in some growth characteristics of the vicia faba plant grown in gypsiferous soil

KEY WORDS:

Rhizobium bacteria, gypsiferous soils, Azolla primaeva, vicia faba.

ARTICLE HISTORY:

Received: 31/05/2022
Accepted: 07/07/2022
Available online: 31/12/2022

ABSTRACT

A field experiment was carried out in a randomized complete block design (R.C.B.D) to study the effect of inoculation with R. leguminosarum at two levels (with and without inoculation) and The compost made from the azolla plant and the interaction between them in efficiency of the root nodule formation and growth and yield of the faba bean plant grown in gypsiferous soil after being isolated and identificated by phenotypic, biochemical and molecular methods, the results showed that there was a significant increase in all plant characteristics of the fertilized and inoculated treatments compared to the non-inoculated treatment, as outperformed the compost treatment with azolla on the control treatment in all growth and yield traits, and significantly superior the inoculation treatment with Rhizobuim leguminosarum in all studied traits compared to the control treatment, and gave the inoculation treatment with Rhizobuim leguminosarum and compost made from the azolla the highest values compared to the control treatment, as the averages were in the number of root nodules, their wet weight and number of the bacteria surrounding the roots (15,000, 2.274 and 6.400 x 104), the plant height and the dry weight of the vegetative part (39,000, 24.995), (gm plant-1 cm plant-1) and concentration of the nitrogen, phosphorous and potassium in the vegetative part (1487, 238 and 1819) ppm respectively.

INTRODUCTION

The bean plant (*Vicia faba* L) is the third most important leguminous crop that grows in many parts of the world after soybeans (*Glycine max L*) and peas (*Pisum sativum L.*), and many studies have shown that adding organic fertilizers to agricultural soil effectively improves the quality of Soil and crop productivity (Manolikaki and Diamadopoulos, 2019), and compost is defined as the final product of the biodegradation process of organic matter under balanced conditions of aeration, humidification, heat and stirring for a period of time. Organic compost to obtain good organic fertilizer through the fermentation process. Compost is prepared under suitable conditions for the decomposition of these residues and activators of microorganisms to ensure the proper decomposition of the materials and the conversion of the rest of them into crumbly materials

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with a soil smell of dark color similar to the color of the soil and a high content of the necessary nutrients for plant growth (Abd El-fatah, 2013), and compost is one of the sources that have an important role in reducing the use of inorganic fertilizers and thus reducing potential pollution and improving soil quality In 1783, Lamarck introduced the word Azolla for the first time. The genus Azolla belongs to the division of symphytes, class Ferns, and order Salviniales) of the family Salviniaceae and has two sub-genera and six viviparous species (Roy et al. 2016) This study demonstrated that the use of azolla fertilizer as a single or combination application can reduce the use of synthetic fertilizers by up to 60% without significant reduction in growth and yield of rice grains. (Seleiman et al. 2022) Azolla is used as an organic fertilizer for its high nitrogen content that enhances crop yield and soil quality as well as being an environmentally friendly practice (Braun-Howland and Nierzwicki-Bauer, 2018). Root nodes and nitrogen fixation, which affects the growth and production of legumes (Al-Saadi 2001), as Al-Kartani et al. (2018) indicated that the inoculation of the mung plant with Bradyrhizobium sp. It led to a significant increase in all studied growth traits in the flowering stage, as the inoculated treatment gave the average number of root nodes, dry weight, root and vegetative dry weight of 19.40 knots.plant^{-1}, 0.11 gm.node^{-1} and 1.20 gm.plant^{-1} and 12. 65 gm plant^{-1} compared to the control treatment, which gave an average of 7.48 knots.plant^{-1} and 0.03gm.node^{-1} and 0.56gm.plant^{-1} and 6.78gm.plant^{-1}, respectively, and the content of the vegetative part of nitrogen, the number of pods and the percentage of protein In the seeds that gave the inoculated treatment an average of 4.90% and 9.46 pods^{-1} and 24.46% respectively compared to the control which amounted to 4.19% and 6.33 pods^{-1} and 20.93% respectively, and Noni mentioned 2012)) in his study On the bean crop that inoculation with R.leguminosarum bacteria gave an increase in the dry weight of the shoot and seed yield which amounted to 10.09 and 41.2%, respectively, compared to the unpollinated treatment. Compost fertilizer made from Azolla plant and inoculation with Rhizobium bacteria in growth and yield of bean plants growing in soil gypsum.

MATERIALS AND WORKING METHODS

How to prepare the organic fertilizer (compost) made from azolla:

The azolla plant was brought from the nurseries of Diyala Governorate to be propagated in the basins to obtain the required amount in preparing the compost to become an organic fertilizer ready for use in the experiment. It was collected in bags, then the organic fertilizer was prepared by collecting the azolla plant in a basin with dimensions of 2 * 2 and a depth of 1 meter. The process of aerobic decomposition was carried out using 280 kg, and it was placed on pieces of polyethylene and nitrogen was added to it by 2% using urea (46%), and phosphorous was added 0.5% of triple superphosphate fertilizer (20%) (Al-Hadithi, 2011) and continuously moistened with coverage and the process of stirring for 5 days until reaching the degree of decomposition and lowering the reaction temperature inside the heap from 65°C to 45°C and following up its daily variation with a thermometer to reach To the end of the reaction, then the decomposing organic waste was spread out to air-dry for three days and lasted for 60 days, starting from 7/24/2020 to 9/24/2020. Then a sample was taken for each decomposing material to conduct some chemical analyzes on it as shown in Table (1) , then estimate the organic carbon according to (Page et al., 1982).

Table (1): Some chemical analyzes of organic fertilizers

Material	pH 1:5	Ec ds.m^{-1} 1:5	N total %	P total %	K total%	moisture content%	C organic %	C/N
Azolla	6.7	4.4	2.75	0.82	0.39	8.74	29.02	10.55

Table (2): Schedule of routine soil analyzes before planting

$CaSO_4.2H_2O$ gm kg^{-1}	O.M gm kg^{-1}	$CaCO_3$ gm kg^{-1}	K available ppm	P available ppm	N available ppm	Ec ds.m^{-1}	pH	Texture
90.6	6.5	230	214.4	4.05	18	3.3	7.5	silty clay

Adding the biological pollen: The biological vaccine containing Rhizobium sp. was added under the name of Rhizo from the manufacturer International panacea limited in India, then the vaccine was added after a week of seedling germination by spraying on the roots of the growing seedlings.

Steps to implement the field experiment: The process of plowing, leveling and smoothing was carried out on 10/12/2020 and was divided into three sectors, each sector was divided into ten plates, and the area of each plate was (3x3) m2 and the distance between one sector and another was (150) cm and between one plate and another (50). cm, then soil samples were taken from a depth of (20-0) cm to conduct physical, chemical and biological analyzes of the soil. The seeds were sown on 10/20/2020 in the panels designated for them in a hole on the lines of the distance between one line and another (60) cm and between one hole and another (40) cm to a depth of 2-3 cm and then diluted to one plant, then nitrogen was added at a rate of 50 $kg.ha^{-1}$, using urea 46%N fertilizer as a source of nitrogen, and phosphorous and potassium were added at once, as phosphorous was added at a rate of 80 $kg.ha^{-1}$, using Triple super phosphate fertilizer 21%P, and potassium was added at a rate of 60 $kg.ha^{-1}$, using potassium sulfate fertilizer K 43% (Abdalgafor and Al-jumaily, 2016), and the fertilizer quantities were mixed and then added to the experiment in a bundle method and adjacent to the planting lines, and the organic fertilizer was added Compost in planting lines is 1% of the soil weight (ie, an average of 40,000 kg ha^{-1}, 36 kg. $plate^{-1}$), and the irrigation method was carried out by tuna Bastard and bush control manually.

Studied traits: Three plants (vegetative part) were randomly extracted from each experimental unit in the flowering stage. The number of root nodes, their wet weight for each plant, the height of plants, the dry weight of the vegetative part, and the numbers of Rhizobium bacteria present in the root periphery of the bean plant were calculated according to (Woomer., 2011). Then, the nitrogen absorbed in the vegetative parts was estimated according to the method described by (Page et al, 1982), the nitrogen content in each plant sample was estimated according to (Bates et al., 1973), and the phosphorous and potassium absorbed in the vegetative parts was estimated by a Spectrophotometer with a wavelength of 882 nm (Page et al., 1982), and potassium with a Flame Photometer according to(Haynes, 1980).

How to calculate the amount of nutrients absorbed by girls

Absorbed by seed = concentration * dry weight * 10 = mg plant $^{-1}$

RESULTS AND DISCUSSION

1- Number of root nodules (node $plant^{-1}$) and their wet weight (gm $plant^{-1}$) and the number of Rhizobium bacteria in the root area of bean plant: Table 3). Evaluation of the effect of organic fertilizer prepared from Azolla and inoculation with *Rhizobium leguminosarum* bacteria on the number and weight of root nodes And the number of Rhizobium bacteria in the root system at the flowering stage of the plant life, as the treatment inoculated with *Rhizobium leguminosarum* bacteria gave an average of 3,333 root knots $plant^{-1}$, while the uninoculated treatment gave an average of 1,666 root knots $plant^{-1}$, and the compost treatment made from Azolla gave an average It reached 5.333 knots. Plant-1, while the comparison treatment gave the lowest average of 1,666 knots. $Plant^{-1}$, and the reason for this is that the addition of organic fertilizer has a significant effect on increasing the number of root nodes because it contains important nutritional components needed by microorganisms such as sugars and enzymes that lead to the support and activation of large numbers of them, which is reflected positively on the increase of root nodes (Kamal et al., 2016). The superiority of the compost made from azolla is also due to the comparison treatment because the azolla plant contains a high percentage of nitrogen, which is the first building block for proteins and amino acids that are easily degraded by organisms. Significantly between treatments, as the interaction treatment (Azolla compost + inoculation with Rhizobium bacteria) gave higher rate of 15,000 knots. $Plant^{-1}$ compared to the comparison treatment, which gave an average of 1,666 knots. $Plant^{-1}$, as for the effect of organic and bio-fertilizer on the wet weight of the root nodes shown in Table (3), the inoculation treatment gave the highest mean of 1.893 $gm.plant^{-1}$, compared to the comparison treatment, which gave 0.000 $gm.plant^{-1}$, and the compost treatment made from Azolla had a higher average of 1.835 gm $plant^{-1}$ compared to the control that gave 0.000 gm $plant^{-1}$. The reason for the increase in the number and weight of root nodes is due to the effect of bio-fertilizer in increasing the number of bacteria in the soil that contribute to the infection of leguminous plants and the formation of root nodes and then the increase in the number of and the weight of the root nodes (Al-Kartani et al., 2018), and the reason for the increase in the weight of the root nodes by adding organic fertilizer is due to the positive effect of these fertilizers on many vital activities of living things and some of the important enzymes for them and the biochemical processes of the plant that provides the root nodule bacteria with energy and food (Al-Tamimi, 1998), As for the bilateral interaction between organic and biological fertilization, it gave clear significant differences between treatments in the dry weight of the vegetative part, as the interaction treatment between compost prepared from Azolla + inoculation with Rhizobium bacteria gave an average of 2.274 gm plant^{-1}, while The comparison treatment gave the lowest average of 0.000 gm plant^{-1}, and the same table indicates the effect of the organic fertilizer prepared from Azolla and inoculation with Rhizobium bacteria on the number of root nodule bacteria surrounding the roots of the plant. Which gave an average of 4.100 x 10^4 c.f.u , and the compost made from Azolla gave the highest average of 6.466 x 10^4 c.f.u , while the non-addition treatment amounted to 4.100 x 10^4 c.f.u . The reason for the increase in bacterial numbers is due to the accumulation of amino acids, organic acids, phenols, sterols, nucleotides, sugars and vitamins that are released in the form of excretions By the roots of the plant and the arrival of carbohydrates to the root nodules acquired by bacteria to use them as a source of energy for their growth, which increases their numbers, activity and vital activities, including their ability to fix nitrogen leading to increasing soil fertility and protecting it from drought and diseases and then stimulating plant growth (Gopalakrishnan et al., 2015), These results are in agreement with (Al-Samarrai 2021), which indicated an increase in the number of bacteria The root nodules present in the soil surrounding the roots of cowpea plant at the flowering stage, while the bilateral interaction between organic and biological fertilization had a significant effect on the numbers of bacteria, as the interaction treatment Azolla compost + inoculation with Rhizobium bacteria gave the highest rate of 6.400 x 10^4 c.f.u compared to the control treatment which amounted to 4.100 x 10^4 c.f.u .

Table (3): Evaluation of the effect of organic fertilizer prepared from Azolla and inoculation with Rhizobium bacteria on the number of root nodules, their wet weight and the number of root nodule bacteria in the root zone of the bean plant

Transactions	Rhizobium bacteria count x 10^4	wet weight (g plant^{-1})	Node number (node plant^{-1})
Control	4.100	0.000	1.666
Azolla	6.466	1.835	5.333
Rhizobium	6.633	1.893	3.333
Azolla+Rhizobium	6.400	2.274	15.000

The plant height (cm plant^{-1}), the dry weight of the vegetative parts of the plant (gm plant^{-1}) and the uptake concentration of the major elements N, P and K in the vegetative part of the plant (mg plant^{-1}): Table (4) shows the effect of organic fertilizer Prepared from Azolla and inoculated with the bacteria Rhizobium leguminosarum in the height of the bean plant at the flowering stage, as the treatment inoculated with the bacteria Rhizobium leguminosarum gave an average of 36,500 cm plant^{-1} compared to the control treatment, which gave an average of 35,000 cm. Azolla had the highest average of 37,333 cm.plant-1 compared to the control agent who gave 35,000 cm plant^{-1}. The reason is due to the role played by organic fertilizers because they contain organic acids that lower the pH and increase the availability of nutrients in the soil and then increase their absorption by Roots and increase vegetative growth, and also due to the activity of root nodes that are more evident in the advanced stages of the plant life and which have a major role in the process of nitrogen fixation, as a state of balanced nutrition is achieved that is positively reflected in encouraging the characteristics of plant growth, especially A characteristic of plant height (Al-Kartani, 1995). As for the bilateral interaction between organic and biological fertilization, it had a significant effect on plant height, as the interaction between compost prepared from Azolla + inoculation with Rhizobium bacteria gave an average of 39,000 cm. The comparison is 35,000 cm Plant^{-1} The same table indicates the evaluation of the effect of the organic fertilizer prepared from Azolla and inoculation with Rhizobium leguminosarum bacteria on the dry weight of the vegetative part at the flowering stage of the plant's age. Plant^{-1}, and the increase in the dry weight of the vegetative mass in treatments inoculated with Rhizobium bacteria may be due to the fact that

Rhizobium bacteria have an important role in increasing the amount of fixed nitrogen and converting it into amino acids and compounds that benefit the plant in tissue formation and thus improving plant growth and increasing the vegetative total (Bashan et al. , 2004), and the azolla compost treatment gave an average of 20,728 gm plant^{-1} compared to the control treatment which gave 14.424gm plant^{-1}, and the reason for this is that the organic matter has an important role in increasing the dry weight in all organic fertilizer treatments. Leading to an increase in plant height and an encouraging carbon metabolism to increase the products of photosynthesis and their accumulation in the plant, which is reflected in an increase in the dry weight of the vegetative part of the plant (Gonzalez et al 2008), and These results are in agreement with (Gomaa et al., 2010) who found that the addition of organic matter led to an increase in the dry weight of the vegetative part of the bean plant, and the results of the binary interaction between organic and biological fertilization showed significant differences between treatments, and the interaction between Azolla compost + inoculation with Rhizobium bacteria gave higher The average was 24,995 gm plant^{-1}, while the control treatment was 14,424gmplant^{-1}.

Table (4): Evaluation of the effect of organic fertilizer prepared from Azolla and inoculation with Rhizobium bacteria on plant height, dry vegetative weight, and the amount of nitrogen, phosphorous and potassium in the vegetative part of the bean plant

Transactions	Amount of potassium absorbed (mg plant^{-1})	Amount of phosphorous absorbed (mg plant^{-1})	Amount of nitrogen absorbed (mg plant^{-1})	dry weight (g plant^{-1})	plant height (cm)
Control	1117	109	706	14.424	35.000
Azolla	1530	173	1060	20.728	37.333
Rhizobium	1613	164	1187	20.237	36.500
Azolla+Rhizobium	1819	238	1487	24.995	39.000

The same table refers to the evaluation of the effect of organic fertilizer prepared from Azolla and inoculation with bacteria *Rhizobium leguminosarum* on the concentration of nitrogen, phosphorous and potassium in the vegetative part of the bean plant at the flowering stage of the plant's age. 1613 mg.plant^{-1} on the sequentially compared to the non-inoculated treatment that gave an average of 706, 109 and 1117 mg.plant^{-1} on the sequentially, and the reason for the increase in nitrogen content in the vegetative part is due to the high efficiency of Rhizobia bacteria in forming effective root nodes in nitrogen fixation because they contain a high density High bacteria, and then increase nitrogen fixation and benefit from it by the plant (Noni 2012,). The reason for the increase in phosphorous content in the shoot is that inoculation with Rhizobium bacteria improves the readiness of nutrients and increases their absorption by the plant, including phosphorus (FAO, 2000) The reason for the increase in potassium may be attributed to the increase in nitrogen absorption, which in turn led to an increase in potassium absorption due to a cooperative relationship Between nitrogen and potassium upon absorption, as for the compost treatment made from azolla, the amount of nitrogen, phosphorous and potassium in the vegetative part was 1060, 173 and 1530 mg plant^{-1}, in order, compared to the comparison treatment, which amounted to 706, 109, and 1117 mg plant^{-1}, respectively, which may be attributed to the increase in the concentration of The nitrogen in the leaves indicates the role of organic fertilizer in improving the physical properties of the soil through its ability to hold and retain water, reduce the loss of nutrients and increase vital activity, in addition to the decomposition and mineralization of organic matter that provides the soil with nitrogen in its various forms, which increases the efficiency of the plant and its ability to absorb nitrogen. Then increasing its concentration in the vegetative system (Abd-Wahab and Said, 2004), and the reason for this is that the decomposition of the organic matter leads to the secretion of organic acids that help in dissolving insoluble nutrients and make them soluble and ready for absorption through the roots of plants, especially phosphorous and increasing its concentration. In the vegetative part (Abou El-Magd et al. 2006).

As for the bilateral interaction between organic and biological fertilization, it gave a significant and clear increase in the amount of major elements between treatments, as the interaction between compost prepared from Azolla + inoculation with Rhizobium bacteria gave an average amount of nitrogen, phosphorous and potassium in the vegetative part of the plant amounted to 1487, 238 and 1819 mg plant^{-1} respectively, while the comparison treatment averaged the amount of elements 706, 109 and 1117 mg plant^{-1} respectively, and the reason may be due to the importance of organic additives in improving soil properties and the ability of organic matter to hold mineral elements in a chelating form such as fulvic acid, humic, citric, and oxalate oxalate And acetate, carbonic and nitric acid, which stimulate the dissolution of potassium minerals necessary to increase soil fertility and improve plant production (Abouel-magd et al. 2006; Khatoon et al. 2020). For absorption, especially the major elements such as phosphorous and potassium (Havlin et al. 2005).

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تقييم تأثير السماد العضوي المحضر من الأزولا Azolla primaeva والتلقيح ببكتريا Rhizobium في بعض صفات النمو لنبات الباقلاء النامية في تربة جبسية

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الكلمات المفتاحية:
بكتريا رايزوبيوم , ترب جبسية , ازولا, الباقلاء

الخلاصة

نُفذت تجربة حقلية بتصميم القطاعات العشوائية الكاملة (R.C.B.D) لدراسة تأثير التلقيح ببكتريا R.leguminosarum بمستويين (مع التلقيح وبدون التلقيح) والكومبوست المصنع من نبات الأزولا والتداخل بينهما في كفاءة تكوين العقد الجذرية ونمو وحاصل نبات الباقلاء النامية في تربة جبسية بعد عزلها وتشخيصها بالطرائق المظهرية والكيموحيوية والجزيئية, وأظهرت النتائج وجود زيادة معنوية في جميع صفات النبات في المعاملات المسمدة والملقحة مقارنة بالمعاملة غير الملقحة ,إذ تفوقت معاملة التسميد بالكومبوست المصنع من الأزولا على معاملة المقارنة في جميع صفات النمو والحاصل , وتفوقت معاملة التلقيح ببكتريا Rhizobuim leguminosarum معنوياً في جميع الصفات المدروسة قياساً بمعاملة المقارنة , وكذلك أعطت معاملة التلقيح ببكتريا Rhizobuim leguminosarum والكومبوست المصنع من الأزولا أعلى القيم مقارنةً بمعاملة المقارنة, إذ بلغت المتوسطات في عدد العقد الجذرية ووزنها الرطب وعدد البكتريا المحيطة بالجذور (15.000 و2.274 و6.400 x 104) وفي ارتفاع النبات والوزن الجاف للجزء الخضري (39.000 و24.995) وتركيز النتروجين والفسفور والبوتاسيوم في الجزء الخضري (1487و238و1819)% على التتابع .