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PREPARING AND SELECTION MICROBIAL CRRIER FOR BACILLUS MEGATERIUM AND AZOSPIRILLUM LIPOFERUM AND ITS IMPACT ON GROWTH AND YIELD OF POTATO

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Abstract

A factorial experiment was conducted at the Research Station of the College of Agriculture, Anbar University, using the RCBD factorial experiments to determine the effect of the type of carrier and bacterial inoculum on the growth and yield of potatoes. Three carrier materials were used: pre-ripened date seeds, molasses, and zeolite as a control. *B. megaterium* and *A. lipoferum* carriers and inoculum were prepared and incubated for five days. The treatments were distributed randomly, adding the full fertilizer recommendation to the field, except P and N, which were added at a rate of 60% of the recommendation. The results showed that the carrier made from pre-ripe date seeds was superior to the zeolite, as the highest plant height and potato yield were recorded at 73.22 cm and 42.95 tons ha^{-1}, with a significant difference from the lowest rate recorded for zeolite, 67.17 cm and 38.18 tons ha^{-1}, respectively. Dpbm and molasses carriers recorded the highest percentages of N and P in soil, reaching 47.0, 45.98, 11.77, and 11.18 mg kg^{-1} soil, respectively. *B. megaterium* recorded the highest yields of potato and phosphorus in the soil, reaching 44 ton ha^{-1} and 9.86 mg Pkg^{-1}. The interaction DpbB-*B. megaterium* recorded the highest average soil microbial content at 8.65 log cfu g^{-1}. The molasses-A followed the *lipoferum* interaction with an average microbial content of 6.831 and 6.35.65 log cfu g^{-1}.

Keywords: Date pits before maturity, Molasses, Zeolite, Bacillus, Azospirillum, Potato.

تحضير واختيار الحامل الميكروبي لبكتيريا Bacillus megaterium وAzospirillum lipoferum وتأثيره في نمو وحاصل البطاطا

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الخلاصة

أجريت تجربة عاملية في محطة أبحاث كلية الزراعة جامعة الانبار باستخدام التجارب العاملية RCBD لمعرفة تأثير نوع الحامل واللقاح البكتيري في نمو وحاصل البطاطا. تم استخدام ثلاث انواع من المواد الحاملة وهي نواة التمر قبل النضج والمولاس والزيولايت مع السيطرة. تم تحضير وخلط المواد الحاملة واللقاحات *B. megaterium* و*A.lipoferum* وحضنت لمدة 5 ايام. وزرعت المعاملات عشوائيا مع اضافة التوصية السمادية الكاملة للحقل باستثناء الفسفور والنتروجين اضيفا بمعدل 60% من التوصية. اظهرت النتائج تفوق الحامل المصنوع من نواة التمر قبل النضج على حامل الزيولايت، اذ سجلت اعلى ارتفاع للنبات وحاصل البطاطا 73.22 سم و42.95 طن هكتار$^{-1}$، بفارق معنوي عن اقل معدل مسجل للزيولايت 67.17 سم و38.18 طن هكتار$^{-1}$ بالترتيب. سجل كل من Dpbm والمولاس اعلى نسبة من النتروجين والفسفور في التربة بلغت 47.00 و45.58 و11.8 و11.7 ملغم كغم$^{-1}$ بالترتيب، كما سجلت *A.lipoferum* اعلى ارتفاع وتركيز للنتروجين في التربة بلغا 75.12 و45.69 ملغم كغم$^{-1}$، كما سجل *B. megaterium* اعلى حاصل وجاهزية للفسفور في التربة بلغا 44 طن هكتار$^{-1}$ و9.86 ملغم كغم$^{-1}$. سجل التداخل *B. megaterium* DpbB– اعلى معدل للمحتوى المكروبي في التربة 8.65 log cfu g^{-1} وDpbB تلاهما تداخل المولاس–A.lipoferum بمعدل للمحتوى المكروبي بلغ 6.831 و6.350 log cfu g^{-1}.

كلمات مفتاحية: نواة التمر قبل النضج، المولاس، باسلس، الازوسبيريلوم، البطاطا.

Introduction

The carrier is the substance that can carry microbial inoculant cells in sufficient quantities and keep them alive, actively and effectively, under certain conditions until reaching and colonizing the root zone (2, 10 and 11). It works to protect cells and provide the appropriate environment for their growth, which results in microbial strains that are more effective in facing biotic and abiotic stresses in the soil and increase their competitiveness with the rest of the soil microorganisms, and thus the continuation of the plant's supply of nutrients by bio-fertilizers. Also (13 and 21) defined the process of loading microbial inoculants as the use of carrier materials from various sources of organic or organic origin (mineral) that have a high ability to

carry microbial inoculants without the slightest impact on them; this process is of great importance by keeping the microbial inoculant effective and active for the longest period possible (15 and 18), securing the necessary protection for the survival of the microbial inoculant as a result of competition from other microorganisms or in unsuitable growth conditions or the presence of toxic and growth-inhibiting substances secreted by other microorganisms (1 and 3). Also, providing energy and carbon sources for the inoculant cells enough to continue it actively and effectively during the storage period or even after a period of use in cultivation. The loading process is a preliminary stage that the microbial inoculant goes through until it is adapted and integrated into the soil medium when applied (9 and 19). Many materials were used to load bacterial inoculants as biofertilizers, including peat moss, charcoal, peat, corn waste, wheat and barley straw, sugarcane straw, sand, clay minerals such as (bentonite, vermiculite), polymers such as sodium alginate (8). Many studies have proven the efficiency of these materials in being good loading materials for most vital inoculants (20). The genera of bacteria used as biofertilizers vary in terms of their uses as they are fixed to atmospheric nitrogen, which needs a host such as Rhizobia and its different types. Also, free-living bacteria such as *Azotobacter sp, Azospirillum sp* (2 and 13) and bacteria that contribute to dissolving complex phosphorous compounds and releasing phosphorous, such as *Bacillus sp, Pseudomonas sp, Arthrobacter sp, Streptomyces sp* (2, 4, 5, 6 and 19). It also leads to the liberation of potassium elements such as *Thaiobacillus sp Enterobacter sp,* the decomposer of some organic compounds, and the release of some plant growth regulators such as Xanthomonas (1 and 7). The mechanism underlying the production process of bio-fertilizers based on PGPR (plant growth promoting rhizobacterial) is to isolate and purify these organisms and their propagation in private farms until they are used as a biological inoculant that is added directly to the soil and near the roots of plants or mixed with seeds before planting (14). These inoculants are usually loaded on natural materials, whether organic or inorganic, to maintain the viability and vitality of the inoculant cells well until they reach the area near the roots of the plants and achieve the desired goal (12). Several studies indicated the role of bio-bacterial fertilizers in enhancing the growth and productivity of potatoes and reducing the use of mineral fertilizers by up to 25%, thus improving the quantitative and qualitative characteristics of the crop in addition to reducing environmental pollution problems and reducing production costs (17).

Therefore, this study aimed to improve the efficiency of biofertilizers prepared from bacterial isolates in promoting the growth and production of potatoes by selecting an appropriate inoculant carrier that provides the appropriate environment for the inoculant to play its primary role.

Materials and Methods

Preparing the carrier: The zeolite, molasses, and Date pits before maturity were selected as carriers, and some treatments were performed on them before mixing with the bacterial culture.

The date pits were sufficiently cleaned to remove impurities and dry them in the air. The materials were ground to obtain a suitable size by passing them through a sieve with holes of 2 mm in diameter.

Ten grams of each type of material were placed in 100 ml of sterile water for sterilization. 0.5 gm of $CaCO_3$ was sterilized in an autoclave at 121 C for 15 min and dried to reach a humidity of 50-60% (9).

Preparing bacterial isolates: Two isolates of *Bacillus megaterium* and *Azospirillum lipoferum* were brought from the Soil and Water Resource College of Agriculture laboratory at the University of Anbar. The activation process was carried out by growing them on NB culture medium (nutrient broth); the isolates were grown in a planning method on plates containing (nutrient agar) NA and incubated in the incubator at 28±2 C for 48 hrs. Bacterial isolates were diagnosed based on culturing, microscopic, and biochemical characteristics mentioned in (16).

Preparation of primary culture from bacterial isolates: a portion of each bacterial culture was transferred to 250 ml flasks containing 100 ml of sterile NB (nutrient broth medium) and incubated in a vibrating incubator at 28 °C ± 2 for four days (7).

Biofertilizer combination preparation: The vials containing the carrier materials were inoculated with young B. megaterium and A. lipoferum isolated by adding 2 ml to each vial containing at least 33×10^8 cfu.ml^{-1}. One vial was left for each type of carrier as a comparison treatment without inoculation (2 ml of distilled and sterilized water was added). It was incubated in the incubator at 28±2°C for seven days, and the contents were mixed by stirring the bottles daily (1).

The following symbols are given for combinations by carrier 3 type and inoculant:
First Factor: Date pits before maturity, molasses, zeolite, and without carrier =4
Second Factor: *B. megaterium*, *A. lipoferum,* and without inoculant =3
Replicates: 3 times = 4×3×3= 36 experimental units using the factorial with RCBD design.

Implementation of experiment: A field experiment was conducted on 24-9-2023 in A research station at the College of Agriculture Anbar University, north of Anbar Governorate. Table 1 shows some physical, chemical, and biological characteristics of soil and experiments carried out using the factorial with RCBD design. The land was divided into three equal sectors, each divided into 12 experimental units. The transactions were randomly distributed over the sectors. The fertilizer recommendation was added to all sectors except for P and N, and only 60% of it was added. Potato seeds of the Safran variety were planted at a distance of 25 cm between one tuber and another, and after planting was completed, they were irrigated fields.

Table 1: Some Physical, Chemical, and Biological Characteristics of Soil.

Characters	Unit	Value
EC	$ds.m^{-1}$	2.80
pH	---------	7.62
OM	$Mg\ kg^{-1}$	14
CEC	$C\ mol\ kg^{-1}$	16.14
Available N	$Mg\ kg^{-1}$	30.7
Available P	$Mg\ kg^{-1}$	10
Available K	$Mg\ kg^{-1}$	200
Sand	$g\ kg^{-1}$	260
Silt	$g\ kg^{-1}$	488
Clay	$g\ kg^{-1}$	252
Texture	-------	Loam
Total NO. Bacteria	$cfu\ g^{-1}$	38×10^{5}

Results and Discussion

Table 2 shows that plant height was not significantly affected by the change in the type of bacterial inoculant carrier. The results were close, as the highest rate of plant height was recorded in the treatment of carrier Date pits before maturity. It reached 73.22 cm, a significant difference from the zeolite treatment, which recorded 67.17 cm. As for the type of bacterial inoculation, the A. lipoferum inoculant was superior by recording the highest rate of plant height of 69.82 cm, with a significant difference from the treatment of B. megaterium inoculant and the comparison treatment (without inoculant). The interaction treatment between the type of carrier and the bacterial inoculum DPM- A. lipoferum recorded the highest value for plant height, which amounted to 78.25 cm, while the lowest value for plant height without inoculum-carriers was 54.22 cm.

Table 2: Effect of type of carrier, inoculation, and interaction on high plant (cm).

Carriers	Without Inoculum	*B. megaterium*	*A. lipoferum*	Average
Date pits before maturity DpbB	**66.38**	**75.03**	**78.25**	**73.22**
Molasses	**64.00**	**71.41**	**76.80**	**71.17**
Zeolite	**63.20**	**68.01**	**70.31**	**67.17**
Without Carriers	**54.22**	**54.93**	**53.89**	**54.34**
Average	**61.95**	**67.42**	**69.82**	
LSD 0.05	**In=1.252**	**C=0.922**	**Ic=1.541**	

The results of Table 3 indicate that the bacterial carrier type affected the yield rate. The carrier (DpbB) recorded the highest rate of 42.95 $Mg\ ha^{-1}$, with a significant difference from (molasses, zeolite, and without carrier). As for the type of bacterial inoculant, B. megaterium inoculant recorded the highest rate of total yield of 39.62 $Mg\ ha^{-1}$, with a slight difference from the treatment of A. lipoferum inoculant, which recorded 38.74 $Mg\ ha^{-1}$, while the lowest rate of production in the comparison treatment (without inoculation- carrier) was 32.63 $Mg\ ha^{-1}$. The interaction treatment between the type of carrier and the bacterial inoculum DpbB - B. megaterium recorded the highest value for yield rate, which amounted to 46.50 $Mg\ ha^{-1}$.

Table 3: Effect of type of carrier, inoculation, and interaction on yield Mg ha^{-1}.

Carriers	Without Inoculum	*B. megaterium*	*A. lipoferum*	Average
Date pits before maturity- DpbB	36.70	46.50	45.67	42.95
Molasses	34.60	44.20	43.07	40.62
Zeolite	32.83	41.30	40.42	38.18
Without Carriers	26.40	26.50	25.80	26.23
Average	32.63	39.62	38.74	
LSD 0.05	In=1.112	C=0.521	Ic=1.75	

The results of Tables 4, 5 and 6 indicated that the concentrations of N and P recorded significant differences according to the carrier type used if the results were close between the two carrier types (DpbB and molasses), with a significant difference from the zeolite that recorder 47.0, 45.98 and 41.73 mgNkg^{-1}, and 11.77, 11.18 and 10 mgPkg^{-1}, respectively. As for the bacterial inoculant type, A. lipoferum inoculant recorded the highest available N rate (45.69 mgNkg^{-1}) with a significant difference from the comparison treatment, which recorded the lowest average N concentrations amounting to 38.28 mg N kg^{-1}. As for the interaction between the carrier type and the inoculant used, combination DpbB- A. lipoferum recorded the highest nitrogen concentration rate of 53.20 mgNkg^{-1} soil, and combination DpbB- B. megaterium recorded the highest rate of phosphorous of 14.13 mgP kg^{-1} soil, while combination molasses with A. lipoferum recorded the highest rate of nitrogen, which amounted to 50.43 mg kg^{-1} soil.

Table 4: Effect of type of carrier, inoculation, and interaction on available N in soil (mg kg^{-1}).

Carriers	Without Inoculum	*B. megaterium*	*A. lipoferum*	Average
Date pits before maturity DpbB	40.00	47.80	53.20	47.00
Molasses	40.22	46.10	50.43	45.58
Zeolite	39.27	40.10	45.84	41.73
Without Carriers	33.64	32.80	32.90	33.11
Average	38.28	41.70	45.69	
LSD	Inoc=1.81	Car=1.85	Ic=2.12	

Table 5. Effect of type of carrier, inoculation, and interaction on available P in soil (mg kg^{-1}).

Carriers	Without Inoculum	*B. megaterium*	*A. lipoferum*	Average
Date pits before maturity- DpbB	8.98	14.13	12.20	11.77
Molasses	8.53	12.83	11.51	11.18
Zeolite	7.93	11.48	10.60	10.00
Without Carriers	7.02	9.86	9.03	8.09
Average	8.09	12.07	10.83	
LSD 0.05	In=0.72	C= 0.27	Ic=0.41	

Table 6 indicates that the bacterial carrier type affected the total microbes in the soil; the carrier (DpbB) recorded the highest rate of 7.153 log cfu g^{-1} soil, with a significant difference from (molasses, zeolite, and without Carrier). As for the bacterial inoculant type, B. megaterium inoculant recorded the highest rate of 7.028 log cfu g^{-1} soil, while the lowest rate of TM in the comparison treatment (without inoculation- carrier) was 5.525 log cfu g^{-1} soil. The interaction treatment between the

carrier type and the bacterial inoculum DpbB - B. megaterium recorded the highest value for TM, which recorded 8.650 log cfu g^{-1} soil.

Table 6: Effect of type of carrier, inoculation, and interaction on TM in soil (log cfu g^{-1} soil).

Carriers	Without Inoculum	*B. megaterium*	*A. lipoferum*	Average
Date pits before maturity DPbM	**5.980**	**8.650**	**6.831**	**7.153**
Molasses	**5.930**	**7.312**	**6.350**	**6.530**
Zeolite	**5.320**	**7.130**	**6.020**	**6.156**
Without Carriers	**4.872**	**5.021**	**4.982**	**4.985**
Average	**5.525**	**7.028**	**6.046**	
LSD 0.05	**Incul=4.8**	**Carr=5.9**	**Ic=8.7**	

Conclusions

The suitability of Date pits before maturity as a carrier of bacterial inoculum and its superiority over zeolite in all the studied characteristics may be due to its good content of essential nutrients, proteins, some oily acids and fibers, and its good preservation of moisture content suitable for the bacteria cells growth and the duration of its stay in the soil is longer. This was reflected in the activity and effectiveness of inoculant bacteria isolates. In promoting plant growth and production and improving the availability of nutrients N and P in the soil (21), this was confirmed by (3, 10 and 14). They referred to the success of the date seed material in carrying *Rhizibium* inoculate cells and maintaining a good population density during the storage period and when applied in the field. Also, (1 and 13) confirmed the date seeds' suitability as an inoculant carrier for Azospirilum bacteria and improved plant growth in all traits**.** This reduces the cost of mineral fertilizers used in potato production and increases the plant's nutritional requirements. Applying bio-fertilizers increased soil nutrients and the number of microorganisms in the soil, which led to an increase in enzyme dehydrogenase and increased availability of nutrients.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Conceptualization, Idham A.A. and Alya Aljuaid Funding Acquisition and Investigation, Waqas; Data Curation, Hassan M.A. Formal Analysis and, carrying out research, providing services, preparing biofertilizers, recording field data, M.T. Yaqub conducting measurements and analyses, Idham A. A writing the work and sending it for publication.

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