

INFLUENCE OF PHOSPHATE SOLUBILIZING MICROORGANISMS ON SOIL PHOSPHORUS AVAILABILITY AND FABA BEAN YIELD UNDER CALCAREOUS SOIL CONDITIONS.

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ABSTRACT

Availability of phosphorus to plant is impaired in the calcareous soils due to the formation of poorly soluble calcium phosphate minerals. This work aimed to investigate the influence of phosphate solubilizing microorganisms on the availability of soil phosphorus and faba bean yield under calcareous soil. Accordingly a pot experiment was carried out during the winter season of 2010-2011 under the greenhouse of the Experimental Farm of the Faculty of Agriculture, Mansoura University. The experiment was conducted in a split plot design; which consisted of six main treatments of microorganisms (Control, Microbin, Mycorrhiza, Phosphorin, Microbin + Mycorrhiza, Microbin + Phosphorin) and four levels of P-fertilization (in the form of calcium super phosphate) in the sub plots. The P-rates were 0, 50, 75, and 100% of recommended dose. The obtained results indicated that inoculation of seeds either alone or in combination with any of the P-levels especially 50% and 75 % increased yield and yield components, crude protein, as well as the N, P, and K contents when compared to uninoculated plants. The highest significant values were observed under the treatment of (Microbin + Mycorrhiza) and (Microbin + Phosphorin). It could be also indicated that biofertilization could play a significant role in converting the fixed form of phosphorus into an available form to plant uptake especially under calcareous soil conditions where P is fixed due to high pH and significant quantities of free excess lime.

Keywords: Faba bean, biofertilizers, soil phosphorus, calcareous soil, chemical composition.

INTRODUCTION

Phosphorus is one of the essential chemical elements required for plant growth and reproduction. It is often referred to phosphorus as the "energizer" since it helps store and transfer energy during photosynthesis. Also, it is a vital component of ATP, the "energy unit" of plants. Under normal soil conditions especially calcareous ones, phosphorus is often found in fixed chemical forms that cannot immediately be absorbed by plants. Sodic and calcareous soils are defined as soil with pH values greater than neutral, typically 7.5 to 8.5. They have significant quantities of free excess lime (calcium or magnesium carbonate). Lime dissolves in neutral to acidic soils, but does not readily dissolve in sodic soils and instead serves as a sink for surface adsorbed calcium phosphate precipitation (Bryan and Jason, 2005).

Faba bean (*Vicia fwaba L.*) is considered one of the most important green leguminous crops grown in winter season at different types of soils in Egypt. Also, it is considered as one of the basic sources of protein for human consumption, so it is important to obtain a clean product of faba bean. In this

respect, many attempts took place in the recent years in order to avoid the harmful effects of chemicals and pesticides. Bio-organic farming systems are the recent trend, newly introduced in the agricultural practice for getting clean products. Application of these systems in the agriculture regimes became inevitable to put an end to the unwise doses of harmful chemical fertilizers that are used for increasing crop productivity. This is in addition to controlling the toxic pesticides that hide enormous amounts of metals and hydrocarbon compounds that negatively affect the surrounding environment in which humans, animals and plants are living. Soil application of bio-fertilizers is the most common method used in bio-organic farming systems (Hoda and Gomaa, 2005). Growing faba bean in newly reclaimed soils usually needs integration between the bacterial inoculation with mineral fertilization for producing high quality and quantity yield (El - Habbasha *et al*; 2007).

Biofertilization generates plant nutrients such as nitrogen and phosphorus through their activities in the soil or rhizosphere and make them available to plants in a gradual manner. Phosphate solubilizing biofertilizers (PSB) can be used to turn soil phosphorus to available forms which can be absorbed by plants. It can be used either for seed treatment or soil application. Mycorrhizal fungi and P-dissolving bacteria have an important role in solubilizing of P and enhancing its absorption as well as enhancing crop productivity (Habashy, 2005). Thus, recent researches aim to confirm that the best way to utilize native soil phosphorus is by biofertilization especially phosphate solubilizing microorganisms, which can solve the problem of phosphorus fixation and reduce pollution resulted from chemical fertilizers.

The objectives of this work are to determine the positive effects of biofertilizers on phosphorus solubilization in calcareous soil and to make a comparison among certain kinds of commercial biofertilizers and their influence on both NPK contents and yield of faba bean crop.

MATERIALS AND METHODS

A pot experiment was carried out during the winter season of 2010-2011 under the greenhouse condition at the Experimental Farm of the Faculty of Agriculture, El-Mansoura University to investigate the influence of phosphate solubilizing microorganisms on soil phosphorus availability and faba bean yield under calcareous soil condition.

Experimental Design: The experiment was conducted in a split plot design; which consisted of six main treatments of different biofertilizers compounds and four levels of phosphorus fertilization in the subplots.

The treatments of biofertilizers were as follow (A):

1. (Co) Control (un-inoculated seeds)
2. (Mi) Microbin (Rhizobium sp.)
3. (My) Mycorrhiza (Glomus macrocarpium)
4. (Pho) Phosphorin (Bacillus megatherium var. phosphaticum)
5. (Mi+My) Microbin + Mycorrhiza
6. (Mi+Ph) Microbin + Phosphorin

Levels of P-fertilization were as follow (B):

1. (P0) without P-fertilization
2. (P1) 50% from the recommended dose
- 3- (P2) 75% from the recommended dose
- 4- (P3) 100% from the recommended dose

Each treatment was replicated 3 times and the total number of studied pots was 72. The interaction effects among the treatments were also studied.

Preparation of Pots: 72 polyethylene pots of 10 cm diameter and 60 cm length were used. Each pot was filled with six kg of air dried soil (collected from the surface layer (0-30 cm) of a calcareous soil located near El-Nobararia city, El-Behera Governorate. The studied soil was sandy clay loam in texture. Soil physical and chemical properties of the studied soil before cultivation are presented in Table 1.

Table 1: Initial soil physical and chemical properties of the studied soil.

Physical analysis	Particle size distribution					Sp%	OM%	CaCO ₃ %
	Coarse sand	Fine sand	silt	clay	texture			
	8.65	58.28	11.70	21.37	Sandy Clay Loam	26	0.78	17.88
Chemical analysis	E.C.dS.m ⁻¹ (1:5)	pH(1:2.5)	Available nutrients(ppm)			Total nutrients (ppm)		
	0.74	8.17	P		K	N		
			3.87		274.5	28.9		
	Soluble ions (meq.100g ⁻¹ soil)							
	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
	0.98	0.56	2.18	0.07	---	1.05	2.16	0.58

Cultivation of faba bean seeds: On the 7th of December 2010, faba bean seeds (C.V. Giza716) acquired from the seeds production unit, Agriculture Research Center, Egypt, were divided into six parts and mixed with Co, Mi, My, Ph, MiMy, and MiPh; respectively. These biofertilizers were produced and distributed commercially by the General Organization of Agriculture Equalization Fund (GOAEF), Ministry of Agriculture in Egypt. Arabic gum (40%) was used as an adhesive material. Five seeds were sown at equal distances in each pot. Two weeks later, the grown plants were thinned to the most three uniform plants per each pot. All pots were irrigated to the field capacity. Faba bean plants were harvested after 120 days from sowing.

Chemical fertilization: recommended dose of N and K for faba bean were applied in the form of ammonium nitrate and potassium sulfate, respectively. Four levels of mineral P (0, 50, 75, and 100% of the recommended dose (14 Kg P fed⁻¹) were applied to the studied pots before sowing in the form of calcium-super phosphate (7% P).

Chemical analysis: - particle size distribution was determined using the international pipette method as described by Piper (1950). Soil pH was measured in 1: 2.5 soil water suspensions as described by Jackson (1967). Saturation percentage was determined as described by the US Salinity

Laboratory Staff (1954). Electrical conductivity (EC) was measured in the 1:5 soil water extract using the electrical conductivity meter (Jackson (1967). Organic matter was determined according to Walkley and Black method (Black (1965). Calcium carbonate was measured volumetrically using collin's calcimeter according to Piper (1950). Soluble cations and anions were measured in 1;5 soil water extract according to the methods described by Jackson (1967).

Available phosphorus in the studied soil was extracted by sodium bicarbonate and determined following the method of Olsen *et al.*, (1954). Available potassium was extracted by ammonium acetate and measured flame photometrically according to Black (1965). Oven dry plant samples were ground and wet digested by sulphuric and perchloric acid mixture as described by Peterburgski (1986).

Total nitrogen was determined by using microkjeldahl method as described by Pregle (1945). Total phosphorus was determined colorimetrically using cholrostanus-reduce molybdo-phosphoric blue color method in sulphuric acid as described by Jackson (1967). Potassium was determined in the digested plant materials using the flame photometer as described by Black (1965). Crude protein in dry seeds was calculated by multiplying the N concentration by 6.25 according to AOAC (1970).

Statistical analysis: Statistical analysis of the collected data was done according to the methods described by (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Effect of biofertilizers on yield and yield components:

Data in Table 2 show the mean values of seed yield (g plant^{-1}), weight of 100 seeds "weight of 100 seeds" (g) and crude protein (%) as affected by biofertilization during the experimental season. Data reveal significantly increase in the mean values of seed yield, weight of 100 seeds, and crude protein due to microbial inoculation when compared with the control. However, there were significant differences among the biofertilizers in their impact on the studied parameters. Addition of (Microbin + Microhyza) showed the highest significant increase in seed yield, weight of 100 seeds, and crude protein ($32.43 \text{ g plant}^{-1}$, $70.60 \text{ g. plant}^{-1}$, and 20.99%; respectively). These results are in agreement with those obtained by Elghandour *et al.* (1996) and El-Wakeil and El-Sebai (2007).

Effect of phosphorus fertilization on yield and yield components:

The mean values of seed yield, weight of 100 seeds and crude protein were also significantly increased as a result of P-fertilization. The highest values were recorded under the addition of 75% from the recommended. These values were $28.10 \text{ g plant}^{-1}$, $61.15 \text{ g plant}^{-1}$, and 18.06% for seed yield, weight of 100 seeds, and crude protein; respectively. These results are in harmony with those obtained by Sorour (1993), Abdel-Haleem (1994), and Fageria and Cavalho (2002).

Table 2: Effect of biofertilization and P-fertilization on seed yield (g plant⁻¹), weight of 100 seeds (g plant⁻¹) and crude protein (%) of faba bean plants at harvest.

Biofertilizers	Seed yield (g plant⁻¹)	Weight of 100 seeds (g plant⁻¹)	Crude protein (%)
Co	19.64	42.75	13.14
Mi	29.56	64.33	19.43
My	25.36	55.18	15.71
Pho	22.16	48.23	14.83
Mi+My	32.43	70.60	20.99
Mi+Pho	27.19	59.19	18.58
LSD at 5%	0.02	0.15	0.41
P-Fertilization	Seed yield (g.plant⁻¹)	Weight of 100 seeds (g plant⁻¹)	Crude protein (%)
P0	24.15	52.56	15.77
P1	26.62	57.95	17.52
P2	28.10	61.15	18.06
P3	25.37	55.20	17.10
LSD at 5%	0.02	0.11	0.33

Co: Control Mi: Microbin My: Mycorrhiza
 Ph: Phosphorin
 MiMy: Microbin + Mycorrhiza MiPh: Microbin + Phosphorin
 P0: without phosphorus P1: 50% from the recommended dose
 P2: 75% from the recommended dose P3: 100% from the recommended dose

The interaction effect between biofertilization and phosphorus fertilization:

Data presented in Table 3 show the interaction effect between biofertilization and phosphorus levels on faba bean yield and its components. Data indicate that, seed yield, weight of 100 seeds and crude protein were significantly increased when biofertilizations were used in combination with P-fertilizer. The highest value was recorded under 50% from recommended dose of calcium super phosphate and (Microbin +Phosphorin) when compared with the other treatments. These results could be enhanced with those obtained by El- Assiouty and Abo-Sedera (2005) and Talaat, Neveen and Abdalla, Amany (2008).

Table 3: The interaction effect of biofertilization and P-fertilization on seed yield (g plant^{-1}), weight of 100 seeds (g plant^{-1}) and crude protein (%) of faba bean plants at harvest.

Treatments		Yield and yield components		
Biofertilizers	P-Fertilizer	Seed yield (g plant^{-1})	Weight of 100 seeds (g plant^{-1})	Crude protein (%)
Co	P0	17.85	38.85	12.25
	P1	19.10	41.57	12.63
	P2	20.24	44.05	13.44
	P3	21.38	46.54	14.25
Mi	P0	20.55	44.73	17.31
	P1	21.80	47.44	18.00
	P2	23.67	51.51	19.94
	P3	22.63	49.25	19.06
My	P0	24.91	54.22	14.75
	P1	27.71	60.32	16.19
	P2	29.58	64.38	16.75
	P3	26.57	57.83	15.13
Pho	P0	23.46	51.05	13.44
	P1	25.43	55.35	15.31
	P2	28.34	61.67	16.00
	P3	24.19	52.63	14.56
Mi+My	P0	27.82	60.54	17.11
	P1	30.21	65.74	20.30
	P2	31.66	68.90	20.94
	P3	28.58	62.16	19.38
Mi+Pho	P0	30.31	65.96	19.75
	P1	33.94	73.87	22.75
	P2	33.50	72.97	21.31
	P3	31.97	69.58	20.13
LSD at 5%		0.05	0.27	0.81

Co: Control Mi: Microbin My: Mycorrhiza Pho: Phosphorin
 Mi+My: Microbin + Mycorrhiza Mi+Pho: Microbin + Phosphorin
 P0: without phosphorus P1: 50% from the recommended dose
 P2: 75% from the recommended dose P3: 100% from the recommended dose

Effect of biofertilizers on N, P and K contents of faba bean seeds:-

Data in Table 4 represent the mean values of N, P and K contents (mg plant^{-1}) in faba bean seeds at harvest as affected by biofertilization. Significant increase in the mean values of N, P and K contents can be noticed under the microbial inoculation treatments when compared with the control. Microbial inoculation with (Microbin + Microhyiza) showed the highest values of N, P and K contents in seed yield (62.31, 4.50, and 56.82 mg plant^{-1} ; respectively).

Effect of phosphorus fertilization on N, P and K contents of faba bean seeds:-

The mean values of N, P and K contents were also significantly increased as a result of P-fertilization. The highest values were observed under the addition of 75 % from the recommended dose of calcium superphosphate. These values were 50.00, 3.58 and 45.55 mg plant^{-1} for N, P and K; respectively. Similar results were recorded by El-Shamma *et al.*,

(2000), Ismael (2001), Abou Hussien *et al.*, (2002), Abdalla (2002), and Wenxue *et al.*, (2003).

Table 4: Effect of biofertilization and P-fertilization on N, P and K contents (mg plant⁻¹) in faba bean seeds at harvest.

Biofertilizer	Nutrient contents in seeds (mg plant ⁻¹)		
	N	P	K
Co	27.25	1.97	25.06
Mi	44.19	3.18	40.17
My	46.56	3.33	42.70
Pho	39.28	2.83	36.08
Mi+My	62.31	4.50	56.82
Mi+Pho	57.91	4.19	53.45
LSD at 5%	1.75	0.14	1.56
P-Fertilizer	Nutrient contents (mg plant ⁻¹)		
	N	P	K
P0	39.61	2.87	36.52
P1	45.91	3.32	42.19
P2	50.00	3.58	45.55
P3	49.48	3.57	45.26
LSD at 5%	1.46	0.12	1.49

Co: Control Mi: Microbin My: Mycorrhiza Pho: Phosphorin
 Mi+My: Microbin + Mycorrhiza Mi+Pho: Microbin + Phosphorin
 P0: without phosphorus P1: 50% from the recommended dose
 P2: 75% from the recommended dose P3: 100% from the recommended dose

The interaction effect between biofertilization and phosphorus fertilization:

Data in Table 5 illustrate the interaction effect between biofertilization and P-fertilization on N, P and K contents in faba bean seeds. Data indicate that contents of N, P, and K were significantly increased at the time of using biofertilization in combination with the addition of P-fertilizer. The highest values were recorded at the rate of 75% from the recommended dose of calcium super phosphate and inoculation with (Microbin + Mycorrhiza) treatment. These results were in agreement with those obtained by Han and Lee (2005) and Rafaat and Radwan (2006).

Table 5: The interaction effect between biofertilization and P-fertilization on N, P and K contents (mg plant⁻¹) in faba bean seeds at harvest.

Treatments		Nutrient contents in seeds (mg plant ⁻¹)		
Biofertilizers	P-Fertilization	N	P	K
Co	P0	24.09	1.78	22.11
	P1	25.42	1.83	23.17
	P2	27.71	2.03	25.62
	P3	31.78	2.24	29.34
Mi	P0	37.86	2.69	34.68
	P1	42.46	3.06	38.54
	P2	47.44	3.48	43.59
	P3	49.02	3.48	43.88
My	P0	40.13	2.96	36.82
	P1	46.13	3.26	42.48
	P2	52.13	3.69	47.48
	P3	47.85	3.42	44.03
Pho	P0	32.61	2.33	31.18
	P1	38.63	2.78	34.69
	P2	43.03	3.15	39.26
	P3	42.83	3.06	39.18
Mi+My	P0	53.63	3.82	48.23
	P1	61.64	4.54	57.32
	P2	68.38	4.87	61.65
	P3	65.59	4.78	60.08
M+iPho	P0	49.34	3.63	46.08
	P1	61.19	4.43	56.97
	P2	61.32	4.29	55.68
	P3	59.80	4.43	55.07
LSD at 5%		1.10	0.26	3.16

Co: Control Mi: Microbin My: Mycorrhiza Pho: Phosphorin
 Mi+My: Microbin + Mycorrhiza Mi+Pho: Microbin + Phosphorin
 P0: without phosphorus P1: 50% from the recommended dose
 P2: 75% from the recommended dose P3: 100% from the recommended dose

Available phosphorus in calcareous soil:-

Data presented in Table 6 show the effect of microbial inoculation under P-fertilization on the availability of soil phosphorus. Availability of soil P in the studied calcareous soil was highly affected by microbial inoculation. Available P was significantly increased with inoculation with (Microbin + Phosphorin) followed by (Microbin + Mycorrhiza). Data also reveal that values of available P were increased in the surface layer (0-15 cm) and decreased in the subsurface layers (15-30 cm) and (30-45 cm). These data are in harmony with those obtained by Rafaat and Radwan (2006).

Table 6:- Available P in calcareous soil as affected by the interaction between biofertilization and P-fertilization.

Treatments		Available P (ppm)		
Biofertilizers	P-fertilization	(0-15 cm)	(15-30 cm)	(30-45cm)
Co	0	5.40	3.20	1.90
	P1	7.56	4.16	2.28
	P2	9.83	4.99	2.62
	P3	11.79	5.74	2.88
Mi	0	8.10	5.67	3.24
	P1	12.15	7.75	4.08
	P2	13.77	8.26	5.12
	P3	14.58	10.33	5.42
My	0	8.64	5.18	2.59
	P1	13.82	6.91	3.32
	P2	15.55	7.09	3.41
	P3	15.98	7.42	3.50
Pho	0	8.37	5.02	2.51
	P1	12.97	7.26	3.27
	P2	14.65	8.06	3.37
	P3	15.07	7.93	3.40
Mi+My	0	9.18	6.21	3.69
	P1	15.15	8.29	4.13
	P2	16.98	8.51	4.26
	P3	17.44	8.88	4.38
Mi+Pho	0	9.72	7.43	4.32
	P1	16.51	9.94	4.85
	P2	17.99	10.17	5.06
	P3	18.14	10.65	5.15

Co: Control Mi: Microbin My: Mycorrhiza Pho: Phosphorin
 Mi+My: Microbin + Mycorrhiza Mi+Pho: Microbin + Phosphorin
 P0: without phosphorus P1: 50% from the recommended dose
 P2: 75% from the recommended dose P3: 100% from the recommended dose

The previous data can be discussed as follow:-

- Treating seeds with (Microbin+Mycorrhiza) and (Microbin + Phosphorin) increased yield, yield components, and crude protein as well as N, P and K contents. This could be due to the large amounts of microorganisms in the studied biofertilizers; which may result in increasing soil fertility and availability of soil nutrients.
- Microbin provides nitrogen and increases plant growth and yield. It also helps plants to absorb nutrients in available forms leading to increasing yield and yield components as well as the uptake of N&K.
- The benefit of mycorrhiza to plants is mainly attributed to increase contents of nutrients, especially phosphorus. This may be due to the increase in surface area of soil contact, movement of nutrients into mycorrhiza, modification of the root environment, and increase in nutrient storage. Mycorrhiza can be much more efficient than plant roots at taking up phosphorus.

- Phosphorin contains phosphate solubilizing bacteria (PSB) that can convert triple calcium phosphate from unavailable form to a soluble one that can be easily absorbed by plants. It also decreases the addition of mineral fertilization to the soil.
- Biofertilization increases the availability of phosphorus in the soil especially calcareous soil where P is fixed due to high pH and significant quantities of free excess lime (calcium or magnesium carbonate). It also reduces soil, plant, and water pollution resulted from mineral fertilizers

CONCLUSION

It could be concluded that microorganisms (Mycorrhiza, Phosphorin and Microbin) have a significant role in converting unavailable forms of soil phosphorus into a readily available form to plant uptake especially under calcareous soil conditions. Biofertilizers also resulted in a significant increase in seed yield, weight of 100 seeds and crude protein in faba bean seeds as well as N, P, and K contents. Biofertilizers may contribute by about 50% from the required amount of super phosphate. However, further investigation is required under field condition.

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تأثير الكائنات الحية المذيبة للفوسفات على الفوسفور الميسر ومحصول الفول البلدى تحت ظروف الأراضى الجيرية.

خالد حسن الحامدى ، عبد الحميد أحمد النجار و منى أحمد الجندى
قسم علوم الأراضى - كلية الزراعة - جامعة المنصورة

تعانى الأراضى الجيرية والصودية من نقص الفوسفور وذلك لتكون صور قليلة الذوبان من معادن فوسفات الكالسيوم ، ويهدف هذا العمل إلى دراسة تأثير الكائنات الحية المذيبة للفوسفات على تيسر الفوسفور بالتربة ومحصول الفول البلدى تحت ظروف الأراضى الجيرية ولهذا الغرض :-
أجريت تجربة أصص خلال الموسم الشتوي 2011/2010 تحت الصوبة الخاصة بمزرعة كلية الزراعة جامعة المنصورة. واشتملت التجربة على 24 معاملة موزعة فى تصميم قطع منشقة. وهذه المعاملات هى محصلة ست معاملات من التسميد الحيوى وهى (الكتنول ، ميكروبيين ، ميكروهيزا ، فوسفورين ، ميكروبيين + ميكروهيزا ، ميكروبيين + فوسفورين) وأربعة مستويات من السماد الفوسفاتى فى صورة سويفر فوسفات الكالسيوم وهى (0 ، 50 ، 75 ، 100 %) من الموصى به موزعة فى ثلاث مكررات وبذلك يصبح عدد الأصص 72 أصيصاً للتجربة.

ويمكن تلخيص النتائج كما يلى :-

- يتضح أن التسميد الحيوى سواء كان بمفرده أو بالإضافة لمعدلات السماد المعدنى خاصة عند 50% و 75% من الموصى به يزيد من المحصول ومكوناته ونسبة البروتين الخام زيادة معنوية كما أنه يزيد محتوى البذور من عناصر (النتروجين ، الفوسفور والبوتاسيوم) بصورة أكبر مقارنة بالبذور غير المعاملة بالسماد الحيوى .
- أثبتت النتائج أن التسميد الحيوى وخاصة إضافة (الميكروبيين + الميكروهيزا) و (الميكروبيين + الفوسفورين) أدى إلى وجود أعلى زيادة معنوية فى القيم .
- إضافة السماد الحيوى له دور فعال فى تيسير عنصر الفوسفور مما يزيد من صلاحيته لإمتصاص النبات خاصة تحت ظروف الأراضى الجيرية والتي تتصف بوجود كربونات كالسيوم بنسبة عالية كما أن رقم الـ pH بها أعلى من 8.5 مما يزيد تثبيت الفوسفور بها.

الإستنتاج :-

إستخدام السماد الحيوى خاصة (الميكروبيين + الميكروهيزا) ، (الميكروبيين + الفوسفورين) بالإضافة إلى السماد المعدنى للأراضى الجيرية يزيد من صلاحية العناصر وبالتالي زيادة محصول نبات الفول ومكوناته.

قام بتحكيم البحث

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