IMPROVEMENT OF RHIZOBIAL INOCULANTS OF FABA BEAN IN EGYPTIAN SOILS

By

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B.Sc.Agric.Sc. (Agric. Microbiology), Ain Shams University, 2010

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ABSTRACT

Samar Al Refaey Abd El Salam Ahmed. "Improvement of Rhizobial Inoculants of Faba Bean in Egyptian Soils". Unpublished M.Sc. Thesis, Department of Agric. Microbiology. Faculty of Agriculture, Ain shams University, 2017.

This study aims to improve the quality of *Rhizobium* spp. biofertilizers by using polymeric alginate formulation inocula as an alternative support and selected rhizobiophages resistant mutant rhizobia isolates. Obtain results revealed that, more than twenty five *Rhizobium* isolates were collected fresh young nodules developed on faba bean (*Vicia faba*) seedlings from six different regions in Egypt. All isolates were confirmed as rhizobia after the authentication test in growth pouch and pot experiment under controlled environmental conditions. Amongst these 25 isolates, five representative isolates were selected to examine their growth characteristics. Measurements *in planta* showed that the five rhizobia isolates induced the formation of the effective nodules on the roots of the original host grown in sand culture after days of inoculation were selected to evaluate the effects of the inoculant formulation on performance of Faba bean plants. Different types of inoculants were prepared.

On the other hand rhizobiophages were also isolated and purified as single plaques, from rhizospheric soil samples representing different leguminous plants collected from Qalubia governorate using Egyptian local bacterial isolates as test organisms. The occurrence of phages in the field was studied on indicator isolates and the crude lysate of rhizobiophages was assayed qualitatively and quantitatively. High titer rhizobiophages suspension was prepared and the host range specificity and the morphological characteristics of rhizobiophages particles were also studied.

A pot experiment was carried out to evaluate different inoculant formulation in faba bean growth, nodulation, N_2-fixation and their susceptibility to rhizobiophages. Obtained results prove that using QR19 or GR24 *Rhizobium* alginate polymer inoculant had increase effect on plant

growth parameters; shoot and nodules dry weights, total nitrogen and protein contents, N_2-ase activity and leghemoglobin content that compared to non-inoculation, but using GR24 *Rhizobium* better than using QR19 *Rhizobium* alginate polymer inoculant. Data shows no differences between the plant growth, the N_2-fixation activity parameters, between the *Rhizobium* alginate plus charcoal polymer inoculant with or without rhizobiophages. However, *Rhizobium* inoculant in polymer alginate plus charcoal, with or without Rhizobiophages, has a positive effect on the protection of *Rhizobium* cells from rhizobiophages attack. This finding was also detected with fresh *Rhizobium* liquid inoculant. Using *Rhizobium* alginate polymer inoculant without rhizobiophages had non increase effect on plant growth parameters compared to witht rhizobiophages. This may be due to a mechanical increase in bacteria defend themselves against rhizobiophages, which is considered an oxidizer vital (biological) factor.

Key words: *Rhizobium* spp., *Vicia faba*, inocula formulation, alginate, rhizobiophages.

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LIST OF ABBRIVIATIONS

Abbriviate	Name
BNF	Biological nitrogen fixation
SNF	Symbiotic nitrogen fixation
N_2-ase	Nitrogenase
IRA	Intrinsic resistance antibiotics
PGPR	Plant growth promoting rhizobacteria
IAA	Indole acetic acid
MLSA	Multilocus sequence analysis
AMF	Arbuscular mycorrhiza fungi
PSB	Phosphate solubilizing bacteria
CFU	Colony forming units
PFU	Plaque forming units
EC	Electrical conductivity
YEMA	Yeast extract mannitol agar
YEMB	Yeast extract mannitol broth

INTRODUCTION

Faba bean (*Vicia faba* L.) is a major leguminous crop grown around the world and is most intensively cultivated in the North East Africa **(Bond, 1976)**. In Egypt, faba bean is considered as one of the most important food legumes and plays a major role in the Egyptian diets as a source of protein. Its seeds have 35% protein, 45% carbohydrate and 2 % fat. Moreover, it is used as forage crop for animals, and for available nitrogen in the biosphere **(Crépona *et al.*, 2010)**. The cultivated area of faba bean decreased in the last 3 years in Egypt from 34871 to 26700 ha. *Rhizobium* is able to form nitrogen-fixing root nodules as result of symbiosis with legumes and this permit plant growth in the absence of exogenous N fertilizers. This symbiosis is very important and accounts for 50% of 175 million tons of total biological nitrogen fixation used in agriculture. Inoculation of legumes with rhizobial strains selected for high N_2-fixing capacity can improve nitrogen fixation in agriculture, particularly when native rhizobial strains are absent from soils or ineffective. However, newly introduced strains often fail to compete with well-adapted indigenous populations **(Sessitsch *et al.*, 2002b)**. Most rhizobia research focused on the bacterial genetics and physiology and less than 1 % of research articles on rhizobia have focused on formulation aspects of inoculants. However, there is a real need for improved formulations of inoculants, to create and commercialize new bio fertilizers that will be more effective, more stable over time, of better quality, and meeting farmers' needs **(Malusá *et al.*, 2012)**.

Rhizobium-based biofertilizers, available in liquid form, are commonly used as liquid inoculants are more suitable for mechanical planting. On the other hand, worldwide, the use of peat as a bacterial carrier has decreased due to its fossil origin and that is extracted from a fragile natural environment. Therefore, alternative materials such as polymers are being widely studied to improve the quality and efficiency

of rhizobial inoculants and to reduce production costs, as well as their impact on the environment **(Herrmann and Lesueur 2013)**. Polymers and individual compound formulations have been assessed as innovative carriers for rhizobia **(Girgis and Mostafa 2002)**, as well as related bacterial species. Rhizobia can show wide variations in numerous characteristics; abiotic and biotic i.e., phage susceptibility stress factors. Phages are believed to be responsible for the intensity and spatially varying selection pressure on their host and vice versa. The presence of rhizobiophages in soils suggests that through selection or elimination of certain types of *Rhizobium* bacteria, the rhizobiophages influence the evolution of bacterial populations. The common presence of rhizobiophages in soil suggests that they can affect the outcome of *Rhizobium*-legume symbiosis. However, presence of rhizobiophages in bacterial inoculum mixture did not affect the symbiotic efficiency. **Herrmann and Lesueur, (2013)** reported that one of the key issues in formulation development and production is the quality control of the products, at each stage of the process.