MIXED BIOFERTILIZATION IN RELATION TO GROWTH OF SOME CROPS UNDER EGYPTIAN AGRICULTURAL CONDITIONS

BY

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DEDICATION

I dedicate this work to the dearest people in my life.

*To my dear father ,may **GOD** rest his soul, and my dear mother ,for every thing they have done for me and their prayers.*

*To my husband ,my daughter and my son for their patience ,continuous care and love. I am thanking **GOD** for their being in my life.*

To beloved brothers and sisters for their love and support.

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Name of Candidate: Mounira Moawad Bishara **Degree:** Ph.D.
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ABSTRACT

Seed and grain germination, plant growth under controlled conditions in a sandy soil or under field conditions as affected by single or mixed biofertilizers viz cyanobacteria (Cy) *Azolla* (AP) yeasts (*Saccharomyces cerevisiae* and *Kluyvromyces marxianus*) and Arbuscular mycorrhizae (AMF) or effective microorganisms (EM) were the main target of this work.

Germination percentages and development of radicle and epicotyl were raised and improved by inoculation with the biofertilizers, however, the magnitude of stimulation depended both of organism introduced and plant genotype. Wheat was the most responsive to biofertilizers followed by faba bean, where as the onion was the poorest in this respect. Plant growth parameters, plant heights, dry weights of shoots and roots, percentages and contents of plant components of either macro (N, P, K) and microntrients (Fe, Mn, Zn & Cu) as well as mycorrhizal root colonization and their spore numbers in addition to soil biological activities (dehydrogenase and CO_2 evolution) were all estimated at different growth periods on wheat grown in pots. The same estimations in addition, yields of grains or seeds and straws of both wheat and faba bean were all determined in plants kept to harvest stages.

The highest values of all growth parameters were obtained by plants supplied with the mixture of Ap + Cy + AMF + Y followed by other dual or triple inocula, which , in most cases, superseded the stimulative of either individually.

The mixture of both N_2-fixers (Ap + Cy) induced richness of N-percentages and contents in plant tissues. The biofertilization was most effective on wheat followed by faba bean in the field. Coinciding this behaviour,AMF either alone or in conjunction with other microorganisms significantly raised the percentages and contents of P and ,micronutrients in plant tissues as well as root colonization and mycorrhizal spore counts in resulted the rhizospheric soil. The mixture of (AMF + Y) in different levels of increases in N, P, K contents as well as the various micronutrients (Fe, Zn, Mn, Cu).

Key words: *Azolla* , Cyanobacteria, Yeast , Arbuscular mycorrhizae fungi , Effective Microorganisms , Biofertilization , N_2 fixers.

CONTENTS

INTRODUCTION

Cultivation of economic crops particularly those genetically engineered with high yields consume a major share of the chemical fertilizers that lead to accelerated exhaustion of macro-and micronutrients leading to nutritional imbalance and depletion of soil fertility in terms crop productivity. The heavy application of either chemical fertilizers result in an environmental pollution, in addition to their expensive costs of their prices, handling and application in field. Among the plant nutrients, N and P are the two key plant nutrients. Nitrogen is the first among nutrients, however it is exposed to removal from soil by different biological activities, *e.g.* plant absorption, microbial transformation to a leachable or volatile forms. Phosphorus represents an important macronutrient either in plant nutrition, or in the different microbial activities. Therefore, phosphatic fertilizer as P sources are applied to soil to meet the crop demands and all other biological activities. After its application in tropical soil like Egypt, a major part of P is fixed and becomes less available for uptake by the growing plants. Therefore, sufficient quantities of N and P must be introduced to soil to keep or increase its crop productivity. Such practice leads to accumulation of environmental hazards, either in soil, air or water, most of which results in human risks through the wide distribution of versatile diseases.

To overcome these problems, or at least most of them, the latter part of the last century witnessed the emergence of concept of “organic agriculture” advocating minimum use of chemical fertilizers and increasing dependence on biological inputs like compost, farmyard manure, green manure and biofertilizers. Amongst the array of biofertilizers developed for different crops are *Azolla* - *Anabaena* and cyanobacteria as biological nitrogen fixing systems which can replace or at least reduce application of nitrogenous fertilization. Arbuscular mycorrhizal fungus, facilitates the plant, absorption of P and some other plant nutrients *e.g.* Fe, Mn, Zn and Cu …..*etc.* Yeasts, play an important role in producing certain growth promoting substances as hormones, amino acids, vitamins and enzymes. Effective microorganisms (EM), enhance the soil biological and chemical activities, improve the source of essential nutrients supply and some positive interaction with chemical fertilizers through increasing their efficiency and thereby reducing the environmental hazards. The product may have a role in humus formation and in controlling of certain plant diseases and pathogens (Myint ,1999).

The utilization of some biofertilizers either individually or in different combinations was the main goal of this work. The biofertilizers include, *Azolla pinnata*, N_2- fixing cyanobacteria, *Saccharomyces cerecisiae*, *Kluyveromyces marxianus*, Arbuscular mycoorhizal fungi and/or effective microorganisms. The influence of each or their mixtures was determined in seed-germination as well as on plant growth under controlled conditions in pots or under field conditions.

REVIEW OF LITERATURE

Biofertilizers as bacteria, fungi, yeasts and actinomycets play a crucial role in the reduction of inorganic fertilizers or chemical pesticides during growth of various crops.

The multiple benefits may include one or more of: increasing fungicide tolerance, increasing the production of chitinases, B-gluconases, antibiotics, siderophores, induction of systemic resistance in plants increasing phosphate solubilization, enhancing N_2-fixation or plant growth promoting substances .

1. Effect of inoculation with some biofertilizers on plant growth and yield production of some crops

There are many organisms having the capability of N_2-fixation either in soil, aquatic habitats or sharing with certain plants. For this, the review will be concentrated on those types which have been utilized as biofertilizers in our work, *i.e. Azola* and N_2-fixation Cyanobacteria.

a. *Azolla pinnata* (Ap)

Azolla is a genus of free small aquatic heterosporous petriodophytes: *Azolla* plant (green gold mine) (Wagner,1997) is triangular or polygonal in shape and bear deeply bilobed leaves and adventitious roots.

Azolla sporophyte consists of horizontal to vertical main rhizome bearing individual roots or root bundles at branch points and alternately arranged bilobed leaves with an endophytic cyanobacterium .The dorsal lobes are chlorophyllous and contain the symbiotic cyanobacterium

Anabaena Azollae within an ovoid cavity connected to the atmosphere by a pore. The translucent ventral lobes resting on the water surface support the frond and are nearly achlorophyllous (Peters *et al,*.1976 and Wagner, 1997).

The *Azolla* – *Anabaena azollae* symbiosis is an important N_2-fixating between eukaryotic fern and prokaryotic cyanobacterium. This host symbiont combination is exploited as biofertilizer for many economic crops (Singh, 1998 and Sharma *et al.,*1999).

Anabaena contain cells known as heterocysts that are specifically responsible for N_2-fixation. It has been found that in non symbiotic species *Anabaena* that have the capability of N_2-fixation. The heterocyst frequency never makes up more than 3 to 5% of the cells and yet in nature *Azolla* leaves, they compose up to more than 30% of the cell numbers of the symbiont (Van Hove, 1989).

Favilli *et al.* (1988) stated that in pot experiment,wheat,sunflowers and maize were given 100.80 or 40kg $N.ha^{-1}$ respectively, without *Azolla* or *Azolla* incorporated at the rate equivalent to 8 or 16 t. fresh weight. ha^{-1}, with a concomitant reduction in meniral N rate of 20 kg N per each 8 t. *Azolla*. *Azolla* increased roots and shoots and their N-contents after 60 days in the three crops.

Mahapatra and Sharma (1989) found that the application of *Azolla* with *Sesbania* had beneficial residual effects on subsequent wheat crop raising grain yield by 56 – 69 % over control.

Kolhe and Mittra (1990) applied *Azolla* as a mono crop between the wheat and rice crops, or added as an intercrop with rice. It has significant beneficial effects on subsequent wheat crop. They found that, *Azolla* with urea –N gave average grain yield of rice as well as maximum residual effect on wheat by 63% greater grain yield than the control.

Marwaha *et al.* (1992) found that, *Azolla* increased grain and straw yields of wheat, however, number of tillers per plant were largely unaffected Ram *et al.* (1994), observed that the incorporation of *Azolla* into the soil significantly increased its water holding capacity, organic carbon ammonium and nitrate nitrogen, and available P, K, Ca and Mg, while it decreased pH and bulk density, meanwhile significantly raised the yield of mung-beans.

El- Bassel and Ghazi (1996) reported that using *Azolla* as a biofertilizer, substituted 50% of the inorganic N-fertiliizer and resulted in a marked improvement in the rice crop and higher quality than the control. Sharma and Jain (1997) used *Azolla* or cyanobacteria with green manuring for transplanted rice, a practice that can partially replaces N-fertilizer and improves the soil properties. Integrated use of organic and inorganic sources of nutrients increased the production and returns in the rice wheat sequence and maintained sustainable production on a long-term basis.

Adel *et al.* (2000) observed that, the highest grain yield, 1000 grain weight and crude proteins of wheat were obtained by applied mineral N (75 $kg.fed^{-1}$) and *Azolla* (25 $kgN.fed^{-1}$). While, the highest yield of straw and its micronutrient contents were obtained with mineral N(75kg. fed^{-1}) and *Azolla* (50kg $N.fed^{-1}$) respectively.

Ripley *et al.* (2003) studied the response of wheat to soil application of varying proportions of *Azolla filiculoides* (Af) subjected to 3 pretreatments (heated, fresh of dry). Wheat grown in acid – washed sand mixed with 80% (v/v) air dried (Af) recorded a significantly out performed plants. When the top soil was mixed with 20 or 80% Af with the N P K fertilizer higher wheat yields were gained. The authors added that, the invasive aquatic fern (Af) can be considered a potentially biofertilizer.

Gowda *et al.* (2004) indicated that the application of 10 t. ha^{-1} fresh *Azolla* and supplementary chemical N or recommended dose of chemical. N, recorded significantly maximum yield of rice, plant height, tillers and panicles during kharif as well as during rabi season.

Samal (2004) reported that, *Azolla* (10t. ha^{-1}) with 60 kg N. gave maximum grain yields with an increase of 56% over the uninoculated ones and gave higher number of tillers and panicle.

Kar *et al.* (2005) reported that, most of the physiological and biochemical putative traits excelled in *Azolla* treated rice plants compared with other treatments. However, number and weight of panicles, number of spikelets and filled grains were increased in *Azolla.* Treated plants compared to those that received recommended dose of chemical N-Grain –protein was increased by 5 to 7% and 29% with *Azolla* + 50% N + Full PK compared to that of recommended dose of chemical fertilizers and control received farmyard manure respectively.

El. Shahat *et al.* (2006) revealed that the use of *Azolla pinnata* either as dry of fresh enhanced most of rice yield components specially grain and straw yields.

Girgis *et al.* (2006) reported that , the use of both dry and fresh *Azolla pinnata* alone or combined with urea had no significant effect on height, number of panicle, and straw of wheat, fresh *Azolla* achieved slightly higher values. Both forms of *Azolla* led to decrease in pH and E.C., but increased organic matter content compared with control but soil water holding capacity was increased in the dry treatment. Fresh *Azolla* increased the density of total viable bacteria, azotobacters and azospirilla as well as CO_2 evolution.

b. Cyanobacteria (Cy)

Gupta and Seth (1990) revealed that, inoculation with cyanobacteria increased the crop yield of *Triticum aestivum,Zea mays* and *Brassica compestres, Cicer arientnum, Vigna mungo* and *Pisum sativum.*

Gantar *et al.* (1991 a and b) reported that, the heterocytous cyanobacteria is able to form tight association with wheat roots and to penetrate both root epidermis and cortical intracellular space.

Abd-Alla *et al.* (1994) demonstrated that live cyanobacteria alone or with K, P and S significantly increased wheat growth parameters. Cyanobacteria either live or killed led to a significant increase in dry matter accumulation and N-contents.

Inderjit and Dakshini (1997), found that, cyanobacterial inoculated soils, sometimes had significantly different values for