Role of Biofertilizers in Maintaining and Restoring Soil Fertility as a Main Aid for Sustainable Increase in Crop Production

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Recently, higher doses of mineral fertilizers are applied to all soil types of different fertility. Nobody can doubt that this practice in modern agriculture has led to significant increases in the yields of various crops, and is worthwhile from the economic point of view, rather than for the quality of such crops or hygiene.

The lack of organic manuring, specially with farmyard manure, coinciding with the dense application of mineral fertilizers, significantly affected the fertility status and the distribution of microbial activity in various soils. The author pointed out this fact as early as 1964, when he recorded a close relation between organic matter content and microbial activity. Soils which received a continuous application of farmyard manure showed the highest index of fertility, i.e. total microbial count and specific groups, production of CO_2, ammonification, nitrification and cellulose degradation rates as compared with soils receiving a continuous application of N, NP and NPK.

The beneficial role of organic manuring in maintaining and restoring soil fertility and in rendering insoluble nutrients available to plants is well-known. This phenomenon is clear, and in this respect, mention can also be made of the softness of vegetables and fruits due to the depletion of soluble calcium in soils, leading to the rapid deterioration of such crops.

Biofertilizers with different commercial names are now produced and applied in many countries. They are mainly either nitrogen fixers (Rhizobia, Azotobacter, Azospirillum and Algae) or phosphate solubilizing microorganisms. The use of these biofertilizers could be a cheap means of supplying plants with their nitrogen and phosphorus needs during growth, which in turn would decrease the doses of expensive mineral fertilizers applied, thus leading to a significant decrease in the production costs of various crops. In addition, pollution rates in the soil, water and air could be lowered as a result of this practice.

The present investigation summarized a series of experiments carried out in the laboratory, greenhouse and in the field on phosphate solubilizing microorganisms and their role in maintaining soil fertility, and the growth and yield of some crops.

Phosphorus is well-known to be one of the most important major elements. It is second only to nitrogen as a mineral nutrient required by both plants and microorganisms. It plays a major physiological role in certain essential steps in the accumulation and release of energy during cellular metabolism.

Several hundred strains of phosphate solubilizing bacteria isolated by IBRAHIM (1988) from the soils and rhizosphere of different plants showed great variations with respect to their activities. Among the most active strains 140 were identified as *B. megatherium,* 56 as *B. subtilis,* and 18 as *B. cereus.* A modified broth medium was recommended for culturing these strains. The log phase started early, within 4 hours of inoculation, and the peak of growth was reached after 72-96 hrs for shaken cultures incubated at 30 °C. The rate of P released reached up to 50 ppm during 24-hr intervals. An inverse relationship was recorded between the pH values and the amounts of phosphorus released, and a highly positive correlation ($R = 0.95$) was calculated between total acidity and soluble phosphorus released. This is in accordance with TAHA et al. (1969), who found negative and positive correlations between the pH and acidity, respectively, and the phosphorus released by P dissolving bacteria.

A chromatographic analysis of the organic acids formed in the culture media of *B. megatherium* showed citric, malic and fumaric acids, confirming the results of many authors (PAJPAI & RAO, 1971; MISHUSTIN, 1972).

Phosphorine

A phosphate solubilizing biofertilizer named Phosphorine has been prepared on the basis of the results recorded from experiments carried out for more than five years in the Faculty of Agriculture, Al-Azhar University. This biofertilizer is now available to farmers sponsored by the General Authority of the Agricultural Fund, Ministry of Agriculture. The price of one sachet containing 200-250 g inoculum (10^{-9-10} viable cells) is 1 Egyptian pound, equal to about 30 cents.

The method used to prepare Phosphorine biofertilizer can be summarized as follows:

Starter - The most active strains of *B. megatherium* are maintained on nutrient agar slants kept in the refrigerator and in sterilized soil culture. The strains are examined every three months for their activity in solubilizing rock phosphate. Starters are prepared in 250 ml conical flasks on modified medium amended with asparagine, incubated at 30 °C for 48-72 hrs using a stationary shaker.

Inoculum - The modified broth medium amended with asparagine is prepared in amounts of 5 L and in 10 L jars, where the pH is adjusted to 7.2-7.4

after sterilization using volumetric solutions of NaOH and $CaCl_2$. One conical flask of the starter (100 ml) is added to each jar. A plate count of the starter is used to give 10^{-10} to 10^{-12} viable cells per 1 ml medium.

A simple aeration technique is adopted using air pumps similar to those used in ornamental fish tanks. In addition, a stationary shaker with a capacity of 24 (10-litre) fermentor jars is also prepared for inocula preparation.

Carrier - Irish moss peat amended with different concentrations of activated charcoal, calcium carbonate and gum arabic proved, in our investigation, to be the most suitable carrier. Laboratory experiments showed the survival of the bacterial inoculum at high counts (10^{-8} - 10^{-10} viable cells) after more than 2 months of storage at room temperature.

Polyethylene bags supplied with the carrier (200 g) are prepared and sterilized with gamma irradiation by the Atomic Energy Authority, and stored ready for bacterial inoculation. This is carried out by adding 20 ml of the inoculum (72-96 hr -old culture) to each bag using a plastic syringe.

Application - In practice, the wetted seeds of various crops must be thoroughly mixed with Phosphorine just before cultivation. When growing trees, it is recommended to spread the biofertilizer around the root system, and for mulshed plants, the root system must be dipped for several minutes in emulsion previously prepared from the biofertilizer. In all cases, the contents of the bags must be mixed with a suitable amount of fine soil before use.

The commercial production of Phosphorine biofertilizer bags started in January 1991. A total of 154,200 bags were produced that year, and used by farmers.

The results of pot and field trials with Phosphorine as compared with fertilization with superphosphate are recorded in Tables 1-2. In this connection, it is worth mentioning that deteriorated 3-year-old apple trees cultivated in a clay loam soil near Tanta, Gharbia Governorate, showed good growth and development as a result of Phosphorine application. Naturally, this could be attributed to the stimulating effect of the growth promoting substances released by P solubilizing bacteria on root initiation and formation, rather than to the effect of soluble phosphorus. This was also proved by earlier investigations where higher amounts of GA and Indoles were chromatographically and biologically detected in the culture filtrates of *B. megatherium*, confirming the results previously recorded by EWEDA (1976).

Gains due to the inoculation of soybeans with Phosphorine reached 0.33, 0.41, 0.60 and 0.75 tons/feddan. Inoculation with Phosphorine alone gave a yield of 1.36 tons as compared with 1.09 tons due to the application of 200 kg superphosphate per feddan alone. Gains due to treating clover seeds with Phosphorine, and due to fertilization with 100 kg superphosphate reached 10.93 and 5.89 g dry matter yield, respectively, amounting to 55.7 and 30.0% increases. In a pot treatment of broad beans with Phosphorine and 100 kg superphosphate,

Table 1

Effect of inoculation with biofertilizer "Phosphorine" on mean yield of soybean (ton/feddan) and mean dry matter yield of wheat (g/pot)

Phos-phorine	Superphosphate, kg/feddan				Mean
	0	50	100	200	
Soybean					
- Inoc.	1.03	1.14	1.22	1.09	1.12
+ Inoc.	1.36	1.55	1.82	1.84	1.64
Mean	1.19	1.34	1.52	1.46	
Gain due to inoculation	0.33	0.41	0.60	0.75	
$LSD_{0.05}$	Superphosphate		0.12		
	Phosphorine		0.17		
Wheat					
- Inoc.	7.66	-	9.32	10.26	
+ Inoc.	9.30	-	9.88	11.48	
$LSD_{0.05}$	1.12				
Gain due to	1.64		0.56	1.22	
inoculation	21.4%		7.3%	15.9%	

dry matter yields were increased by 44.6 and 81.2% due to fertilization and inoculation, respectively. Similarly, the gain due to inoculation in wheat reached 49.4% as compared with 16.5% due to the application of 100 kg superphosphate per feddan. Maize yields showed significant increases of 18.2 and 47.2% due to phosphate fertilization and the application of Phosphorine, respectively. In other experiments, inoculation with Phosphorine increased the dry matter yields of wheat by 21.4, 7.3 and 15.8% and of broad beans by 46.9, 40.7 and 42.1% in treatments receiving 0, 100 and 200 kg superphosphate /feddan, respectively.

Conclusion

The application of Phosphorine is strongly recommended as seed dresser before the cultivation of various crops; this biofertilizer can also be spread around the root system of growing trees. Vegetative parts however, must be immersed in an emulsion of Phosphorine for a few minutes before planting. This practice will supply the growing plants with their phosphorus needs during growth, which in turn will decrease the necessary doses of expensive mineral phosphate fertilizers, leading to a significant decrease in the production costs of various crops. In addition, the pollution rates in soil, water and air could be lowered to a certain degree as a result of this practice.

Table 2

Effect of inoculation with biofertilizer "Phosphorine" on mean yield of maize, wheat and dry matter yield of clover and broad bean

	- Superphosphate - Phosphorine	+ Superphosphate - Phosphorine	- Superphosphate + Phosphorine
A. Maize			
Yield, kg/plot ($10m^2$)	1447	1711	2130
LSD $_{0.05}$	240		
Gain due to	fertilizer	264 kg	18.2%
	inoculation	683 kg	47.2%
B. Clover			
Yield, g/pot 1st cutting	6.12	7.80	8.34
2nd cutting	8.15	9.02	10.82
3rd cutting	5.36	8.70	11.40
Total yield	19.63	25.52	30.56
LSD $_{0.05}$	2.48		
Gain due to	fertilizer	5.89 g	30.0%
	inoculation	10.93 g	55.7%
C. Wheat			
Grain yield, g/pot	9.96	11.61	14.89
LSD $_{0.05}$	2.10		
Gain due to	fertilizer	1.65	16.5%
	inoculation	4.93	49.4%
D. Broad bean			
Yield, g/pot	8.05	11.64	14.59
LSD $_{0.05}$	3.01		
Gain due to	fertilizer	3.59	44.6%
	inoculation	6.54	81.2%

Superphosphate: 100 kg/feddan

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