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Effect of FZB 24® *Bacillus subtilis* as a biofertilizer on cotton yields in field tests

A. V. YAO[1], H. BOCHOW[2], S. KARIMOV[1], U. BOTUROV[3], S. SANGINBOY[3], & A. K. SHARIPOV[4]

[1]*German Agro Action, Dushanbe, Tajikistan,* [2]*Phytomedicin, Institute of Horticultural Science, Humboldt University Berlin, Germany,* [3]*Institute of Soil, Tajikistan, and* [4]*Dekhan Farm Sharif Sherin, Tajikistan*

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Abstract
In 2004, two field trials with cotton were conducted at two locations in Tajikistan to estimate the effectiveness of FZB 24® *Bacillus subtilis* (ABiTEP GmbH Berlin) as a biofertilizer for increasing the cotton yield in comparison to the utilization of the conventional fertilizer containing nitrogen (N), phosphorus (P) and potassium (K), with an application rate per ha 180 kg N, 120 kg P, 60 kg K. The following treatments of cotton cultivated as test variants each on 1 ha field under the same conditions were carried out and the effect on the plant growth, the development of number of bolls/plant and mainly the cotton fibre yield were calculated: (i) Seed treatment with water and only crop application of the NPK fertilizer; (ii) Only seed treatment with spore suspension of FZB 24 WG *Bacillus subtilis,* without application of the NPK fertilizer; (iii) Seed treatment with spore suspension of FZB 24 WG *Bacillus subtilis* plus crop application of the NPK fertilizer; and (iv) Only seed treatment with the comparable plant-growth-promoting rhizobacteria product Extrasol 55 (ARRIAM St Petersburg), without application of the fertilizer. Comparing cotton growth and yield of the variants in both field tests, there appeared a remarkable yield and growth increase (up to 30%) by the use of FZB 24 alone compared to the application of the NPK fertilizer. The additional application of the NPK fertilizer (Variant: FZB 24 + NPK) could not enlarge this plant productivity-promoting effect. Apparently under the test conditions the increase of the root system in size and capacity as well as the additional enzymatic mobilizing of organic bound phosphorus for the plant as activities from the *Bacillus subtilis* introduction was more important for the plant system than the limited direct input with N, P and K. The plant treatment alone with Extrasol 55 resulted in similar promotion of cotton growth and yield as FZB 24 when compared with the NPK plant fertilizing, showing that the activity of FZB 24 was not very specific. However the cotton field tests generally demonstrate for FZB 24 *Bacillus subtilis* a high ability to improve plant growth and yield based on increasing the capacity of roots to mobilize and take up nutrients and substances for overall reproductive plant fitness. The use and value for part-substitutions of some environmentally problematical mineral fertilizers in other and intensive plant production systems is discussed.

Keywords: Bacillus subtilis*, FZB 24 biofertilizing effects, cotton, yield, NPK fertilizing effects*

Introduction

Currently, there is an increasing interest in testing biologicals containing Plant Growth Promoting Rhizobacteria (PGPR) in many countries. The benefits from such root colonizing

Correspondence: Dr. Helmut Bochow, Springborn str. 136, 12487 Berlin, Germany. Tel: +4930 6369644.
E-mail: bochow-030@versanet.de or profbochowberlin@aol.com

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bacteria are the general stimulating effects on root and plant growth resulting in increasing fitness and yields and additionally some specific antagonistic influences on soil-borne pathogenic organisms. Thus, these biologicals act as very useful biofertilizers and biocontrol agents (Kloepper 2004). They are able to substitute, at least partly, applications of environmental problematical non-organic chemicals like specific nitrogen fertilizers and fungicides and also bring monetary advantages for the farmers since they are less costly than the conventionally used chemicals.

Some of the most effective PGPR are isolates of the genus *Bacillus*, mainly of the group *Bacillus subtilis* (Backman et al. 1994; Kloepper 2004). An internationally used biofertilizer on that base is FZB 24® *Bacillus subtilis*, produced formerly by FZB Biotechnik GmbH, Berlin, and now by ABiTEP GmbH, Berlin. The mode of action of FZB 24 in its activities for plant growth promotion and as a biocontrol agent are well studied and many successful practical experiences with applications in agricultural (potatoes, cereals, corn) and horticultural (vegetables, ornamentals) crops have been collected (Krebs et al. 1998; Schmiedeknecht et al. 1998; Bochow & Dolej 1999; Grosch et al. 1999; Kilian et al. 2000; Schmiedeknecht et al. 2001; Bochow et al. 2001; Foos 2005).

Besides the production of antibiotics and plant resistance inducers as activities in disease and pathogen-controlling effects (Kilian et al. 2000; Koumoutsi 2004), it has been recently shown that phytohormon (auxin)-like actions and an extracellular phytase activity, mobilize phosphorus from the organic compound phytate, in FZB 24 and related *Bacillus* isolates as the main modes of action in plant growth-promotion and biofertilization (Idris 2002; Idris et al. 2002, 2004).

Of course, the effectiveness of such plant fertilizing activities depends upon the soil and climatic conditions where the product is used. As a base for estimation, the value of a comparison can be made between the application of the biofertilizer and the use of conventional chemical fertilizers in a given agro-system. With FZB 24, we have carried this out in cotton fields of Tajikistan. The relative juvenile soil from the view of cultivation in Tajikistan, which has been characterized as a phosphorus poor soil, presents a great opportunity for testing *B. subtilis* since the bacteria are able to enhance the availability of limited organically-created nutrients such as carbon, nitrogen, phosphorus and amino acids to the plant.

Two field trials were conducted in 2004 in Tajikistan, one in Dangara district and the other in Rudaki district. This was done in order to test the efficacy of cotton seed pretreated with *B. subtilis* FZB 24 upon plant yields as well as the average number of bolls formed per plant, in comparison to the control treatment yield increase resulting from only an application of conventional fertilizer consisting of nitrogen, phosphorus and potassium. In the Rudaki field test additional Extrasol 55, containing nitrogen-fixing bacteria and their metabolites and active in biocontrol plus plant growth promotion (produced by ARRIAM, St Petersburg, Russia) was applied for comparison.

Material and methods

Field trial in Dangara district in 2004

The test crop cotton was cultivated in the trial area on soil, which can be characterized as lightly grey soil with humus horizon of about 1.8%. As a variety, a local one called Fergona 3 was used, which was selected as reproduction seed material for this experiment, due to characteristics making it suitable to local Tajik conditions. Seed amount was 70 kg/ha, sown on 15 April 2004. During the growth period, the plants were regularly watered by metered channel irrigation 4 times. In order to keep the trial field clean from weed plants during the vegetation period, 9 cultivations were carried out on 27 June, 29 June, 3 July, 7 July, 11 July, 15 July, 20 July, 18 August and 22 August, 2004, which correspond to phenological phases.

Standard chemical fertilization was implemented by application of a conventional fertilizer in the cotton seedling vegetation period at following rate: 180 kg N, 120 kg P and 60 kg K. After 4 months' cultivation, the cotton harvest was carried out in three different periods: 21 August, 27 September and 11 October, 2004. The average yield in $t\,ha^{-1}$ was calculated and the number of bolls per randomly selected plants was counted.

The *B. subtilis* preparation FZB 24 WG with a titer 10^{11} spores g^{-1} was applied as seed dressing. Some 250 g of FZB 24 WG were diluted in 100 l water in a container, 70 kg cotton seed (amount for 1 ha) were added and left for 24 h in the suspension, then air dried and used for planting. As part of the experimental design, three variants of different treated cotton, (each variant 1 ha in size, under the same conditions of cultivation) were compared with the parameter average yield in $t\ ha^{-1}$ and average number of bolls per cotton plant as mean of 20 randomly selected plants in each variant.

- Variant 1: NPK – Cotton only treated with the conventional NPK fertilizer, seeds water dressed;
- Variant 2: FZB 24 – Cotton only with FZB 24 seed dressed, without chemical fertilizing;
- Variant 3: FZB 24 + NPK – Cotton with FZB 24 seed dressed and treated with the conventional NPK fertilizer.

The variant comparison of the measured yield values was calculated without special statistical analyses because the results were absolutely clear.

Field trial in Rudaki district

The test crop cotton was cultivated in the trial on soil characterized as dark serazium with low profile of humus about 1.5%. The selected variety was Mergon, also suitable for the local conditions. Seed amount was 70 kg ha^{-1}, sown on the 6 May 2004. During the growth period the plants were watered and weeded in the same manner as in the Dangara district. Also, the standard chemical fertilization was in agreement with the trial in the Dangara district using the same conventional NPK fertilizer with the same amount per ha and application method.

After 5 months' cultivation, the cotton harvest was carried out during three different periods: 5 October, 21 October 4 November, 2004, by calculating the average yield $t\ ha^{-1}$ and the mean number of bolls per plant during the maturity stage (9 October 2004). Additionally, the average height of the cotton plants was evaluated by measuring the main stalk length during various growth stages of the plants.

The preparation *B. subtilis* FZB 24 WG containing 10^{11} spores g^{-1} was applied as seed dressing in the same manner as described for the trial in Dangara.

Additionally, for comparison in the test, the bioagent Extrasol-55 biocontrol plus plant growth promotion (titre about 10^{8} bacteria/ml) was also applied by seed dressing. In a container filled with 100 l water, 140 ml Extrasol-55 were prepared, after mixing 70 kg of cotton (1)
seed were added and left for 2 h in the suspension. Following this, seeds were air dried and sown in the field.

The experimental design consisted of three variants of differently treated cotton, sized each 1 ha and cultivated under the same conditions to compare the productivity parameter mean plant height, average yield t/ha and the mean number of bolls per plant after enumerating in 20 randomly selected plants.

- Variant 1: NPK – Cotton only treated with the conventional NPK fertilizer, seeds water dressed;
- Variant 2: FZB 24 – Cotton only with FZB 24 seed dressed, without chemical fertilizing;
- Variant 3: Extrasol 55 – Cotton only with Extrasol 55 seed dressed, without chemical fertilizing.

Special statistical analyses were not conducted because the results were absolutely clear.

Results and discussion

Table I shows the yield and the productivity of cotton in three differently treated variants in the field test in the Dangara district. The data of the variants clearly demonstrates a remarkable yield and growth increase by the application of FZB 24 *B. subtilis* compared to the application of the conventional NPK fertilizer. The stimulating effect of the introduction of *B. subtilis* into the cotton plant system could not be enhanced by the additional application of the chemical fertilizer; the combination of both treatments brought the same result as the use of FZB 24 alone.

This fact indicates that under the given conditions on the juvenile Tajikistan soil with low phosphorus content or phosphorus which is embedded in the soil like other important plant nutrients (not easy accessible to the plants), the specific action of the biofertilizer FZB 24 with the PGPR was obviously more important for plant nutrition than the direct application of the fertilizer with nitrogen, phosphorus and potassium. The well-known action of *B. subtilis* after root colonization to stimulate the root growth by production of phytohormones like auxins (Kilian et al. 2000; Idris 2002; Idris et al. 2004), resulted in an increase of the root system in dimensions – size and activity, e.g. for uptake of nutrients, water and also interactions with other helpful root colonizers or inhabitants like mycorrhizal fungi. Additionally the observed ability of *B. subtilis* and related species (*B. amyloliquefaciens*) to produce the enzyme phytase, not existing in plants, can desegregate organic embedded phosphorus (phytate) in the soil liberating it for plant use and remarkably supporting the nutrition (Idris et al. 2002). Elemental phosphorus, for example, is responsible for plant growth rates and overall reproductive health since it is a basic component of nucleic acids and phospholipids. In conclusion, the test shows that biofertilizing with FZB 24 enhances growth and physiological activities of plants, resulting in improved use of important nutrients, including positive influences of its availability. This, FZB 24 plays a very important role for the general plant productivity and capacity, besides the direct plant fertilizing with nutrients.

The data of growth and yield of the cotton treatment variants in the Rudaki field test are given in Table II. A lower general level of cotton yield and productivity (bolls/plant) can be

Table I. Growth and yield of the differently treated cotton in the Dangara field test.

Variants	Average yield t ha^{-1}	Relative	Bolls/plant mean number	Relative
V. 1. NPK	1.85	100	9.0	100
V. 2. FZB 24	2.42	131	11.5	128
V. 3. FZB 24 + NPK	2.49	135	11.1	123

Table II. Growth and yield of the differently treated cotton in the Rudaki field test.

Variants	Average yield t ha^{-1}	Relative	Bolls/plant mean number	Relative	Mean plant height cm	Relative
V. 1. NPK	1.72	100	7.7	100	78.8	100
V. 2. FZB 24	2.25	131	9.2	119	87.2	111
V. 3. Extrasol 55	2.14	124	8.6	112	87.8	111

seen in that test, but in comparison of the variants, the sole use of FZB 24 to the sole application of the conventional NPK fertilizer, the remarkable yield-increasing effect of plant biofertilizing with FZB 24 is evident. With only a few lower degrees, the additionally tested bioagent Extrasol 55, containing other PGPR species with different mode of actions, induced a similar yield and plant growth increasing effect as FZB 24 when compared to the NPK variant.

This leads to the conclusion, that the specific biofertilizing activities of FZB 24, for instance, the phytase production desegregating phosphorus from organic bound phytate, which is unknown for Extrasol 55, are not absolutely dominant in its action. It seems that the activities of the PGPR, and consequently of the corresponding bioproducts, are more complex in nature and combinations, in which single actions can agree in different products.

But for FZB 24® *B. subtilis*, both cotton field trials clearly demonstrate a high value as biofertilizer improving plant growth and yield based on increasing the capacity of roots to mobilize and take up nutrients and substances for overall reproductive plant fitness. The degree of that value depends – beside climatic conditions – on the soil, its type and fertility and the presence and availability of plant nutrients. Under the Tajikistan conditions, the degree of this value was relatively high −30% more than a convenient and normal high NPK mineral fertilizer. But there are a number of experiences, also in very intensive European plant productions (e.g. potatoes) with a higher and optimized mineral plant fertilization, that under such conditions a yield increase can be actually induced too by additional applications of FZB 24, in lower degrees, of course (around 2–8%), but this clearly indicates that the assimilation of chemical plant fertilizers can be remarkably improved in that way (Bochow & Junge 2005).

In future, therefore, we aim to study the details of the actions in the biofertilizer containing FZB *B. subtilis* strains or related ones to find out to what degree environmental difficult mineral fertilizers (e.g. nitrogen) can be substituted by this biofertilizer in specific plant production systems.

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