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Associative Effect of the Rhizobacteria *Streptomyces chibaensis* and Commercial Biofertilizers on the Growth, Yield and Nutritional Value of *Vicia faba*

Maha A. Hewedy
Botany Dept., Women's College for Arts, Sciences and Education, Ain Shams Univ., Cairo, Egypt.
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ABSTRACT

A pot experiment was carried out to investigate the effect of the commercial biofertilizers Biogen (B) and Potassiomage (PG) as well as the rhizobacteria *Streptomyces chibaensis* on the growth of faba bean (*Vicia faba* CV. Giza 843). The experiment was undertaken through two successive growing seasons (2008/09, 2009/10). In the first season, three different doses of each inoculant were used to select the profitable dose while in the second season; the co-inoculation by the three profitable doses was used as inoculant for faba bean seeds. Data presented as, growth, yield parameters, seed content of minerals (N, P and K), protein; vitamins (ascorbic acid, riboflavin and thiamin) and carbohydrates, were estimated by applying the co-inoculant of the three biofertilizers. The highest values of all these items were obtained from the application of the Biogen + *S. chibaensis,* followed by Potassiomage + *S. chibaensis,* which also showed a biological control activity against fungal diseases.

Key words: Biofertilizers, *Vicia faba*, *Streptomyces chibaensis*, fungal diseases.

INTRODUCTION

Symbiotic nitrogen fixation is the result of a delicate balance between a higher plant and specific bacteria. Most of nitrogen–fixing prokaryotes are free–living microorganisms found in soil (Elahi *et al.*, 2004). Use of soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate or stimulate plant growth through synthesis of growth promoting substances by deliver a number of benefits including plant nutrition (Kaci *et al.*, 2005).

Legume seeds are one of the most important sources of food in the world, have made a significant contribution to the diet since ancient times. They are known to be a good and inexpensive source of protein, also rich in carbohydrates, some vitamins (thiamine, riboflavin ,niacin) and certain minerals (phosphorus, potassium and sodium), this was reported by Ejigui *et al.* (2005).

Faba bean is a legume which is used as a green vegetable dried, fresh or canned in the Middle East, Mediterranean region, China and Ethiopia. It is considered as a cash crop in Egypt and Sudan (El Wakiel and El Sebai, 2007). Several soil-borne fungi attack *Vicia faba* during its various growth stages. Disease control measures which have been applied to minimize infection with fungal disease usually include the use of biological control (Sallam *et al.*, 2008).

Biological nitrogen fixation is the key to sustain agricultural productivity through the application of bio-fertilizers in the field. Application of bio-fertilizers is an acceptable approach for higher crops with good quality and safe to human health.

The objectives of this study were to investigate the effect of bio-fertilizers, with the plant growth promoting rhizobacteria (PGPR) *Streptomyces chibaensis*, on growth and yield of faba bean as well their role in controlling soil-borne fungal diseases.

MATERIALS AND METHODS

Two pot experiments were conducted in sandy soil at the experimental garden of the Botany Department, Women's College, Ain Shams Univ., Heliopolis, Cairo, Egypt during the two growing seasons 2008/09 and 2009/10. Faba bean seeds, cultivar Giza 843, were obtained from the Agriculture Research Center (ARC), Ministry of Agriculture, Giza. Egypt.

Bio-fertilizers Used

Three types of bio-fertilizers were used; the two commercials Biogen and Potassiomage, obtained from Biofertilizers Unit, General Organization of Agriculture Equalization Fund (GOAEF), ARC, Giza, Egypt, and *S. chibaensis* which was isolated from Toushka's region Egyptian soil and maintained on starch-nitrate agar medium, characterized by Hewedy (2003).

Bio-fertilization treatments

Faba bean seeds were sown in pots 35cm diameter and 40 cm depth, each contained 5kg loamy–sandy soil. Five seeds/ pot (6 replicates) were used for each treatment.

First pots experiment was carried out during the winter season, 2008/09, consisted of four main groups:

- Un-inoculated seeds (Control).
- Seeds inoculated with Biogen (B), using three doses (B1 0.08g, B2 0.16g and B3 0.32g/pot).
- Inoculation with Potassiomage (PG), using three doses (PG1 0.04g, PG2 0.08g and PG3 0.16g/pot).

- Inoculation with the rhizobacteria *S. chibaensis* (S1 10^{-4}, S2 10^{-5} and S3 10^{-6} CFU/ ml) (20ml/pot). Biofertilizers inoculation treatments were undertaken post planting. The plants were irrigated weekly using tap water; supported dose was given after 2 weeks later for each pot.

Data were recorded at 60 days post sowing, 3 plants were randomly uprooted from each pot to determine: shoot fresh weight (SFW), shoot dry weight (SDW), number of flowers (NF) and number of pods (NP)/plant. At harvest (120 days after sowing), a random sample of 3 plants was taken from each treatment to determine: number of pods (NP), pods length (PL), fresh and dry weight of pods (FWP, DWP) and dry weight of seeds (DWS). In the second growing season (2009/10), the best doses of the previous treatments (B:0.32g and PG 0.16g.) were mixed and used as co-inoculant for faba bean seeds, in addition to the best conc. 10^{-4} of rhizobacteria (*S. chibaensis*) to determine the following growth parameters after 50 days: shoot and root dry weight (SDW and RDW), number of nodules (nN), dry weight of nodules (DWN) and acetylene reduction (ARA) (Hardy *et al.*, 1973). At plant maturity (120 days), the following data were determined: number of pods (NP), dry weight of pods (DWP), dry weight of seeds (DWS), and number of seeds (NS) all by ten plants.

Chemical analysis

Seed contents of N, P and K were determined according to AOAC (1975). Crude protein% of seeds was obtained by multiplying N% X 6.25, extraction and determination of total carbohydrates content were carried out according to Dubois *et al.* (1956).

Quantification of vitamins

Concentration of three vitamins (riboflavin, thiamine and ascorbic acid) was determined by using KNAUER Smartline HPLC system in the ARC, Plant Pathology Research Institute, Center Laboratory of Biotechnology, Giza, Egypt.

Statistical analyses

Obtained data were subjected to Analysis of Variance (ANOVA). L.S.D. test was used to compare the treatment means according to the procedures outlined by Snedecor and Cochran (1980) using MSTAT computer program software program (MSTAT Ver., 1.42).

RESULTS AND DISCUSSION

This work showed that application of bio-fertilizers, either single or mixed inocula, gave positive response to the studied plant parameters. Highest concentration at each treatment gave the best results; shoot fresh weight (SFW), shoot dry weight (SDW), number of flowers (NF) and number of pods (NP) at 60 days (Table 1) and NP, pod length (PL), fresh weight of pods (FWP), dry weight of pods (DWP) and dry weight of seeds (DWS) after 120 days (Table 2).

Nitrogen deficiency, indicated by brown pigment of leaves, was not observed at the high concentrations (0.33g of Biogen and 0.16g of Potassiomage and 10^{-4} CFU/ ml of *S. chibaensis*) but it was observed clearly on the untreated plants and lightly at the other plants treated with other concentrations of both bio-fertilizers and *S. chibaensis*.

A small round, slightly raised, cream colored spots occurred in late season only on leaves of untreated plants (control). The plants treated with different concentrations of *S. chibaensis* were found resistant to these fungal spots. Data illustrated in fig. (1) showed that all the applied concentrations of Biogen, Potassiomage and *S. chibaensis* led to decrease the number of non-healthy leaves resulted from soil-borne plant pathogens and highest values were obtained from the application of *S. chibaensis* 10^{-4}, while Biogen was the least effective. Thus, it is clear that *S. chibaensis* might play an important role in reducing chances of survival of pathogens and primary infections in the field. This is in accordance with the results obtained by Hewedy (2003) which indicated that *S. chibaensis* produced chitinase enzyme and HCN which played a major role against *Fusurium oxysporum* which significantly improved plant health and promoted the growth and yield of *Lupinus termis*.

Plants treated with different concentrations of *S. chibaensis* surpassed Biogen and Potassiomage and gave significant high results in the majority of parameters. Growth parameters of *V. faba*, treated with the best concentration of each treatment individually or in combination, gave promotive effect compared to the control in the two seasons. These results are on line with Gomaa *et al.* (2002) and Hewedy *et al.* (2006) who cleared that the biofertilizers were used to stimulate plant growth by producing plant growth regulators.

Nodules were formed on all plants (either inoculated or not), which indicated the presence of the native rhizobia. Root nodulation was characteristically promoted in plants treated with B+*S. chibaensis,* followed by plants treated with Biogen, respectively (Figs. 2a, b). These treatments gave also the highest results for shoot and root dry weights (Fig. 3).

The competitiveness among *S. chibaensis*, Biogen and Potassiomage gave results in which B + *S. chibaensis* treatment was the best, followed

Table (1): Effect of individual inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on certain growth parameters with different concentrations after 60 days from planting

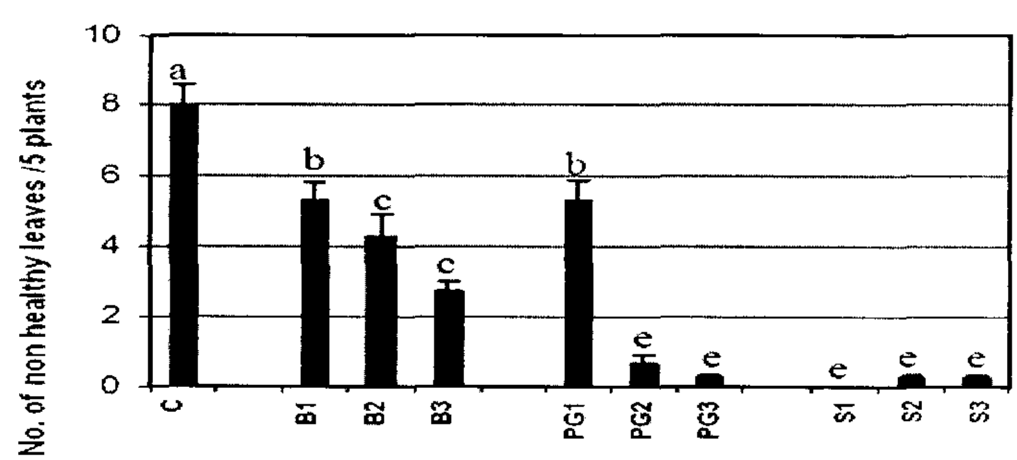
Treatments	Shoot fresh wt./3 plants (g)	Shoot dry wt./3 plants (g)	No. of flowers/3 plants	No. of pods/3plants
C	112.8h	18.1e	27g	20f
B1	134.0fg	20.1d	30fg	27de
B2	149.8eg	20.9cd	47e	33cd
B3	159.8de	29.4a	70c	37bc
PG1	218.0bc	21.4cd	37f	0g
PG2	176.3d	24.7b	57d	23ef
PG3	202.2c	26.2b	103a	40b
S1	256.4a	25.6b	103a	53a
S2	232.4b	22.0c	80b	30d
S3	188.5cd	12.3f	50de	27de
LSD at 5%	20.5	1.8	9.5	6.2

Values in the same column with the same letter are not significantly differed at p<0.5%.

Table (2): Effect of individual inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on the yield of *Vicia faba* plants after 120 days

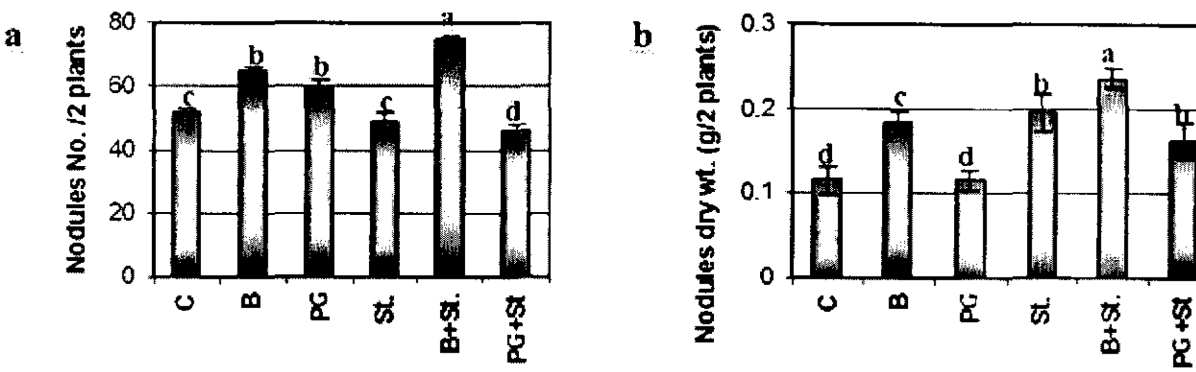
Treatments	No. of pods/3 plants	Pod length (cm)	Fresh wt. of pods/3 plants	Dry wt. of pods/3 plants	Dry wt. of seeds/3 plants
C	23f	6.3c	16.8e	9.44e	7.05e
B1	30e	7.0bc	16.2e	15.18d	11.89bc
B2	33de	7.2b	20.2d	15.75d	11.98bc
B3	47c	7.3b	33.2b	20.43b	14.84a
PG1	30e	7.3b	11.2f	7.02f	4.27f
PG2	37d	8.2a	20.1d	14.96d	9.56d
PG3	53b	8.5a	27.2c	18.78c	14.42a
S1	77a	9.0a	37.5a	22.96a	15.71a
S2	53b	8.7a	30.5b	21.28b	13.78ab
S3	47c	9.0a	20d	15.77d	10.45cd
LSD at 5%	5.6	0.8	3.2	1.5	2.0

Values in the same column with the same letter are not significantly differed at p<0.5%.



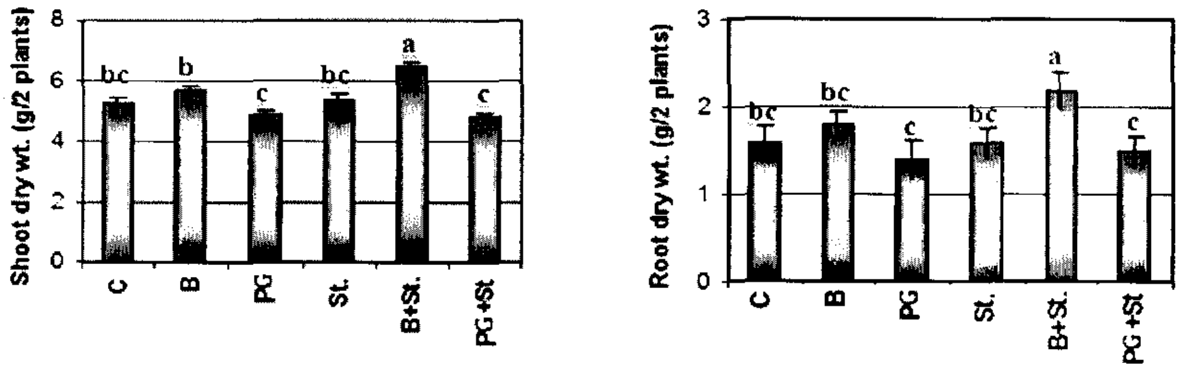
LSD at 5% → 0.56

Fig. (1): Effect of different concentrations of Biogen, Potassiomage and *Streptomyces chibaensis* on the presence of non-healthy leaves in *Vicia faba*.



LSD at 5% → 6.3 LSD at 5% → 0.015

Fig. (2): Effect of individual and mixed inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on shoot and root dry weight of *Vicia faba*.



LSD at 5% → 0.70 LSD at 5% → 0.32

Fig. (3): Effect of individual and dual inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on shoot and root dry weight of *Vicia faba*.

Columns with the same letter are not significantly differed at $p < 0.5\%$.

Table (3): Effect of individual and dual inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on the yield of *Vicia faba* plants

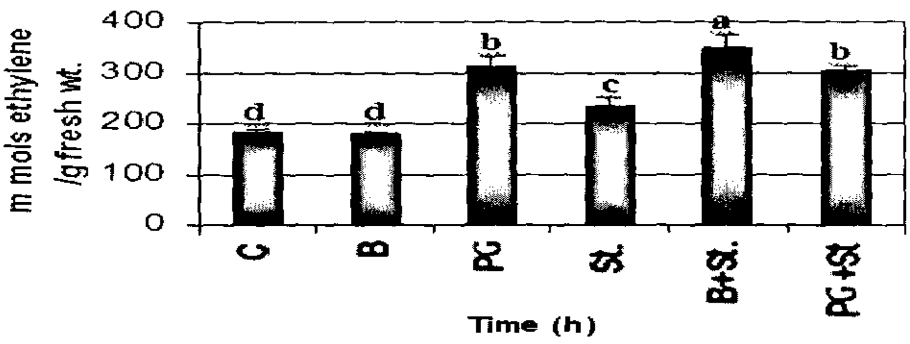
Treatments	No. of pods /10 plants	Dry wt. of pods (g/10 plants)	No. of seeds/10 plants	Dry wt. of Seeds g/10 plants)
C	70c	7.10c	100d	4.36c
B	80a	11.81a	190a	9.47a
PG	77a	7.01c	100d	5.72c
St.)	73bc	10.14b	100d	7.83b
B+St.	80a	11.56a	153b	9.55a
PG+St	73bc	6.94c	135c	7.41b
LSD at 5%	5.0	1.4	15.0	1.5

Values in the same column with the same letter are not significantly differed at $p < 0.5\%$.

by Biogen. This result improved the ability of *S. chibaensis* to compete for *V. faba* nodulation and exhibition for acetylene reduction than in the other treatment (Fig. 4), which means that *S. chibaensis* had an indication to promote N_2 fixation. Hewedy (2003) indicated that a neutralism relationship between *S. chibaensis* and N_2 fixing bacteria, which are widely distributed in the Egyptian soil, might be the result of positive dual treatments.

Concerning yield parameters in the present study, the used biofertilizers could improve the growth of the economic crop, faba bean (Table 3), but in the presence of *S. chibaensis*. This improvement might be due to the inoculants filament bacteria which degraded organic matter and supplied to the plants, also converted atmospheric nitrogen into available form, and stimulated plant growth directly through the production of phyto-stimulating compounds (Hewedy, 2006 and Zaied *et al.*, 2007). This may be also due to their ability to produce antibacterial and antifungal compounds (Hewedy, 2003 and Hosseny & Ahmed 2009).

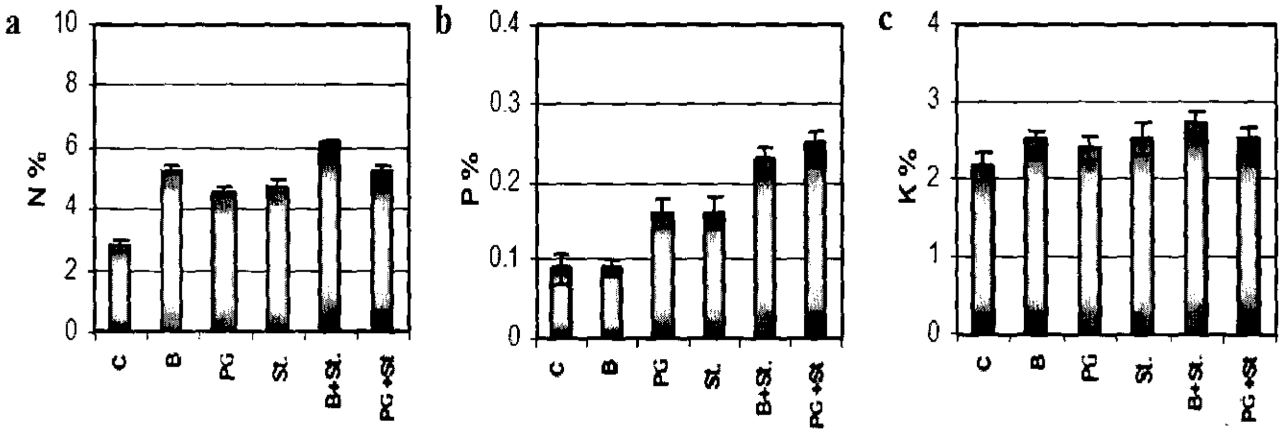
NPK uptake in seeds was significantly affected by different treatments. The dual inoculations gave



LSD at 5% → 18.0

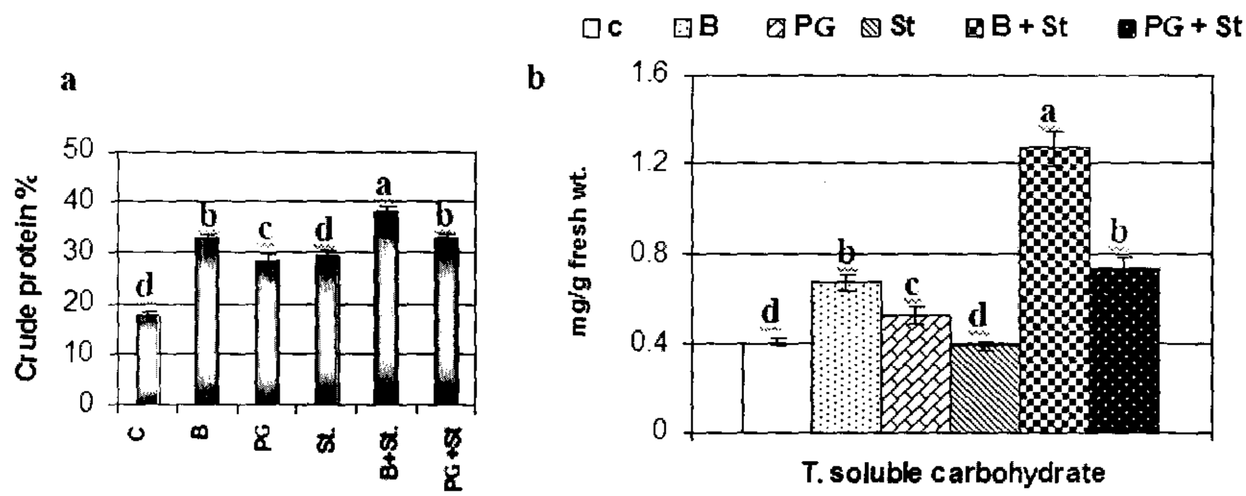
Fig. (4): Effect of individual and dual inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on acetylene reduction activity of *Vicia faba*.

Columns with the same letter are not significantly differed at p < 0.5%.



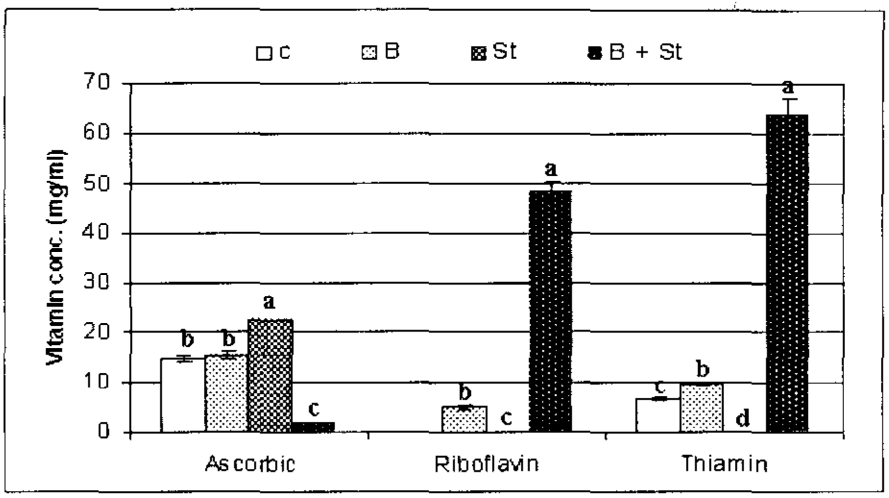
LSD at 5% → 0.32 LSD at 5% → 0.04 LSD at 5% → 0.11

Fig. (5): Effect of individual and dual inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on the NPK of *Vicia faba* seeds.



LSD at 5% → 3.68 LSD at 5% → 0.042

Fig (6): Effect of individual and dual inoculants of Biogen, Potassiomage and *Streptomyces chibaensis* on crude protein and total soluble carbohydrate of *Vicia faba* plants.



LSD at 5% → **3.54** **1.26** **1.44**

Columns with the same letter are not significantly differed at p<0.5%.

Fig. (7): Effect of single and dual inoculants of Biogen and *Streptomyces chibaensis* on vitamin concentration of *Vicia faba* seeds.

the greatest of N, P and K% values. Plants treated with B. + *S. chibaensis* gave the highest uptake of N & K, followed by PG + *S. chibaensis,* which gave the highest uptake of P. (Fig. 5). This could be explained as both biofertilizers and PGPR filamentous bacteria might improve the rhizosphere conditions which are mainly due to the bacterial production of N, P and indole 3–acetic acid (Wu *et al.*, 2005 and Rothballer e*t al.*, 2006). Also, it might be due to the capability of *S. chibaensis* to dissolve P and its affectivity to fix N_2 (Hewedy, 2003 and Hewedy *et al.*, 2006).

It is obvious from the data recorded in Figs. (6 a and b) that the seeds produced from the plants treated with PGPR, *S. chibaensis* individually or combined with Biogen and Potassiomage had significant increase in seeds protein and total carbohydrates content. Biogen + *S. chibaensis* gave the highest protein content, followed by Potassiomage+ *S. chibaensis*. This result is in agreement with Abu-Zekry (2000) and Hewedy *et al.* (2006) who recorded that soybean treated with biofertilizers gave the highest yield and seed protein and carbohydrates.

Because of its promising results, treatment of *S. chibaensis* + Biogen was chosen for studying its effect on the content of vitamins in seeds (Fig. 7). This combination increased the production of both thiamine and riboflavin than in the control. On the contrary, ascorbic acid production was increased in the presence of *S. chibaensis* individually, followed by Biogen > control > Biogen + *S. chibaensis*. Atta *et al.* (2008) reported that the spectroscopic analysis (UV, IR and HPLC spectrum) was used to perform comparative studies between vitamin B12 and two mixed cultures of *S. halstedii* AZ-8A and *Bacillus firmus* AZ-78B, which showed an increase in the mixed culture (peak was at 2.503) than the standard (peak was at 2.496). Isabel *et al.* (1988) found that thiamine and riboflavin were found in high abundance, early in the development of lupine and pea, but their contents decreased during maturation The thiamine content of faba bean seeds was increased from 33 to 47 days after flowering (DAF), then a decrease was observed until 61 DAF, the riboflavin was increased after 54 DAF.

In conclusion, this work may prompt further screenings of Actinomycetes, especially Streptomycetes as potential plant growth promoters for use in nutrient impoverished soils. The isolate of *S. chibaensis* examined in this study, in addition to being a native of Egypt soils, is the most suited for seed inoculation because of its high levels of rhizosphere competency.

Therefore, this work recommends the utilization of the commercial inoculants in combination with *S. chibaensis,* which gave a good response as a PGPR commercially for improving production of faba bean with its competitive ability to survive and affect growth of inoculated plants at the presence of indigenous microflora to protect the environment from chemical pollution and its harmful effect on nature. Also, *S. chibaensis* may be utilized as a biocontrol agent to help in protecting plants against non-induced fungal infection and to minimize the risk and hazard of toxic fungicides. This might help in modern agriculture through the increase use of microorganisms, which is an advantageous alternative to chemical treatments and may contribute substantially to the goal of environmental friendly agriculture.

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