RESPONSE OF SEXUAL EXPRESSION AND PRODUCTIVITY OF SQUASH PLANTS TO SOME BIOFERTILIZER TREATMENTS

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ABSTRACT

Two field experiments were conducted during 2004 and 2005 summer seasons at the Experimental Farm of the National Research Center at Shalakan (Qalubia Governorate) to investigate the response of squash plants to bio-fertilizers i.e., Microbein(2 and 4 kg/1kg seeds), local media (2 and 4 kg/1kg seeds), FZB24 (1 and 2 gm/1kg seeds) and Biogein (2 and 4 kg/1kg seeds). on plant growth, sex expression and fruit yield of squash plant, Iskandrani cultivar.

The obtained results showed that the maximum increments of vine length and leaf number as well as fresh and dry weight of shoots were recorded by the inoculation of seeds with FZB24. Moreover, FZB24 at both levels significantly enhanced the induction of female flowers of squash plants. All bio-fertilizer treatments increased N percentage in plant leaves compared to check untreated seeds, but it was only significant for Biogein treatment.Treating the seeds with FZB24 at both concentrations was found the best treatment, since it increased the number of fruits, early and total fruit yield. On the other hand, check plants gave the lowest values, while plants from inoculated from seeds with local media, Biogein and Microbein produced the intermediate values.

INTRODUCTION

Squash (C*ucurbita pepo* L.) is one of the most popular vegetable crops grown in Egypt. In most monoecious cucurbit plants, the ratio of staminate to pistillate flowers greatly varies when the plants are grown under different environmental conditions, including photoperiod, temperature, nutrient availability, or treated with auxins and plant hormones (Lau and Stephenson, 1993; Swiader *et al*., 1994; Yin and Quinn, 1995). Consequently, any means of regulating environmental factors influencing the ratio of staminate to pistillate flowers is valuable, since yields of high-grade fruits depend, within limits, upon this ratio.

Evidence has accumulated from a number of experiments indicating that nitrogen level may modify the sex expression of many cucurbits (Brantley and Warren, 1960). In general, high levels of nitrogen appear to promote

female sex expression. This effect has been most clearly demonstrated in monoecious cucurbits (Lau *et al*., 1995). Most if not all the conventional N-fertilizers are rapidly lost by different ways; sixty percent or more of the applied fertilizer is mainly lost by either volatilization or by leaching in drainage water (Hayes, 1971). The problem does not only stop at losing amounts of nitrogen and then causing reasonable economic losses, but it extends also to other dangerous environmental pollutions.

Biological fertilization of plants by N2 fixing bacteria gained importance in the last years. The significant effect of bio-fertilizers may be due to the effect of different strain groups such as nitrogen fixer, nutrient mobilization microorganisms which help in increasing the availability of minerals and their forms in the composted materials and increase levels of extractable of macro or micronutrients (El-Karamany *et al*., 2000)

Biogein has high amounts of symbiotic and non symbiotic bacteria responsible for atmospheric nitrogen fixation. Application of Biogein reduced the required mineral nitrogen by 25%, increased the availability of various nutrients, enhances the resistance of plants to root disease and reduces the environmental pollution from chemical fertilizer application (Rizk and Shafeek, 2000 on *Vicia faba).* Application of bio-fertilizer encouraged plant growth and productivity of many crops, was studied by some investigators (El-Metwaly, 1998; Abdalla *et al*., 2001 on pepper; Adam *et al.* 2002 on Cantaloupe). Abd El-Fattah and Sorial (2000) on squash, indicated that bio-fertilizer treatment (Halex2) significantly enhanced the induction of female flowers, which was reflected afterward on the increase of fruit yield, and reduced the male, and consequently reduced the sex ratio of squash plants.

Utilization of bio-fertilizers in the form of Microbein is very successful in minimizing chemical fertilizer to half of the recommended dose. Many investigators reported that Microbein affected plant growth and total yield of plant (Abdel-Mouty *et al.,* 2001; Abdalla *et al*., 2001).

It's well Known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect on plant growth. The bacteria have been called 'plant growth regulator promoting rhizobacteria (PGPR), and include strains in the genera Bacillus, Pseudomonas, Rhizobium and etc, (Bloemberg and Lugtenberg, 2001). PGPR bacteria can stimulate growth and yield of crops including potato, radish, tomato, lettuce, beans, cucumber (De Silva *et al*., 2000; Sudhakar *et al*., 2000). All the monitored activities and formulation properties suggest an effective use of *Bacillus subtilis* as a plant –strengthening agent and for biocontrol of diseases.

This study was carried out to investigate the influence of some bio-fertilizer treatments on growth, sex expression and productivity of squash plants.

MATERIAL AND METHODS

Two field experiments were performed at the experimental station of the National Research Center (Qalubia Govenorate), during the summer seasons of 2004 and 2005, to evaluate the response of four types of biofertilizers viz., Microbein, Biogein, Local media and FZB24 as compared to the check treatment (without bio-fertilizers) on growth, flowering and yield of squash plants. Seeds of summer squash Iskandrani cultivar were sown on March 8 and 10 in 2004 and 2005, respectively.

The experimental treatments and design

Microbein is a nitrogenous bio-fertilizer containing nitrogen fixation bacteria like Rhizobium, Biogein is a nitrogenous bio-fertilizer containing nitrogen fixation bacteria like Azotobacter. Microbein and Biogein are produced and distributed commercially by the General Organization for Agriculture Equalization Fund, Ministry of Agriculture, Egypt. The Local media prepared by the National Research Centre contain Mychorrizae, Peseudomonas, Putrde, *Bacillus megatherium*, and Fungi mixture. FZB24 is a strain of *Bacillus subtilis* which was tested with the registered granular preparation *Bacillus subtilis*, formulated on corn starch with 10^{11} spores/g, originated from FZB Biotechnik GmbH Berlin .

Squash seeds were mixed with the biofertilizers, i.e., Microbein, local media, and biogein, just before sowing using Arabic gum as an adhesive material. FZB24 was applied by dissolving the tested rates in 1L water and soaking the squash seeds for 10 minutes before drying. The experiment included nine treatments as follows:

1- Check (untreated seeds).
2-Inoculation of squash seeds with Microbein at the rate of 2 kg/ 1kg seeds.
3-Inoculation of squash seeds with Microbein at the rate of 4 kg /1kg seeds.
4-Inoculation of squash seeds with local media at the rate of 2 kg /1kg seeds.
5-Inoculation of squash seeds with local media at the rate of 4 kg /1kg seeds.
6 -Seed was treated with FZB24 at the rate of 1 g / 1kg seeds.
7- Seed was treated with FZB24 at the rate of 2 g / 1kg seeds.
8-Inoculation of squash seeds with Biogein at the rate of 2 kg/ 1kg seeds.
9-Inoculation of squash seeds with Biogein at the rate of 4 kg /1kg seeds.

The treatments were arranged in a complete randomized block design with three replicates. The plot area was 11.2 m^2 consisted of four ridges of 4

m long and o.7 m wide. Plant spacing was 40 cm apart on one side of the ridge under surface irrigation. The Agriculture practices were done in accordance with those advised for summer squash. The soil of the experimental sites was silty loam texture. The chemical analysis of the soil is shown in Table (1).

Table(1):Chemical analysis of the soil used (depth 0-30 cm)

Soil properties		First season	Second season
PH	(1:2.5)	8.12	8.16
EC 1:5	(dsm^{-1})	0.87	0.94
$CaCo_3$	(%)	2.5	2.3
Organic mater	(%)	2.83	3.26
Total N	(%)	0.15	0.17
NH4-N	(mg/kg^{-1}soil)	25	23
NO3-N	(mg/kg^{-1}soil)	42	44
Available P	(mg/100g soil)	3.3	2.4
Available K	(mg/100g soil)	0.48	0.65

Data recorded:

Plant growth parameters:

A random sample of three plants was chosen from each treatment, 45 days after sowing, for evaluating vine length (main stem), leaf number and fresh and dry weight of shoots.

Flowering parameters:

A random sample of three plants from each treatment were labeled. Number of staminate and pistillate flowers were counted all over the flowering and fruiting period and the sex ratio was recorded by dividing the average number of staminate by pistillate flowers.

Yield and its components:

Fruits were harvested at two days intervals, upon reaching 12-15 cm length (El-Barkouki *et al*.,1975). During the period of fruiting, fruits were counted and then weighed and number of fruits/plant was recorded. Early yield was counted from the early 4 harvests, whereas the average total yield was recorded during the harvesting period.

Chemical composition:

Nitrogen content was determined in leaves, based on the dry weight using the micro-Kieldahl method as described by Cottenie *et al*. (1982).

The obtained data were statistically analyzed using Costat Software (1985) and treatment means were compared by using Duncans's multiple range test at 5% level according to Snedecor and Cochran (1980).

Results and discussion

Plant growth characters:

It was detected that there were no significant differences between bio-fertilizer treatments and check treatment Table (2). This finding was true in both growing seasons. The only significant increase was observed in FZB24 treatment in fresh and dry weight of shoots compared to check treatment where plants produced from treated the seeds, especially at higher level, fairly produced the highest values in the studied characters. This stimulation of growth characters may be due to the mode of actions of these bacteria which was previously reported by Bochow and Dolej (1999), they suggested that the mechanisms seem to be based on a hormonal push for plant growth due to releasing exogenic bacterial metabolites having precursors of auxin (indole-3-pyruvic acid), or inducers (GA3 fraction) for auxin synthesis. Our results agreed with those of De Silva *et al*. (2000) and Sudhakar *et al*. (2000).

Table (2): Effect of inoculation by bio-fertilizers on growth characters of squash plant during 2004 and 2005 seasons.

Treatment	Vine length cm		Leaf number per plant		Shoots fresh weight g/per plant		Shoots dry weight g/per plant	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Check	54.71a	66.59ab	19.42ab	21.40ab	364.13b	577.46 b	43.80 b	58.50 b
Microbein 1	52.20ab	66.29ab	18.95ab	21.45ab	376.14ab	578.50 b	44.17ab	60.70ab
Microbein 2	54.70a	66.80ab	19.07ab	21.95ab	372.25ab	582.20ab	43.30 b	61.89ab
Local media1	55.04a	66.70ab	20.18ab	21.14ab	370.40ab	581.11ab	45.10ab	63.80 a
Local media2	56.40a	67.40 a	20.98ab	21.90ab	376.20ab	586.13 a	46.16 a	65.48 a
FZB24 1	55.07a	67.60 a	21.89 a	23.14 a	383.18 a	589.24 a	46.86 a	67.17 a
FZB24 2	56.60a	68.30 a	22.40 a	25.11 a	401.27 a	598.62 a	43.50 b	59.03 b
Biogein 1	52.59ab	66.01ab	19.80ab	21.56ab	367.11 b	580.50ab	43.95ab	61.37ab
Biogein 2	51.90ab	66.80ab	19.86ab	21.70ab	378.90ab	581.60ab	42.72 b	57.40 b

Sex expression and sex ratio:

It's obvious from Table (3) that squash plants from seeds treated with FZB24, at both levels, significantly had the highest number of staminate and pistillate flowers compared with check one as well as other treatments. These results were true in both growing seasons, except for the low rate in second season which did not reach the level of significance. However, no significant differences were detected among the other treatments or relative to the untreated one. Many investigators stated that bacterial inoculation of seeds or roots leads to changes in plant growth

which is caused by growth regulating substances especially those of gibberelin, cytokinin and IAA (Goicoechea *et al*., 1995; Noel *et al*., 1996). `These changes may be afterward on reflected the increase in female flowers. These results matched well with those of Bochow and Dolej, (1999), they suggested that mechanisms of *Basillus Subtilis* seem to be based on a hormonal push for plant growth due to releasing exogenic bacterial metabolites having precursors of auxin (indole-3-pyruvic acid), or inducers for auxin synthesis (GA3 fraction).

With respect to sex ratio the results of both seasons did not reflect any significant differences among all treatments.

Table (3): Effect of inoculation by bio-fertilizers on some flowering characters during 2004 and 2005 seasons.

Treatments	Number of flowers / plant				Sex Ratio	
	Male	Female	Male	Female		
	1st season		2nd season		1st season	2nd season
Check	10.34 b	8.11 c	10.60 bc	9.02 b	1.27 a	1.29 a
Microbein 1	10.35 b	8.30 c	10.83 b	9.14 b	1.25 a	1.18 a
Microbein 2	10.32 b	8.66 bc	10.63 bc	9.78 b	1.19 a	1.06 a
Local media 1	10.62 b	8.50 c	10.45 bc	9.92 b	1.25 a	1.05 a
Local media 2	10.71 b	8.80 bc	10.86 b	9.94 b	1.22 a	1.09 a
FZB24 1	11.14 a	9.28 a	10.90 b	10.14ab	1.18 a	1.02 a
FZB24 2	10.16 a	9.62 a	11.32 a	10.60 a	1.16 a	1.10 a
Biogein 1	10.43 b	9.11 bc	10.29 bc	9.24 b	1.21 a	1.13 a
Biogein 2	10.87 b	8.51 c	10.40 bc	9.32 b	1.23 a	1.11 a

1= First rate **2**=Second rate

Nitrogen content

The inoculation of squash seed with Biogein induced significantly resulted in higher values of nitrogen in leaves than those of check as well as other treatments Table (4). These findings were similar in the two experimental seasons. The increase in total nitrogen content could be extended to other factors than N-fixation, e.g., hormone production (Omay *et al*., 1993) or to more efficient N assimilation aided by the bacterial nitrate reductase (Azcon *et al*., 1996).Similar effect and findings about Biogein were reported by Adam *et al.* (2002) on cantaloupe.

Table(5): Effect of inoculation by bio-fertilizers on nitrogen

content % of leaves during 2004 and 2005 seasons.

Treatments		Nitrogen content %	
		First season	Second season
Check		1.57 d	1.61 b
Microbein	**1**	1.60 cd	1.63 b
Microbein	**2**	1.66 c	1.66 b
Local media	**1**	1.60 cd	1.62 b
Local media	**2**	1.62 cd	1.65 b
FZB24	**1**	1.58 d	1.63 b
FZB24	**2**	1.60 cd	1.66 b
Biogein	**1**	1.74 b	1.79 a
Biogein	**2**	1.79 a	1.81 a

1= First rate **2**= Second rate

Yield and its components:

Data in Table (5) show that squash plants grown from seeds treated with FZB24, at both levels increased fruit number compared to those of check as well as other treatments. These increments were significant in the two growing seasons (with only one exception) However, in comparison with check plants, the other three treatments at the two tested levels (microbien, local media and biogein) increased fruit number in both seasons. These increments were significant only in the first season.

Regarding the early yield, FZB24 at both levels significantly increased early yield compared with check as well as other treatments. These results were true in both seasons (except for the lower level in the first season which did not reach the level of significancy). In the same time, no significant differences have been detected among the other three biofertilizers treatments as well as to check treatment (with one exception).

Fruit yield could be considered the out put of all growth and flowering features of the plant. Therefore, it was though advisable to investigate the yield as a net expression for the response of squash plants to bio-fertilizer treatments. Comparing the effect of all different treatments the results revealed that, in the two successive growing seasons, FZB24 at both levels significantly increased squash yield with clear superiority at higher level over those of other treatments as well as the check treatment. The significant increase over control induced by FZB24 at lower and higher levels were 10% and 17%, respectively, in first season and 12% and 16%, respectively, in second season. Moreover, in both growing seasons, the other three biofertilizer treatments insignificantly improved the total yield compared with the check treatment.

The trend of fruit yield response to bio-fertilizer treatments was almost the same as growth characteristics and flowering responses. When the results of the two seasons were discussed, it was detected that the key to all high yields is the early and continued development of a large healthy growth and enough plant nutrient supply for the crop needs, will lead to increased squash yield, this was readily available in FZB24 treatment. These explanations may account for the superior fruit yield of squash plants grown from seeds treated with FZB24 which overyielded that of plants treated with other treatments as well
as those of the check one.

Table(5):Effect of inoculation by bio-fertilizers on fruit yield and it's component during 2004 and 2005 seasons.

Treatments	Number of fruits per plant		Early yield kg per plot		Total yield Kg per plot	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Check	6.60 c	8.42 bc	7.96 b	9.20 b	20.95 bc	22.86 bc
Microbein 1	6.95 b	8.65 b	7.90 b	8.72 c	19.55 c	20.18 c
Microbein 2	7.03 b	8.70 b	8.35 b	8.85 bc	20.46 c	21.10 bc
Local media 1	7.70 b	8.90 b	8.40 b	9.55 b	21.13 bc	22.96 bc
Local media 2	7.80 b	9.01 b	9.45 ab	9.85 b	22.50 b	23.65 b
FZB24 1	8.01 ab	9.76 a	9.50 ab	10.70 a	23.02 a	25.31 a
FZB24 2	8.30 a	9.88 a	10.35 a	11.20 a	24.40 a	26.50 a
Biogein 1	7.20 b	8.50 bc	8.15 b	9.01 b	21.15 bc	22.26 bc

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إستجابة التعبير الجنسى وانتاجية نباتات الكوسة لبعض معاملات التسميد الحيوى

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**** بيو تكنيك برلين المانيا**

اجريت تجربتين حقليتين خلال العروة الصيفية لموسمى 2004 , 2005 بمحطة المركز القومي للبحوث بمحافظة القليوبية لدراسة تاثير التسميد الحيوى بكل من الميكروبين (4،2 كجم / 1 كجم بذرة) والبيئة المحلية (2، 4 كجم / 1 كجم بذرة) واف زد بى 24(1, 2 جم / 1 كجم بذرة) والبيوجين (2،4 كجم / 1 كجم بذرة) بالاضافة الى الكنترول (بدون تسميد حيوى) على النمو الخضرى والتعبير الجنسى وكذلك المحصول االثمرى لنبات الكوسة صنف الأسكندرانى.

وقد اوضحت النتائج ان معاملة البذرة بال اف زد بى 24 أعطت أعلى قيم لكل من طول الساق وعدد الاوراق وكذلك الوزن الطازج والجاف للاجزاء الهوائية. كذلك أدت المعاملة نفسها الى زيادة معنوية فى عدد الأزهار المؤنثه مقارنة بباقى المعاملات (باستثاء حالة واحدة) بينما لم توثر معاملات التسميد الحيوى المختلفة على النسبة الجنسية.

أظهرت النتائج زيادة معنوية فى محتوى الاوراق من النتروجين باستخدام البيوجين فقط خلال موسمى التجربة بينما ادت باقى المعاملات الى زيادة غير معنوية مقارنة بالكنترول.

ادت معامل البذور بال اف زد بى 24 بكلا التركيزين الى زيادة عدد الثمار بالنبات والمحصول المبكر والكلى فى حين أعطت نباتات المقارنة اقل قيم للصفات السابقة بينما ادت معاملة البذور بكل من البيئة المحلية والميكروبين والبيوجين الى وجود قيم متوسطة .