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Original paper

Variability assessment of garden pea (*Pisum sativum*) production by using two-way ANOVA analysis

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Abstract

The aim of the paper was to determine the effects of seed bacterization and soil fertilization (Universol Blue, Ferticare I/3 weeks), upon production parameters of Bördi variety pea. The garden peas were randomly sown on 36 plots, which formed 12 experimental variants. The V1-V6 variants were unbacterized and V7-V12 variants were bacterized (factor A). Variants V1 and V7 were unfertilized controls. Variants V2, V3, V4 and V8, V9, V10, were fertilised with Universol (337.5 g; 421.875 g; 506.25 g/variant), while V5, V6 and V11, V12 were fertilised with Ferticare (540 g; 607.5 g/variant) (factor B). Dispersional analysis for the bifactorial experiment (2×6) placed in subdivided plots, highlighted that there were significant differences between the peashells production (t/ha) in bacterized variants (experimental F distribution 532.411 > theoretical F 98.5; p < 0.01). Significant differences were also obtained between the production of peashells (t/ha) in fertilized variants (experimental F distribution 53.55 > F theoretically 4.10; p < 0.01). The interaction between the two bacterization-fertilization (AxB) factors was not significant. In all variants, Ferticare fertilizer was found to be significantly more effective than Universol. The bacterization effect was very significant, the production of peashells (t/ha) increased by 1.002 t/ha and 116.542%, respectively, compared to unbacterized variants (7.059 t/ha vs. 6.057 t/ha).

Keywords

Bacterization, factorial analysis, fertilization, *Pisum sativum*.

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Introduction

Garden pea (*Pisum sativum L.*) is a very valuable crop from an economic, nutritive and agrotechnical point of view (S. PALCU [17]). Pea was one of the earliest crops cultivated by humans, since 9000 BC in the Crescent Fertile area. The world main producers are: Canada, United States, India, Russia, France and China [GOVERNMENT OF SASKATCHEWAN PUBLICATIONS [11]]. At this point, it is estimated that between 6 and 8 million ha are cultivated worldwide, producing between 10-12 million t/year pea beans. In Romania, the area sown with pea was estimated at about 31543 ha in 2015 and the average productivity was around 1749 kg of peabeans/ha (total production of 55179 tonnes). In 2016, the area cultivated with pea grew by 7700 ha, and the average production increased to 2649 kg/ha [I. VĂDUVA [28]].

From the nutritional point of view, the green pea seeds are characterized by a rich carbohydrate content (12.5-14.0%), protides (6.0-8.4%) lipids (0.6%) and fibers (6.0% of which cellulose 2.6-2.7%). The content of mineral substances is over 0.9%, being mainly represented by: K (300-400 mg/100 g), Ca (25 mg/100 g), Mg (33-40 mg/100 g), P (100-126 mg/100 g), Fe (2 mg/100 g). The main vitamins represented in the peabeans are: ascorbic acid (25-40 mg/100 g), complex B (1 mg/100 g), PP (over 2 mg/100 g), E (0.2-3 mg/100 g). The water content of peabeans is estimated to be on average 74-76% and the energy value is estimated to 780-790 kcal/kg (D. BECEANU & al [3]).

Also, pea is a good predecessor plant for the majority of crops, especially for autumn wheat, because it is harvested early and has a particularly favorable influence on soil structure and soil fertility (leaves 30-100 kg nitrogen/ha in the soil), being able to fit easily into the crop rotation system (C. SAMUIL [22]). It is estimated that for a production of 10 tons/ha, pea extracts from the soil amounts of 125-170 kg of nitrogen, 45 kg of phosphorus, 100-125 kg of potassium, 60-150 kg of calcium and 12-30 kg of magnesium (S.I. BRUMĂ [5]). Last but not least, Agricultural pea shells (*Pisum sativum*) can be used as a potential source for bio-ethanol production [A. REHMAN [20]].

Applying bioinoculants (bacteria) to leguminosae seeds is an effective way to get production increases at minimal costs. The bacterization is intended to supply the plants roots with a sufficient number of symbiotic bacteria.

These bacteria are involved in providing a significant amounts of nutrients to the plant. Of these, we include: nitrogen, phosphorus, substances that stimulate growth or protect the plant from a series of infections, by antibiotic action etc. Antibiotic action occurs as a result of the secondary metabolites synthesis, during the development

of bacterial colonies (T. SIVAKUMAR [25]). The bacterization contributes to the creation and maintenance of an ecosystem at the level of the rhizosphere microbiome, in which other symbiotic forms, such as mycorrhizae, are involved. As a result, the amount of nutrients and soil water supplied to the plant is increased, as well as its protection by a number of pests, such as nematodes (Z.A. SIDDIQUI & al [23]).

The use of bioinoculants influences the production increase and the inputs reduction of non-environmentally friendly substances (fertilizers from fossil fuels, pesticides etc.). It is also a way of lowering overall costs across the product line, concerning Life Cycle Assessment and of delivering benefits consistent with resource saving and sustainable development policies (D. GOLEMAN [10]).

Nitrogen fixation bacteria generally belong to the genera: *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium*, *Azotobacter* and *Mesorhizobium*. To these are added a number of bacterial genera that are part of the soil spontaneous flora and which make free associations with the crop plants, namely: *Azospirillum*, *Enterobacter*, *Klebsiella* and *Pseudomonas* (R. HAYAT [12]). A series of bacteria (*Bacillus megaterium* var. *phosphaticum*, *Bacillus subtilis*, *Bacillus circulans*, *Pseudomonas striata*) may be involved in the mobilization of bound phosphorus in calcareous soils, in insoluble forms. These bacteria produce a series of organic acids (oxalic, succinic, citric, glutamic, malic, fumaric etc.) which cause the release of phosphorus (I.A. JEHANGIR & al [13]; P. PONMURUGAN & al [18]; A. ROKHZADI & al [21]). A number of bacteria involved in potassium solubility, such as *Fracteuria aurentia*, can be added to nitrogen-binding bacteria (V. SINGH & al [24]).

Production increases, by using bacterization, are significant for a large number of crops, including: cereals, root vegetables, leguminosae, solanaceae or technical plants (5% in rice, 8-10% in wheat, 15-20% in sorghum and maize, 16% in potato, 40% in carrot, 7-74% in tomatoes and up to 24% in cauliflower and cotton) (S.S. DUDEJA & al [7]).

The bacterization of the leguminosae seeds, namely garden pea, with *Rhizobium*, can provide up to 80% of the nitrogen requirements of the crop, the rest of the nitrogen being supplied by the soil, or by the application of fertilizers (S.T. ALI-KHAN & al [2]; K.E. BOWREN & al [4]). Nodulation and nitrogen fixation is dependent on nitrogen sources in the soil or on fertilizers application. Thus, small amounts of nitrogen in the soil can stimulate the formation of nodules (U.K. KAUSHIK & al [16]), while the presence of more than 40 kg nitrogen/ha can inhibit nodules formation (A.K. K. ACHAKZAI [1]). The ideal quantities of nitrogen, with which the pea culture from bacterized seeds must be supplemented, vary from 9 to 20 kg/ha to various authors (K.E. BOWREN & al [4]; M. ERMAN & al [8]). A series of studies have also

shown that the application of fertilizers with P and K stimulates vegetative growth and pea beans production (J.A. CUTCLIFFE & al [6]; S.P. KANAUIA & al [14]; S.P. KANAUIA & al [15]; V.A. VOROB'EV [26]).

The present research has been designed to address a matter of practical interest in the assessing of garden pea (*Pisum sativum*) production parameters, following seeds bacterization and soil fertilization.

Materials and Methods

Experiences made with garden pea, Bördi semi-early variety, were placed in a temperate climate region (46° N; 21° E). The effect of seed bacterization and soil fertilization on production performances (peashellst/ha) was studied. Experiences were located on the ground with respect to the experimental technique requirements.

The experimental variants were analyzed in three repetitions (plots) in randomized blocks with protective strips between them. The total experimental area, seeded with garden peas, which was 229 m², had the following characteristics:

- number of variants 12;
- number of repetitions/variant 3;
- total number of plots 36;
- length, width and surface of a plot:
2.0 m × 1.5 m = 3.75 m²;
- actually cultivated area of the experiment:
36 × 3.75 m² = 135 m²;
- area of access ways and protective strips: 94 m².

Each plot was labeled and contained approximately 409-412 plants (with an average of 110 plants/m²). Randomized pattern of experimental variants is presented in Table 1.

Unbacterized variants		
Control V1	V2	V3
V4	V5	V6
V3	Control V1	V2
V6	V4	V5
V2	V3	Control V1
V5	V6	V4
Bacterized variants		
Control V7	V8	V9
V10	V11	V12
V9	Control V7	V8
V12	V10	V11
V8	V9	Control V7
V11	V12	V10

To the first 6 variants (V1-V6), the seeds have not been bacterized, and to the following 6 variants the seeds were bacterized (V7-V12). The bacterization was made with the Biotrophin product, a bacterial growth stimulator, containing two biological components, namely: *Bacillus megaterium* and *Azotobacter chroococcum*.

Both bacteria successfully solubilize tricalcium phosphate, making it accessible to plants. *Azotobacter chroococcum* is a bacterium that fixes atmospheric nitrogen in the soil. Biotrophin was applied at a rate of 10 l/ha, 10 days before sowing, and was incorporated by disking.

Variants V1 and V7 represent unfertilized controls. The rest of variants have been fertilized with two fertilizers containing microelements, including nitrogen. Variants V2, V3, V4 (unbacterized), as well as variants V8, V9, V10 (bacterized), were fertilized with Universol Blue. Variants V5, V6 (unbacterized), and variants V11, V12 (bacterized) were fertilized with Fercicare I.

As a manner of administration, fertilization treatments were applied for 3 weeks, extraroot, in all variants. The quantities of applied fertilizers have respected the maximum application limits specified in the instructions [9, 27].

The experimental model and fertilizers doses are outlined in Table 2.

Variant	Treatments applied to seeds	Fertilizations			
		Fertilizers	No. of fertilizations	Dose (g/m ²)	
				Dose/fertilization	Total dose
V ₁ (control)	Unbacterized (A ₁)	Control (B ₁)	-	-	-
V ₂		Universol (B ₂)	3	10.0	30.0
V ₃		Universol (B ₃)	3	12.5	37.5
V ₄		Universol (B ₄)	3	15.0	45.0
V ₅		Fercicare I (B ₅)	3	16.0	48.0
V ₆		Fercicare I (B ₆)	3	18.0	54.0
V ₇ (control)	Bacterized (A ₂)	Control (B ₇)	-	-	-
V ₈		Universol (B ₈)	3	10.0	30.0
V ₉		Universol (B ₉)	3	12.5	37.5
V ₁₀		Universol (B ₁₀)	3	15.0	45.0
V ₁₁		Fercicare I (B ₁₁)	3	16.0	48.0
V ₁₂		Fercicare I (B ₁₂)	3	18.0	54.0

The soil in which the sowing was done was of chernozem type. The garden pea was seeded in early April, it grew in the second half of April and was harvested in mid-June, from all over the experimental field. Research results, namely production performances and variability study, were done using the professional IBM SPSS Statistics Program.

Results and Discussions

The descriptive statistics of the production results obtained after the pea harvesting period of time are shown in Table 3. The experimental pattern shows the production of peas ha/variant, depending on two factors, namely: factor A-bacterization treatment, factor B-fertilization treatment.

Table 3. Variability estimators of peashells weight (t/ha) (n=3)

Factor A Bacterization treatment	Factor B Fertilization treatment (kg/ha)	Mean (X)	Stand. dev. (s _x)	Variab. coeff. (CV, %)
(A ₁) Unbacterized	(B ₁) Control	5.116	0.367	7.173
	(B ₂) Universol (3×100)	6.007	0.122	2.031
	(B ₃) Universol (3×125)	5.822	0.194	3.332
	(B ₄) Universol (3×150)	6.250	0.186	2.976
	(B ₅) Fercicare I (3×160)	6.533	0.161	2.464
	(B ₆) Fercicare I (3×180)	6.614	0.160	2.419
(A ₂) Bacterized	(B ₇) Control	6.033	0.120	1.989
	(B ₈) Universol (3×100)	6.721	0.284	4.225
	(B ₉) Universol (3×125)	6.832	0.254	3.717
	(B ₁₀) Universol (3×150)	7.396	0.094	1.271
	(B ₁₁) Fercicare I (3×160)	7.703	0.165	2.142
	(B ₁₂) Fercicare I (3×180)	7.674	0.111	1.446

Concerning the unbacterized harvest, the control showed the lowest value of peashells weight t/ha, and fertilized Fercicare I variants showed the best results. At the same time, the variability coefficients had low values for fertilized variants (2.031%-3.332%), compared to the control, for which the variability coefficient of was 2-3 times higher (7.173%).

Concerning the bacterized harvest, the control also showed the lowest value of peashells weight t/ha, and fertilized Fercicare I variants had the largest peashells production. The coefficients of variability were small, including that of the control.

The results of the experiment, with the above-mentioned structure, organized into randomized blocks with 3 repetitions, respectively the variance analysis, had been evaluated by the two-way

ANOVA method. “Differences errors” were calculated (s_d) and the “Least significance difference” (LSD) test was applied to:

- differences between two means of “A” factor, respectively bacterization;
- differences between two means of “B” factor, respectively fertilization;
- differences between two means of “B” at the same level of “A” (AxB, interaction (bacterization – fertilization)).

The calculation of sums of peashells weight/plot, taking into account the factors A and B, namely bacterization and fertilization variants, is presented in Table 4, being the first step of the two-way ANOVA analysis.

The obtained data defines the meaning of peashells weight changes, depending on the twofactors, the bacterization of the seeds and the fertilization with complex microelements fertilizers.

The data in the table above were further used in the variance analysis of the bifactorial experiment (Table 5).

Table 4. The peashells sums of weight t/ha for all treatments

Factor A Bacterization treatment	Factor B Fertilization treatment (kg/ha)	Peashells weight (t/ha)			Sums	Means (t/ha)
		Plot 1	Plot 2	Plot 3		
(A ₁) Unbacterized	1 (Control)	5.486	5.110	4.752	15.348	5.116
	2 (Universol)	5.872	6.109	6.040	18.021	6.007
	3 (Universol)	5.821	6.017	5.628	17.466	5.822
	4 (Universol)	6.387	6.038	6.325	18.750	6.250
	5 (Ferticare)	6.501	6.708	6.390	19.599	6.533
	6 (Ferticare)	6.577	6.789	6.476	19.842	6.614
	TOTAL	$\Sigma_I = 36.644$	$\Sigma_{II} = 36.771$	$\Sigma_{III} = 35.611$	$\Sigma_{IV} = \Sigma_{I+II+III} = 109.026$	Mean = 6.057
(A ₂) Bacterized	7 (Control)	6.072	5.898	6.129	18.099	6.033
	8 (Universol)	6.980	6.766	6.417	20.163	6.721
	9 (Universol)	6.590	7.098	6.808	20.496	6.832
	10 (Universol)	7.401	7.488	7.299	22.188	7.396
	11 (Ferticare)	7.699	7.540	7.870	23.109	7.703
	12 (Ferticare)	7.802	7.606	7.614	23.022	7.674
	TOTAL	$\Sigma_I^I = 42.544$	$\Sigma_{II}^I = 42.396$	$\Sigma_{III}^I = 42.137$	$\Sigma_{IV}^I = \Sigma_{I+II+III} = 127.077$	Mean = 7.059
R	$\Sigma_I + \Sigma_I^I = 79.188$	$\Sigma_{II} + \Sigma_{II}^I = 79.167$	$\Sigma_{III} + \Sigma_{III}^I = 77.748$	$\Sigma_{IV} + \Sigma_{IV}^I = 236.103$	6.558	

Table 5. Dispersional analysis for the bifactorial experiment (2×6) placed in subdivided plots

The source of variation	MSS(mean sum of squares)	df (degrees of freedom)	MSE (mean square for errors)	F distribution
Large plots (6 variants)	9.200	5		
Repetitions	0.114	2		
Bacterization (A)	9.051	1	9.051	532.411(18.51; 98.5)
Error (a)	0.035	2	0.017	
Small plots	20.926	35	0.597	
Fertilizers (B)	10.713	5	2.142	53.55 (2.71; 4.10)
Interaction AxB	0.212	5	0.042	1.06 (2.71; 4.10)
Error (b)	0.801	20	0.040	

Analyzing the dispersional distribution of the variation effects in the synthesis, according to the data in Table 5, we noticed that there were significant differences between the means production of pea t/ha in the bacterized variants. The calculated F distribution value was 532.411 and is higher than the theoretical F distribution value (98.5 for $p < 0.01$ and 18.51 for $p < 0.05$). It can be stated, with a small probability of 1% to be wrong, that there are real differences between experimental variants regarding the bacterization (99% confidence).

Also, there are significant differences between the means of pea production t/ha in fertilized variants. The calculated F distribution is 53.55, being higher than the theoretical F distribution (4.10 for $p < 0.01$ and 2.71 for $p < 0.05$). It can be stated with a probability of less than 1%

to be wrong, that there are real differences among the experimental variants regarding the fertilization (99% confidence).

If the experimental value of F distribution (532.411) would have been less than the theoretical F distribution value, for the 5% probability, then going on with the analysis concerning bacterization, would not have been justified.

At the same time, if the experimental value of F distribution (53.55) would have been less than the theoretical F distribution value, for the 5% probability, then further analysis concerning fertilization would not have been justified. In fact, at least one of the means of peashells

weights/variant (t/ha) was statistically different from the others, both in terms of bacterized and fertilized plots.

The F distribution value of 1.06, calculated for AxB interaction (comparison of two averages “B” at the same level of “A”), was less than tabular F distribution value (4.10 for p<0.01 and 2.71 pt p<0.05), so in this case, AxB interaction was not significant and had no influence on pea production (t/ha).

Since the experimental F distribution was higher than theoretical F distribution, both for bacterization and fertilization, the LSD test (least significant difference) was applied to compare the differences of adjacent media, for different probabilities. The differences errors s_d (s_d = square root of $2 \times MSE^2$ /experimental units receiving treatments) and LSD have been calculated, and are highlighted in Table 6.

Table 6. Differences errors (s_d) and Least significant differences (LSD)

s_d	Units receiving treatments	df	t	LSD
For comparing 2 means of factor A				
0.042	18	2	4.30 (5%)	0.180 (5%)
			9.93 (1%)	0.417 (1%)
			31.60 (0.1%)	1.327 (0.1%)
For comparing 2 means of factor B				
0.114	6	20	2.09 (5%)	0.238 (5%)
			2.84 (1%)	0.323 (1%)
			3.85 (0.1%)	0.438 (0.1%)
For comparing two means of B to the same level of A (AxB)				
0.161	3	20	2.09 (5%)	0.336 (5%)
			2.84 (1%)	0.457 (1%)
			3.85 (0.1%)	0.619 (0.1%)

A centralized situation of the results, regarding the significance of means differences in production of

peashells t/ha, influenced by the interaction of bacterization and fertilization factors, is presented in Table 7.

Table 7. The significance of peashells production (t/ha) means differences

Bacterization \ Fertilization		Unbacterized (A 1)				Bacterized (A 2)				Fertilized (B)				
		t/ha	Differences t/ha	Percentages %	Signific.	no. variant t/ha	Differences t/ha	Percentages %	Signific.	t/ha	Differences t/ha	Percentages %	Signific.	
6	Ferticare I 3x180 kg/ha	6.614	1.498	129.280	***	12	7.674	1.641	127.200	***	7.144	1.570	128.166	***
			0.557	109.195	**			0.615	108.712	**		0.586	108.935	***
5	Ferticare I 3x160 kg/ha	6.533	1.417	127.697	***	11	7.703	1.670	127.681	***	7.118	1.544	127.700	***
			0.476	107.858	**			0.644	109.123	**		0.560	108.539	***
4	Universol 3x150 kg/ha	6.250	1.134	122.165	**	10	7.396	1.363	122.592	***	6.823	1.249	122.407	***
			0.193	103.186	*			0.337	104.774	*		0.265	104.040	*
3	Universol 3x125 kg/ha	5.822	0.706	113.799	**	9	6.832	0.799	113.243	**	6.327	0.753	113.509	***
			- 0.235	96.120	0			- 0.227	96.784	0		- 0.231	96.477	
2	Universol 3x100 kg/ha	6.007	0.891	117.415	**	8	6.721	0.688	111.403	**	6.364	0.790	114.172	***
			- 0.050	99.174	0			- 0.338	95.211	0		- 0.194	97.041	
1	Unfertilized (M ₁)	5.116	-	100		7	6.033	-	100		5.574	-	100	
			- 0.941	84.464	00			- 1.026	85.465	00		- 0.984	84.995	000
Mean (M ₂)		6.057	-	100		7.059	-	100		6.558	-	100		

Bacterization	Unbacterized	Bacterized	LSD (t/ha)	A	B	AxB
t/ha	6.057	7.059	5 %	0.180	0.238	0.336
Differences t/ha	-	1.002	1 %	0.417	0.323	0.457
Percentage %	100	116.542	0.1 %	1.327	0.438	0.619
Significance		**				

The differences between means in Table 7 (t/ha), in bacterized and fertilized variants, in first row, are calculated as differences between the production means and the mean of the control and in second row, as differences between the production means and the total mean of the entire column M₂. The LSD (least significant differences) values in the table (A, B, AxB) were compared to the differences in Table 7, in order to determine the degree of significance.

Concerning the “unbacterized A 1” column, which represents the effect of fertilization in the absence of bacterization, all the variants means were higher and different (different degrees of significance) from the production mean of the control, but not significant different from the total mean M₂.

The extremely significant differences in pea production, fertilized with Ferticare I, compared to the control and very significant differences compared to the M₂ total mean, showed the increased efficiency of Ferticare I, compared to Universol Blue fertilizer.

Concerning the “bacterized A 2” column, which represents the bacterization effect, it was noted that all the production means were different and higher (different degrees of significance) than the mean of the control. Even the efficiency of the Universol Blue fertilizer, at 3×150 kg/ha level increased, because the difference in the production mean compared to the control was extremely significant higher (the level of significance went from p<0.1 to p<0.01), compared to the unbacterized variant (Figure 1).

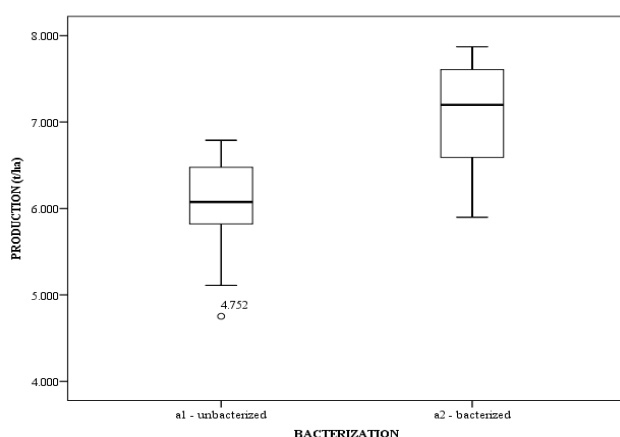


Figure 1. The effect of bacterization on peashells production (t/ha)

The “fertilized B” column represents the variation explained by the fertilization factor B (it shows the means between the bacterized and unbacterized variants production). It was also seen an increased efficiency of fertilization treatments with Ferticare I, compared to Universol Blue (Figure 2).

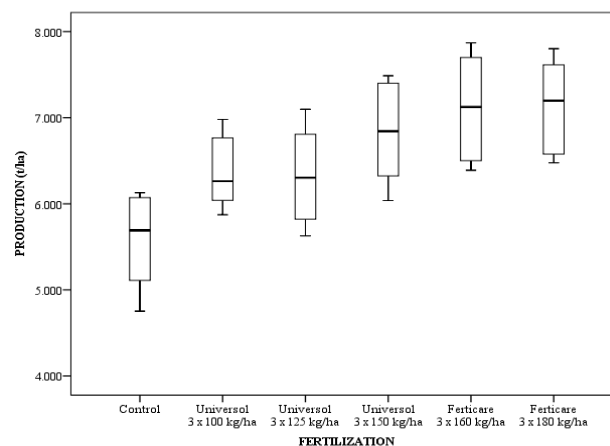


Figure 2. The effect of fertilization on peashells production (t/ha)

Percentages estimates of pea production t/ha, showed an increase of 127.697% (3×160 kg/ha) respectively 129.280% (3×180 kg/ha) for unbacterized variants (V5 and V6, fertilized with Ferticare I, versus unfertilized control. Also, the production on bacterized variants, fertilized with Ferticare I (V11 and V12), increased by 127.681% (3×160 kg/ha), respectively 127.200% (3×180 kg/ha), compared to the unfertilized control.

Basically, the best results were obtained on bacterized plots. On these plots, the production of peashells t/ha increased on average with 116.542% (7.059 t/ha), compared to the average production on the unbacterized plots (6.057 t/ha). The difference of 1.002 t/ha was very significant. Our results confirmed the fact that bacterization is a very important treatment, which enhances the yield in t/ha, even if the plots were initially fertilized. At the same time, the addition of fertilizers containing nitrogen, within certain limits, favor the growth of peashells production (t/ha), in plants with bacterized seeds.

Conclusions

In the fertilized variants, the variability coefficients of peashells production (t/ha) had lower values (2.031%-3.332%), compared to the unfertilized control (7.173%).

According to the F distribution results, there were significant differences between the peashells production (t/ha) in bacterized variants (experimental F 532.411 > theoretical F 98.5; p<0.01).

Significant differences were also obtained between the production of peashells (t/ha) in fertilized variants (experimental F distribution 53.55 > F theoretically distribution 4.10 for p<0.01).

In bacterized and fertilized variants the peashells production (t/ha) was significant higher, compared to unbacterized or unfertilized controls.

For AxB interaction (bacterization-fertilization), experimental F distribution was 1.06, less than theoretical F distribution value (2.71 for $p < 0.05$). So basically, the interaction between the two bacterization-fertilization (AxB) factors was not significant, in terms of changing the production parameter of peashells (t/ha).

In all variants, fertilizer Ferticare I was found to be significantly more effective than Universol Blue. The bacterization effect was very significant, the production of peashells (t/ha) increased by 1.002 t/ha and 116.542%, respectively, compared to unbacterized variants (7.059 t/ha vs. 6.057 t/ha).

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