

Efficiency of phytomeliorants and bacillus biologicals in increasing alfalfa yield

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ABSTRACT

The study was conducted to examine the effect of alfalfa-based compost and *Bacillus* biologicals on alfalfa (*Medicago sativa*) yield in the arid climate of the Syrian Arab Republic. The main research objective was to identify opportunities to increase crop productivity with fertilizers and biologicals. We conducted a field experiment over a three-year period (2020-2022), including four variants: control, compost, biologicals, and combined use of compost and biologicals. The data were subjected to a two factor analysis of variance. We also used pairwise and multiple regressions to analyze how the factors influence the yield. The results proved that compost and biologicals significantly increased alfalfa yield compared to the control. The greatest effect was observed with the combined use of compost and biologicals, which confirms the hypothesis of a synergistic effect. The analysis of variance showed the significance of the differences between the experimental tests and the cultivation years at the 5% and the 1% significance levels. Pairwise comparisons using the Tukey's criterion also confirmed the presence of statistically significant differences between the experiment variants. The regression analysis with dummy variables allowed us to develop a model explaining the dependence of the yield on the cultivation year and the ameliorant type. The high value of the coefficient of determination ($R^2 = 0.982$) and the significance of the coefficients of the regression equation confirm the model reliability. The results can be used to develop recommendations for the use of organic and biological ameliorants in arid climates, which helps improve agrotechnical characteristics and increase the yield of agricultural crops.

Keywords: Field experiment, Alfalfa yield, Phytomeliorants, Biologicals, Statistical modeling, Dummy variables.

Article type: Research Article.

INTRODUCTION

Improving agrotechnical characteristics of crops with organic fertilizers and biologicals is an important aspect of modern agriculture. Using composts and biologicals to increase crop yields and plant resistance is a promising sphere. This study examines the effect of alfalfa-based compost and *Bacillus* genus biologicals on alfalfa yields. The Syrian Arab Republic (SAR) faces numerous challenges in agriculture, especially in the animal feed manufacturing. The main challenges include:

Climate conditions: SAR is characterized by an arid climate with hot and dry summers and cold winters. The average annual precipitation is about 150 mm, which is extremely insufficient to support productive agriculture without additional land reclamation measures. High summer temperatures (45 °C and above) create unfavorable conditions for many crops.

Soil conditions: A significant part of agricultural land in SAR suffers from salinization. The soils in the experiment area are classified as “typical Calcigypsids” and “typical Haplocalcics”, which are characterized by a high content

of calcium carbonate and gypsum, which worsens their structure and water permeability. Weakly saline soils occupy about 35% of the total area of saline lands, moderately saline (about 45%), and highly saline ones (about 20%).

Water resources: Lack of water resources is a key problem to the agriculture in SAR. Main water sources, such as the Euphrates River, are used for irrigation but this is not enough to cover all the needs of the crops.

Economic and political factors: The prolonged conflict in SAR has led to the economic instability and destroyed the infrastructure, which negatively affects the agriculture. The lack of financial resources and equipment also hinders the introduction of modern agricultural practices.

Selection of alfalfa

Alfalfa, *Medicago sativa*, was chosen as an object of the study for several reasons.

Alfalfa is an important forage crop, rich in proteins and nutrients, which makes it indispensable in animal feed production. It improves animal health and productivity.

As a representative of the legume family, alfalfa has an ability to fix atmospheric nitrogen through symbiosis with nitrogen-fixing bacteria. This reduces the need for synthetic nitrogen fertilizers, which is especially important in the resource-limited conditions.

Alfalfa has deep roots, which allows it to use available water resources efficiently and to demonstrate drought-resistant qualities. This makes it suitable for cultivation in arid regions such as SAR.

Alfalfa growing improves soil structure, its aeration and water permeability. This is especially important for saline and low-fertile soils, which are prevalent in SAR.

Alfalfa is important in a crop rotation, it helps restore soil fertility and prevents its depletion.

Thus, the choice of alfalfa as an object of the study is justified by its high feed value, the ability to improve soil conditions and its resistance to adverse climatic conditions, which makes it a promising crop for increasing agricultural productivity.

The study of the effect of organic fertilizers and biologicals on the legume yield, as well as alfalfa one, is an important and relevant topic. This is linked to the need to increase the productivity of agricultural crops and improve soil conditions, especially in arid climates. The publications over the last decade confirm the relevance of the research in such area.

The yield of agricultural crops in arid climates depends on many factors, the assessment of their influence was provisionally divided into six thematic areas:

The impact of biomeliorants on soil condition. The research in this sphere assesses the impact of various biological ameliorants, such as biologicals, organic fertilizers and micronutrients, on crop yields and better soil conditions. These studies demonstrate how the use of biomeliorants helps enhance humus content, improve soil structure and reduce heavy metal toxicity, which ultimately increases crop productivity (Hanc *et al.* 2008; Yakovleva *et al.* 2020; Gong *et al.* 2020; Pathariya *et al.* 2021; Ali 2023).

The influence of agrotechnical methods on alfalfa yield. This research field is devoted to the influence of agronomic technics aiming to increase alfalfa yield. It includes the studies analyzing the impact of fertilizers, soil cultivation and using ameliorants on alfalfa growing conditions, its productivity, and reduced weed infestation (Solovyeva *et al.* 2012, 2013; Aleinikova & Dyachenko 2015; Nand *et al.* 2020; Azzawi *et al.* 2021; Ali *et al.* 2021; Kumar *et al.* 2022; Abro *et al.* 2022).

Using inoculants and biologicals to increase crop yields. This publication field is devoted to the use of inoculants and biologicals to enhance crop yields and improve soil condition. The works show how seed inoculation and using biologicals stimulate plant growth, increase biomass and improve nutrient content in soil (Berendsen *et al.* 2012; Calvo *et al.* 2014; Backer *et al.* 2018; Rouphael & Colla 2020; Ivanova 2023; Kalin *et al.* 2023).

Soil salinization and its impact on crop yields. This field includes studies that examine the impact of soil salinization on the various crop yields. The authors analyze how salinization worsens the water regime, disrupts plant physiological processes and reduces their productivity (Munns & Tester 2008; Flowers & Colmer 2008; Shrivastava & Kumar 2015; Cheeseman 2016; Acosta-Motos *et al.* 2017).

Agrotechnical methods and water management for a crop yield increase. This field discusses the issues related to the assessment of soil factors, such as water regime and soil cultivation methods, to increase crop yields. The influence of soil moisture and its distribution on plant productivity draws particular attention (Bezborodov 2000; Hatfield *et al.* 2001; Turner 2004; Rockström *et al.* 2009; Blum 2009; Li & Provansal 2020; Ermolaeva & Zeiliger 2021; Kurenkova 2021).

The influence of soil factors on crop yield. Various soil factors such as fertility, moisture content, soil structure and their influence on the soybean and alfalfa yield are considered in this research field. The studies highlight the necessity of a complex approach to the soil condition assessment of the optimal crop growth and the increase in their productivity (Jha *et al.* 2019; Tilman *et al.* 2002; Kaspar & Singer 2011; Pagliai & Vignozzi, 2002; Ferreira *et al.* 2020).

Basic scientific principles for the issue under consideration

The influence of organic fertilizers on the crop yield

Organic fertilizers such as compost play a crucial role in a better soil structure, its fertility and a higher crop yield. Compost improves physical and chemical soil properties, contributes to a higher organic matter content and a better microbiological activity.

The role of biologicals in agriculture

The biologicals based on the genus *Bacillus* bacteria are used for a better plant growth and development, as well as their higher resistance to stressful conditions. *Bacillus* bacteria can make a better access for nutrients producing ferments and organic acid, which has a positive influence on the crop yield.

Synergistic effect

The combined use of organic fertilizers and biologicals can provide a synergistic effect, which can significantly increase crop yields. This is linked to the complex effect on soil processes, its improved structure and fertility, in addition to the stimulation of the plant growth and development.

The study aims to determine the effect of alfalfa-based compost and the *Bacillus* genus biologicals on alfalfa yield in arid climates. Research objectives:

To conduct an analysis of variance of the effects of different compost and biologicals apply on alfalfa yield over three years of blue alfalfa growing.

To evaluate the differences in yield between the experiment variants by years of crop cultivation using the Tukey's statistical criterion.

To evaluate the synergistic effect of the compost and biologicals combined use.

To construct pairwise and multiple regressions of the correlation between alfalfa yield and the year of alfalfa cultivation considering the used type of ameliorants.

MATERIALS AND METHODS

The study was conducted in the Al-Asharrah area, located approximately 60 km east of Deir ez-Zor, at an altitude of 203 meters above sea level. The coordinates of the experimental site were 34°55'13" N and 40°33'34" E (Fig. 1).

Natural and climatic conditions

The climate of the Al-Asharra region can be characterized as arid with hot and dry summers and cold winters. In summer, temperatures can reach 45 °C, while in winter they can drop below zero. The main winds in this region are westerly with occasional eastern sand ones. The average annual precipitate is about 150 mm, which is too little to support productive agriculture without additional land reclamation measures.

Soil characteristics

The experimental site is located in the zone of modern terrace deposits of the Euphrates River. The soils are poorly developed and classified as “typical Calcigypsid” and “typical Haplocalcic”, the formers are characterized by the calcium carbonate (CaCO_3) and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) accumulation within one meter of the soil surface. They have a sandy and loamy texture, however, they are of little use for irrigated agriculture because of high gypsum content, which worsens the soil structure and its water permeability. The latters are characterized by the calcium carbonate (CaCO_3) accumulation within one meter of the soil surface. They have a sandy and loamy texture, moderate potential for irrigated agriculture, however, they require careful water management. The general picture of changes in the main climatic indicators by months over three years of observations (precipitation, average and maximum air temperature) and alfalfa yield is presented in Fig. 2. Given to the data presented in Fig. 2, it should be noted that during the three-year experiment, there were no abnormal fluctuations in the main climatic indicators, which contributed to maintaining the purity of the experiment.

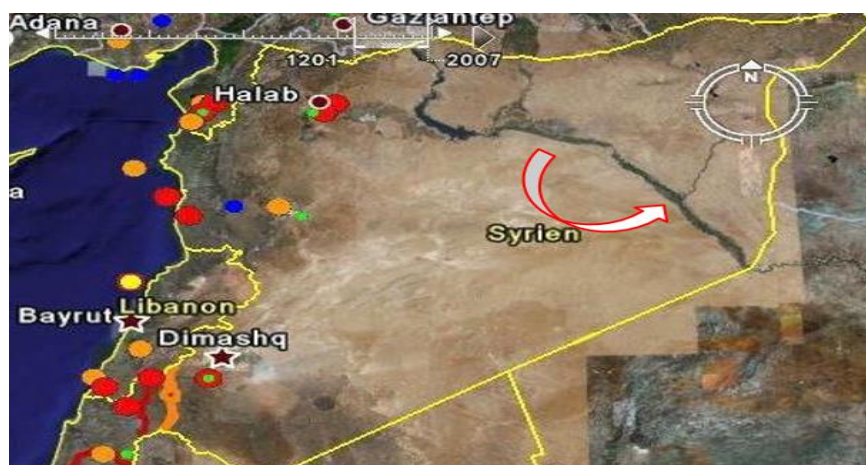


Fig. 1. The site of the research on the map of the Syrian Arab Republic.

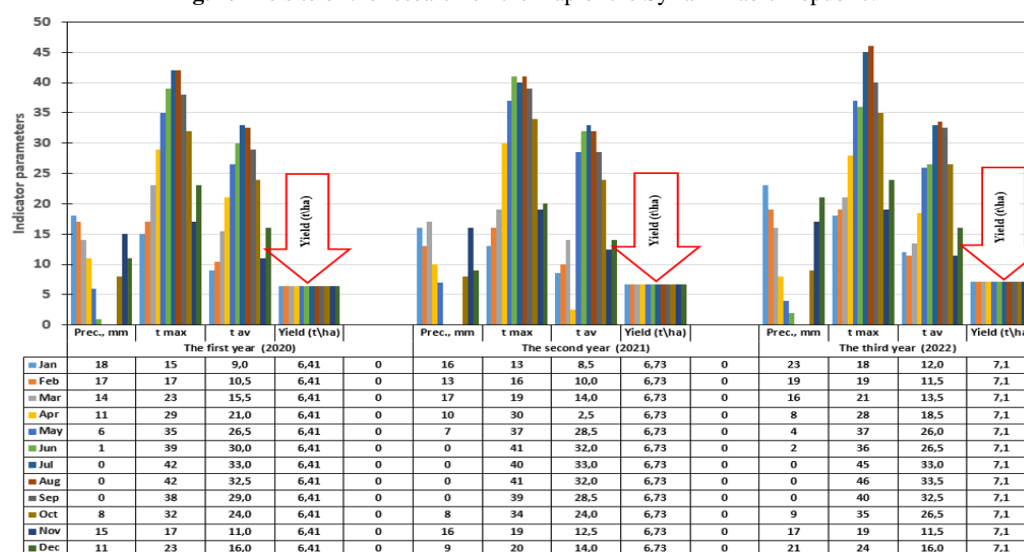


Fig. 2. Calendar change in climatic factors limiting alfalfa yields for three years of cutting (2020, 2021, and 2022).

Research methodology

The agronomic part of the study related to alfalfa yield was organized with the purpose of conducting a two factor experiment with replications by the years of growing three-year-old alfalfa. The experiment was completely randomized in three variants and a control. Each variant had three replications. Three land plots with an area of $2 \times 3 \text{ m}^2$ each were prepared. Compost and biologicals were added to the soil before plowing at the rate of 2 kg compost per 1 m^2 of soil as biocatalysts. Afterward, alfalfa was sown manually to the 5 cm depth at the rate of 30-35 kg of seeds per hectare. Flooding along strips was chosen as an irrigation method. Watering with a 10-mm layer was carried out daily after sowing for 10 days, and then once every 20 days with a layer of 50-80 mm. The layout of the field experiment and the plots are shown in Figs. 3 and 4.

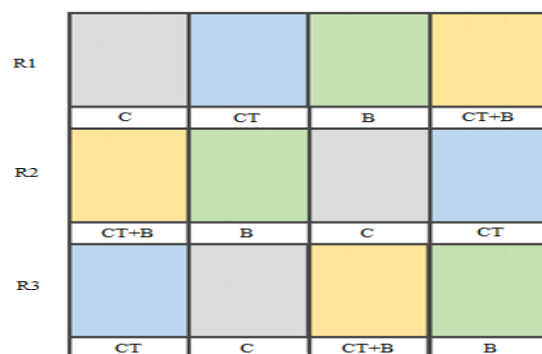


Fig. 3. Scheme of a field experiment with randomized placement of variants in 3-fold replication. R1, R2, R3 – replications
C: control, CT: compost, B: biological, CT + B: compost and biological.



Fig. 4. External view of the field experiment.

RESULTS AND DISCUSSION

A two factor analysis of variance with replications was conducted to assess the reliability of the field experiment results. The HSD criterion (Tukey's Honest Significant Difference) was used to perform a pairwise comparison of differences between the experiment variants. The analysis of yield changes over the experiment variants and the years of alfalfa cultivation was performed by pairwise and multiple regression analysis with coding of qualitative predictors with dummy variables. The results of the three-year experiment are presented in Table 1.

Table 1. Alfalfa yield (tons ha⁻¹) by years of cutting depending on the experiment variant.

Year of growth	Control	Compost	Biologicals	Compost+biologicals
The first (2020)	6.31	7.26	7.10	9.02
	6.49	7.21	7.24	9.20
	6.43	7.25	7.17	9.14
The second (2021)	6.43	7.45	7.12	9.68
	7.04	7.70	7.81	9.90
	6.73	7.80	7.63	9.77
The third (2022)	6.90	9.24	9.10	9.21
	7.00	9.16	9.50	9.53
	7.40	9.38	9.30	9.52

As a first approximation, the data for statistical processing, collected over the experiment variants, are presented graphically in the form of box plots (Fig. 5).

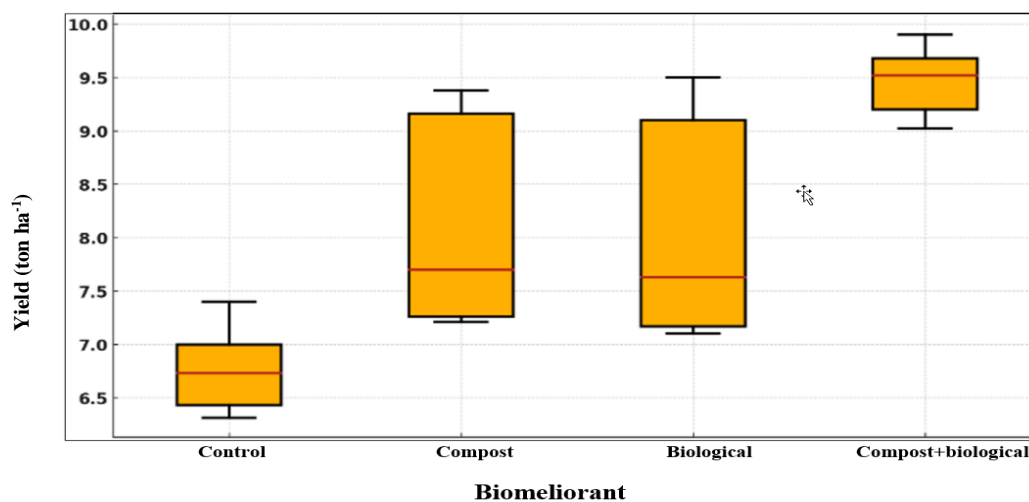


Fig. 5. Box plots to estimate an average alfalfa yield and its variation indexes over the experiment variants.

A box plot is a graphical method to display the distribution of numerical data and its variability with quartiles. There is a description of the main elements of a box plot:

Median (Middle line of the box): A vertical line inside the box showing the average value of the dataset.

The upper and lower boundaries of the box represent the first (Q1) and the third (Q3) quartiles, respectively. Q1 and Q3 are the values below which 25% and 75% of the data lies respectively.

Interquartile range (IQR): The difference between the first and the third quartiles ($Q3 - Q1$). This shows the spread of the middle point of the data. Whiskers are the lines that go from the start of the box to the minimum and maximum data values within 1.5 IQR of the first quartile and the third one, respectively. Whiskers help visualize the data range except for the outliers. Outliers are the data points that lie outside the whiskers. They are labeled separately (usually as small circles or stars) and show values that are significantly different from the rest of the data. Box plots are useful to compare distributions across multiple datasets because they provide a visual representation of the data spread and their symmetry, as well as of the presence of outliers. Statistical processing of the data organized in the experiment, presented in Table 1 by the method of a two-factor analysis of variance with replications, allowed us to obtain the result presented in Table 2. The results of the analysis of variance show that using compost and biologicals significantly increases alfalfa yield compared to the control. The null hypothesis is rejected since the calculated values of the Fisher F-criterion in all the sources of variants (by cultivation years, by experiment variants, and by their interaction) were higher than the theoretical criterion values at the 5% and 1% significant levels. The highest yield was observed with the combined utilizing of compost and biologicals, which confirms the hypothesis of the synergistic effect of these variants. *Bacillus* bacteria, acting as biocatalysts, improve the nutrient availability, which has a positive effect on the plant growth and development. Pairwise comparisons of average values were carried out after conducting the analysis of variance and proving statistical differences between the experiment variants and the number of alfalfa cultivation years. The Tukey's criterion (Q) was used, which is a statistical method for conducting multiple comparisons of average values. It corresponds to the standard (Table 3) value at the 5% significant level with the known replications of the experiment variant and the number of mean squared error (MSE). In our case, for 4 experiment variants with the number of MSE equal to 24, it is 3.9. The main purpose of the Tukey's test is to identify which specific variants differ from the other, ensuring the control of the error level in multiple comparisons.

Table 2. Analysis of variance of alfalfa yield.

Indicators	Experiment variants				Total
	Control	Compost	Biologicals	Compost + biologicals	
The first year (2020)					
Replications	3	3	3	3	12
Sum	19.230	21.720	21.510	27.360	89.820
Average	6.410	7.240	7.170	9.120	7.485
Dispersion	0.008	0.001	0.005	0.008	1.092
The second year (2021)					
Replications	3	3	3	3	12
Sum	20.200	22.950	22.560	29.350	95.060
Average	6.733	7.650	7.520	9.783	7.922
Dispersion	0.093	0.032	0.128	0.012	1.443
The third year (2022)					
Replications	3	3	3	3	12
Sum	21.300	27.780	27.900	28.260	105.240
Average	7.100	9.260	9.300	9.420	8.770
Dispersion	0.070	0.012	0.040	0.033	1.046

Total					
Replications	9	9	9	9	-
Sum	60.730	72.450	71.970	84.970	-
Average	6.748	8.050	7.997	9.441	-
Dispersion	0.132	0.866	1.022	0.096	-
Analysis of variance					
Variety source	SS	df	MS	Fcalc.	F _{0.01}
Years of growth	10.246	2	5.123	138.54	3.40/5.61
Experiment variants	32.702	3	10.901	294.76	3.01/4.72
Interaction	5.799	6	0.967	26.14	2.51/3.67
Inside	0.888	24	MSE = 0.037	-	-
Total	49.635	35	-	-	-

To calculate HSD (Tukey's Honest Significant Difference – Exact and required difference according to the Tukey's test), the following formula is used:

$$HSD = Q \times \sqrt{\frac{MSE}{n}} = 3.9 \times \sqrt{\frac{0.037}{3}} = 0.433 \quad (1)$$

HSD values comparison with the yield difference between the experiment variants leads to the conclusions about the differences at the 5% significance level. A histogram of alfalfa yield was constructed over the experiment variants and over the years of alfalfa cultivation based on the analysis results (Fig. 6).

Table 3. Results of multiple comparisons of alfalfa yield with the Tukey's test over the experiment variants and the cultivation years.

Experiment variants to compare		Difference of average	HSD	p-value	Differences
The first year (2020)					
Control	Compost	0.830	0.433	<0.05	Present
Control	Biologicals	0.760	0.433	<0.05	Present
Control	Compost+ Biologicals	2.710	0.433	<0.05	Present
Co+mpost	Biologicals	0.070	0.433	>0.05	Absent
Compost	Compost+ Biologicals	1.880	0.433	<0.05	Present
Biologicals	Compost+ Biologicals	1.950	0.433	<0.05	Present
The second year (2021)					
Control	Compost	0.917	0.433	<0.05	Present
Control	Biologicals	0.787	0.433	<0.05	Present
Control	Compost+ Biologicals	3.050	0.433	<0.05	Present
Compost	Biologicals	0.130	0.433	>0.05	Absent
Compost	Compost+ Biologicals	2.133	0.433	<0.05	Present
Biologicals	Compost + Biologicals	2.263	0.433	<0.05	Present
The third year (2022)					
Control	Compost	2.160	0.433	<0.05	Present
Control	Biologicals	2.200	0.433	<0.05	Present
Control	Compost + Biologicals	2.320	0.433	<0.05	Present
Compost	Biologicals	0.040	0.433	>0.05	Absent
Compost	Compost + Biologicals	0.160	0.433	>0.05	Absent
Biologicals	Compost+Biologicals	0.120	0.433	>0.05	Absent

The results of the field experiment showed that the highest alfalfa yield was achieved with a combined using compost and biologicals. This confirms the hypothesis of their synergistic effect on alfalfa yield. The analysis of variance showed that using compost and biologicals significantly increases alfalfa yield compared to the control. The largest share of the total yield variation is assigned to the “Experiment variants” factor, which indicates a significant effect of the fertilizer type on the yield. The “Years of cultivation” and “Interaction” factors also make

a significant contribution, while the internal variation “Inside” is relatively small (Table 2). Along with the analysis of variance, statistical modeling of the yield relations was performed by constructing pairwise regressions and a multivariate model combining qualitative and numerically expressed predictors. Thus, Fig. 7 presents pairwise regressions that show changes in alfalfa yields depending on the cultivation year for each of the four experiment variants: control, compost, biologicals, and compost + biologicals.

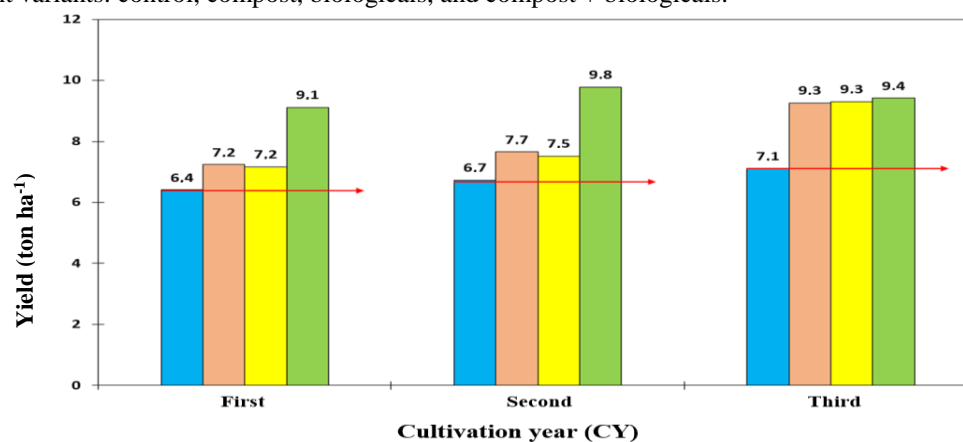


Fig. 6. The change in alfalfa yield over the cutting years according to the experiment variants.

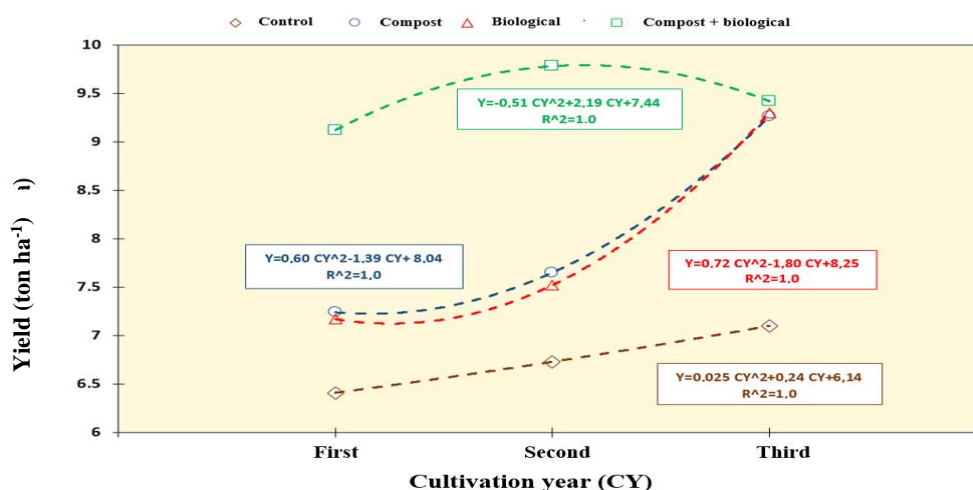


Fig. 7. Pairwise regressions of alfalfa yield changes for 4 experiment variants depending on the cultivation year.

The regression lines help visualize the trends in yield changes over time and demonstrate how the application of biomeliorants affects yield. Thus, the regression line for the control shows that the alfalfa yield in the control variant tends to increase slightly over time. The regression line for the compost variant indicates a slight increase in yield by the second year and a more significant effect by the third year. The regression line for the biologicals shows a similar trend towards increasing yield with a positive effect of a biological by the third cultivation year. The regression line for the combination of compost and biologicals demonstrates the greatest increase in yield in the second cultivation year, which confirms the synergistic effect. Multivariate regression models are of the greatest interest for identifying the patterns of alfalfa yield. The models considering qualitative predictors coded for analysis with block dummy variables are the most suitable among them. In our case, these are the experiment variants representing the control and the three experiment ones (Table 4).

Table 4. Coding experiment variants with dummy variables.

Experiment variant	Block dummy variables		
	X_1	X_2	X_3
Control	0	0	0
Compost	1	0	0
Biologicals	0	1	0
Compost + Biologicals	0	0	1

In the result of the regression analysis we obtained the following model –

$$Y = 6,05778 + 1.97222X_1 + 2.19222X_2 + 1.37222X_3 + 0.34500Y - 1.73500YX_1 - 2.14000YX_2 + 1.85833YX_3 + 0.60000Y^2X_1 + 0.71500Y^2X_2 - 0.51333Y^2X_3$$

$$R^2 = 0.982; ES = \pm 0.19; F = 137.16 \text{ at } p < 0.05; t = [36.4; 3.9; 4.4; 2.7; 4.5; 3.2; 3.9; 3.4; 4.5; 5.4; 3.9]$$

$$> t_{05} = 1.96.$$

where

Y: yield (ton ha⁻¹);

Y: cultivation year (vegetation);

Xi: dummy variable encoding experiment variants;

R²: determination index;

ES: standard error of the regression equation, ton ha⁻¹;

t: significance of numerical coefficients of the equation;

F: the Fisher criterion of regression reliability.

The theoretical yield values obtained from the equation were compared with the actual values (Fig. 8).

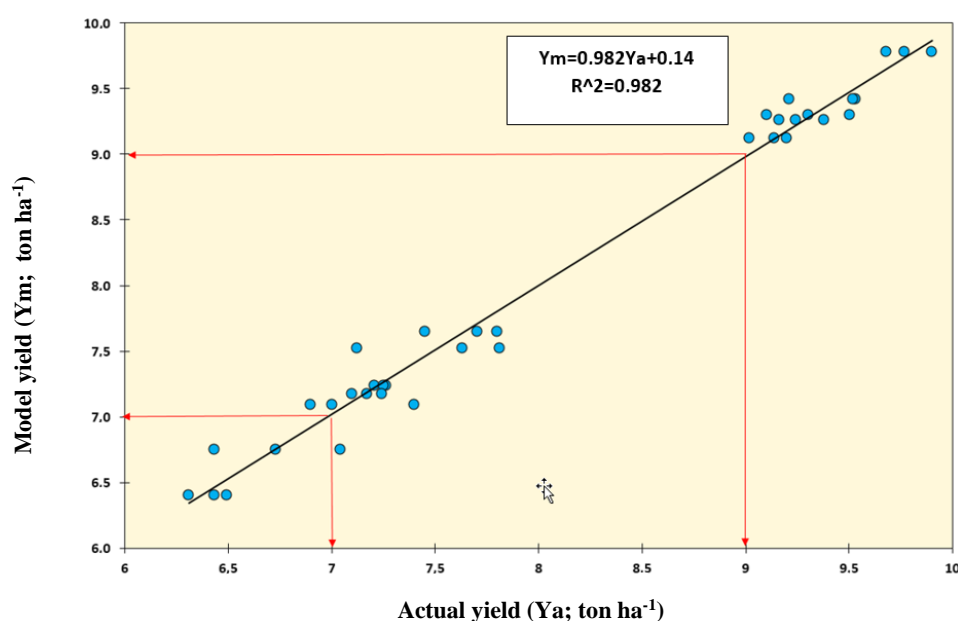


Fig. 8. The square of the assessment for the regression model adequacy.

The graph above demonstrates the compliance of the regression model with the actual data. The closer the predicted values (Ym) obtained by the regression model to the actual data (Ya), the more accurate the model is. Along with the assessment of the model adequacy, an analysis of the residuals (the difference between the observed and predicted values) was performed depending on the observation number, shown in Fig. 9.

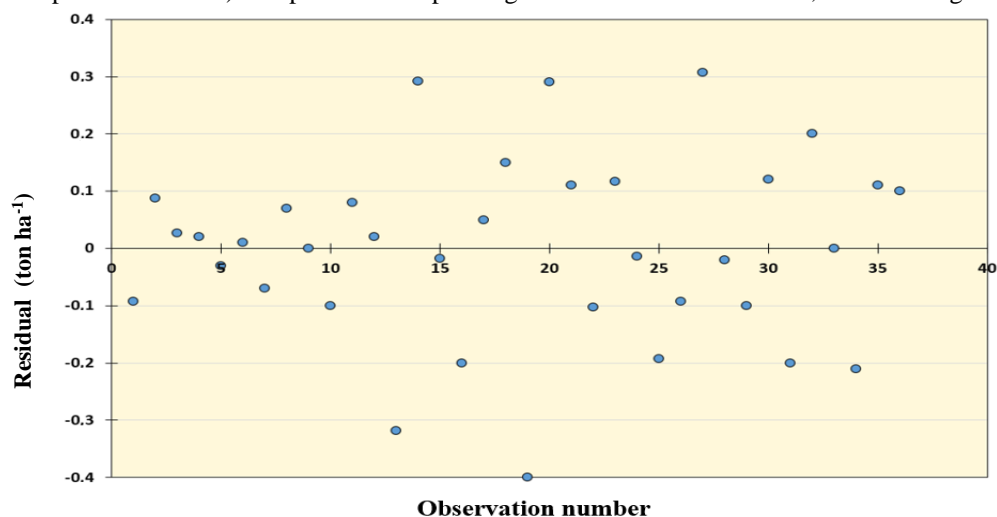


Fig. 9. Analysis of residuals for model 1.

The horizontal line at the zero level “0” indicates the absence of deviations from the regression line obtained according to the model. The visualization of 36 residuals indicates their random distribution over the entire range of variations (from 0.31 to - 0.40 ton ha⁻¹). The final graphical interpretation of the obtained results of the yield changes is shown in Fig. 10.

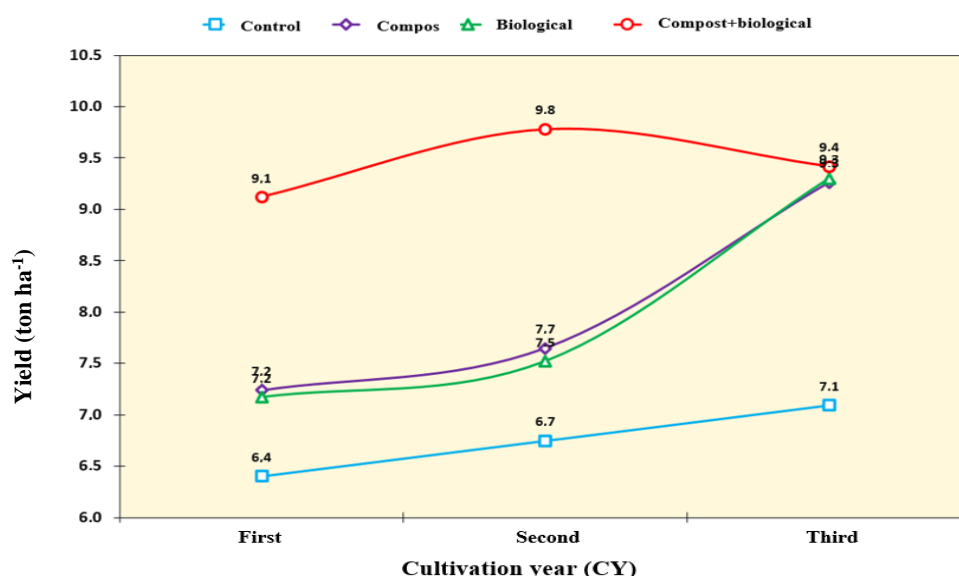


Fig. 10. Change in alfalfa yield with two cuts over the experiment variants and the cultivation years.

This graph visualizes the change in alfalfa yield for each experiment variant and cultivation years. The X-axis represents the cultivation years (2020, 2021, and 2022), and the Y-axis shows the alfalfa yield in tons per hectare. The regression lines show the change in the yield for each of the four experiment variants (control, compost, a biological, and compost + biological). Thus, the control variant line shows a slight increase in the yield caused by more favorable indicators of the atmospheric moisture and the temperature regime in 2022 (Fig. 2). The line of the variant with compost indicates a slight increase in the yield in the second and a significant increase in the third year of cultivation. The line of the variant with a biological is slightly below the line of the variant with compost. The greatest increase in the yield in all the observation years is demonstrated by the variant with combined use of compost and a biological.

CONCLUSION

The use of alfalfa-based compost and *Bacillus* genus biologicals significantly increases alfalfa yields in arid climates. The combined use of compost and biologicals provides the greatest effect, which confirms the synergy hypothesis. The two factor analysis of variance with replication showed that the use of compost and biologicals significantly enhances yields compared to the control. The calculated values of the Fisher criterion (F-criterion) indicate that the differences between the experiment variants and the cultivation years at the 5% and 1% significant levels are considerable. Pairwise comparisons of average yield values over the experiment variants using the Tukey's criterion confirm the presence of statistically significant differences between the experiment variants, especially between the control and the combination of compost and biologicals. The regression analysis with coding of experiment variants with dummy variables allowed us to obtain an equation explaining the dependence of the crop yield on the cultivation year and the type of applied ameliorants. Statistical parameters ($R^2 = 0.982$; $ES = \pm 0.19$; $F = 137.16$) and the significance of the equation numerical coefficients ($t > t_{05} = 1.96$) confirm the model reliability. The results can be used for developing recommendations to increase the agricultural crop productivity in arid climates, since they offer some effective combinations of phytomeliorants and biologicals for increased crop yields. Thus, the conducted study demonstrates the significant potential of using compost and biologicals for improved agrotechnical characteristics and an increased alfalfa yield in arid climate conditions.

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