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CLIMATE ANALYSIS AND EFFECTS OF ABIOTIC STRESS ON SALAD GROUNDED IN UNDERGROUND GREENHOUSE AND OUTDOOR AND EFFECTS OF ORGANIC FERTILIZERS IN THE FIGHT WITH STRESS FACTORS

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Abstract

In the study was determined the impact of organic fertilizers as anti-stress agents and their impact on the particular species. Also was carried out an evaluation of the plant anti-stress response, by applying different methods of reporting and looking for ways to overcome it. Climate change and coming with them abiotic stressors, a consequence of extreme weather conditions, especially affect on salad grown outdoors. What are the climate fluctuations in hydrothermal conditions and how they can affect the growth and development conditions? This question is very relevant at the moment. Therefore a general hydrothermal characterization of the climate in the Plovdiv region for the period 1990-2019 has been made. In the study were made analyses of the basic conditions during the period investigated, the presence of stressors, as well as an assessment of the impact of major meteorological factors on salad crop productivity, on three different varieties, using four different formulations of organic fertilizers, and also control variants with mineral fertilizer and without fertilizing. The goal was to increase plant resistance to abiotic stress and to guarantee the yield by optimizing nutrition.

Key words*: Lettuce (Lactuca sativa), organic fertilization, climate change, Plovdiv region.*

INTRODUCTION

In the face of climate change, organic production is among the leading modern strategies for adapting green systems to changing conditions of growth and development. Agricultural production, including leaf and head lettuce, fed with organic fertilizers, guarantees the safety and purity of food while at the same time having the function of preserving and enriching soil biodiversity. The high interest in the use of organic fertilizers of different concentrations is justified due to the fact that their use leads both to the conservation of soil resources and to an increase in soil fertility. Organic fertilizers combined with soil temperature increase the usability of nutrients. The rhizosphere is the area around the roots of the plants where maximum microbial activity occurs. Bacteria that improve plant growth and health are known as rhizobacteria that stimulate plant growth (PGPR). On the combinations and mode of action of plant growth-promoting rhizobacteria (PGPR) are studies by Vessey, 2003. The use of PGPR in agriculture is definitely a priority, as synthetic chemicals pose a serious threat to agro-ecosystems (Verma et al., 2019).

The agricultural production is mainly performed outdoors, and the importance of the basic meteorological elements - temperature and precipitation - is well known. Despite the existing mechanisms for sustainability and environmental plasticity, the cultivation of early spring crops, including lettuce, is determined by the climatic conditions of the individual territories. The salad is grown in our country, both in unheated greenhouses and in the open air, with any change in hydrothermal conditions affecting the economic efficiency in the first case and directly on the productivity in the second. The analysis of the multiannual data of the major meteorological elements over

the last century in Bulgaria shows a tendency to increase temperatures and decrease or change the distribution of rainfall by seasons (Kazandjiev, 2008; Koleva & Alexandrov, 2008). There has been a change in humidity through the index I_{DM}DeMartonne (1926) from moderately moist to moderately dry, with a proven decrease in the productivity of non-irrigated crops (Kazandjiev et al., 2009). There is a change in the duration of the potential growing season (Slavov et al., 2006) to a faster accumulation of the required temperature sums and shortening of the interphase periods, which adversely affects the crop yield. Moisture conditions are directly related to the amount of precipitation. Trends in declining annual precipitation in the Danube Plain and the Thracian Plain, as well as an increase in the frequency of dry years over the last century, have been identified by Koleva & Alexandrov, 2008; Alexandrov et al., 2011. In the first 15 years of this century, some weather stations have experienced an increase in rainfall during the autumn season and moisture accumulation of up to 10% (Georgieva et al., 2017). In recent years, the frequency of extreme weather events has caused damage to varying degrees and loss of agricultural production, both worldwide and in our country. More recent climate studies in Bulgaria show a clear trend of warming after the mid-1980s. In the period 1988-2016, the average annual air temperature in areas up to 800 m above sea level increased by an average of 0.8°C compared to the climatic norm for the reference period 1961-1990, and the trend for precipitation amounts is inversely determined by the previous climatic period. Quantities have increased and heavy rainfall has caused severe flooding harmful to various socio-economic sectors, including outdoor agriculture (Marinova et al., 2017). On the other hand, the process of water distribution and irrigation presupposes rationality and balance in the guaranteed human right to have water and protect ecosystems in the face of climate change (Kolcheva, 2019).

The salads are grown for fresh consumption and have a great variety, color and taste. They are characterized by a relatively short growing season, reaching economic maturity 50-60 days after planting.The optimum temperature for growth and development is 23°C during the day and 7.0°C at night. At high temperatures, the plants bloom, deform and become bitter. Freezing causes damage to varying degrees, especially on the wrapping sheets and reduces their resistance to storage and transport. The recommendations for their cultivation, the timing of planting in the different regions of the country, nutrition and drifts, depend on the climatic conditions. As leafy plants, they are susceptible to nitrate accumulation, which makes them organically grown extremely important and promising. With this publication, the authors aim to make a hydrothermal analysis of the Plovdiv area for the last 30 years. For a period of 3 years an attempt has been started to test the behavior of three varieties of salad in conditions of transition to organic fertilization. Data are currently being collected, but the experiment has not been completed and some preliminaryresults will be analyzed here. In the course of the study the influence of biotors as anti-stress agents and their impact on the specific species was determined. The anti-stress response of the plants has also been evaluated by applying different methods of reading it and looking for ways to overcome it. One of the main goals is to optimize nutrition and increase the resistance of salads to abiotic stress.

MATERIALS AND METHODS

Daily air temperature data, rainfall (mm), air humidity (%), etc. were used from the weather stations in Plovdivaccording to the recommendations of WMO and the methodological guides of NIMH (Stanev, 1969). For the analysis and calculation of the climatic norm for the reference period of the De Martonne Index (1926), daily values of air temperature of 2m above the earth's surface and 24 hours of precipitation from the NIMH database available at the AU Plovdiv were used. Annual and monthly indices are calculated that are appropriate for both planetary and regional analysis using the formulas (Mitkov & Topliyski, 2018):

$$J_{DM} = \Sigma R / T + 10),$$

where ΣR is sum of the rainfall in mm and T is the average temperature °C; J (mm/°C) index (De Martonne, 1926) by months.

$$J_{DM} = 12 * \Sigma R / T + 10),$$

where ΣR is the monthly sum of the rainfall in mm and T is the average monthly temperature

°C using the index and rating scale appropriate for the index.

A professional agrometeorological station was installed for the purpose of the experiment and immediately to the observed plantsMeteobot® Pro (https://meteobot.com/meteostancii/), with a set of sensors: for rainfall, air temperature, soil temperature at planting depth, etc. Attempts have been made to plant plastic greenhouses with planting respectively - before winter and outdoors - in the spring of 2018-2019. The plants were planted in phenophase 4-5 leaves in November 8 in the polyethylene greenhouses of AU-Plovdiv in 4 rows according to the scheme 70+30+30+30/30 cm with a profile of the soil surface a high level bed (100+60 cm.) The experiment was based on the block method in four repetitions in 28 plants per repetition, with a plot size of 3.36 m^2. Organic seeds were provided for seedling production using container technology using 150-hole Styrofoam boards.

Organic seedlings are used - 80%, Perlite - 20%, Lumbricompost, developed by us for bio-production of seedlings(Kostadinov & Filipov, 2013). The following fertilization options are being studied: 1. Control-non-fertilization; 2. Control-MT (mineral fertilization-NPK); 3. Italpollina; 4. Arkobaleno; 5. Lumbricompost; 6. Ekoprop NX. Granular fertilizers were introduced as basic fertilization, with pre-transplantation of soil, into the following norms: N- 12.5 kg/da, P_2O_5 - 1.25 kg/da, + K_2O - 4.75 kg/da, Italpollina- 25 kg, Arkobaleno - 100 kg/da, Lumbricompost - 400 l/da. Ekoprop NX is applied by double treatment at a dose of 100g/dka, the first in the seedling phase, the second after planting and planting.

The salads are three varieties of Batavia variety 'Maritima', Leaf lettuce-type Lolo rosa variety 'Tuska', Head lettuce variety 'Winter Oil Head', 3000 plants (1000 plants of each variety). The greenhouse experience is based on 6 variants with a total area of 450m^2, of which 375m^2 with organic fertilization. A drip system was used for watering in the greenhouse and outdoors.

Biometric studies include three-fold measurement of phenophase commercial maturity every 7-10 days for each variety and variant of: Fresh whole plant mass (g); Socket - D - diameter (mm) and mass (g); Head - arrangement, shape, coloring of the rosette leaves (superficial and internal); Leaf count and fresh mass (g); Stem mass (g), diameter and length. The total yield, quality, appearance, texture, organoleptic evaluation, was reported by repetition.

Statistical Package for the Social Sciences (SPSS) (SPSS Inc., 2007) and Microsoft® Office products were used for statistical data analysis. The visual evaluation of the smoothed curves is subjective, so the existence of a trend is investigated using the nonparametric test of Mann-Kendall (WMO, 1990).

RESULTS AND DISCUSSIONS

What are the current climate studies in Plovdiv, what are the climate fluctuations in hydro-thermal conditions and how they can affect the growth and development conditions?

Given the climate fluctuations discussed, a general hydrothermal characterization of the climate in the Plovdiv region has been made.

The air temperature, precipitation and soil temperature were studied, as well as the De Martonne (1926) index, which gives an estimate of the humidity conditions.

For the period 1990-2019. a positive, statistically significant trend (Test Z 3.55; Signific. ***) in annual air temperature was observed in the study area (Figure 1). The deviation from the reference period (1961-1990) in degrees is significant +0.9ºC. It should be noted that the highest values occur after 2000, with the two warmest years being 2000 and 2019, with values of 14.6ºC and 14.2ºC, respectively, with an average value for the whole period of 12.9ºC (Figure 1).

In this sense, it is necessary to conclude that there is a change in temperature conditions, i.e. a statistically significant trend, an increase in the new period, and that the increase is most significant at the beginning of our century.

The deviation from the period 1990-2019 is between + 1.6ºC and -1.2ºC, but after 2010 is only positive (Figure 2). For January the deviation from the reference period is positive + 0.7ºC.

For February the deviation for the period 1990-2019, compared to the reference period is positive + 0.5ºC.

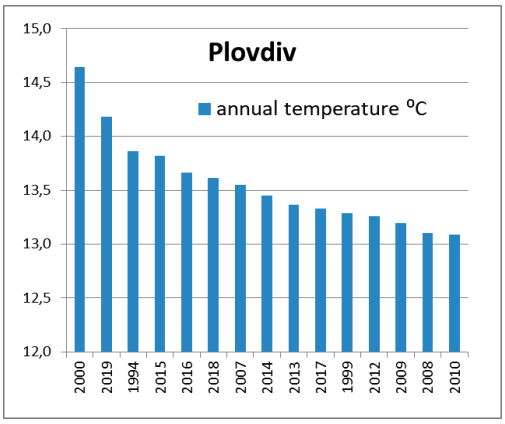

Figure 1. Highest annual temperature °C since 2000

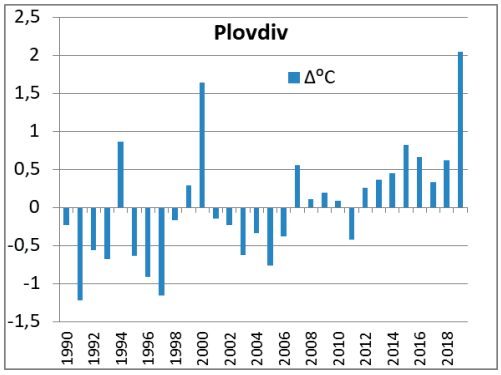

Figure 2. Deviations from 1990-2019

A positive trend (Z 1.89 +) was observed at the average monthly temperature for March, and the deviation of the period 1991-2019 from the reference 1961-1990 was 0.9°C.

A positive trend was observed with a level of significance (Z 1.74 +) and a deviation for April 1991-2019, compared to the reference 1961-1990 of 0.5°C. The trend in May is significant (Z 1.71 +) and the deviation from the reference period is 0.7°C.

In June, the increase over the reference period was 1.2°C, in July 1.4°C, August 1.9°C. For September, the increase compared to the reference period is 0.7°C. Mann-Kendall tests report a positive trend (Z 2.82**) for the period 1990-2019.

For October the deviation is 0.6°C. November deviation from the reference period is 0.5°C positive trend with significance level (Z 2.36*). The only month with a slight, negative deviation of less than one-tenth is December (-0.1°C) (Figure 3). Previous surveys of the area, but for the period 1986-2015 they give a negative course of two winter months and it with a greater deviation (Georgieva et al., 2017).

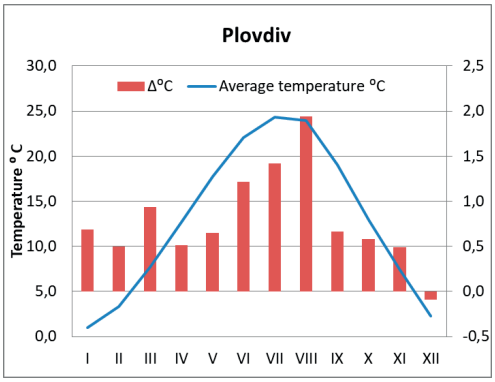

Figure 3. Average temperature by months and deviations from 1961-1990

The annual rainfall shows a significant increase over the period 1990-2019 (Z 2.50*), with a deviation from the reference period 1961-1990 of 34 mm.

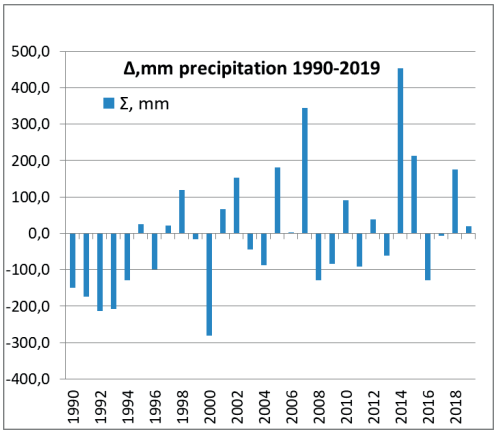

Figure 4. The annual rainfall deviations (Σ mm) 1990-2019

The highest amounts are predominantly after 2000, the two largest being in 2014 and 2007, respectively, 993.9 mm and 884.1 mm (Figure 5). Also, the highest negative deviation (annual ∑ mm) is during the same period, which shows an increase in the frequency of extreme events (Figure 4).

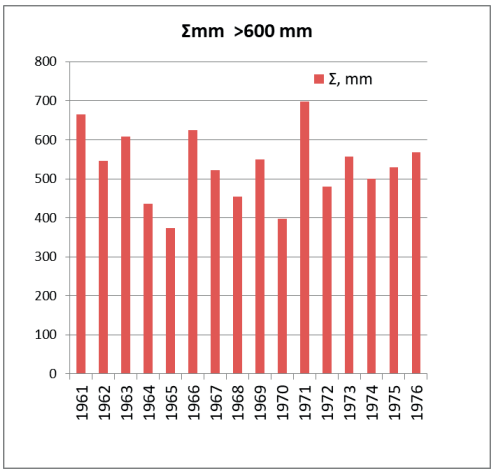

Figure 5. The highest annual rainfall (Σ mm) 1961-2019

The monthly analysis shows negative deviations in the spring - April, May and November and positive during the other seasons, with the highest values in September and October, about 30%, respectively 12.2 mm and 13.3 mm (Figure 6). Here, Georgieva et al., 2017, found a 5-10% increase in rainfall outside active vegetation over the period 1986-2015, compared to the reference 1961-1990. There is a statistically significant trend for the period 1990-2019 (Z 1.96*) and a deviation of 3.9 mm in June, compared to the value for the same month during the reference period.

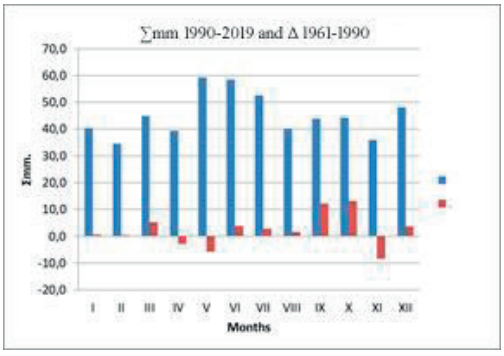

Figure 6. Monthly rainfall and deviations (Σ mm) 1990-2019 from the reference period

Soil temperature is no less important element in the development of salads. An analysis of the period 1990-2019 has been made (Figure 7). Here, the values of 20 cm are investigated here, because at this depth the planting is carried out and the basic root system of the observed species develops. The great importance of PGPR in the rhizosphere has already been mentioned. In addition to their activity, soil temperature also affects the speed of biochemical processes in the growth and development of plants and through the absorption of biological fertilizers. The soil temperature by months shows a positive trend throughout the period, including the winter months. The most pronounced and of the highest statistical significance are summer and autumn - July, August, September and October (March 20 cm (Z 1.76 +); April 20 cm (Z 2.26*); May 20 cm (Z 3.25**); June 20 cm (Z 2.36*); July 20 cm (Z 3,55***); August 20 cm (Z 3,40***); September 20 cm Z 4 , 04 S***); October 20 cm (W 4.04***); November 20 cm (Z 2.80**); December 20 cm (Z 2.16*). Only three are months with a temperature of 20 cm <5.0ºC - December, January and February.

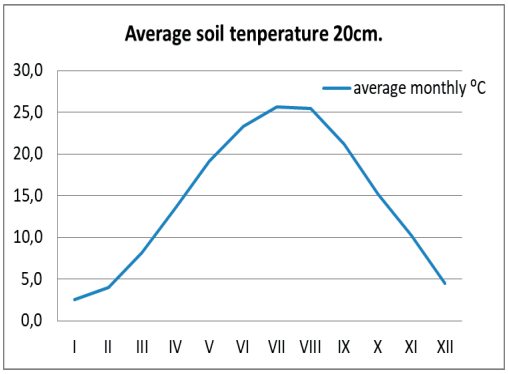

Figure 7. Average monthly soil temperature (1990-2019) at a depth of 20 cm in °C

The humidity conditions were estimated using the De Martonne Index (1926). The calculations show a difference of 1.7 compared to the period 1961-1990 and a positive trend (Test Z 3.46; Signific. * * *) For the period 1990-2019. The values for the two periods are respectively 23.2 and 24.9, which on the respective scale (Figure 8) characterizes the area as Semiarid, with the second period very close to the beginning of moderately arid.

All highest values were found in the period after 2000, and the highest values were in 2018, 2014, 2017 and 2019 as follows: 52, 42, 40, and 39 and characterize the years as Very humid, Humid and Moderately humid (Figure 9).

J(mm/°C)	Climate classification
<10	Arid
10-25	Semiarid
25-30	Moderately arid
30-35	Slightly humid
35-40	Moderately humid
40-50	Humid
50-60	Very humid
60-187	Excessively humid

Figure 8. The classifications by DeMartonne,1926

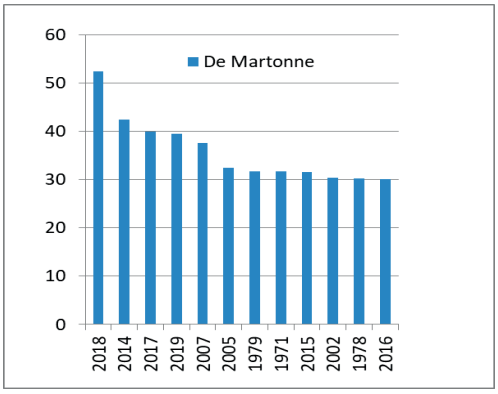

Figure 9. Highest De Martonne Values 1961-2019

An analysis of the De Martonne Index (1926) noted an improvement in the humidity conditions during the period 1990-2019 in the Plovdiv area. At the same time, extreme phenomena such as intense droughts and intense rainfall are observed (Figure 10).

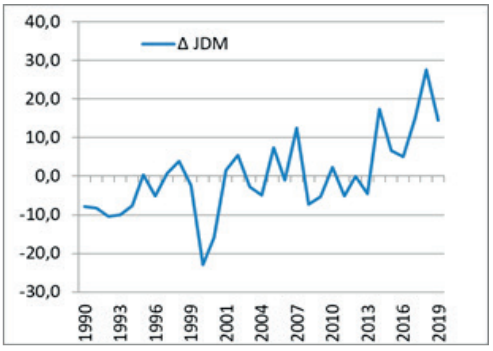

Figure 10. De Martonne indexes (1990-2019 deviations)

Greenhouse experience

Biometric Characteristics of Harvesting Plants –'Winter Oil Head' Variety - 2019. More significant differences are reported in the biometric parameters of the vegetative organs. In the first harvesting date (March 13th) With a higher average mass of the whole plant than the control - mineral fertilization is only option 6 (Ekoprop NX) with an excess of 12.7%. On the second date (March 20th), the trend from the first is preserved. Similar values to NPK control values were reported in Option 5. At the last harvest, Option 3 and Option 5 had similar values to those of the mineral fertilization control, with Option 6 exceeding 6.2%. Although most organic fertilizer options are inferior to mineral fertilization control, they meet the requirements of the standard (300-400 g) for individual harvests. The number of leaves showed more significant differences in the variants studied. They are more pronounced in the second and third harvest. The excess of 7 leaves is reported for option 5 and option 6 for the second date and from 4-5 sheets for the last harvest. The average results on this indicator give an advantage to the studied variants over the control - mineral fertilization. The organic fertilizers used stimulated the formation of 2 to 4 leaves more per plant. The average values of the stem indices do not differ significantly between the variants except for variant 6. It exceeds the control (variant 1) by 4.4% in diameter and by 16.7% by mass. Fertilizing had the least impact on the rosette diameter. The differences between the variants are insignificant in the harvests and in the average data. They are within 2-3 cm. In conclusion, it can be noted that fertilization had the most significant effect on the number and size (mass) of the leaves. A smaller effect is recorded with the size of the leaf rosette (diameter) and the size of the stem. Given that the salad consumption part is the leaves, a positive effect is observed in all organic fertilizer variants tested.

Morphology and biometrics of the 'Tuska' variety B. The biometric indices of the individual organs were less affected by the fertilization applied. The average mass of the whole plant in the variants with biofertilization for the 1st and 2nd harvest is inferior to the control - mineral fertilization. At the 3rd harvest date, Option 6 exceeds it. All the variants tested have better values than the control - not rough. Other leaf, stem and rosette indices show a similar trend to that of the control over the mean mass of the whole plant.

The values of the individual indices for the 1st and 2nd harvest are the highest for the control - mineral fertilization. At the 3rd harvest date, Option 6 slightly outperforms it. All biofertilizer variants have slightly better values than the nonfertilized rosette variants. However, the excess is insignificant within 1-2 cm.

Morphology and biometrics of grade A 'Maritima'. The effect of applied biofertilization on the morphological characteristics of plants follows the trends, as in the other two varieties. The highest values for the indices of the individual organs are reported in the mineral fertilization control. The highest number of harvested leaves and average for the growing season shows option 1 - control. The variants tested had lower values, with only Option 6 approaching the Option 1 control. Stem diameter values, averaged and harvested, show an advantage of the mineral fertilization control over most of the variants tested. This tendency also persists with the mass of the stem. A better stimulating effect of the biotors is accounted for by the diameter of the leaf rosette. The difference between the control and the individual variants is small within 1.0-1.5 cm. An important indicator affecting the quality of the salad and the consumer rating is the average mass of the whole plant. With the highest average harvest weight and average during the harvesting period, the mineral fertilizer version is about 800g. With close weight Option 6 about 775g. The other variants tested, with the use of different biotors, are lagging between 136g - 165g. Despite the lower weight, the plants cover and exceed the requirements of the standard (300-400 g).

Outdoor experiment

Biometric measurements. In order to determine the influence of different biotrains on the growth and development of lettuce plants, the indicators characterizing vegetative growth were monitored. Two observations were made and measurements were made on the plants after planting. The impact of individual biotores on the growth and development of salads was determined. Fertilization (with mineral and organic fertilizers) has an extremely strong impact on overall biology, growth and development. This effect is especially pronounced in plants with a consumptive part of the vegetative organs, such as salad. Fertilizers have a complex effect, greatly stimulating the productivity of the salad. In order to assess this impact, two readings of the basic morphological parameters were conducted twice during the growing season in accordance with the salad standards. The first reading took place when entering maturity. The following is contacted - as the vegetation progresses, the positive effect of the fertilizers tested on the consumption part increases, and on the non-consumption part, this effect weakens. These indicators are reported at anomalous in terms of humidification year conditions and higher than usual average temperatures. In all the variants examined, plants meeting the requirements of the lettuce harvesting standards developed. The field experiment was planted on April 17thand harvested on June 24th. Due to the extreme meteorological conditions (floods), the C-head lettuce entered premature phenophase full flowering 50% on May 24th.

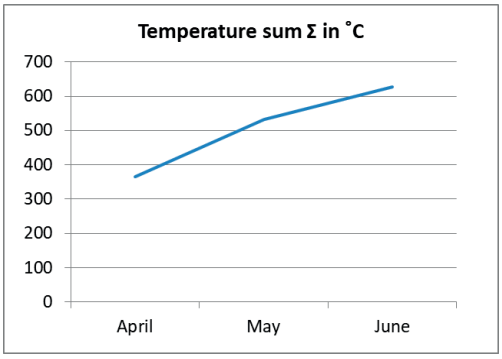

Figure 11. Accumulated temperature sums during thefield experiment

A very high correlation was found in all three varieties to the temperature sum by months (Figure 11), with the correlation coefficient R=0.97 and the determination coefficient R2=94.1%, respectively, indicating that 94% of the changes in the resulting variable were the result of the changes of the factor variable. The number of leaves in the different varieties is in direct proportion to the accumulated temperature sums per month for the trial period (Figure 12).

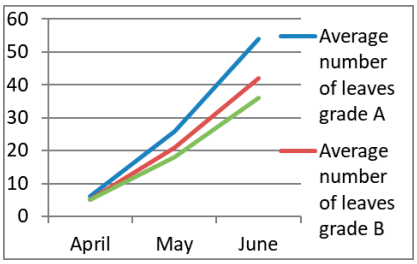

Figure 12. Average number of leaves at the beginning is the end of the trial for the different varieties

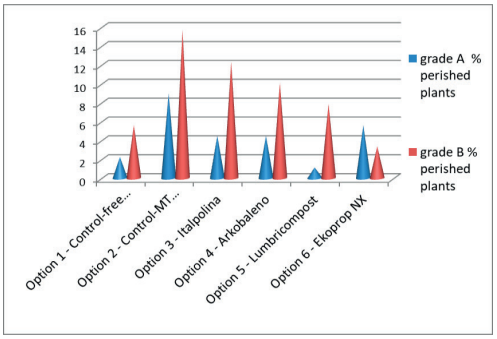

Figure 13.Average number of leaves at the beginning is the end of the trial for the different varieties

Different survival rates are observed for the different fertilization options (Figure 13). It should be emphasized that in some of the variants with biofertilization, the survival of plants subjected to extreme weather conditions is significantly higher. In the case of variety A Option 5 - Lumbricompost gives the lowest percentage of dead plants only 1.1%, in variety B the best result with Option 6 - Ekoprop NX only 3.4% of dead plants. These percentages are lower than in the non-fertilized control variants, which are 2.2% for cultivar A and 5.6% for cultivar B. It is crucial to note, however, that the dead plants in the mineral fertilizer variants in both the varieties are 8.7% higher for variety A and 15.7% higher for variety B, respectively, which is a statistically significant difference.

CONCLUSIONS

When comparing the values of the air temperature for the period 1990-2019 to the reference 1961-1990, the average deviation in the annual value is 0.9ºC. Checking the trend from 1990-2019 with the Mann-Kendall test shows a statistically significant increase (Z 3.55; Signific. ***). The highest value 14.6ºC was established in 2000, the second highest in the period and above 14ºC in 2019. The variations during the summer are the highest - August, July and June with values of 1.9ºC, respectively 1.4ºC and 1.2ºC. Negative deviation in air temperature is observed in winter - in December. In the remaining months, the distribution is relatively uniform, between 0.5ºC and 0.7ºC. Compared to previous surveys for the period 1986-2015, which found negative deviations during the two winter months - November and December, in the period 1990-2019 the negative value remained only in December, but was significantly mitigated.

The trend in the amount of precipitation is opposite to that observed during the 1971-2000 period. There is a positive trend (Z 2.50; Signific. *) and an increase of 34 mm (annual Σ mm) according to the period 1961-1990. Extreme events have also intensified - intense droughts, intense torrential rainfall, with both highest amounts since 2000.

At a soil temperature of 20 cm, which is a factor in the development of lettuce, the activity of rhizobacteria and the absorption of organic fertilizers, statistically significant trends are observed, with the most pronounced positive increase in the summer and autumn months. Statistical significance was not taken into account only in the two winter months - January and February.

An analysis of the De Martonne (1926) Index noted an improvement in the humidity conditions over the period 1990-2019. At the same time, it can be seen that extreme phenomena are observed, with intense droughts and intense rainfall.

Fertilizers tested for outdoor salad cultivation show a good overall stimulating effect, combined with rapid initial effects, under conditions of temperature and water stress per year, with extreme rainfall and temperature variations between 2.0ºC and 4.0ºC. The study shows the importance and benefits of biofertilization, both for the ecology and for the survival of plants exposed to abiotic stress. An adverse effect mitigation effect has been identified, which implies that the study should be continued outdoors. The experience goes on and these are initial results that are very good and give us hope for the future. The results

obtained show the advantage of organic fertilizers and warrant being tested in a longer study covering different meteorological conditions.

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