SUMMARY OF PhD THESIS

The influence of sulphurous water from the Jibou area on blackcurrant fruits

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INTRODUCTION

The mineral nutrition of plants is a physiological process of supplying plants with nutrients. Nutrients absorbed from the environment can be inorganic (ie minerals) or organic. When the chemical elements are missing, insufficient or in excess in plant nutrition, physiological diseases appear, accompanied by the slowing down or stopping of the growth of the root, the stem, the leaves, or the fruits.

In the present paper, we proposed to carry out an extensive study of the soil and sulphurous mineral waters in the Jibou area, as well as their influence on blackcurrant fruits from the agricultural lands adjacent to the springs.

CURRENT STATE OF KNOWLEDGE

Description of the blackcurrant plant (*RibesNigrum* L.). Blackcurrant (*RibesNigrum* L.) belongs to the genus Ribes, family Grossulariaceae, order Saxifragales, class Magnoliopsida, phylum Magnoliophyta, kingdom Plantae. It is an erect shrub, 1–2 m tall, with glabrous branches.

The leaves are petiolate and alternate on the stem. They have a cordate base, are palmately lobed, having five lobes, are wide, 3–5 cm long. The flowers are grouped in 5–10-flowered racemes with pubescent rachis and pedicels and pubescent, ovate or lanceolate bracts. Calyx with elongated, pubescent, recurved sepals; hypanthium subcampanulate with spreading or reflexed lobes. The petals are ovate, half as long as the sepals, white to reddish; nectary disc prominent, green or purple, circular, covering the ovary; stamens slightly longer than the petals. The fruits are black, globose, grouped in racemes, juicy, with a sour taste, 6 – 11 mm in diameter, each containing several seeds.

The importance of the blackcurrant plantation. More and more often we are told that "we are what we eat", and functional foods are becoming more and more interesting for consumers. Blackcurrants are recognized as a rich source of polyphenols (especially anthocyanins, phenolic acid derivatives, flavonols and proanthocyanidins) useful for vascular and metabolic health. Among the wide range of bioactive components of these fruits, a main position is occupied by anthocyanins, which are polyphenolic glycosides and belong to the flavonoid family.

Biochemical composition of blackcurrant fruits. The nutritional composition of raw Ribesnigrum fruits per 100 g edible portion was reported as: water 81.96 g, energy 63 kcal (264 kJ), protein 1.40 g, total lipid 0.41 g, ash 0.86 g, carbohydrates 15.38 g.

Therapeutic properties. Black currant is used successfully in the prevention of osteoarthritis, as a remedy for colds and flu, and the juice is used to stop diarrhea and stabilize digestion.

The spread of blackcurrant plantations. In the early to mid-1900s, black currants were discovered to be high in vitamin C (ascorbic acid) and spread rapidly in Europe, where citrus fruits could not be grown and were expensive to import

(HUMMER and DALE, 2010). Black currants are native to the N hemisphere, mainly in the colder regions of Germany, England and France (https://www.frutas-hortalizas.com/Fruits/Origin-production-Black-currant.html). According to FAO data, among the ten main producers of currants in the world, in 2020, the leader in production is Russia (436,900 t), followed by Poland (146,500 t) and Ukraine (25,790 t).

The current state of blackcurrant cultivation technology. The blackcurrant is easy to grow, forming, in mid-summer, clusters of dark purple fruits, rich in vitamin C, which, thanks to their aroma, can be used in pies and jams, but also in other preparations (https://www.rhs.org .uk/fruit/blackcurrants/grow-your-own). Blackcurrants can also be grown successfully in containers.

Black currants, in general, require irrigation only during periods of drought, but abundant watering should be avoided during the ripening phenophase of the berries (BABUC, 2012).

At the end of winter (February), it is recommended to apply a potassium-rich fertilizer, and in the case of poorly developed plants, it is recommended to apply a nitrogen-based fertilizer.

The history of the use of sulphurous waters in Romania. Mineral water represents underground water that is formed at different depths in the earth's crust naturally, when it meets appropriate geological conditions, being extracted on the surface of the earth spontaneously or by technical methods (TURHAN, 2021; Official Journal, 2004).

PALKO KARASZ specified, in 2019, for The New York Times that, in Romania, mineral water is deeply rooted in local culture and mythology, being the favorite drink of Romanians. Mineral waters are unevenly distributed on the territory of Romania, most of them are found in Transylvania. Currently, Romania, known as the homeland of mineral waters, covers more than half of Europe's mineral water reserves (BODOR et al, 2021).

The springs of sulphurous chlorinated mineral water in the Jibou area, Sălajcounty. In the sector called "Sărătura", on the road leading to Zalău, 1.5 km from the city center, on a plot of land of 1.40 ha, there is a spring with sulphurous-chlorinated mineral water. PărțulFiriza is at the property boundary with the land where the soil samples were taken and where blackcurrant bushes are grown, in ecological conditions.

PERSONAL CONTRIBUTION

The objectives pursued. The purpose of the research carried out during the doctorate period was the physico-chemical characterization of the soils on the lands adjacent to the sulphurous springs and of the sulphurous water samples, as well as the research of the quality of the blackcurrant fruits grown on these lands, in the period of 2017-2020.

The objectives of the doctoral thesis were the following:

- Analysis of the particularities of the natural environment of the Jibou area where the experimental research took place;

- Organization of a natural experimental field to study the influence of sulphurous water from the Jibou area, on the soil and together with the blackcurrant fruits, with 5 experimental variants, characterized by soil and water analyzes (5 soil samples, 5 water samples); the analysis of the quality and production indices of currant fruits was carried out for each parameter tested, in three repetitions, for each year studied: 2017-2020.

- Carrying out physical-chemical and granulometric analyzes of soil samples:

Determination of soil texture; Determination of total nitrogen from soil samples; Determination of carbonates in soil samples; Determination of potassium; Phosphorus determination; Determination of humus content in soil samples.

- Performing enzymological analyzes of soil samples:

Determination of dehydrogenase activity; Determination of phosphatase activity; Determination of catalase activity; Determination of the enzymatic soil quality indicator (IECS).

- Carrying out chemical analyzes of water samples:

Determination of pH; The redox potential (Eh); Electrical conductivity; Salinity; The amount of oxygen.

- Carrying out microbiological analyzes of water samples:

Determination of the number of aerobic heterotrophic bacteria; Determination of the number of aerobic sulfur-reducing bacteria; Determination of the number of anaerobic sulfur-reducing bacteria; Determination of the number of sulfate-reducing (desulfurizing) bacteria; Determination of the number of sulfur-oxidizing bacteria; Determination of the bacterial indicator of soil quality (IBCS)

- Carrying out biochemical analyzes of blackcurrant fruits in the Jibou plantation:

The dry substance; Sugar content; Acidity; Detection of tanning and coloring substances; Vitamin C; total phenol content; antioxidant activity.

- Analysis of correlations and regressions between soil properties, sulphurous water quality and the characteristics determined in blackcurrant fruits from the Jibou area.

The particularities of the natural environment in which the experiment took place. The research was carried out in Jibou City, which is part of the category of the youngest urban centers of the country, being located in the northeastern part of Sălaj County, on the left bank of Someț, at its confluence with Agrijul. It is crossed by the parallel 47 16 5 North latitude and the meridian 23 16 10 east longitude.

Material and method. The research within the doctoral thesis was carried out in the Jibou area, in 4 experimental years: 2017, 2018, 2019 and 2020, and the physical and chemical analysis of the water and soil samples were carried out in the Laboratory of the Office for Pedological and Agrochemical Studies Cluj-Napoca, respectively in the Pedology Laboratory of the USAMV, Cluj-Napoca, according to the methods proposed by BORLAN and RĂUȚĂ, 1981. In the Microbiology Laboratory of Babeș-Bolyai University Cluj-Napoca, the microbiological, enzymological and molecular analyzes of the experimental variants were carried out .

From the agricultural lands adjacent to the sulphurous springs, water and soil samples were taken, from 5 distinct plots whose coordinates were established with the help of a GPS. The 5 experimental variants were analyzed from a physico-chemical and biological point of view.

Both water and soil samples were taken in special, sterile, hermetically sealed containers and were kept in the laboratory at a constant temperature of +4°C. Both the taking of the samples and their processing were done in compliance with the rules provided in SR EN ISO 5667-1:2007/AC, 2007, SR EN ISO 5667-3, 2013, and SR EN ISO 19458, 2007.

The enzymological and microbiological determinations of the soil samples were carried out immediately after their sampling.

Results and discussions. Due to the benefits it has on the human body, blackcurrant culture has become more and more interesting for growers and consumers. The cultivation of this species is limited, however, by the cultivars currently on the market, genotypes that fail to capitalize on varied pedoclimatic conditions and that show a high sensitivity to specific diseases and pests. Also, the nutritional qualities of blackcurrant fruits are strongly influenced by the genotype, the environment and the interactions between them, the knowledge of these factors being essential for the success of the culture.

Results regarding the physico-chemical analyzes of the water samples. The results indicated that the number of bacteria in each group varied according to the sampling sites. Actual acidity (pH) is very important because it influences the physiological groups of bacteria. In the water samples, the pH ranged from 6.73 to 6.88. These values belong to the very weakly acidic reaction classes.

Sulfur bacteria are critical mediators of redox transformations that occur within the biogeochemical sulfur cycle of the biosphere. The redox potential values in our water samples were very low. The electrical conductivity values of the five water samples varied between 845 and 867 µS/cm, the minimum being recorded in the first sample and the maximum of the sample being recorded in the fifth sample.

A salinity of 0.2% was measured in all water samples, which means that the sulphurous waters of the Jibou area contain salts. In the five experimental samples, the amount of dissolved oxygen took values between 0.1 and 0.3 mg/l, with a variation amplitude of 0.2 mg/l.

Results regarding the physico-chemical analyzes of the soil samples. The pH parameter had a small variation amplitude of 0.07 and took values in the range of 7.98 (value obtained for the second soil sample) and 8.05 (value obtained for the third soil sample).

According to the methodology for pedological studies (FLOREA et al., 1987), the data obtained show a low humus content, no higher than 1.7%.

The conductivity of the five soil samples taken varied very little, taking values between 0.21 and 0.28 mS. Based on the obtained values, we can state that nitrogen absorption is very low in all analyzed soil samples. Also, the nutrients in the tested soil, such as phosphorus and potassium, did not exceed the value of 129 ppm and 612 ppm, respectively. Regarding the carbon in the five soil samples collected, it reached a maximum of 6.72, in sample 5.

The content of SO4 in the soil samples is between 19.02-19.23 mg/100 g soil

Microbiological analyzes of water samples. From the obtained results it appears that the predominant bacteria involved in the sulfur cycle are aerobic sulfur-reducing bacteria, followed by sulfur-oxidizing ones. The number of anaerobic bacteria is quite low in all analyzed water samples. Aerobic heterotrophs constituted the largest eco-physiological group of bacteria and is used as a global indicator of microbiological water quality.

Results regarding the enzymological analyzes of the soil samples. ADHA was more intense in soil samples 3 and 5, followed by samples 4, 1 and 2. The lowest actual dehydrogenase activity was displayed by soil from sample 2, where the pH was also lower and probably influenced the development. of microorganisms. It can be seen that PDHA shows higher values in all samples compared to ADHA. The addition of glucose has a stimulatory effect on dehydrogenase activity. These enzymes that catalyze the oxidation of many organic compounds through the exchange of electrons and protons are located only in living, intact cells. The phosphatase activity of the soil samples was expressed in mg. phenol/g soil by measuring absorbance at 600 nm (CARPA et al., 2014).

Phosphatase activity was detected in all soil samples. Phosphatase activity was more intense in sample 3, and the weakest activity was recorded in sample 2. Catalase activity was detected in all analyzed soil samples. Especially in samples 1 and 5, catalase activity was more intense. The studied soils have a fairly high enzymatic activity. EISQ values ranged from 0.574 to 0.633.

Results regarding the production and quality of blackcurrant fruits. In the framework of the experiment carried out in the Jibou area, the production of black currants varied a lot in the 4 experimental years, the maximum of almost 20 kg/ha being reached in 2018. On average, in the 4 years, a yield of 17 was obtained, 91 kg/ha, the lowest production, of 16.47 kg/ha, being recorded in 2020. The weight of the fruits varied slightly from one year to another, with an average, in the 4 years, of 1.48 g. The variation amplitude was 0.2 g and took values between 1.4 and 1.6 g.

Although the highest production of black currants was recorded in the second experimental year, the highest values for fruit mass were recorded in 2019.

The sugar content over the 4 experimental years varied slightly, being, on average, 8.36%, the lowest content being registered in 2019 (7.96%), at the opposite pole being the sugar content obtained in 2017 (8.64%).

The acidity detected by titration in blackcurrant fruits during the study was fairly uniform and varied between 1.47-1.58%, with an average of 1.52% over the 4 experimental years.

The coefficient calculated for the ratio between sugar content and acidity showed a slight upward trend in 2020. On average, in the 4 experimental years it varied slightly and took values between 4.59 and 4.82.

The variation of vitamin C content in blackcurrant fruits, in the 4 experimental years, indicates a lower value of this quality index in 2018 (22.69 mg/100 g), compared to the other 3 experimental years.

The total content of tanning substances is variable from one year to another, registering a maximum of 73.29 mg/100 g in 2017 and a minimum of 69.58 mg/100 g in 2020.

Total polyphenol content reached values between 0.492 mg/ml in 2017 and 0.552 mg/ml in 2020.

The anti-free radical activity of the analyzed extracts is directly correlated with the content of polyphenolics, thus the free radical scavenging capacity of the analyzed extracts increases with the increase of the polyphenolic content. The differences recorded in the research are mainly due to the content of polyphenols in blackcurrant fruits.

Analysis of the correlations and regressions between the properties of the soil, the quality of sulphurous water and the characters determined in blackcurrant fruits from the Jibou area. The calculation of correlation coefficients is a statistical analysis frequently used in agricultural research, highlighting strong positive or negative links between the different characters studied, which can be better highlighted by calculating regressions.

Correlation coefficients are indicators of the relationship between two different variables. A correlation coefficient that is greater than zero indicates a positive relationship. A value less than zero means a negative relationship. The closer the values of the coefficients are to 1, respectively to -1, the stronger the identified link is.

Following the calculation of the correlation coefficients, more or less close positive or negative links were identified between: the physico-chemical properties and enzymatic activities in the soils present in the Jibou area, the bacteria in the water samples, the quality characteristics and productivity elements, the indicator bacterial quality of sulphurous water in the Jibou area, the production and biochemical composition of blackcurrant fruits, the enzymatic indicator of soil quality in the Jibou area, the production and biochemical composition of blackcurrant fruits.

Statistical analysis using linear regression allowed exemplifying the existing link between the studied parameters, helping to better understand them.

CONCLUSIONS

The following conclusions can be drawn from the results of the physical-chemical analyzes of the water samples:

1. The number of bacteria in each group varied according to the sampling sites.
2. A salinity of 0.2% was measured in all water samples, which means that sulphurous waters in the jibou area contain salts.

From the results regarding the physico-chemical analyzes of the soil samples, it can be concluded that:

1. The reaction of the analyzed soils (pH) is weakly alkaline. The pH parameter had a small variation amplitude of 0.07 and took values in the range of 7.98 (value obtained for the second soil sample) and 8.05 (value obtained for the third soil sample).
2. The data obtained show a low humus content, not higher than 1.7%.

From the results of microbiological analyzes of water samples, it can be concluded that:

1. The predominant bacteria involved in the sulfur cycle are aerobic sulfur-reducing bacteria, followed by sulfur-oxidizing bacteria.
2. The number of anaerobic bacteria is quite low in all the water samples analyzed.

From the results regarding the enzymological analyzes of the soil samples, it can be concluded that:

1. The studied soils are of good quality, having a fairly high enzymatic activity. EISQ values ranged from 0.574 to 0.633. Based on the obtained results, we can consider that the analyzed soils have a wide biological potential.

In the framework of the experiment carried out in the Jibou area, the production of black currants varied a lot in the 4 experimental years, the maximum of almost 20 kg/ha being reached in 2018. On average, in the 4 years, a yield of 17 was obtained, 91 kg/ha, the lowest production, of 16.47 kg/ha, being recorded in 2020.

From the analysis of the correlations between the physico-chemical properties and enzyme activities in the soils present in the Jibou area, it can be concluded that:

1. The pH correlates strongly and positively with: the real activity of dehydrogenases (r=0.72) and the activity of phosphatase (r=0.97);
2. electrical conductivity with phosphatase activity (r=0.76);

3. nitrogen content with: the real activity of dehydrogenases (r=0.71) and with the activity of phosphatase (r=0.80);
4. phosphorus content with phosphatase activity (r=0.82);
5. potassium content with phosphatase activity (r=0.86);
6. carbonates with phosphatase activity (r= 0.81).
7. the phosphorus content correlates negatively with the potential activity of dehydrogenases (r=-0.81).

By studying the correlations between the bacterial communities in the water samples present in the Jibou area, the following were identified:

1. strong positive link between aerobic sulfur-oxidizing bacteria and anaerobic sulfur-reducing bacteria (r=0.70), respectively the bacterial indicator of water quality (r=0.70);
2. there was a positive relationship between aerobic sulfur-reducing bacteria and anaerobic sulfur-reducing bacteria (r=0.91), respectively the bacterial indicator of water quality (r= 0.91);
3. inverse relationship between anaerobic sulfur-oxidizing bacteria and anaerobic sulfur-reducing bacteria (r=-0.79), respectively the bacterial indicator of water quality (r=0.79).

From the analysis of the correlations between quality traits and productivity elements, in the black currant culture, in the Jibou area, the following conclusions were drawn:

1. between the acidity identified by titration and the content of tanning substances, with a correlation coefficient value of -0.94, a strong negative relationship was identified;
2. with a correlation coefficient r=0.71, it is noted, as expected, the connection between the sugar content and the sugar/acidity coefficient;
3. positive links were also identified between: fruit mass and acidity identified by titration (r=0.63), respectively between production and sugar;
4. negative links were also established between: fruit mass and sugar content (r=-0.58), currant production and vitamin C content (r=-0.65), sugar content and substance content tanning substances (r=-0.49), the content of tanning substances and the content of vitamin C (r=-0.60).

By analyzing the correlations between the bacterial quality indicator of sulphurous water in the Jibou area, the production and biochemical composition of blackcurrant fruits, it was observed that:

1. the bacterial indicator correlates positively with and closely with the content of blackcurrant fruits in bronzing substances (r=0.96). In general, negative links were observed between the studied parameters;
2. as expected, the presence of a large number of bacteria, from each ecological group in sulphurous waters, leads to a decrease in the blackcurrant harvest and implicitly in its quality.

The conclusions drawn from the study of the correlations between the enzymatic soil quality indicator in the Jibou area, the production and the biochemical composition of blackcurrant fruits are the following:

1. the enzymatic activity existing in the soils of the Jibou area has a positive influence on the quantity and quality of the blackcurrant culture;
2. a strong connection was identified between the enzymatic soil quality indicator and the coefficient determined by the ratio between sugar content and acidity (r= 0.99);
3. a strong positive connection was also observed between the enzymatic activity present in the soil and the sugar content of currant fruits (r=0.64) and their vitamin C content (r=0.35);
4. with a negative Pearson correlation coefficient (r=-0.77), it would seem that this ezymatic activity present in the soils leads to a decrease in the mass of currant fruits, but the production is not affected much.

By analyzing the positive and negative regressions between the main indices determined on soil, water and blackcurrant fruits, the following conclusions were drawn:

1. in 93% of cases, the increase in soil pH value causes an increase in phosphatase activity;
2. in more than half of the cases ($R2=0.52$), a higher pH by one unit leads to an increase of 2.48 mg formazan/g soil of this parameter characteristic of enzymatic activity;
3. in 73% of cases, increasing the potassium content by 1 ppm leads to an increase in phosphatase activity by 0.27 mg phenol/g soil;
4. phosphatase activity correlates positively with the phosphorus content in the soil, when its increase by 1 ppm causes an increase in phosphatase activity by 0.5 mg phenol/g soil;
5. it would appear that an increase of approximately 45 mg phenol/g soil occurs with a 1 mS increase in conductivity in more than half of the cases;
6. the linear regression exemplified between the carbonate content of the soil in the Jibou area and the phosphatase activity indicates an increase of 15.92 units of the latter when the carbonates in the soil increase by 1 ppm;
7. phosphatase activity depends in more than half of the cases on the supply of soil with phosphorus, potassium and carbonates as well as on its electrical conductivity, all the previously mentioned links being described by regression lines with a strong upward trend.
8. the sugar content of fruits depends in a percentage of 42% on fruit production. Thus, increasing the currant harvest by 1kg/ha would remove a higher sugar content by 0.15%.
9. a high sugar content of the fruit causes, in 39% of cases, an increase in acidity determined by titration by 0.3%.

10. the increase by 1% of the concentration of carbohydrates in blackcurrant fruits contributes to the increase by 0.24 of the coefficient calculated for the ratio between sugar and acidity.
11. the experimental data obtained for the bacterial indicator of water quality indicate the presence of a different number of bacteria in the sulfur water samples analyzed;
12. it would seem that this water quality indicator influences in a percentage of 92% the content of fruits in tanning substances;
13. the variation of the sugar/acidity coefficient depends in proportion to 98% on the enzymatic activity present in the soil, while the variation of sugar and vitamin C content are less dependent on this soil indicator, the coefficients of determination calculated in this case being R2= 0.41, respectively R2=0.12;
14. the less close relationship between the enzymatic activity of the soil and the vitamin C content also emerges from the smooth slope of the regression line.
15. an increase of one unit in the enzymatic activity of the soil causes a 3.9 increase in the sugar/acidity ratio and a 7.6 increase in the sugar in blackcurrant fruits.
16. regarding the vitamin C content of blackcurrant fruits, a 4.9% increase in quality occurs with an increase of one unit of the enzyme activity present in the soil;
17. The exemplification of the relationship between the water quality indicators and the main production and quality characteristics identified in the studied fruits, provides valuable information regarding the forecast of the quantity and quality of the blackcurrant harvest as well as the impact of sulphurous waters on them.
18. an increase in phosphorus by 1 ppm leads to a decrease in potential dehydrogenase activity by 0.14 units, this decrease being explained in a percentage of 66%.
19. the decrease in fruit quality due to the increase in production or their mass is explained in a percentage lower than 50%, the coefficients of determination being: R2=0.33 respectively R2=0.42. The increase by 1 g of the fruit mass causes a decrease in sugar by 1.89%, and the increase in production by 1 kg/ha reduces the vitamin C content by almost 2 mg%.
20. an increase in bronzing substances from fruits explains in a percentage of 89% the decrease by 1% of the acidity determined by titration;
21. in a percentage lower than 50%, a reduction in the vitamin C and sugar content of blackcurrant fruits is due to the increase of tanning substances in these fruits;
22. the contamination of water with bacteria, as expected, produces a decrease in the quality of blackcurrant fruits, the values identified for the sugar content, for the vitamin C content, respectively for the acidity obtained by titration, being lower when the bacterial indicator water quality showed superior values;
23. the vitamin C content would seem to be most strongly influenced by the increase in the community of bacteria present in the water, in 71% of cases, a deterioration of this quality index occurring when higher values are recorded for the bacterial indicator calculated.

RECOMMENDATION

Based on the results obtained regarding the influence of sulphurous waters in the Jibou area on the quality of blackcurrant fruits, as well as the physico-chemical characterization of the soils on the lands adjacent to the sulphurous springs and of the sulphurous water samples, we recommend the successful cultivation of blackcurrants in this cultivation area, managing to capitalize on the pedo-climatic conditions through good and quality harvests.

SELECTIVE REFERENCES

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3.***https://www.frutas-hortalizas.com/Fruits/Origin-production-Blackcurrant. html)