ECHOPHYSIOLOGICAL REACTION OF SOME VINE VARIETIES FROM IASI, TARGU BUJOR AND COTNARI IN WINTER 2010-2011 CONDITIONS

CERCETĂRI PRIVIND REACŢIA ECOFIZIOLOGICĂ A UNOR SOIURI DE VIŢĂ DE VIE DIN PODGORIILE IAŞI, COTNARI ŞI TÂRGU BUJOR, ÎN PERIOADA DE REPAUS VEGETATIV

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Abstract. *The determinations made in this paper are part of a comprehensive study conducted on some vine varieties (White Feteasca, Royal Feteasca, Italian Riesling, Grey Băbească, Francusa, Cotnari Grasa, Romanian Tamaioasa), vines grown in three regions of Moldova: Iaşi, Cotnari and Târgu Bujor. The research conducted in the climatic conditions of November 2010 - February 2011 enabled us to highlight, in the case of the studied varieties, aspects of tissue aging (wood maturation) - premise of resistance to negative temperatures in winter and bud viability. The maturation degree of the wood can be assessed by determining the water content of the shoots, which varies depending on variety and the wood / bone ratio. The water content of the cells is correlated with the amount of osmotic pressure - another indicator for maturation and assessing of the frost resistance - led by the carbohydrate content, free ions or amino acids, especially proline.*

Key words: vine varieties, frost resistance, starch, protein

Rezumat. *Determinările realizate în prezenta lucrare fac parte dintr-un studiu complex realizat asupra unor soiuri de viţă de vie (Fetească albă, Fetească regală, Riesling italian, Băbească gri, Frâncuşă, Grasă de Cotnari, Tămâioasă românească) cultivate în trei zone viticole ale Moldovei: Iaşi, Cotnari şi Târgu Bujor. Cercetările efectuate în condiţiile climatice ale lunilor noiembrie 2010 - februarie 2011, ne-au permis să evidenţiem la soiurilor luate în studiu, aspecte referitoare la maturarea ţesuturilor (maturarea lemnului) - premiză a rezistenţei la temperaturile negative din timpul iernii, precum şi viabilitatea mugurilor. Gradului de maturare a lemnului poate fi apreciat şi prin determinarea conţinutului de apă al lăstarilor, care variază în funcţie de soi şi de raportul lemn/măduvă. Conţinutul de apă al celulelor este corelată cu valoarea presiunii osmotice - un alt indicator al gradului de maturare şi de apreciere a rezistenţei la îngheţ - determinat de conţinutul în glucide, ioni liberi sau aminoacizi liberi, în special prolina.*

Cuvinte cheie: ecofiziologie, viţă de vie, rezistenţă ger, amidon, proteine

INTRODUCTION

In order to establish the areas where grapevine can be cultivated on the one side and the appropriate wine assortments on the other side, an important element

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that must be bared in mind is represented by the ecologic characteristics of the areas. The pedoclimatic conditions influence the length of the vegetation period (precociousity), conditions the quantity and the quality of crops, offering originality to the grapes and the wines resulted in a specific area (Jitareanu Carmen-Doina, 2007).

The capacity to resist to negative temperatures is obtained over a long and complex process that starts before the first frost; it is related to the accumulation of the reducing glucides, the amidines' hydrolysis, the modification of the tissues' resistance to dehydration and their capacity to regenerate (Howell, G.S. 2003).

The grapevine's capacity to adjust and its strength to resist to low temperatures is related to a series of physiological and biochemical changes that are influenced by the accumulation and the dynamic of some chemical compounds that protect the cellular protoplasm from the irreversible coagulation of proteins, that is caused by the mechanical and destructive action of intracellular freezing (Burzo, 1999; Fennell Anne, 2004).

Starting with the second part of October, the enzymatic hydrolysis process of amidines intensifies, leading to the growth of the content of soluble glucides, growth that is related to the decrease of the frost temperature. In December and January, the content of soluble glucides reaches its maximum (10-12% s.u.), period in which there are also recorded the lowest temperatures. In February – March, the concentration level of glucides decreases as they take part at the respiratory process (the respiratory process intensifies in this period).

MATERIAL AND METHOD

Determining the vine buds viability was used to the sectioning method.

Each bud is sectioned with a blade or a very sharp knife, starting from the base of the tendril towards its top (Rotaru Liliana, Petrea Gabriela, 2006).

Wood maturation. To determine the starch presence in the annual branches it was used the color reaction/test with the help of the Lugol reactive, I in KI. The branches were sectioned with the microtome and the materials were analyzed at the microscope.

Water content and its forms: free water – bound water.

The tendrils for fruit production, recently harvested and were stored into a drying stove, up to a constant weight to a temperature of 40^{o}C to determine the content of free water and at 105^{o}C for the total content of water.

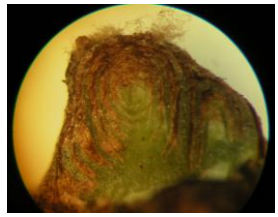
Determining the quantity of nitrogen and calculating the amount of raw protein from the grapevine's tendrils in December 2010 – was performed after a standard identical to the International Standard Project ISO 5983:1992, which replaces STAS 9597/3-74.

RESULTS AND DISCUSSIONS

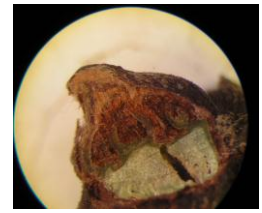
Determining the grapevine buds viability

The bud's viability was appreciated according to the color of the tissues. If the entire group of buds that form the winter bud was green, color characteristic to living tissues, the bud was considered to be viable. The bud was considered non-

viable and lost if the tissues of the main bud were grey – black, color characteristic to dead tissues. (fig. 1 a and b).



a) Viable buds



b) Unsustainable buds

Fig. 1 - Image at the Binocular magnifier of the grapevine bud complex

After analyzing the buds from the grapevines species included in this study, in the conditions of the winter of 2010 – 2011, it was noticed that the "White Feteasca" variety had a high percent of viable buds in all the three vineyards. (82-100%), and it was followed by "Italian Riesling", "Frâncușa" and "Royal Feteasca", the lowest potential fertility being present at the "Grey Babeasca" variety (58-60%) (fig. 2).

A distinct case is represented by the variety "Grasa de Cotnari" that in the winter of 2010-2011 presented a high percent of viable buds in the Cotnari vineyard (94%) but a very low percent (38%) in Iași.

Referring to the areas from where the analyzed species come from, the Cotnary vineyard results to be very favorable since both "White Feteasca" and "Romanian Tămâioasă" presented 100% viable buds, potentially fertile. Thee other two species included in the study have high percentage of potentially fertile buds.

Wood maturation – the amount of starch in the grapevine shoots

Starch accumulates mainly in the inner layers of the xylem, the starch level starts decreasing in autumn and continues until January; this period is followed by growth with a spring maximum. The moment temperature decreases, the starch level diminishes because of hydrolysis and the glucides level grows correspondingly (fig. 3).

Observing the starch level in the shoots during dormancy, it was noticed that all grapevines varieties presented lower quantities of starch in February 2011. The differences were due to temperature values and to the metabolic characteristics of the species.

Water content and its forms: free water – bound water

One clue regarding the grapevines' resistance to frosty weather is offered by the proportion of the two types of water that exists in the shoots, free water – bound water, the superior values of this relation indicating the grapevine's sensibility to low negative temperatures.

In November, free water – bound water report presented values that were superior in the middle parts of the shoots, comparatively with the basal internodes that are generally more resistant to frost.

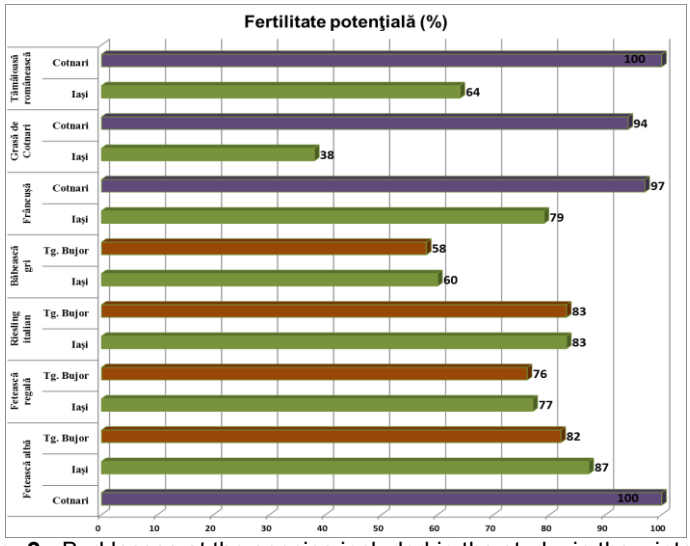


Fig. 2 - Bud losses at the species included in the study, in the winter of 2010-2011

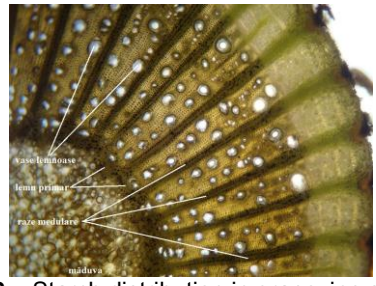


Fig. 3 – Starch distribution in grapevine shoots

The lowest values of this parameter were recorded at the grapevine species cultivated at Cotnari, demonstrating that these species adapted better to the difficult conditions of winter than the species cultivated in Iași. This fact was also confirmed by the analysis of the viability of the fruit buds that reached values of 100% (fig. 4).

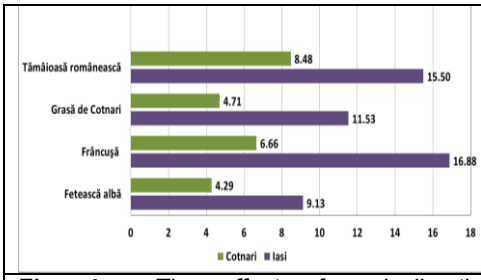


Fig. 4 - The effect of pedoclimatic conditions on the free water – bound water report in November, in the shoots of the grapevine species cultivated in the vineyards from Iași and Cotnari

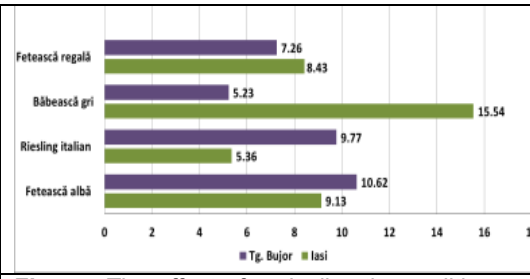


Fig. 5 - The effect of pedoclimatic conditions on the free water – bound water report in November, in the shoots of the grapevine species cultivated in the vineyards from Iași and Târgu Bujor

In the case of the species cultivated at Târgu Bujor, just like in the case of those cultivated at Cotnari, the free water – bound water report is lower if we compare it with that from Iași, and this shows that they are more resistant to freezing (fig. 5). The free water – bound water report showed in November values between 16.88% - 26.43 % at the "Frâncușă" variety that was cultivated in Iași and the lowest values at "White Feteasca" from Cotnari vineyard.

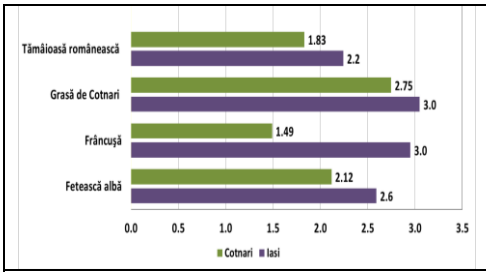


Fig. 6 - The effect of pedoclimatic conditions on the free water – bound water report in February, in the tendrils of the grapevine species cultivated in the vineyards from Iași and Cotnari

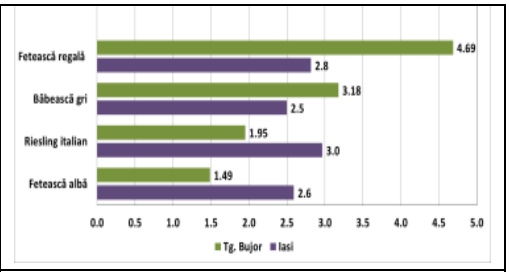


Fig. 7 - The effect of pedoclimatic conditions on the free water – bound water report in February, in the tendrils of the grapevine species cultivated in the vineyards from Iași and Târgu Bujor

In February, after the annealing process, after the shoots got through the period with negative temperatures it can be seen a decrease of the total amount of water at all the species included in the study (fig. 6).

All species presented lower values at the free water – bound water report comparatively to those from November and this proves that they have moved on to the 3rd phase of the annealing process that consists in cell dehydration, the transfer of free water to the intracellular areas and water loss during transpiration (fig. 6 and 7).

The total protein content from the grapevine tendrils in December 2010

After determining the total protein level at the species included in the study, the results of the analysis show that in the shoots, the grapevine species from the vineyard situated in Iași, the raw protein level varies from 2,9% at "Grasa de Cotnari" to 3.9% at "White Feteasca" (fig. 8). Comparing the grapevines cultivated in Iași to those cultivated at Cotnari resulted that the latter one stored more raw protein than the first one, the values varying from 4,1% at "Grasa" and 6,0% at "White Feteasca". Since the concentration level was higher, this contributed to improving the buds capacity to resist to cold and these species had practically no bud losses during winter.

Analyzing the species cultivated at Târgu Bujor and comparing them to those cultivated in Iași results that only between two species - "Royal Feteasca" and "White Feteasca" – there are significant differences as far as the total amount of protein is concerned. These differences are positive in the case of "Royal Feteasca" where the values varied from 3.5% at Târgu Bujor and 2.8% at Iași and negative for "White Feteasca" with values of 3.9% in Iași and 2.9% at Târgu Bujor. For the "Italian Riesling" and "Grey Babeasca" there were not significant differences from one vineyard to the other (fig. 9).

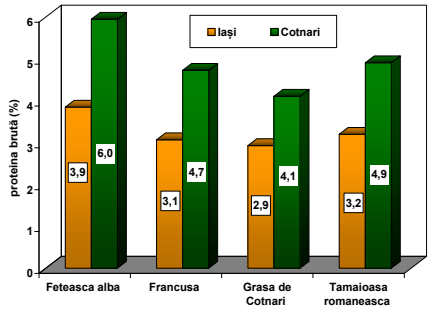


Fig. 8 - Raw protein concentration level in the tendrils from the grapeyards in Iași and Cotnari

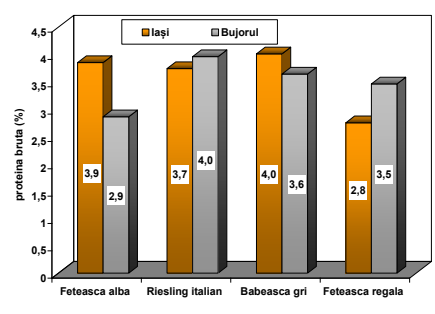


Fig. 9 - Raw protein concentration level in the tendrils from the grapeyards in Iași and Târgu Bujor

CONCLUSIONS

1. After analyzing the buds from the grapevines species included in this study, in the conditions of the winter of 2010 – 2011, it was noticed that the "White Feteasca" variety had a high percent of viable buds in all the three vineyards. (82-100%). Referring to the areas from where the analyzed species come from, the Cotnari vineyard presents itself as a very favorable one for both species.

2. The species cultivated at Cotnari presented the lowest values of report free water – bound water, if we compare them to those cultivated in Iași; this proves that the species cultivated at Cotnari adjusted better to the inauspicious winter conditions, fact that was also confirmed by the analysis of viability of the fruit buds that presented values of 100%. In February, after annealing and after the shoots got over the negative temperatures it can be seen a decrease of the total amount of water for all the species included in the study.

3.The results for determining the total quantity of protein for the species included in the study from the three vineyards mentioned, point out that the grapevines cultivated at Cotnari presented more raw protein, the higher concentration influencing the buds capacity to face the cold and these species did not present bud losses during winter.

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PRELIMINARY STUDIES CONCERNING THE AMINOACIDS INFLUENCE ON SOME DEHYDROGENASES AT *MONILINIA LAXA* (ADERH. & RUHL.) HONEY PARASITE ON PLUM TREE

STUDII PRELIMINARE PRIVIND INFLUENŢA UNOR AMINOACIZI ASUPRA UNOR DEHIDROGENAZE LA SPECIA *MONILINIA LAXA* (ADERH.& RUHL.) HONEY PARAZITĂ PE PRUN

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Abstract. *This study aimed the evaluation of the activity of some dehydrogenases from the Krebs cycle, as the principal energy supplier that assures the fabrication of mediators and products so that they may maintain the equilibrium between cells and to avoid the uneconomic supraproduction of metabolites and, respective, of glucose-6-phosphate dehydrogenase, ubiquitous enzyme that catalysis the conversion of glucose-6-phosphate in glucono-lactone-6-phosphate with $NADP^+$. The experiments were made using cultures of Monilinia laxa (Aderh.&Ruhl.) Honey on mediums supplemented with different types of aminoacids. The enzymes activity was determinated using the spectrophotometric method of Sîsoev and Krasna (modified by Artenie) and some semnificative differences were recorded, variations influenced by the age of the culture and the aminoacid type used in working samples, compared with the control sample.*

Key words: dehydrogenases, amino acids, *Monilinia laxa*

Rezumat. *Studiul de faţă a urmărit evaluarea activităţii unor dehidrogenaze ale ciclului Krebs, ca principal furnizor de energie ce asigură producerea de intermediari si produşi astfel încât sa menţină starea de echilibru a celulelor si pentru a evita supraproducţia neeconomica de metaboliţi şi, respectiv, a glucozo-6-fosfat dehidrogenazei, enzimă ubicuitară ce catalizează conversia glucozo-6-fosfatului în glucono-lacton-6-fosfat în prezenţa $NADP^+$. Experimentele s-au derulat în condiţiile cultivării fungului Monilinia laxa (Aderh.& Ruhl.) Honey pe medii suplimentate cu diferite surse de aminoacizi. Activitatea enzimelor, urmărită în dinamică, a fost determinată prin metoda spectrofotometrică Sîsoev şi Kasna (modificată de Artenie), fiind constatate diferenţe semnificative în funcţie de vârsta culturii şi de tipul aminoacidului la variantele de lucru în comparaţie cu martorul.*

Cuvinte cheie: dehidrogenaze, aminoacizi, *Monilinia laxa*

INTRODUCTION

Ubiquitous organisms, the fungi, have developed along time various nutritional strategies which allowed them to adapt in all conditions of the environment and this, due to the genetic background of the fungal cell which enabled the expression a phenotype that allows use of any organic compounds or inorganic nitrogen from the living environment. Data from literature indicates, however, that the mixtures of

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amino acids generally allows a greater and more rapid growth than a single amino acid (Griffin, D.H., 1996), but the basis for this phenomenon are unknown. Some fungi cannot use the inorganic nitrogen sources, requiring glutamate, asparagine and other amino acids for growth and development (Griffin, D.H., 1996).

The tricarboxylic acid cycle is the central point of the metabolism–related to processes that are not part of energy production. One of its enzymes (α-ketoglutarate-dehydrogenase) is a gateway to one of these processes, allowing entry of amino acids in the Krebs cycle, with energy production. Sometimes, the amino acids entering in the structure of some enzymes of the citric acid cycle, as is the case of the isocitrat-dehydrogenase which has in the active site some amino acids like tyrosine, arginine, serine, threonine, aspartic acid (Tadhg, D.B., McMurry, J., 2005; Cox, M. *et al*., 2005). Some enzymes involved in Krebs cycle occur on some intermediary products resulting from the conversion of carbon chains and of some amino acids to further decomposition: i.e. - malate dehydrogenase attacks the oxaloacetate resulted from the conversion of aspartate and asparagine, the succinate dehydrogenase attacks the fumarate derived from tyrosine, phenylalanine and asparatat (McNurry, J., Begley, T.P., 2005, Storey, K.B., 2004) and the α-cetoglutarat is a precursor in the biosynthesis of other amino acids (so-called glutamate family, represented by proline, glutamine, arginine and histidine (Grow, N.A.R, Gadd, G.M., 1995, Griffin, D.H., 1996, Owen, O.E., 2002, Brody, T., 1999).

Going on the line of other researches (Manoliu, Al. *et al*., 2003, 2004, 2007), this study concentrates on the analysis of the influence that some amino acids have on the activity of some dehydrogenase as glucose-6-phosphate dehydrogenase, α-ketoglutarate-dehydrogenase and malate dehydrogenase from *Monilinia laxa* (Aderh. & Ruhl.) Honey fungus, an ascomycetous fungus responsible for the appearance of brown rot on some stones fruits belonging to the *Prunus* genus.

MATERIAL AND METHOD

The microbial strain used in this study, represented by the *Monilinia laxa* (Aderh.&Ruhl.) Honey fungus has been isolated from the mummified fruits collected from varieties of the *Prunus domestica* from Experimental Orchard for Pomiculture Research Station, Miroslava, Iasi County. The "in vitro" cultivation of the fungus involved the use of acidified streptomycin 2% malt medium (Constantinescu, O., 1974; Malvarez *et al*., 2001) formula's without agar, distributed in Erlenmeyer flasks, over which were added 0,125 mg each of the amino acids: glutamic acid, alanine, asparagine, histidine, leucine, lysine, methionine, serine, valine. We used a control sample, devoid of amino acids. The ten culture medium were seeded with disk cut-out from a culture of *Monilinia laxa* (Aderh.&Ruhl.) Honey aged 7 days and incubated under alternating light - dark conditions and variable temperature. The experimental measurements performed at 7days and respectively 14 days, were made from the mycelium of the fungus, for each treatment in part three parallel determinations were performed, and the enzymes activity, followed as dynamics, was determined using the Sîsoev and Kasna spectrophotometric method (Cojocaru, D.C., 2009).

RESULTS AND DISCUSSIONS

The graphical representation of the statistically processed results of the dehydrogenases activity in *Monilinia laxa* (Aderh. & Ruhl.) Honey specie's mycelium shows at a rigorous analysis that the glucose-6-phosphate dehydrogenase, the α-ketoglutarate-dehydrogenase respectively, malate - dehydrogenase activity was different, being influenced by culture age of mycelia and the type of amino acid that has been added to the culture medium.

The activity of glucose-6-phosphate dehydrogenase is shown graphically in figure 1, where it can be seen that at 7 days after inoculation of the culture, the value of the enzyme activity in control sample was 0.6600 μg formazan/g, while the peak of enzyme activity in the mycelium at this time was detected for V9 variant - 1.1778 μg formazan/g and the minimum point of the enzyme activity was found in V3 (asparagine) - 0.1439 μg formazan/g. Between the two limits of activity, we found in descending order: V8 variant (serine) - 0.8164 μg formazan/g, V2 variant (alanine) - 0.4385 μg formazan/g, variant V7 (methionine) - μg formazan/g, variant V4 (histidine) - 0.3306 μg formazan/g, V1 (glutamic acid) - 0.2397 μg formazan/g, V6 (lysine) - 0.1941 μg formazan/g. respectively, V5 (leucine) - 0.1890 μg formazan/g.

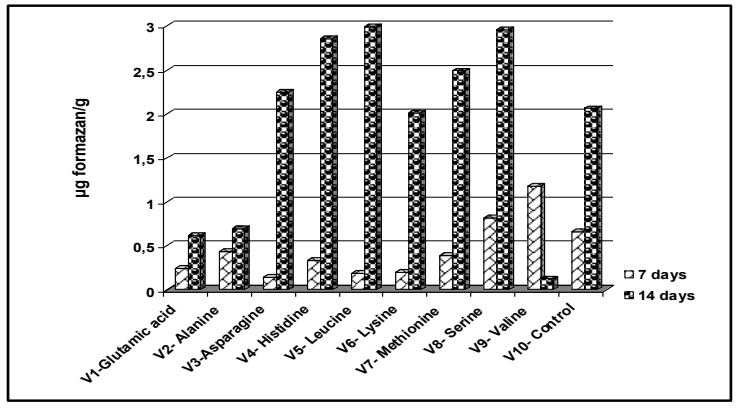

Fig. 1 - The glucose-6-phosphat- dehydrogenase activity in *Monilinia laxa* (Aderh.&Ruhl.) Honey specie's cultivated on medium with different amino acids

In the parallel series of tests conducted at 14 days, the value of the activity of glucose-6-phosphate dehydrogenase in the mycelium, in the control sample was 2.063 μg formazan/g. Valine inhibited the enzyme activity, which reached the lower limit at this time - 0.1257 μg formazan/g. Reporting to the value that had a control variant, the strongest activity of glucose-6-phosphate dehydrogenase was intensified by leucine - 2.9937 μg formazan/g, followed by serine - 2.9596 μg formazan/g, histidine - 2,8611 μg formazan/g, methionine - 2.4907 μg formazan/g, asparagine - 2.2475 μg formazan/g. As for the test where we administered lysine we observed that it inhibited the activity of glucose-6-phosphate dehydrogenase compared to control value - 2.0150 μg formazan/g,

followed by alanine and glutamic acid (0.6934 μg formazan/g, respectively 0.6135 μg formazan/g).

Critical analyzing the evolution of the glucose-6-phosphate dehydrogenase, it can be seen that it increased in all medium variants, except V9 variant in which the enzyme activity decreased between the two series of tests, ranging from1.778 μg formazan/g. to 0.1257 μg formazan/g.

Data on the evolution of the α-ketoglutarate dehydrogenase activity in *Monilinia laxa* (Aderh. & Ruhl.) Honey specie's cultivated on medium enriched with various amino acids are shown in Figure 2. Diagram analysis shows that for the first time measurements of the enzyme activity in the mycelium, the value the control sample was 0.3421 μg formazan/g. The peak of α-ketoglutarate-dehydrogenase activity at 7 days after inoculation was found in variant V9 - 2.4442 μg formazan/g and the smallest value of this enzyme activity was noted in version V3 - 0.0318 μg formazan/g, at the rest of the medium variants ranged from relatively uniform activity values, as follows: leucine - 0.5723 μg formazan/g, serine - 0.5685, alanine - 0.4187 μg formazan/g, histidine - version V4 - 0.4045 μg formazan/g, methionine - 0.3915 μg formazan/g, glutamic acid - 0.3286 μg formazan/g.

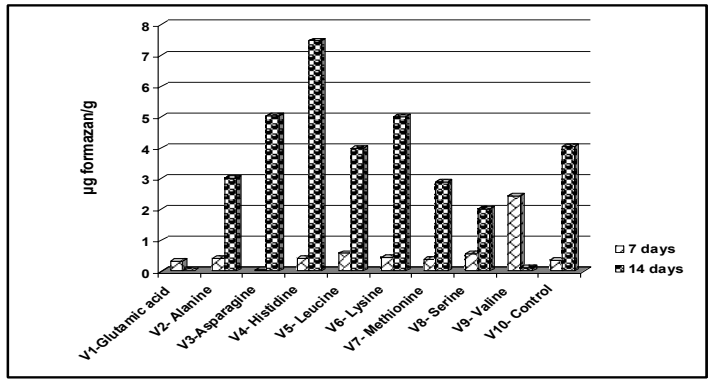

Fig. 2 - The α-ketoglutarate dehydrogenase activity in *Monilinia laxa* (Aderh. & Ruhl.) Honey specie's cultivated on the medium with different amino acids

By measuring α-ketoglutarate-dehydrogenase in *Monilinia laxa* specie's mycelium after 14 days of incubation we noted that the enzyme shown in the control variant, an average activity of 4.0554 μg formazan/g., while histidine, asparagine and lysine have stimulated a greater or lesser extent of their activity (7.4823 μg formazan/g.- V4; 5.0549 μg formazan/g - V3; 5.0074 μg formazan/g. - V6). In this case it is shown a strong inhibitory effect of α-ketoglutarate-dehydrogenase activity, the most notable effect being detected in glutamic acid case - 0.0256 μg formazan/g. The same inhibitory effect were, in descending order, leucine (3.9912 μg formazan/g), alanine (3.0087 μg formazan/g), methionine (2.8902 μg formazan/g), serine (2.0224 μg formazan/g) and valine (0.1117 μg formazan/g). Studying the dynamics of α-ketoglutarate-dehydrogenase activity in time, it appears that between the two series of determination thee

enzyme activity in the mycelium has escalated to all medium variants, except the V9 variant where it decreased from 2.4442 μg formazan/g to 0.1117 μg formazan/g and variant V1, where it decreased from 0.3286 μg formazan/g to 0.0265 μg formazan/g.

The malate dehydrogenase activity chart from *Monilinia laxa* specie's cultivated on medium supplemented with various amino acids indicates at the control sample at 7 days after inoculation, an endoenzyme activity with a value of 0.5285 μg formazan/g. The minimum point of it's activity was recorded in the medium variant containing asparagine - 0.0467 μg formazan/g and the maximum activity was recorded in the medium variant supplemented with valine - 2.4536 μg formazan/g, a change in its values was ound in the rest of the medium variants supplemented with different amino acids, as follows: serine - 0.6828 μg formazan/g, leucine - 0.4667 μg formazan/g, lysine - 0.4311 μg formazan/g, histidine – 0.3870 μg formazan/g, glutamic acid - 0.2594 μg formazan/g.

In this case also was determined the enzyme activity at 14 days after inoculation. Experimental results have shown, for the control sample, that the malate-dehydrogenase activity in the mycelium of the *Monilinia laxa* fungus what reached for 2.0637 μg formazan/g. The maximal limit of the enzyme activity was recorded in the V4 variant - 9.3863 μg formazan/g and the minimum was noted in V1 variant (glutamic acid) - 0.0246 μg formazan/g. Amplification of the enzyme activity was found in the mycelium when the medium variant is supplemented with lysine (7.2475 μg formazan/g), asparagine (3.2755 μg formazan/g), leucine (3.2718 μg formazan/g), methionine (2.8231 μg formazan/g) and the reduced values at the time, for malate - dehydrogenase activity compared with the control sample, were recorded in mediums supplemented with alanine (1.5922 μg formazan/g), valine (0.4785 μg formazan/g) and serine - 0.0290 μg formazan/g.

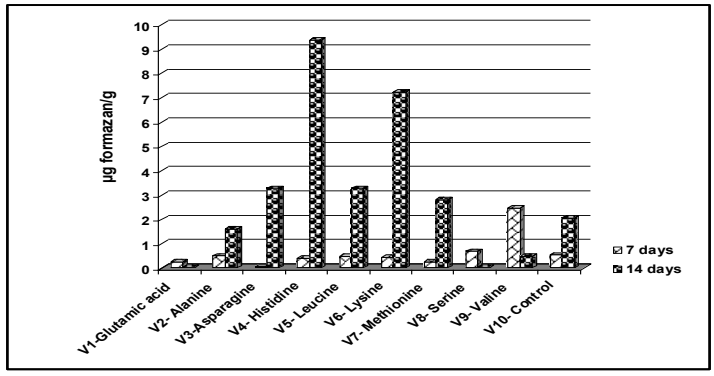

Fig. 3 - The malate-dehydrogenase activity in *Monilinia laxa* (Aderh.&Ruhl.)Honey specie's cultivated on the medium with different amino acids

The dynamic's analysis of the malate-dehydrogenase activity in the two time intervals show that in all medium variants the enzyme activity in the mycelium increased in time, except V8 variant (serine), whose activity decreased from 0.6828 μg formazan/g. to 0.0290 μg formazan/g and V9 variant (valine) which declined from 2.4536 μg formazan/g to 0.4785 μg formazan/g, V8 (serine) - 0.0290 μg formazan/g to

0.0246 μg formazan/g. and V1 (glutamic acid) from 0.2594 μg formazan/g to 0.0246 μg formazan/g.

CONCLUSIONS

The amino acids used have had an important influence on the enzymes studied, the activity of the three studied dehydrogenase was stimulated differently, depending on one hand by the type of amino acid that had been added into the culture medium, and on the other hand by the age of the culture.

At 7 days after inoculation, the activity of glucose-6-phosphate dehydrogenase was stimulates eith valine and serine, the α-ketoglutarate-dehydrogenase activity with valine, methionine, leucine and alanine and malate-dehydrogenase activity with valine and serine.

After 14 days of incubation, the activity of glucose-6-phosphate dehydrogenase was stimulated by serine, methionine, lysine, leucine, histidine, asparagines, the activity of α-ketoglutarate-dehydrogenase by histidine, lysine and asparagine and the malate-dehydrogenase activity by methionine, lysine, leucine, histidine, alanine.

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