

ANALELE ȘTIINȚIFICE
ALE
UNIVERSITĂȚII „ALEXANDRU IOAN CUZA”
DIN IAȘI
(SERIE NOUĂ)
SECȚIUNEA II

a. GENETICĂ ȘI
BIOLOGIE
MOLECULARĂ

TOMUL XIV, fascicula 4

2013

Editura Universității „ALEXANDRU IOAN CUZA” Iași

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TESTING BAYESIAN ALGORITHMS TO DETECT GENETIC STRUCTURE IN TWO CLOSELY RELATED OAK TAXA

CRISTIAN MIHAI ENESCU^{1,2*}, NECULAE ȘOFLETEA²,
ALEXANDRU LUCIAN CURTU²

Keywords: structure, *Quercus*, *Lanuginosae*, oak

Abstract: The aim of this study was to test the Bayesian algorithm implemented in the software STRUCTURE in order to detect the number of clusters, by using microsatellite data from four oak species. Several assignment models, with or without a priori grouping of individuals to species, were proposed. Better results were obtained by using the sampling location information and when only two taxa were analyzed. Particularly, pedunculate oak and sessile oak formed distinct clusters whatever the assignment model we use. By contrast, no separation between the two oaks from series *Lanuginosae* was observed. This can be explained, on one hand, by the small sampling size for Italian oak, or by the genetic similarities of the two pubescent oaks, namely *Quercus pubescens* and *Q. virgiliana*, on the other hand. Our findings support the hypothesis according which Italian oak is an intraspecific taxonomic unit of pubescent oak.

INTRODUCTION

Nowadays, there are a set of software packages developed for delineating clusters of individuals on the basis of their genotypes by using a Bayesian algorithm. The software STRUCTURE (Pritchard et al, 2000) is one of the most used programs which can be applied to multilocus microsatellite data to define the number of clusters corresponding to number of sampled taxa. It can be also used to infer the population structure or to detect the hybrids, especially in species-rich genera, such genus *Quercus* L. (oaks).

It was used in the case of closely related European white oak species, such as *Quercus pubescens* and *Q. frainetto* (Curtu et al, 2011) or *Q. robur*, *Q. petraea*, *Q. pubescens* and *Q. pyrenaica* (Lepais et al, 2009) or the two endemic Podolian oaks, namely *Q. lobata* and *Q. douglasii* (Craft et al, 2002).

The aim of this study was to test the Bayesian algorithm implemented in the software STRUCTURE version 2.3.3 (Pritchard et al, 2000) in order to detect the number of clusters (k), by using microsatellite data for four Romanian oak species, namely pubescent oak (*Q. pubescens*), Italian oak (*Q. virgiliana*), pedunculate oak (*Q. robur*) and sessile oak (*Q. petraea*).

MATERIAL AND METHODS

A total of 162 oak individuals were genotyped at seven microsatellite loci (Table 1). Among them, according to the Dendrological Romanian literature (Șofletea and Curtu, 2007), 61 trees were pubescent oak trees, 18 were Italian oak trees, 37 were pedunculate oaks and 46 were sessile oaks. Special attention was given to certain leaf and fruit descriptors, such as: lamina length, petiole length, basal shape of the lamina and length of the cupula peduncle. According to some recent studies (Enescu et al, 2011; Șofletea et al, 2011), these morphological characters proved to had the highest discriminating power between the Romanian oak species. While the oaks from series *Lanuginosae* were sampled from pure or mixed stands across Romania, the pedunculate oaks and the sessile oaks were sampled from two pure stands, namely Podul Iloaiei (Iași County, NE Romania) for *Q. robur* and Cristian (Brașov County, Central Romania) for *Q. petraea*, respectively.

DNA was extracted from winter buds using the Qiagen DNeasy 96 Plant Kit following the manufacturer protocol, but without liquid nitrogen (Toader et al, 2009). Then, the DNA was kept by -60° C until use. The seven genomic SSRs (gSSRs) were amplified using Polymerase Chain Reaction (PCR). The primers were combined into two PCR multiplexes on the basis of annealing temperature and fluorescent label. The first multiplexing reaction included four gSSRs (ssrQpZAG112, ssrQpZAG96, ssrQpZAG11 and ssrQpZAG110), while the second one only three (ssrQpZAG87, ssrQpZAG20 and ssrQpZAG7). More information about the seven microsatellite loci is given in Table 1. The reactions were performed in a 10 µl volume containing 1 µl template DNA (1:40...1:80; 2 µl DNA : 38 µl H₂O... 2 µl DNA : 158 µl H₂O), 2 µl PCR Buffer 5x, 0.90 µl MgCl₂ (25 mM), 1 µl dNTPs (2mM) and 0.10 µl Promega *Taq* DNA polymerase (5 U/ µl). For primers concentrations see Table 1. Amplification was carried out in an Eppendorf Master Cycler. The PCR profile was as follows: 3 minutes of denaturation at 94° C followed by 30 cycles of 45 s denaturation at 94° C, a 35 s annealing step at 51° C, a 1 min 50 s elongation step at 69° C and a final extension step at 69° C for 15 min. The correct amplification of loci was checked by using 2 µl of PCR products mixed with 3 µL of Dye and migrated on 1.5 % agarose gels for 25 minutes at 100V. Amplification products were run on a Beckman Coulter Genetic Analyser

using Frag-3 method and Size Standard 400. The products were then analyzed using Fragment Analysis Software using default parameters and PA ver 1 dye correction.

Table 1. Characteristics of the seven microsatellite loci

Locus	Nucleotide motif	Linkage group (LG)	Beckman Dye	Primer concentration (uM)	Allele size (bp)
ssrQpZAG112	di	12	D4	0.20	82-112
ssrQpZAG96	di	10	D3	0.80	140-180
ssrQpZAG11	di	10	D3	0.60	242-289
ssrQpZAG110	di	8	D4	0.90	205-243
ssrQpZAG87	di	1	D3	0.55	103-183
ssrQpZAG7	di	2	D4	0.65	116-157
ssrQpZAG20	di	1	D3	0.80	159-213

Species assignments were evaluated using the program STRUCTURE version 2.3.3 and all possible combinations (with two, three or four taxa) were tested (see Table 2). In addition, an extra combination with all four taxa (162 oak trees), by changing the order of four individuals (the pubescent oak no. 5 changed its place with the pedunculate oak no. 95 and the pubescent oak no. 6 changed its place with the sessile oak no. 120, respectively) in the input file was also done.

The admixture model assuming correlated allele frequencies was used. In all cases, two model approaches have been used, namely with or without *a priori* grouping of individuals to species. Three runs were done for each case. In every run the length of *burnin* period was set to 50 000, while the number of MCMC iterations after *burnin* was 100 000. The number of clusters was estimated according to ΔK values (Evanno et al, 2005) by the aid of STRUCTURE HARVESTER software (Earl, 2011).

RESULTS AND DISCUSSIONS

Regarding the eleven possible combinations (Table 2), better results were obtained by using the sampling location information, on one hand, and when only two taxa were analyzed, on another hand. Particularly, pedunculate oak and sessile oak formed distinct clusters, whatever the assignment model was.

By contrast, no separation between the two oaks from series *Lanuginosae*, namely pubescent oak and Italian oak was observed in combinations comprising three or four taxa (cases 7, 8 and 11). Structure clustering results obtained for the latter three cases with *LocPrior* model (with *a priori* grouping of individuals to species) are illustrated in Figures 1, 2 and 3, respectively. Each individual is represented by a vertical bar partitioned into two or three color segments proportional to its membership in each genetic cluster.

Table 2. K values for the 11 combinations (with or without *a priori* grouping of oaks to species)

Case	Combination	K		Case	Combination	K	
		without	with			without	with
1	STP, ST	2	2	7	STP, STV, ST	2	2
2	STP, STV	3	2	8	STP, STV, PET	2	2

3	STP, PET	3	2	9	STV, ST, PET	5	3
4	STV, ST	2	2	10	STP, ST, PET	3	3
5	STV, PET	3	2	11	STP, STV, ST, PET	3	3
6	ST, PET	2	2				
Abbreviations: STP- <i>Q. pubescens</i> , STV- <i>Q. virgiliana</i> , ST- <i>Q. robur</i> , PET- <i>Q. petraea</i>							

If we take into consideration the hypothesis according which the pubescent oak and the Italian oak represent a solely morphological and genetic entity, by analyzing the 2-D 100% Stacked Column Graphs from Figures 1, 2 and 3 we can say that only a few individuals were wrongly assigned. Among them, were the individuals 38 and 54 (identified in the field as being pubescent oaks) and the tree number 106 (from Figures 1 and 3), which was considered a *Q. robur*-like individual according to its twig and leaf morphology.

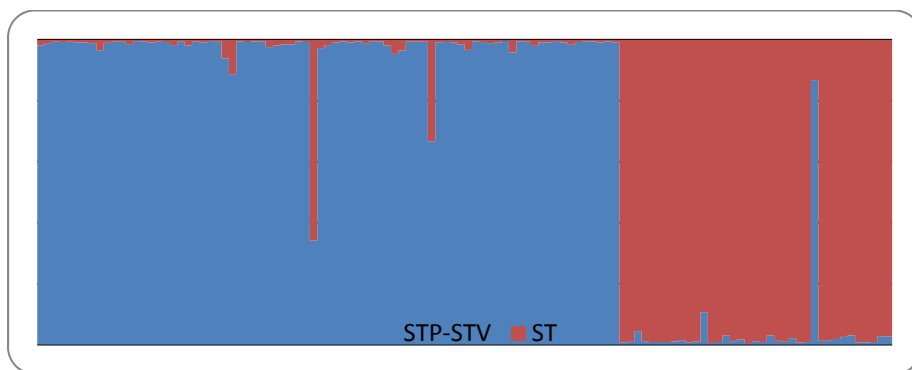


Figure 1. Structure clustering results (K=2) for case 7 (61 STP, 18 STV and 37 ST)

Interesting, regarding the same two pubescent oaks (individuals 38 and 54) different results were obtained in the case number 8, by assigning only the trees from series *Lanuginosae* and the sessile oaks. In this case, the memberships of the two pubescent oaks in the sessile oak cluster were less (Figure 2: 15% and 4%, respectively), compared with those from the pedunculate oak cluster from case 7 (Figure 1: 66% and 33%, respectively). Moreover, for the same two individuals lesser membership values were obtained in the case 11, when four taxa were analyzed (Figure 3).

By contrast, no significant differences were recorded for tree number 106. Its membership values to STP-STV cluster were 87% (case 7) and 84 % (case 11), respectively. Similar results were recorded also for pedunculate oak cluster (ST), namely 13% (case 7) and 14% (case 11), respectively.

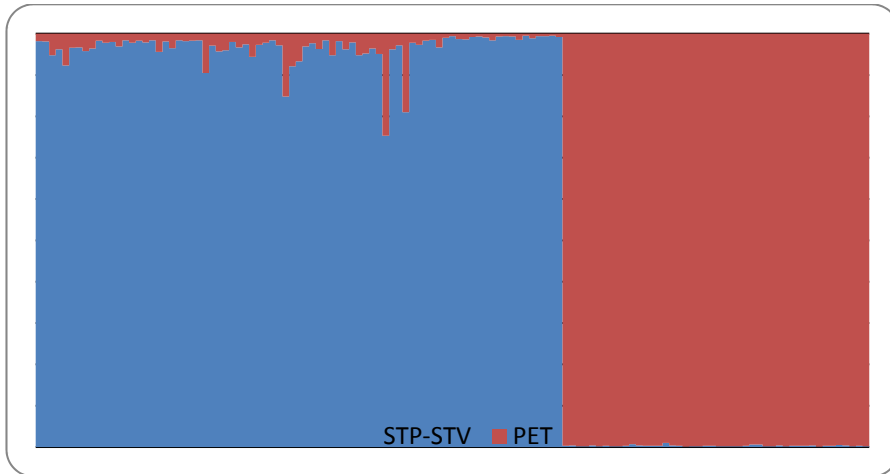


Figure 2. Structure clustering results (K=2) for case 8 (61 STP, 18 STV and 46 PET)

It can be seen from Figure 3 that most of the pubescent oak individuals (no. 1-61) present around 5 to 10 % membership to sessile oak cluster (PET) and only 1 to 3 % to pedunculate oak cluster (ST). Instead, the *Q. virgiliana*-like individuals (oaks no. 62-79) had around 99 % membership to STP-STV cluster.

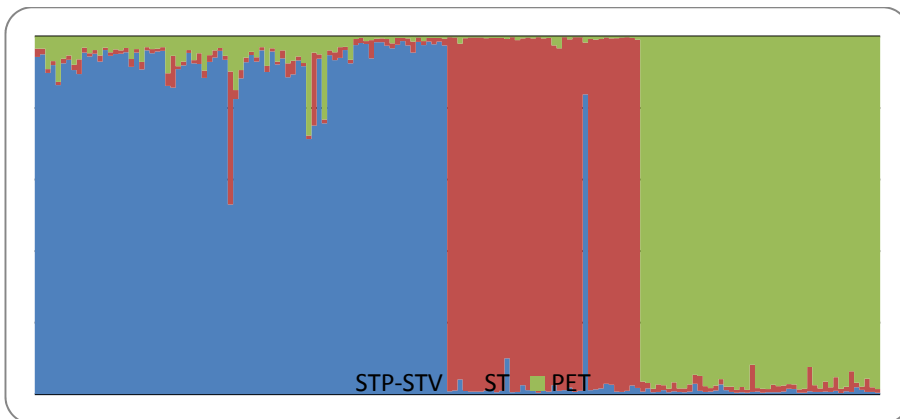


Figure 3. Structure clustering results (K=3) for case 11 (61 STP, 18 STV, 37 ST and 46 PET)

Regarding the extra case (Figure 4), the program identified all the four changes from the input file, being more evident for the first three cases, namely the oaks no. 5, 6 and 95, respectively.

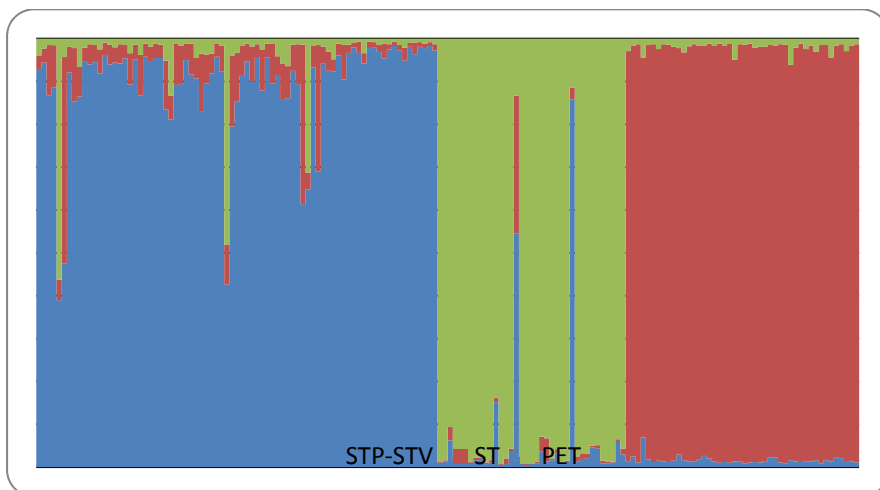


Figure 4. Structure clustering results (K=3) for the extra case

The different results obtained for the oaks from series *Lanuginosae* (cases 1-5, 7, 8 and 11) can be explained, on one hand, by the small sampling size for Italian oak, or by the genetic similarities of the two pubescent oaks, namely *Quercus pubescens* and *Q. virgiliana*, on the other hand. Nevertheless, if we take into consideration the Figure 3 we can say that the pubescent oak individuals shared a bigger part from their genome with the sessile oak, around 5-10%, compared with the pedunculate oak, with only 1-3 %.

By contrast, the distinct clusters formed by the pedunculate oak and sessile oak individuals indicated the existence of two different genetic entities. Same results were communicated by Moldovan (2011) for the two oak species in eastern Romania by using both microsatellite and cpDNA markers or by Neophytou and his colleagues (2010) by sampling individuals from three different European stands (Greece, Bulgaria and Germany).

CONCLUSIONS

Even if we used only seven microsatellite loci only a few individuals were wrongly classified (Figures 1-3). This could be explained, on one hand, by the fact that these individuals could be putative hybrids or, on another hand, by the DNA contamination. It resulted also that the only seven microsatellite loci were able to separate *Q. robur* from *Q. petraea* and the group pedunculate oak – sessile oak from the oaks belonging to series *Lanuginosae*.

Our findings support the hypothesis according which Italian oak is an intraspecific taxonomic unit of pubescent oak. This is in accordance with the results from a morphological survey according which the length of the cupula peduncle was the only descriptor which somehow discriminate the two taxa (Enescu et al, 2012).

It was proven that the software STRUCTURE was able to highlight the changed made in the input file. This could be helpful if someone wants, for example, to determine to which taxa a certain sample belongs. In other words, a bigger number of samples and microsatellite markers will increase the precision of the assignment.

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Acknowledgements: This paper is supported by the Sectorial Operational Program Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/88/1.5/S/59321 and by CNCS-TE-73-2010 project.

VARIABILITY OF ANTHOCYANIN CONTENT AND DRY MATTER AMOUNT IN FRUITS OF SOME *LONICERA CAERULEA* SELECTIONS DEPENDING ON STORAGE CONDITIONS

ZENOVIA OLTEANU¹, LACRAMIOARA OPRICA¹,
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Keywords. berries, edible blue honeysuckle, drying preservation, freezing storage

Abstract. *Lonicera caerulea* var. *kamtschatica* is a species with multiple uses mainly due to the valuable biologically active substances with high antioxidative potential. The anthocyanins occupy an important place in inducing the health-protective benefits of the berries of this species. The aim of our work was to determine the total anthocyanin and dry matter amounts in the fruits collected from Romanian selections of blue honeysuckle, preserved by freezing (three months in refrigerator) and drying at 60°C (to constant weight). The obtained results showed that the storage conditions have generally influenced the anthocyanin content. Thus, in freezing storage conditions, the anthocyanin amount either decreased (C, SL6, SL8, SL15) or increased (SL17, SL57), depending on the biological material, whereas the drying preservation declined the anthocyanin level with ~ 80%, also varying with the selections of blue honeysuckle.

INTRODUCTION

Lonicera caerulea L. var. *kamtschatica* (blue honeysuckle) (Caprifoliaceae family) is a species of shrub largely used in folk medicine because of its rich phytochemical content and which grows across the Northern Hemisphere (Russia, China and Japan) (Paliková *et al.*, 2008). From northern Russia, the plant was introduced and cultivated, although on smaller surfaces, in Central Russia, in Urals, Siberia, and more recently in Canada and USA (Mladin *et al.*, 2011) where the blue honeysuckle was little known for the edible properties of its berries (Svarcova *et al.*, 2007). In Europe, the species occurs rarely in the Alps and Scandinavia (Hummer *et al.*, cf. Svarcova *et al.*, 2007). In Romania, this plant - one of the few species with edible fruits of the *Lonicera* genus - was introduced ~ 30 years ago, at the Research Institute for Fruit Growing, Pitesti - Maracineni, where it was subjected to complex breeding and selection activities with the aim to enlarge the range of the phenotype variability concerning the bio productive and the biochemical traits and to select the forms with the best adaptability to the conditions of Romania (Mladin *et al.*, 2011).

The *Lonicera caerulea* berries have multiple edible and medicinal uses due to the complex biochemical constitution and in literature there are numerous studies dedicated to the knowledge of the biochemical profile of various provenances belonging to this species (Svarcova *et al.*, 2007; Ochmian *et al.*, 2010; Rop *et al.*, 2011; Jurikova *et al.*, 2012). The main group of the polyphenolic compounds showing high antioxidant properties and which confer to the blue honeysuckle berries beneficial effects for human health are the anthocyanins (Oprea *et al.*, 2002; Jordheim *et al.*, 2007). They are secondary plant metabolites, namely water-soluble flavonoids, generally found as glycosidic forms (3-glycosides and 3,5-diglycosides), which are generally linked to glucose, galactose, rhamnose, and arabinose (Svarcova *et al.*, 2007). In ecosystems, the anthocyanins have crucial role; together tannins, they play a major role in the expression of fruit colour and taste, with repercussions on the fruit quality and edible valences (Mladin *et al.*, 2011). Coloured petals of the flowers have role in attraction of the insects during pollination, while the ripe fruits serve as food for animals (distributors of seeds), and for humans, so being contributors to their diet. The maximum anthocyanin content was evidenced during fruit ripening, and it decreased towards the end of this period. In early stage of fruit ripening, the anthocyanins are masked by chlorophyll (Vlahov, 1992). Some authors consider that anthocyanins are protective factors for the photochemical system and contribute to the increase of photosynthesis yield (Lee *et al.*, 2003). Out of the anthocyanins, the fruits of *Lonicera* are rich sources of ascorbic acid, sugars (5 – 10%), lipids (1.52%), organic acids (1.5 – 4%, the most important being citric acid – 90% of the total), and variable quantities of minerals (K, Mg, P, Ca), carotenoids, other phenolic compounds such as proanthocyanidins or phenolic acids which determines their multiple biological activities. Into the anthocyanin level, cyanidin-3-glucoside generally dominates in the berries of most blue honeysuckle berries, with ~84-90% from total amount, followed by peonidin, delphinidin and pelargonidin (Malodobry *et al.*, 2010; Mladin *et al.*, 2011; Kusznierewicz *et al.*, 2012).

The range of the therapeutic effects manifested by the compounds extracted from blue honeysuckle includes the lowering of blood pressure, protection against the risk of heart attack, prevention of osteoporosis and anaemia, diminution of child hyperactivity, amelioration of healthy state in malaria, gastrointestinal disorders and some eye disorders, slowing of the aging processes, modulation of glycemic responses *etc.* (Tarabasanu-Mihaila *et al.*, 1997; Svarcova *et al.*, 2007; Paliková *et al.*, 2009; Hanhineva *et al.*, 2010; Jiao and Wang, 2010; Malodobry *et al.*, 2010; Jurikova *et al.*, 2012). Out of the above mentioned benefits, the antioxidative action of the anthocyanins induces a good protection against serious

diseases such as diabetes mellitus, neurodegenerative conditions and certain types of cancers (Zhao *et al.*, 2004; Gruia *et al.*, 2008). The anthocyanins can be also utilized as natural food colorants (Vendramini and Trugo, 2004), while Palíková *et al.* (2008) reported that freeze-dried fruits of blue honeysuckle and their purified phenolic fraction reduced biofilm formation and adhesion of some pathogenic microorganisms. The impressive variability in biochemical pattern evidenced in blue honeysuckle fruits - both quantitatively and qualitatively - which determines their nutritional valences and health benefits, is strongly affected by the environmental conditions, harvest date, storage conditions and genotype (Hoppula *et al.*, 2006; Ochmian *et al.*, 2010; Truta *et al.*, 2012).

The aim of our work was to quantify the total anthocyanins and the dry matter amounts in the fresh berries collected from Romanian selections of blue honeysuckle, by comparing to the berries preserved by freezing (three months in refrigerator) and drying at 60°C (to constant weight).

MATERIALS AND METHODS

For the quantitative assessment of the anthocyanins, berries of *Lonicera caerulea* var. *kamtschatica* were used. The fruits have been harvested at biological maturity from the experimental fields of Research Institute for Fruit Growing, Pitesti – Maracineni (geographical coordinates: 44°51'N, 24°54'E). The working variants were constituted by different honeysuckle genotypes, namely control (C) and five selections conventionally noted as SL6, SL8, SL15, SL17, SL57. Biochemical analyses were carried out on fresh berries and on plant material preserved by drying (at 60°C, to constant weight) and freezing (three months in refrigerator).

Dry matter (DM) was determined by gravimetric method, which basically consists in maintenance of the biological material at 105°C to constant weight (Boldor *et al.*, 1983). The results are expressed in g dry matter/100g fresh biological material.

The amount of the *total anthocyanins* was estimated by determination of absorbance of the ethanol-acidified extracts at 515 nm. The results are expressed as mg/100g DM (Fuleki and Francis, 1968).

RESULTS AND DISCUSSION

Dry matter content, determined for the experimental model in which the quantification of the biochemical indicator was performed on fresh biological material, is higher than control in all analyzed selections (Fig. 1). The maximum value belongs to SL6 variant (18.03g/100g), while SL57 showed the smallest amount of dry matter (16.05g/100g).

In frozen fruits the behaviour is similar, the selections having amounts of dry matter superior to the control (Fig. 1), with maximum values in SL6 and SL8, and minimum values in SL57.

The drying storage leads to the decrease in water content in the berries harvested from SL6 selection and occurrence of a lower dry matter content (83.9g/100g) as compared to the control, whereas the other selections presented quantities of dry matter higher than the control, with a maximum level of 87.49g/100g registered in SL17.

Therefore, by comparing with the respective controls, with only one exception, the content in dry matter was superior in all analyzed selections, in all experimental models. It is possible that during selection process an increase of the tissue resistance was carried out and, implicitly, the diminution of the diffusion rate of the water vapours. The smallest changes in the dry matter content were observed during freezing storage. The results show that the freezing storage was correctly conducted; as a consequence, the simultaneous alterations supported by the biological material because of freezing of the water present in the cellular solutions were reduced at minimum. According to the results, except for SL8 variant, the other selections – by comparison with the controls – have higher number of intact cells (low variation in dry matter content between experimental variants in fresh condition and after freezing), fact showing an increased capacity of the endocellular colloids to absorb the water.

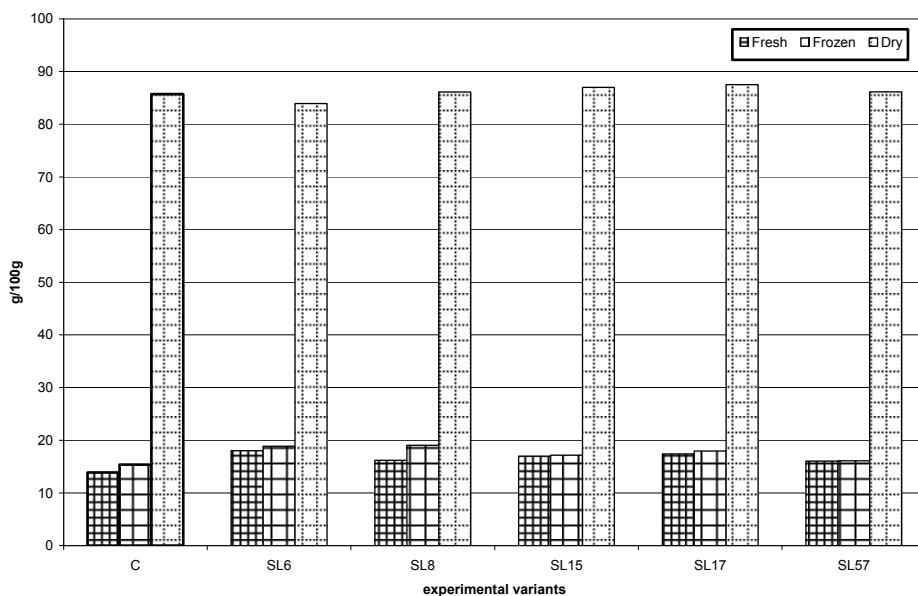


Fig. 1. Variations in the dry matter content in fresh berries, freezing stored berries and drying stored berries harvested from selections of *Lonicera caerulea* var. *kamtschatica*

In literature, Pokorna-Jurikova and Matuskovic (2007) established significant influences of cultivation conditions on dry biomass accumulation, but also variations depending on species and varieties were observed. The amounts of dry matter determined in our selections are generally lower than, for example, those reported by Svarcova *et al.*, (2007) in some Polish varieties (~18.5%).

As concerns the anthocyanins, their amounts in blue honeysuckle fresh berries exceeded the control, with only one exception (SL8) (Fig. 2). The maximum and minimum values belong to the SL15 (2512.71mg/100g DM) and SL8 selections (1784.52mg/100g DM).

The freezing stored fruits had different behaviours by comparing with the controls. So, SL6 and SL8 experimental variants showed diminished anthocyanin levels, while SL15, SL17, and SL57 selections surpassed the control values (Fig. 2).

Regarding the drying stored berries, except for SL8, the other analyzed selections have anthocyanin levels over the control (Fig. 2). SL15 showed the highest content (468.57mg/100g DM), and the SL8 selection had the minimum observed value (296.74mg/100g DM).

The comparative analysis of the obtained results confirms that storage methods exert some influences on anthocyanin content in the tested biological material. Because of their reactivity, the anthocyanins are easily degraded or they interact with other constituents, leading to incoloured or brown-coloured compounds. The instability and the loss of the anthocyanin colour take place as result of their susceptibility to the attack of some nucleophile agents, the long term exposure to oxygen action, activation of some enzymes or enzymatic systems, temperature, *pH*, light etc. Higher decreases in anthocyanin amounts were observed especially in drying stored berries (generally the decrease rate was over 80%): C - 86.27%; SL6 - 84.10%; SL8 - 83.37%; SL15 - 81.35%; SL17 - 83.80%; SL57 - 79.29%).

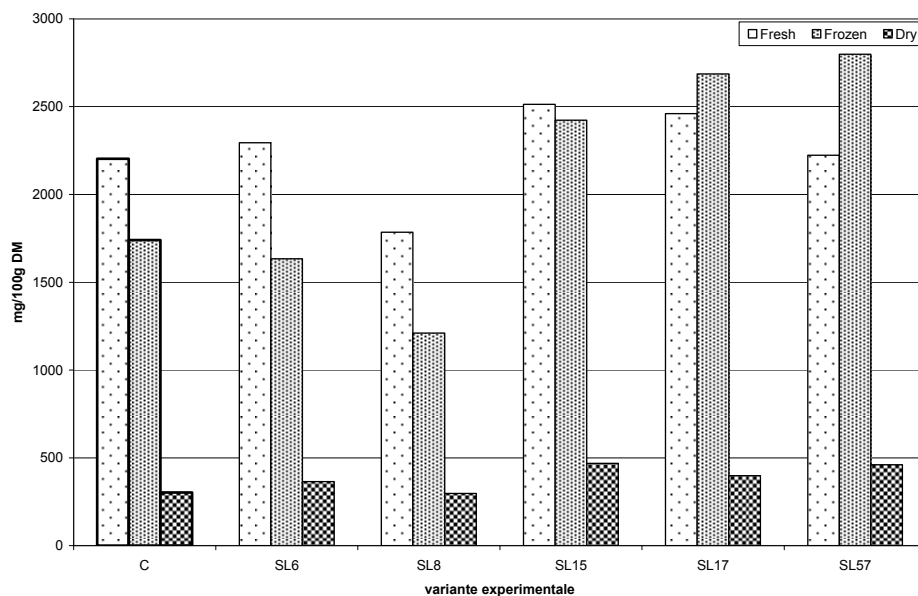


Fig. 2. Variations in the anthocyanin content in fresh berries, freezing stored berries and drying stored berries, harvested from selections of *Lonicera caerulea* var. *kamtschatica*

Although the anthocyanin amount decreased by drying, the fruit colour not greatly changed. This behaviour is due to the co-pigmentation reactions in which the anthocyanins are condensed with each other as well as with other organic molecules, fact resulting in the formation of new pigments.

The method of freezing storage induced different behaviours of the investigated biological material, in that in some experimental variants the anthocyanin content decreased in variable range (C - 21.06%; SL6 - 28.83%; SL8 - 32.16%; SL15 - 3.56%), and in other increased (SL17 - 9.17; SL57 - 25.88).

Mladin *et al.* (2011) also found considerable variations in the anthocyanin content of fresh blue honeysuckle berries, from 206.0mg% to 579.0mg%. In dried fruits, they determined contents by ~6.6-6.8 much higher than in fresh fruits. The content in anthocyanins is subjected to intensive degradation during long storage of the berries, the temperature and the light being decisive factors. Kalisz *et al.* (2013) noted the highest losses occurring at the initial period of storage.

CONCLUSIONS

In conclusion, the results obtained in the present study support the existence of a relatively large extent in the phenotypisation of the studied biochemical quantitative characters, as a reflection of the genotype expression of each variety in the respective environmental conditions. Despite the uniformity of the chromosome features established in our previous studies (Truta *et al.*, 2013) in the selections of blue honeysuckle, the present work shows enough large variability in dry matter

content and anthocyanin levels. It must also note that *Lonicera* berries were harvested from ecotypes having the same geographical provenance (Maracineni-Pitești Agricultural Station) and growing in the same environmental conditions. Considering this facts, we can sustain that these differences in biochemical patterns are the consequence of the differences existing at genic level. For this reason, further detailed researches at molecular levels are needed in order to clarify the relationship between genetic constitution and the range of phenotypisation of some traits of economical interest.

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Acknowledgements. This research was supported by project 52142/15.09.2008 - PNCD II (2009-2011) (National Program for Research in Romania.

„IN VITRO” EFFECT OF SOME INDUSTRIAL BY-PRODUCTS ON *LAVANDULA ANGUSTIFOLIA* MILL. EXPLANT GROWTH

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Keywords: lavender, spruce bark, hemp shives, photoassimilating pigments, deuterium depleted water.

Abstract: After many studies, it was observed that lavender has many therapeutic effects, such as sedation, activities spasmolytic, antiviral, antibacterial. Thus, given the importance of lavender in different areas of human life, in the present study, we studied the influence of natural products bioregulators separated from industrial by-products on some lavender stems explants. These explants were inoculated *in vitro* on MS nutrient media. In these culture media were added polyphenolic extracts obtained from spruce bark and hemp shives, and evaluated their influence on lavender stems explants. The results obtained were compared with those obtained for the control variant, where MS culture medium was used as standard. It was found that the addition of aqueous extract from spruce bark of concentration of 130 mg GAE / L, in the growth of explants of *Lavandula angustifolia* Mill, an increase in the elongation of the main stem, number of leaves formed, the amount of photoassimilating pigments synthesized and causes the phenomenon of shoots formation. At a higher concentration of the extract (26 mgGAE/100g) values are lower.

INTRODUCTION

In the last years, most studies have been directed towards the establishment extraction methods of active principles from fruits and vegetables (Pinelo et al., 2005). Extraction of polyphenolic compounds is a very important step in the isolation, identification and use. The most common techniques for the extraction and isolation of polyphenolic compounds are solvent extraction (Baydar, 2004; Bucic-Kojic, 2007) and supercritical fluid extraction (Bleve et al., 2008; Nahar and Sarker, 2005). A large number of articles in the literature is focused on the extraction and analysis of polyphenols from plant materials (fruits, vegetables, bark, leaves etc.) (Tanase et al. 2011; Ignat et al. 2011; Luthria and Pastor-Corrales, 2006; Balasundram et al. 2006; Naczki and Shahidi, 2006).

The spruce bark aqueous extract was characterized in terms of total polyphenol content, color intensity, pH and was observed to contain a considerable amount of bioactive aromatic compounds, especially catechins and vanillic acid (Tanase et al. 2013). This was revealed by the identification of polyphenolic compounds by HPLC analysis. Regarding, aqueous extract from hemp shives was found that the amount of total polyphenol content expressed in mg GAE/100g is 164 ± 15.23 . The chromatographic analysis were identified and quantified phenolic acids and catechins in hemp shives aqueous extract (catechins - 52.17 mg plant material GAE/100g, vanillic acid - 96.8 mg GAE/100g acid, p-coumaric - 8.06 mg GAE/100g, ferulic acid - 3.2 mg GAE/100g) (Tanase et al. 2013). Regarding the characteristics, DDW (25 ppm isotopic concentration) is a microbiological pure distilled water, obtained by isotopic distillation in vacuum of a natural water with an isotopic concentration of 145 ppm D / (D + H) (Tanase et al., 2013).

Lavender is one the most useful herbs. From commercially point lavender, is an important source of essential volatile oil, which is widely used in the industries of flavors, including soaps, colognes, perfumes, skin lotions and other cosmetics (Paul et al. 2004). In food processing industry, lavender oil is used in flavored drinks, ice cream, candy, baked goods, and chewing gum (Kim and Lee, 2002). Recently, aromatherapy is used for a wide range of growing and lavender is used in aromatherapy as a relaxant (Lis-Balchin and Hart, 1999; Ghelardini et al., 1999). After extensive research, it was found that lavender has many therapeutic effects, such as sedation, activities spasmolytic, antiviral, antibacterial (Gamez et al. 1990; Buchbauer et al. 1991).

Thus, given the importance of lavender in different areas of human life, in the present study we followed the influence of natural products on some lavender stems explants. These explants were inoculated *in vitro* on MS nutrient media. In these culture medium were added polyphenolic extracts (DDW, DDW + M1, M1, M2, P1, P2) and evaluated their influence on lavender stems explants. The results obtained were compared with control, where MS culture medium was used as standard.

MATERIALS AND METHODS

Meristematic explants taken from *Lavandula angustifolia* Mill. obtained under aseptic conditions have been tested "*in vitro*" for regenerative capacity.

Explants were treated with 70% ethanol - 1 min and then with calcium hypochlorite (10%) - 15 minutes. After repeated washing with sterile distilled water, the explants were inoculated on agar nutrient medium (MS medium) solubilized in the polyphenolic extract used for each experimental variant (Table 1). Samples were maintained in growth

chamber at $23 \pm 1^\circ\text{C}$ to 16 hours photoperiod for 30 days. Observations were made at predetermined intervals (1, 5, 10, 20, 30 days), the number of leaves formed, the length of explants, shoot regeneration and photoassimilating pigments content were investigated.

Table 1 - Experimental variants

Experimental variant	Abbreviation	Vegetable material (g)	Total polyphenolic content (mg GAE/L)
Control	Control	0	0
Deuterium depleted water	DDW	0	0
Deuterium depleted water and spruce bark polyphenolic extract (1:1)	DDW+M1	5	96
Spruce bark polyphenolic extract	M1	10	191
Spruce bark polyphenolic extract	M2	5	130
Hemp shives polyphenolic extract	P1	10	164
Hemp shives polyphenolic extract	P2	5	78

Quantification of photoassimilating pigments. 0.05 g fresh vegetal material was extracted in 80% acetone by grinding with a spatula tip of quartz sand. Chlorophyll extract was analyzed spectrophotometrically by reading absorbance at various specific wavelengths: 470, 646, 663 nm. In order to determine the concentration of chlorophyll pigments (chlorophyll a and b) and carotenoid pigments were used formula proposed by Lichtenthaler and Welburn (1983):

Chlorophyll a ($\mu\text{g} / \text{mL}$) = $12.21 (A_{663}) - 2.81 (A_{646})$

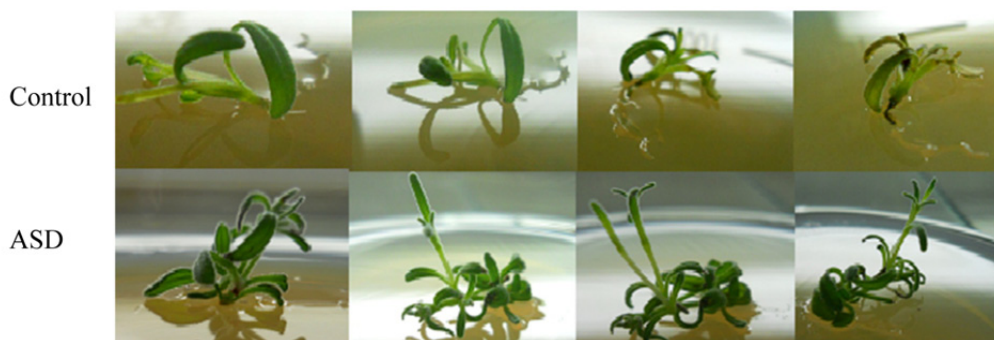
Chlorophyll b ($\mu\text{g} / \text{mL}$) = $20.31 (A_{646}) - 5.03 (A_{663})$

Carotenoids ($\mu\text{g} / \text{mL}$) = $(100 \cdot A_{470} - 3.27 [\text{chl a}] - 104 [\text{chl b}]) / 22$

RESULTS AND DISSCUSIONS

Phenomenon of shoots, number of leaves formed and main stem elongation

Thus, the observations carried out at predetermined intervals, on the stems explants of control samples, revealed no growth and development (any reactions) culminating with partial necrosis explant at 30 days after inoculation (Fig.1)



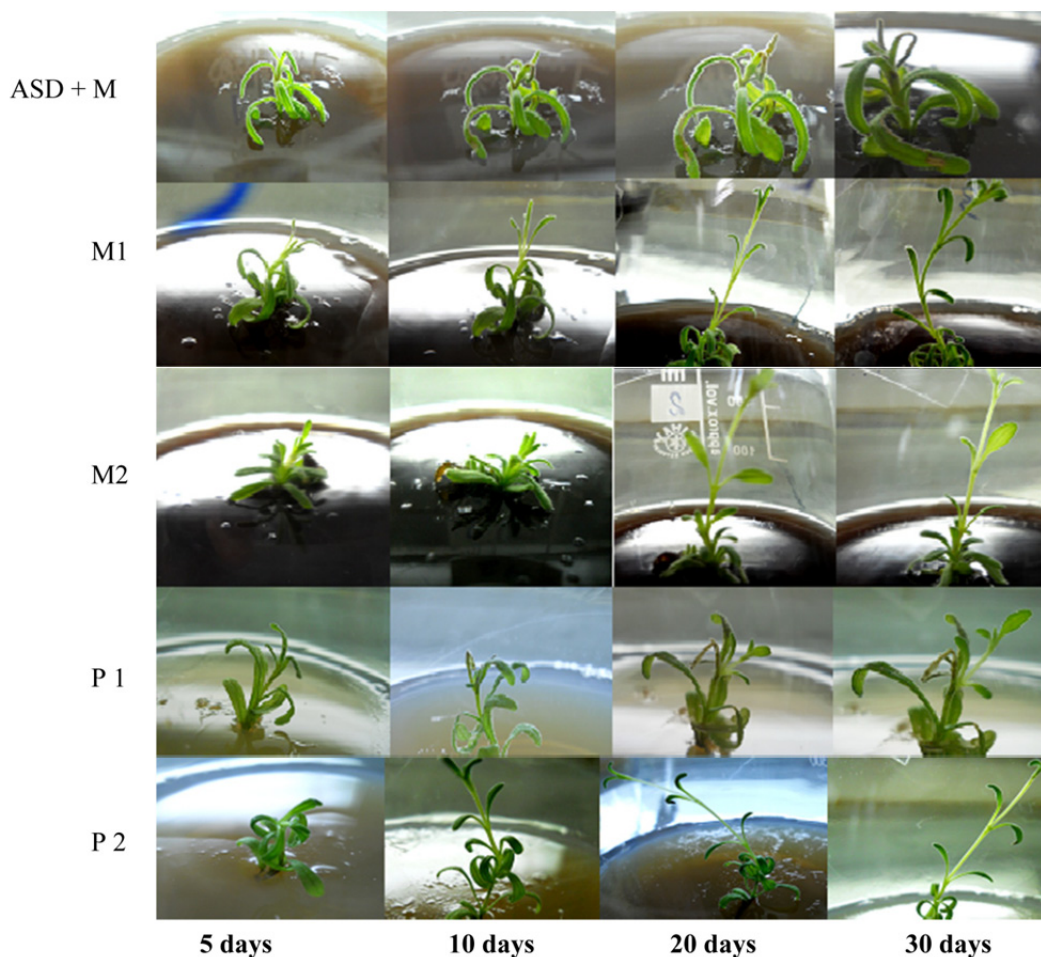


Figure 1 - Aspects of the experimental variants at the analyzing time

In case of DDW version, there was the onset of a process of multiple shoots after 10 days of inoculation, associated with a slight increase in the number of newly formed leaves. In contrast, the variant ASD + M1 were not significant differences in the evolution of the initial explant (Fig. 1).

At meristematic explants that were developed on medium supplemented with spruce bark polyphenolic extract (M2) was found the stimulate growth and shoot elongation after 5 days after inoculation. This process continued spectacular main stem elongation together with the formation of new leaves (Fig. 2). It is also found a multiple shoots (Fig. 3). M1 variant showed the same previous sample in the elongation of the main spindle. However, there is a difference in the number of leaves which is formed which is smaller in the variant mentioned above (Fig. 1).

When the growth medium was supplemented with hemp shives aqueous extract (P1) shows a high number of leaves formed after 5 days from the inoculation, the process is slightly

decrease after 20 days. High frequency of the newly formed leaves (Fig. 2) is associated with the emergence of new shoots after 10 days after inoculation (Fig. 1).

In the variant P2 there is a progressive increase of the main rod together with the emergence of new leaves, predominantly in the range 5-10 days after inoculation. After 10 days from the inoculation, the shoots are observed for P2 variant (Fig. 4).

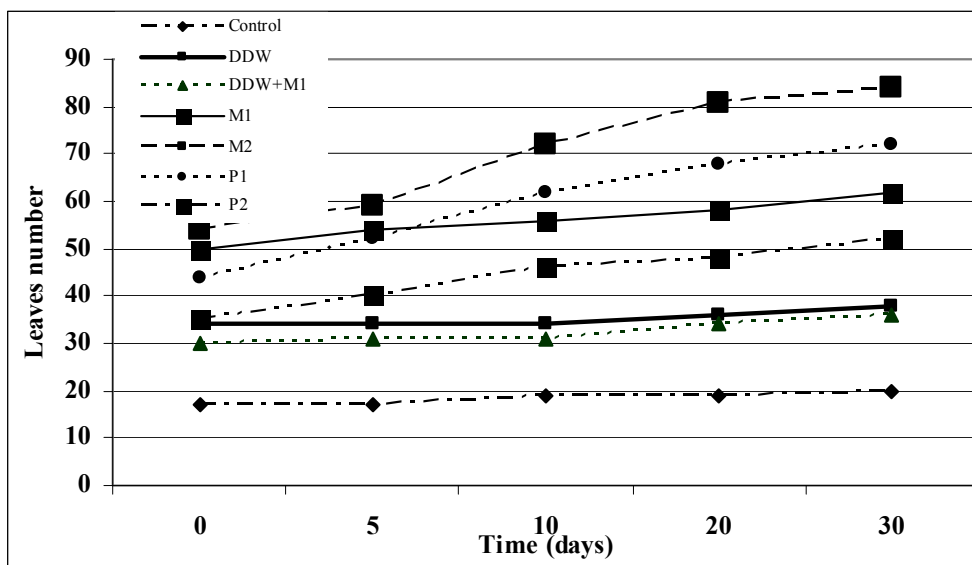


Figure 2 - Influence of polyphenolic extracts and DDW on the number of leaves formed



Figure 3 - Regenerative capacity of shoots (Variant M2)

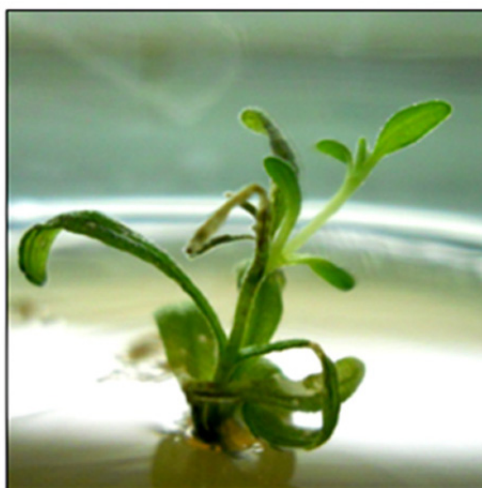


Figure 4 - Regenerative capacity of shoots (variant P1)

The influence of the tested solutions on the photoassimilating pigments content. By analyzing the photoassimilating pigments content at 30 days after inoculation (Table 2), it is found that it is much higher in explants that have developed on the culture medium supplemented with the spruce bark aqueous extract at a concentration of 130 mg / L (M2) and 191 mg / L (M1). At a higher concentration of the extract applied, the percentage of the stimulation is decreased (for spruce bark aqueous extract - M2 and hemp shives extract - P2).

Thus, the growth medium supplemented with hemp shives aqueous extract (in two concentrations) triggers an obvious stimulation for photoassimilating pigments content. Deuterium depleted water increases the the amount of pigments compared with control, but much less than other experimental variants. Moreover and visual observation, direct, there is a depigmentation (relating to the day of inoculation) leaves of explants that developed in control variants, culminating even a start foliar tissue necrosis.

Table 2 - The amount of photoassimilating pigments after 30 days after inoculation

	Chl a $\mu\text{g/g}$	Chl b $\mu\text{g/g}$	Carotens $\mu\text{g/g}$	Chl a+b	Chl a/b
Control	129.81	26.05	36.38	155.86	4.9 8
DDW	239.95	47.12	49.02	287.07	5.0 9
DDW+M1	362.21	67.97	80.05	430.18	5.3 3
M1	1019.11	191.27	322.86	1210.38	5.3 3
M2	1590.05	277.99	372.64	1868.04	5.7 2
P1	889.38	162.47	235.22	1051.85	5.4 7
P2	1550.32	274.04	301.64	1824.36	5.6 6

CONCLUSIONS

It was found that the addition of spruce bark aqueous extract with concentration of 130 mg GAE / L, in the growth of explants of *Lavandula angustifolia* Mill, an increase in the elongation of the main stem, number of leaves formed, the amount of photoassimilating pigments and causes the phenomenon of shoots. At a higher concentration of the extract (26 mgGAE/100g) values are lower.

The hemp shives aqueous extract has a stimulating effect, in particular, the phenomenon of, the shoots, the amount of photoassimilating pigments and the number of leaves formed. Also a higher concentration values of consequently decreases. Deuterium depleted water increases the the amount of pigments compared with control

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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES AT FOLIAR LEVEL INDUCED BY ATMOSPHERIC POLLUTANTS ON SAMPLES OF *AESCULUS HIPPOCASTANUM* L. FROM IAȘI CITY AREA

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Keywords: *Aesculus hippocastanum* L., atmospheric pollutants, physiological changes

Abstract: We present in this paper some physiological changes (photosynthetic and transpiration processes intensity) and biochemical (content of photo-assimilating pigments) induced at foliar level by some pollutants in samples of *Aesculus hippocastanum* L. cultivated for ornamental purposes across the five air quality monitoring stations in Iasi city area. Pollutants monitored by the five stations are represented by gaseous (sulphur dioxide, carbon dioxide, nitrogen dioxide, ozone) and solid pollutants (dust prone to sedimentation). Measurements were made *in vivo*, as well on fresh material covering vegetation periods of years 2012 and 2013. The necrosis and the induced foliar chlorosis by polluting agents represent the clear materialization of some profound physiological modifications which disturb the photo-assimilating structures and assimilator pigments. The results lead to the conclusion that the amount of chlorophyll a and b and the intensity of photosynthesis aren't always correlated, as already known from literature. The most obvious results of pollutants influence occurred for the individuals situated at the traffic station Podul de Piatră, where SO₂ and particulate solids in suspension are the predominating pollutants and this fact states that the traffic pollutants are the most destructive.

INTRODUCTION

Urban air pollution has become a serious environmental problem to trees and crops (Chauhan and Joshi, 2008). Plants are the only living organisms which [3] have to suffer a lot from automobile exhaust pollution because they remain static at their habitat (Mandal, 2006). It has been reported that gaseous forms are absorbed by the leaves, while the particulate forms are absorbed through the outer surface of the plants. Affected plants show some common effects such as chlorosis, necrosis and inhibition in photosynthesis and decreasing plant growth (Davison and Blakemore, 1976). Several studies have shown the impact of automobile exhaust on roadside vegetation throughout their visible and nonvisible effects (Joshi and Swami, 2007).

When exposed to airborne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves (Liu and Ding, 2008). In recent past, air pollutants, responsible for vegetation injury and crop yield losses, are causing increased concern (Joshi and Swami, 2007). Pollutants can cause leaf injury, stomata damage, premature senescence, decreased photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (Tiwari et. al, 2006). Reductions in leaf area and leaf number may be due to decreased leaf production rate and enhanced senescence. The reduced leaf area results in reduced absorbed radiations and subsequently in reduced photosynthetic rate (Tiwari et. al, 2006). In this paper we present the influence of atmospheric pollutants on photosynthetic and transpiration processes intensity and upon the content of photo-assimilating pigments in samples of *Aesculus hippocastanum* L. cultivated for ornamental purposes across the five air quality monitoring stations in Iasi city area.

MATERIALS AND METHODS

The analyzed material consists of leaves belonging to *Aesculus hippocastanum* L. collected from around Iași city's air quality monitoring stations. Vegetal material was collected during the months of May, July and September of 2012- 2013. Control species were collected from the Botanical Garden of "Alexandru Ioan Cuza" University. Collection and measurement "in vivo" were made on leaves situated at the edge of the canopy, of the four cardinal points of each individual, at a distance of 4-5 m above the ground. It was taken into account the height above ground at which were mounted the air quality monitoring station's analyzers and the extent to which measurements of this analysis are relevant. For each foliar test we used 3 parallel probes, and the data was selected as the average value of the results.

Leaf's assimilating pigments were determined by spectrophotometric method of Arnon (1949). They were determined in 80% acetone extract, colorimetric at the following wave length: 663nm, 646nm and 470nm. The results were calculated using formulas developed by MacKinney (1941) and the values were expressed in mg 100g⁻¹ plant material.

To analyze the functional parameters - process of photosynthesis (A), respiration (R) and foliar transpiration (E) it has been used the LCi portable photosynthesis measurement system. Recording parameters was performed *in vivo* on a number of five leaves from the four cardinal points of each individual analyzed (three individuals of the same species in each determination). Therefore the results are the arithmetic mean of readings taken.

RESULTS AND DISCUSSIONS

Net photosynthetic rate is a measure commonly used in the study of the impact of air pollutants on woody species (Woo et al., 2007). Plants are constantly exposed to environmental pollutants that they absorb, integrate and accumulate in their systems. It is reported that, depending on their level of sensitivity, the plants show visible changes that would include biochemical and physiological modification (Agba and Esiefarienrhe, 2009).

In all investigated areas the average of photosynthetic rate at *Aesculus hippocastanum* L. individuals was lower than the control in May, July and September of 2012 and 2013 (fig. 1, 2). The minimum photosynthetic rate was recorded at the individuals from Podu de Piatră area (traffic station). The photosynthesis rate was 46.15% from the control in May 2012 and 32.81% in May 2013, 39.95% from the control in July 2012 and 33% in July 2013, 40.37% from the control in September 2012 and 34.88% in September 2013.

The average of respiration values were very different, either lower or higher than the controls (fig.1, 2). The minimum values were recorded at the individuals from Podu de Piatră area (traffic station). The respiration process values were 52.17 % from the control in May 2012 and 51.85% in May 2013, 56.37% from the control in July 2012 and 49.11% in July 2013, 53.07% from the control in September 2012 and 35.8% in September 2013.

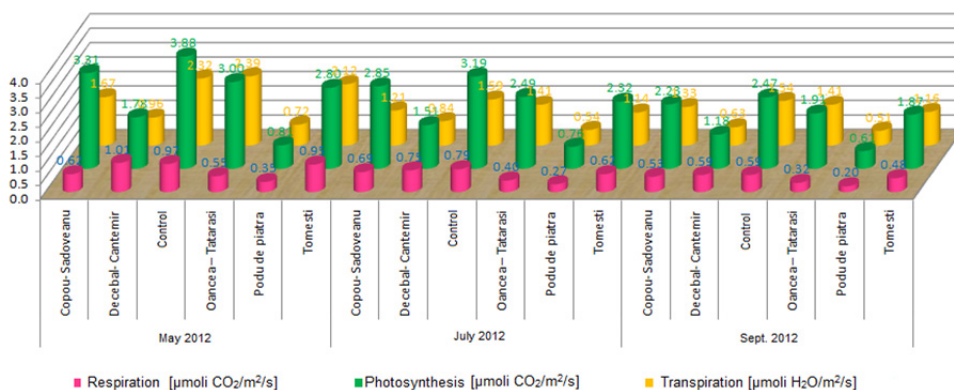


Fig.1 Variation of physiological processes intensity: photosynthesis, respiration and transpiration at *Aesculus hippocastanum* L. individuals derived from the five areas of investigations (May, July and September 2012)

The average of transpiration values was very different, either lower or higher than the controls (fig.1, 2). The minimum values were recorded at the individuals from Podu de Piatră area (traffic station). The transpiration process value was 55.84% from the control in May 2012 and 38.62% in May 2013, 55.78% from the control in July 2012 and 56.91% in July 2013, 51.15% from the control in September 2012 and 50% in September 2013.

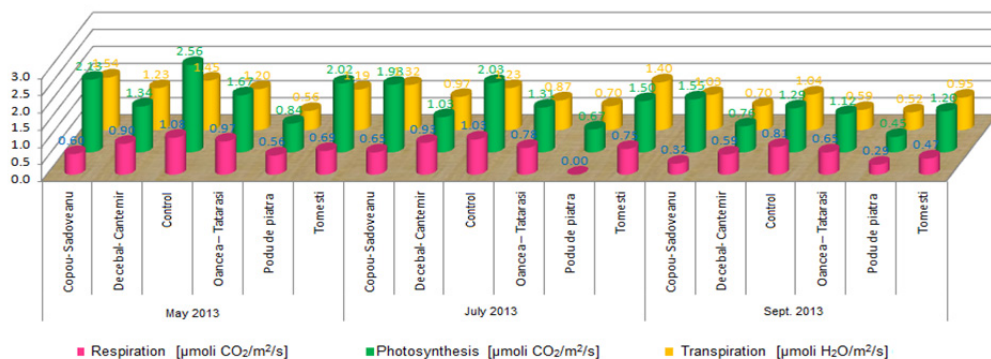


Fig.2 Variation of physiological processes intensity: photosynthesis, respiration and transpiration at *Aesculus hippocastanum* L. individuals derived from the five areas of investigations (May, July and September 2013)

A relationship between traffic density and photosynthetic activity, total chlorophyll content and leaf senescence degree has been studied by Honour et al. in 2009. One of the most common effects of air pollution is the gradual disappearance of chlorophyll and yellowing leaves along with it, which as a consequence reduce photosynthesis (Joshi and Swami, 2007).

In all investigated areas the average amount of chlorophyll a, chlorophyll b and carotenoid pigments at *Aesculus hippocastanum* L. individuals were either lower or higher than the controls in May, July and September of 2012 and 2013 (fig. 3, 4).

The average of chlorophyll a amount was very different either lower or higher than the controls. Minimum values were recorded at the individuals of *Aesculus hippocastanum* L. species from Oancea-Tătărași area (industrial station). The average of chlorophyll a values was 51.70% from the control in May 2012 and 78.24% in May 2013, 53.89% from the control in July 2012 and 57.47% in July 2013, 39.24% from the control in September 2012 and 44.19% in September 2013.

The average of chlorophyll b amount was very different either lower or higher than the controls. Minimum values were recorded at the individuals of *Aesculus hippocastanum* L. species from Oancea-Tătărași area (industrial station). The average of chlorophyll b values was 61.35% from the control in May 2012 and 79.82% in May 2013, 57.60% from the control in July 2012 and 43.52% in July 2013, 27.78% from the control in September 2012 and 21.96% in September 2013.

The average of carotenoid pigments amount was very different either lower or higher than the controls. Minimum values were recorded at the individuals of *Aesculus hippocastanum* L. species from Oancea-Tătărași area (industrial station). The average of carotenoid pigments values was 92.42% from the control in May 2012 and 82.94% in May 2013, 75.05% from the control in July 2012 and 71.95% in July 2013, 62.40% from the control in September 2012 and 61.01% in September 2013.

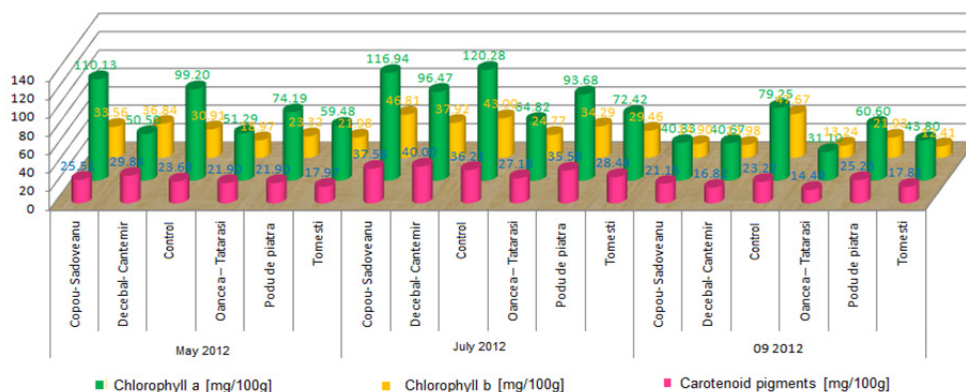


Fig. 3 Variation of foliar pigments content (a chlorophyll, b chlorophyll and carotenoid pigments) at *Aesculus hippocastanum* L. derived from the five areas of investigations (May, July and September 2012)

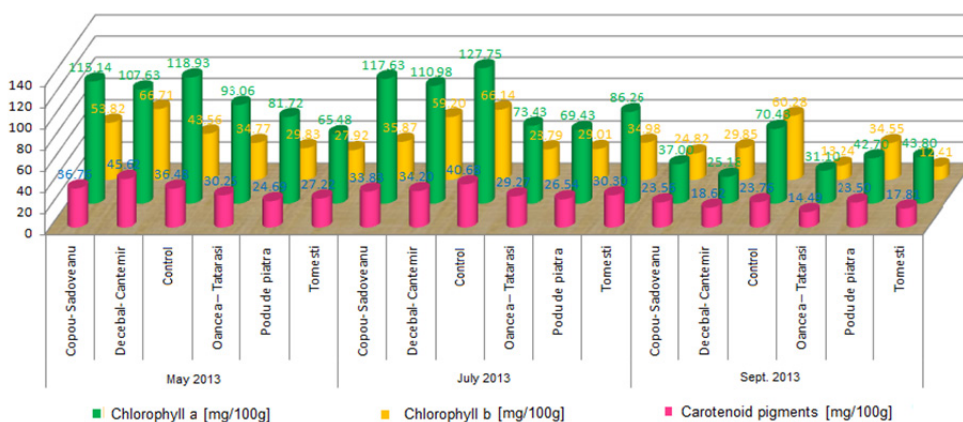


Fig. 4 Variation of foliar pigments content (a chlorophyll, b chlorophyll and carotenoid pigments) at *Aesculus hippocastanum* L. derived from the five areas of investigations (May, July and September 2013)

Under the influence of solid and gas polluting agents the average of chlorophyll a, chlorophyll b and carotenoid pigments quantity can decrease. If the sulphur dioxide (SO₂), nitrogen oxides (NO_x) and CO₂ emissions and particulate matter are absorbed by the leaves, can cause a reduction in the concentration of photosynthetic pigments (chlorophylls and carotenoids) (Joshi and Swami, 2009).

CONCLUSIONS

In this study we investigated the influence of atmospheric pollutants on photosynthetic and transpiration processes intensity and upon the content of photo-assimilating pigments in

samples of *Aesculus hippocastanum* L. cultivated for ornamental purposes across the five air quality monitoring stations in Iasi city area.

Although the polluting agents are different, we can notice similarities regarding the depression location of the two industrial zones, the presence of valleys and the air current circulation, with the greatest concentrations affecting the vegetations around the industrial platforms.

From the correlated interpretation of the obtained data, due to vegetation condition for the years 2012 and 2013 one can conclude that:

The “response” of each individual to pollutant aggression is conditioned by a multitude of genetic factors, pedo-climatic conditions, natural habitat and the nature of pollutant agent distance and it cannot be generalized for the representatives not even for the same genus.

Foliar necrosis and chlorosis are a clear proof of profound physiological modifications that affect the average amount of water, dry substance and assimilating pigments. If the sulfur dioxide (SO₂), nitrogen oxides (NO_x), CO₂ emissions and particulate matter are absorbed by the leaves, can cause a reduction in the concentration of photosynthetic pigments (chlorophylls and carotenoids), which directly lead lower photosynthetic process (Joshi and Swami, 2009).

The minimum rate of photosynthesis, respiration and transpiration recorded at individuals from the Podu de Piatra area is explained by the presence of both coarse and fine particles resulting from heavy traffic. Both, fine and coarse particles, were reported to be responsible for increased leaf temperature that affecting transpiration and decreased light absorption, thus affecting photosynthesis (Tomašević and Aničić, 2012).

The lowest quantity of assimilating pigments was found at the individuals of *Aesculus hippocastanum* L. species from Oancea-Tătărași area (industrial station). The amount of chlorophyll a, chlorophyll b and the intensity of photosynthesis aren't always correlated.

It finds an irregularity regarding the response of this species to pollutants which makes us believe that further investigations are needed to complement the clinical symptomatology.

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MORPHOLOGICAL AND BIOCHEMICAL CHANGES AT FOLIAR LEVEL INDUCED BY ATMOSPHERIC POLLUTANTS ON SAMPLES OF *AESCULUS HIPPOCASTANUM* L. FROM IAȘI CITY AREA

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Keywords: *Aesculus hippocastanum* L., atmospheric pollutants, foliar response

Abstract : We present in this paper some morphological changes (presence and size of the surface both normal and necrotic) and biochemical (water content and dry matter) induced at foliar level by some pollutants in samples of *Aesculus hippocastanum* L. cultivated for ornamental purposes across the five air quality monitoring stations in Iasi city area . These stations monitor the presence of gaseous pollutants (sulphur dioxide, carbon dioxide, nitrogen dioxide, ozone) and solids (powders prone to sedimentation). Measurements were made *in vivo*, as well on fresh material covering vegetation periods of years 2012 and 2013. The results are supporting the fact that the increased values of dry matter content do not correlate directly with the degree of necrosis of the leaves, which entitles us to believe that the biochemical and physiological modifications made by pollutants at this level are fast followed by defoliation events. The most critical situation is found at the samples of *Aesculus hippocastanum* L. grown at the site of the traffic station Podul de Piatră, where SO₂ and particulate solids in suspension are the predominating pollutants.

INTRODUCTION

The air quality from urban areas affects our way of life (Perloff, 1969). If by the 80 major sources of air pollution were domestic heating and industries with high emissions of sulphur dioxide (SO₂), now the main source of pollution in city areas is represented by cars that emit oxides of nitrogen, carbon dioxide , hydrocarbons, particles of dust and heavy metals. On hot summer days, these substances contribute to formation of ozone, a pollutant that is more phytotoxic (Garrec and Rose 1988). Numerous studies have shown that woody plants have a high capacity to reduce the quantities of pollutants in the atmosphere acting as true biological filters. Therefore, their presence in heavily polluted urban areas, proved to be necessary not only aesthetically but also in terms of their decontaminating action. However, air pollution is one of the major stressors of woody plants, which can cause acute damage that is immediately visible, but also chronic damage that can sometimes be asymptomatic thus hindering the ability to determine the cause of their decline. Pollutants are factors with phytotoxic effects in all plant's organs. The symptoms of pollution impact upon trees are investigated in physiological, biochemical, morphological and cytogenetic aspects (Nabais et al. 1999). In this paper we present some morphological changes (presence and size of the surface both normal and necrotic), biochemical (water content and dry matter content of photo-assimilating pigments) and physiological (photosynthetic and transpiration processes intensity) induced at foliar level by some pollutants in samples of *Aesculus hippocastanum* L. cultivated for ornamental purposes across the five air quality monitoring stations in Iasi city area. These stations monitor the presence of gaseous (sulphur dioxide, carbon dioxide, nitrogen dioxide, ozone) and solid (powder prone to sedimentation).

MATERIALS AND METHODS

The biological material represented by leaves of *Aesculus Hippocastanum* L. species was collected from Iași city, from the air quality monitoring stations area. The plant material was collected in the years 2012 and 2013 in May, July and September performing alongside field observations. Control species were selected from the Botanical Garden of "Alexandru Ioan Cuza" University. Collection and measurement "in vivo" were made on leaves situated at the edge of the canopy, of the four cardinal points of each individual, at a distance of 4-5 m above the ground. It was taken into account the height above ground at which were mounted the air quality monitoring station's analyzers and the extent to which measurements of this analysis are relevant.

Determination of leaf area was done with the portable leaf area meter (AM300 apparatus). Optical measurements were made using a simple scanning process.

Water content and dry weight of plant material were determined by gravimetric method (Boldor et al, 1983).

RESULTS AND DISCUSSIONS

Aesculus hippocastanum L. individuals in the five areas studied showed severe foliar symptoms, being a very sensitive to pollutants species.

In Copou-Sadoveanu, Decebal-Cantemir and Tomeşti *Aesculus hippocastanum* L. individuals showed no significant defoliation but showed foliar chlorosis and necrosis since July. (Fig. 1, 2).



Fig. 1 Foliar detail of *Aesculus hippocastanum* L. individual from Decebal-Cantemir (July 2013)



Fig. 2 Foliar detail of *Aesculus hippocastanum* L. individual from Tomeşti (July 2013)

It was found, however, that in Decebal-Cantemir and Tomeşti areas these chlorosis and necrosis widened in late September, occupying significant areas of foliar device (Fig. 3).



Fig. 3 Foliar detail of *Aesculus hippocastanum* L. individual from Tomeşti (September 2013)

In Podul de Piatră and Tătărași-Oancea areas *Aesculus hippocastanum* L. individuals showed significant episodes of defoliation in July. Leaflets showed reddish brown marginal necrosis and, at the end of July, their tops were twisted (Fig. 4). Roadside trees are, to a greater extent, to pollution caused by vehicle emissions (Jităreanu et al., 2010).



Fig 4. Foliar detail of *Aesculus hippocastanum* L. individual from Podul de Piatră (July 2013)

Pollutants can cause foliar injury, stomata damage, premature senescence, reduced photosynthetic activity, disrupts membrane permeability and thus the normal growth and development (Tiwari et al. , 2006). Reducing leaf area and number of leaves may be due to the rate of foliar productivity and early senescence induced by pollutants. Dineva (2004) and Tiwari et al. (2006) recorded reduced leaf area and petiole length under stress due to pollution. Some studies showed changes in leaf area and petiole size in polluted air (Jahan and Iqbal, 1992). Significant reduction in the length of the stem and leaf area was also observed in pollution conditions caused artificially. Reduction in leaf area was observed in five species of trees near areas contaminated with solid particulates and SO₂ (Jahan and Iqbal, 1992). Significant effects of automobile exhaust on phenology, morphology and productivity of tree species on the roadside was also reported (Bhatti and Iqbal, 1988).

Leaf area of all investigated individuals showed lower values compared with the control, in all investigated areas, in both years of study, with minimum values at the individuals from Podu de Piatră area (traffic station) (Fig. 5, 6). Chlorosis and necrosis appearance since early developmental stages of leaves, physiological stress due to pollutants aggression is consequential harm to the degree of development of the plant's leaves. Reduced leaf surface from Podul de Piatră, an intense traffic area, is due to heavy deposits on the leaves or chlorosis and necrosis.

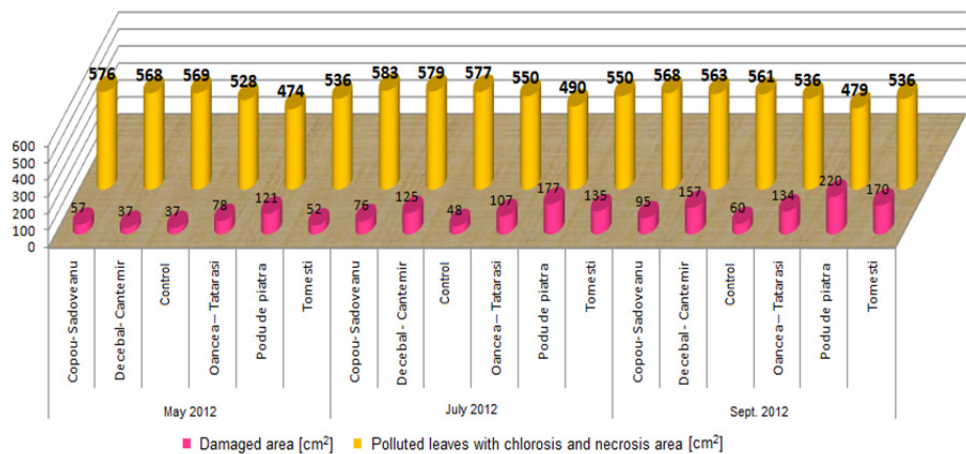


Fig.5 Variation of leaf area at *Aesculus hippocastanum* L. individuals derived from the five areas of investigations (May, July and September 2012)

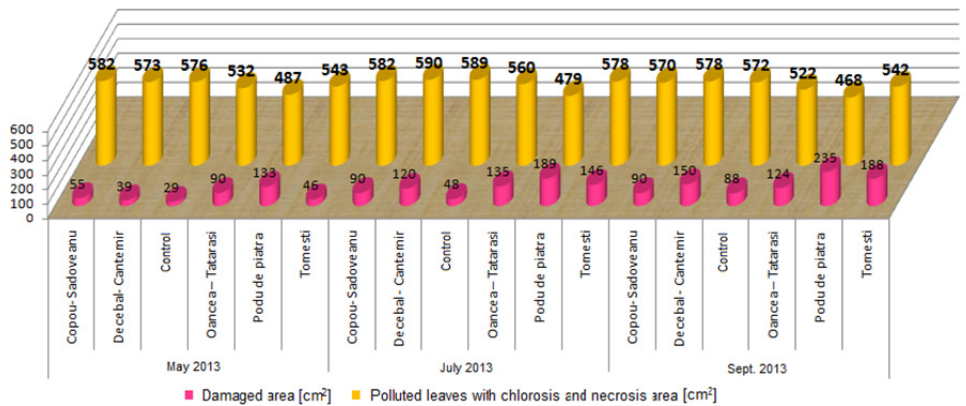


Fig.6 Variation of leaf area at *Aesculus hippocastanum* L. individuals derived from the five areas of investigations (May, July and September 2013)

The dry weight content increased compared to control, in all five areas subject to investigation. Maximum values of dry matter content were recorded in *Aesculus hippocastanum* L. individuals from Tomeşti area (suburban station) (Fig. 7, 8). It should be noted that high dry weight content isn't directly correlated with macroscopically visible necrosis, which means that in some cases disruption of physiological functions is directly followed by defoliation (Ivănescu and Toma, 1999). The decrease of the water amount, combined with the increase of dry substance amount, can be correlated to the close-open stomata mechanism. The solid deposits, the chlorosis and necrosis affect the cuticle perspiration and this affects perspiration, respiration and photosynthesis (Şoltuzu et al., 2012).

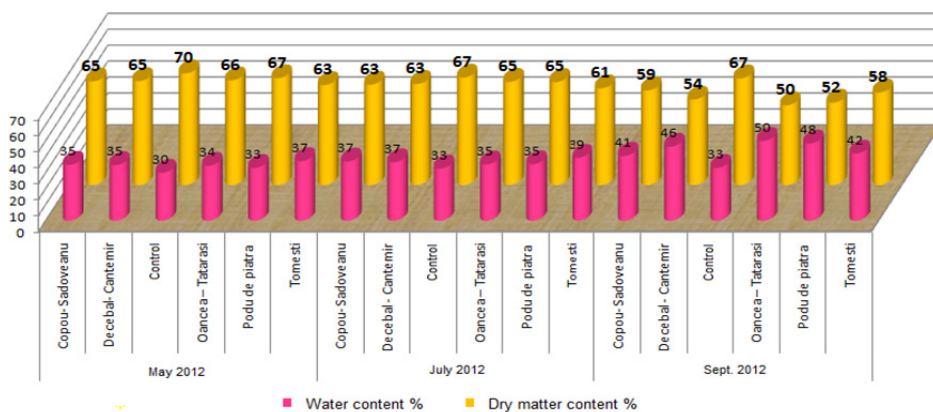


Fig.7 Variation of water content and dry matter at *Aesculus hippocastanum* L. individuals derived from the five areas of investigations (May, July and September 2012)

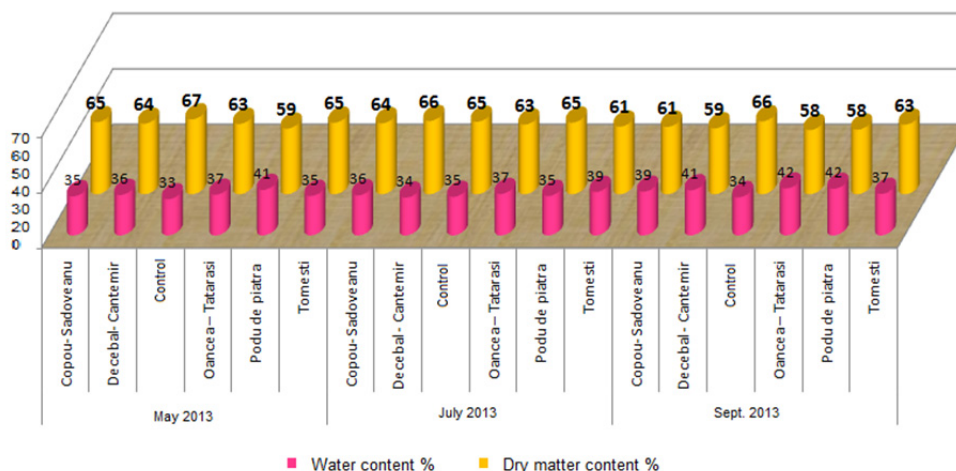


Fig.8 Variation of water content and dry matter at *Aesculus hippocastanum* L. individuals derived from the five areas of investigations (May, July and September 2013)

CONCLUSIONS

On the investigated biological material, due to vegetation condition for the year 2012, following conclusions can be drawn:

At pollution aggression, the responses can be very different even at the individuals from the same species. The foliar chlorosis resulted from deep physiological alterations affect the water and dry substance contents. A large number of chlorosis of the leaf unit accompanied by necrosis of the limb edges were found at the individuals investigated from Podu de Piatră area (traffic station). The lowest values of foliar surface and physiological processes compared to the control, were recorded at the individuals of *Aesculus Hiopocastanum* L. species from Podu de Piatră area (traffic station).

We outline the fact that not always the high dry substance content is related to necrotic leaf surface, which means that in certain cases the disturbance of some physiological functions is straightly followed by defoliation.

Considering all this, we can conclude that there is no uniformity regarding the foliar response to pollutants, not even for individuals from the same species. Because of that, the pollution impact studies on the vegetation should consist of a large range of investigations for each individual in an investigated perimeter.

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ASSESSMENT OF THE ACTION OF DEPOSIT MYCOFLORA ON *TRITICUM AESTIVUM* L. SEEDS FROM SUCEAVA GENE BANK'S COLLECTION

DIANA BATÎR - RUSU

Keywords: micromycetes, CGA medium, blotting paper

Abstract: This study consisted in a phytopathological evaluation of epiphyte and endophyte mycological flora which appeared on *Triticum aestivum* seeds placed on two types of substrates (CGA medium and blotting paper). The 30 populations of wheat resulted from the active collection of Suceava Genebank and conserved for different time intervals (8, 15 and 18 years), in controlled atmosphere storages ($T=+4^{\circ}\text{C}$; relative air humidity = 30 - 40%). Micromycetes were evaluated by counting the infected seeds and the attack frequency was expressed as a percentage, by visual estimation of seeds surface. The target objectives of the study are to establish the influence of the conservation period on the activity of micromycetes placed on stored seeds and to settle the influence of the substrate type - CGA medium (potato - dextrose - agar) and blotting paper - on the development of fungal pathogens. Seeds studied, placed on CGA medium and blotting paper substrate, after incubation, showed a different degree of infection by fungal pathogens, depending on the type of substrate and the age of seeds. The conservation period influenced fungal pathogens longevity, meaning that the more it's higher, the level of infection is reduced. On CGA medium, in comparison with blotting paper substrate, after incubation period, was isolated a greater diversity of fungal pathogens.

INTRODUCTION

Size variation of pathogen colonies kept in constant environmental conditions reflects the differences in quantity, viability and location of inoculums on seed. The development of colony around each seed on growth medium, and the intensity of symptoms on germs in case of blotting paper tests are closely dependent on the amount of inoculum on seed - number of spores or mycelium abundance (Raicu and Baci, 1978).

Generally, the correlation between inoculum (spores load/seed) and colony size (the amount of mycelium) developed on CGA medium (potato - dextrose - agar) is very significant (Hulea et al. 1973).

Micromycetes existing on stored wheat seeds can cause during storage a wide range of changes, with negative consequences from a technological, nutritional, hygienic and commercial point of view (Nagy and Trif, 1998).

Beratlief and collaborators in a study concerning the deposit ecosystem characteristics, revealed the mycological flora evolution and sequence on cereals seeds stored with high moisture content (Beratlief and Oprea, 1994)

The purposes of this study are:

- to establish the influence of the conservation period on the activity of micromycetes placed on stored seeds
- to settle the influence of the substrate type - CGA medium (potato - dextrose - agar) and blotting paper - on the development of fungal pathogens.
- to establish the complementary action of identified micromycetes on *Triticum aestivum* seeds in three storage periods, by determining the correlation coefficients between the action of fungal pathogens identified on the samples taken in study.

MATERIALS AND METHODS

I performed the phytopathological characterization of local germplasm represented by 30 populations of *Triticum aestivum*, conserved for 8, 15 and 18 years at $T = +4^{\circ}\text{C}$, which come from collecting expeditions realized by the collecting department from Suceava Genebank during a term of 18 years (1992-2010).

Lab experiments were carried on Suceava Genebank by using the genetic seminal material from the active collection of the institution, which was placed on the CGA medium and blotting paper.

To make possible the assessment of the micromycetes present on *Triticum aestivum* seeds, I implemented the following research methods:

- macroscopic analyses of the seeds;
- Ulster method (Malone and Muskett, 1941) on CGA medium (potato - dextrose - agar).

Interpretation of results concerning identified micromycetes evolutions on seeds taken in study was achieved by analyzing correlations and regressions accordingly with experimental factors (Ceapoiu, 1968).

RESULTS AND DISCUSSIONS

The seeds of *Triticum aestivum*, placed on CGA medium and blotting paper, presented after the incubation period the following characteristics concerning the presence of fungal microorganisms:

a) CGA medium (potato - dextrose - agar)

On CGA medium, the presence of deposit mycoflora on the 30 samples of *Triticum aestivum* seeds conserved at +4°C temperature, for 8, 15 and 18 years was different, as follows:

On the samples stored for 8 years at +4°C temperature, we identified 9 fungal pathogens (*Penicillium sp.*, *Rhizopus sp.*, *Epicoccum sp.*, *Cladosporium herbarum*, *Alternaria alternata*, *Trichoderma viride*, *Torula herbarum*, *Stemphylium botryosum*, *Chaetomium sp.*) which showed a different attack degree on each sample of the 5 analyzed, registering an infection rate of 97,3%.

On 21 samples stored at +4°C temperature for a period of 15 years we identified 8 fungal pathogens (*Penicillium sp.*, *Rhizopus sp.*, *Epicoccum sp.*, *Cladosporium herbarum*, *Alternaria alternata*, *Trichoderma viride*, *Stemphylium botryosum*, *Chaetomium sp.*). The 630 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 33,3%.

Other 4 seed samples, conserved for a period of 18 years, have been infected by a smaller number of fungal microorganisms (*Penicillium sp.*, *Rhizopus sp.*, *Epicoccum sp.*, *Cladosporium herbarum*, *Alternaria alternata*) and the infection percentage on the 120 seeds analyzed was much lower (35 %).

In table 1 are presented the micromycetes identified on *Triticum aestivum* seeds placed in three experimental conditions (8, 15 and 18 years) at +4°C temperature:

Table 1. Proportion of micromycetes isolated on *Triticum aestivum* seeds placed on CGA medium

Experimental conditions	Seeds stored at T + 4°C, for 8 years	Seeds stored at T+ 4°C, for 15 years	Seeds stored at T+4°C, for 18 years
Isolated micromycets	Attack frequency (%)		
<i>Penicillium sp.</i>	18,6	9,2	3,3
<i>Rhizopus sp.</i>	21,3	8,9	13,3
<i>Epicoccum sp</i>	5,3	2,8	4,2
<i>Cladosporium herbarum</i>	7,3	3,3	4,2
<i>Alternaria alternata</i>	15,3	6,2	10
<i>Trichoderma viride</i>	11,4	0,9	0
<i>Torula herbarum</i>	6,7	0	0
<i>Stemphylium botryosum</i>	12,7	0,6	0
<i>Chaetomium sp.</i>	3,3	1,3	0
TOTAL	97,2	33,2	40,2

Proportion of micromycets isolated on 30 seeds samples of *Triticum aestivum* placed on CGA medium stored at $+4^{\circ}\text{C}$ temperature for 8, 15 and 18 years is represented in figure 1:

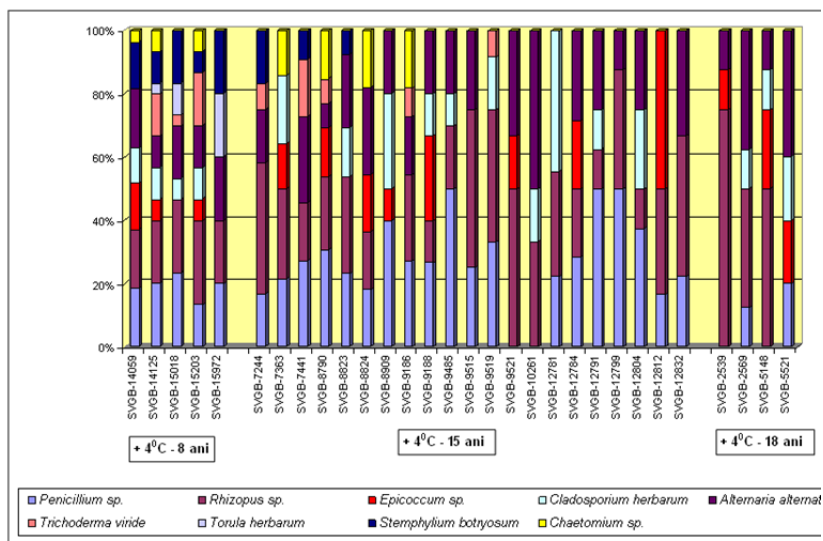


Fig.1. Infection percentages of fungal pathogens isolated on *Triticum aestivum* seeds placed on CGA medium in controlled atmosphere conditions

b) blotting paper

Analyzing the 30 seeds samples of *Triticum aestivum* stored at $+4^{\circ}\text{C}$ temperature for 8, 15 and 18 years, we identified the following infection percentages caused by fungal pathogens:

On the samples stored for 8 years at $+4^{\circ}\text{C}$ temperature we have identified 6 fungal pathogens (*Penicillium sp.*, *Rhizopus sp.*, *Cladosporium herbarum*, *Alternaria alternata*, *Trichoderma viride*, *Torula herbarum*), which had a different attack degree on each sample of the 5 analyzed, registering an infection rate of 41,6% (table 2).

Table 2. Proportion of micromycetes isolated on *Triticum aestivum* seeds placed on blotting paper

Experimental conditions	Seeds stored at T $+4^{\circ}\text{C}$, for 8 years	Seeds stored at T $+4^{\circ}\text{C}$, for 15 years	Seeds stored at T $+4^{\circ}\text{C}$, for 18 years
Isolated micromycets	Attack frequency (%)		
<i>Penicillium sp.</i>	8	3,5	1
<i>Rhizopus sp.</i>	22,4	5,5	4
<i>Cladosporium herbarum</i>	2,8	0,6	0,5

Experimental conditions	Seeds stored at T +4 ⁰ C, for 8 years	Seeds stored at T + 4 ⁰ C, for 15 years	Seeds stored at T+ 4 ⁰ C, for 18 years
<i>Alternaria alternata</i>	3,6	2	1,5
<i>Trichoderma viride</i>	2	0,6	0
<i>Torula herbarum</i>	2,8	0	0
TOTAL	41,6	12,2	7

On 21 samples conserved at +4⁰C temperature for a period of 15 years, we identified 5 fungal pathogens (*Penicillium* sp., *Rhizopus* sp., *Cladosporium herbarum*., *Alternaria alternata*., *Trichoderma viride*). The 1050 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 12,19 %.

The 4 seed samples with a storage period of 18 years have been infected by a smaller number of micromycetes (*Penicillium* sp., *Rhizopus* sp., *Cladosporium herbarum*, *Alternaria alternata*), the infection percentage on the 200 seeds analyzed being more low (7 %).

Analyzing the number of infected seeds, we can observe that all micromycetes genus of wheat seeds were isolated on a smaller number of seeds when samples were incubated on blotting paper, in comparison with the number of seeds placed on CGA medium.

Proportion of micromycetes isolated on 30 seeds samples of *Triticum aestivum* placed on blotting paper stored at + 4⁰C temperature for 8, 15 and 18 years is represented in figure 2.

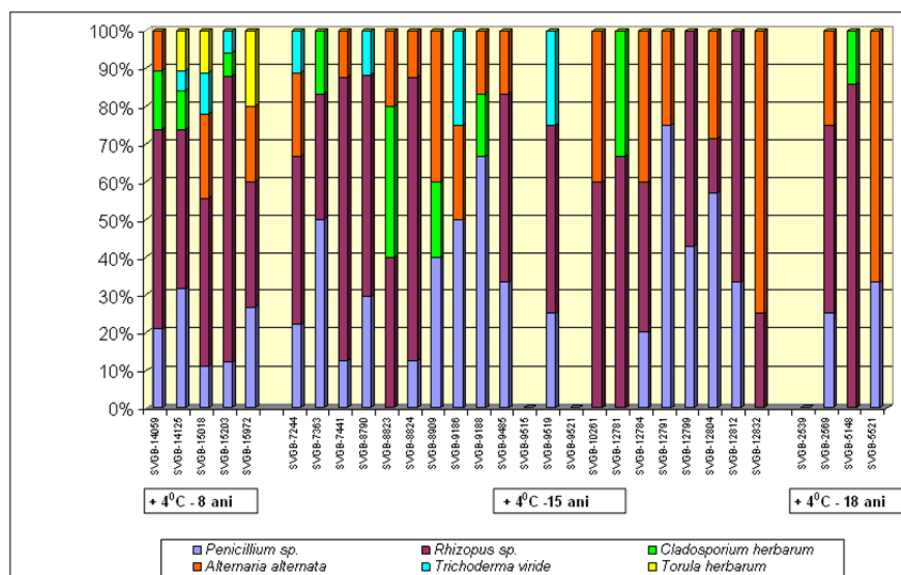


Fig.2. Infection percentages of fungal pathogens isolated on *Triticum aestivum* seeds placed on blotting paper in controlled atmosphere conditions

For establishment complementary action of micromycetes identified on *Triticum aestivum* seeds in three storage periods (8, 15 and 18 years), it was determined correlation coefficients between fungal pathogens action identified on samples taken in study.

In the analyzed samples of *Triticum aestivum*, the results from the table related few statistical correlations in three storage periods.

After 8 years of storage of *Triticum aestivum* seeds at +4°C temperature (table 3), it's noticed that there is few significant positive correlations between micromycetes action: *Stemphylium botryosum* x *Torula herbarum*, *Stemphylium botryosum* x *Alternaria alternata*, *Chaetomium* sp. x *Trichoderma viride*.

Table 3. Correlation coefficients between micromycetes action identified on *Triticum aestivum* samples stored at +4°C, for 8 years

Caracterele corelate	<i>Penicillium</i> sp.	<i>Rhizopus</i> sp.	<i>Epicoccum</i> sp.	<i>Cladosporiu</i> m herbarum	<i>Alternaria alternata</i>	<i>Trichoderma viride</i>	<i>Torula herbarum</i>	<i>Stemphylium botryosum</i> m	<i>Chaetomium</i> m sp.
<i>Penicillium</i> sp.	1								
<i>Rhizopus</i> sp	- 0,230 77	1							
<i>Epicoccum</i> sp.	- 0,628 97	- 0,419 3	1						
<i>Cladosporium herbarum</i>	- 0,437 24	0,100 90	0,733 35	1					
<i>Alternaria alternata</i>	0,230 76	- 0,230 7	- 0,366 9	- 0,7735	1				
<i>Trichoderma viride</i>	- 0,467 47	0,654 46	0,127 41	0,5723 1	- 0,8414 5*	1			
<i>Torula herbarum</i>	0,602 01	- 0,086	- 0,820 4*	- 0,977* **	0,6880 21	-0,543	1		
<i>Stemphylium botryosum</i>	0,693 37	- 0,416 0	- 0,566 9	- 0,8488 *	0,8320 5*	- 0,8760 *	0,868 2*	1	
<i>Chaetomium</i> sp.	- 0,657 79	0,219 26	0,597 61	0,7669 6	- 0,8770 6*	0,8528 0*	- 0,784 4	- 0,9486 **	1

After 15 years of storage of *Triticum aestivum* seeds at +4°C temperature, there is only one very significant positive correlation between fungal pathogens action *Stemphylium botryosum* x *Trichoderma viride* (table 4).

Table 4. Correlation coefficients between micromycetes action identified on *Triticum aestivum* samples stored at +4°C temperature, for 15 years

Caracterele corelate	<i>Penicillium</i> sp.	<i>Rhizopus</i> sp.	<i>Epicoccum</i> sp.	<i>Cladosporiu</i> m herbarum	<i>Alternaria alternata</i>	<i>Trichoderma viride</i>	<i>Stemphylium botryosum</i> m	<i>Chaetomium</i> m sp.
<i>Penicillium</i> sp.	1							
<i>Rhizopus</i> sp.	-0,07395	1						
<i>Epicoccum</i> sp.	0,091247	-0,12263	1					
<i>Cladosporium herbarum</i>	0,224141	-0,09279	-0,03097	1				
<i>Alternaria alternata</i>	0,038123	-0,1964	0,084293	-0,33072	1			
<i>Trichoderma viride</i>	0,153525	0,279145	0,21965	0,28204	0,01066	1		
<i>Stemphylium botryosum</i>	-0,07255	0,407718	-0,26249	-0,15448	0,210195	0,49793*	1	
<i>Chaetomium</i> sp.	0,083883	0,129641	0,250387	-0,09824	-0,14852	0,18999	-0,185	1

Therefore, after 18 years of conservation there is only one very significant positive correlation between fungal pathogens action *Cladosporium herbarum* x *Penicillium* sp. (table 5).

Table 5. Correlation coefficients between micromycetes action identified on *Triticum aestivum* samples stored at +4°C temperature, for 18 years

Caracterele corelate	<i>Penicillium</i> sp.	<i>Rhizopus</i> sp.	<i>Epicoccum</i> sp.	<i>Cladosporiu</i> m herbarum
<i>Penicillium</i>	1			

Caracterele corelate	<i>Penicillium</i> sp.	<i>Rhizopus</i> sp.	<i>Epicoccum</i> sp.	<i>Cladosporiu</i> m herbarum
sp.				
<i>Rhizopus</i> sp.	-0,40825	1		
<i>Epicoccum</i> sp.	-0,30151	-0,73855	1	
<i>Cladosporiu</i> m herbarum	0,904534*	-0,49237	-0,09091	1

Also, it is observed a constant presence of fungal pathogen *Cladosporium herbarum*.

For setting of the two micromycetes action (*Cladosporium herbarum* x *Torula herbarum*) present on seeds after 8 years of storage, it was traced the suitable regression straight (fig. 3).

This regression straight line out negative significant action of the two saprophytic micromycetes, meaning that while maintaining seeds at +4⁰C temperature, after 8 years of storage, *Torula herbarum* inhibit action of fungal pathogen *Cladosporium herbarum*.

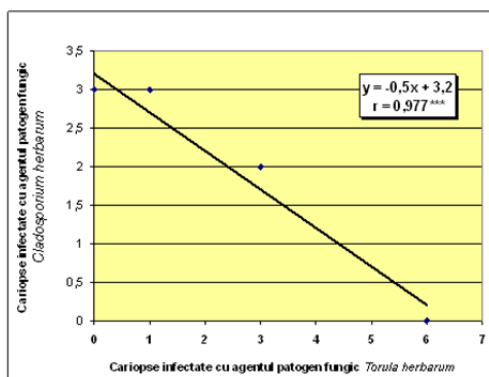


Fig. 3. The regression straight for correlation between number of infected seeds of *Torula herbarum* and number of infected seeds of *Cladosporium herbarum* on *Triticum aestivum* samples stored in controlled environmental conditions (+ 4⁰C) for 8 years

CONCLUSIONS

Deposit mycoflora developed on wheat seeds taken in this study was analyzed according to genotype period of seed conservation and type of substrate used.

The seed samples of *Triticum aestivum* stored in 3 experimental conditions placed on CGA medium were infected in different proportions by fungal pathogens. The species *Torula herbarum* was identified only on the samples conserved for 8 years at +4⁰C temperature and the species *Trichoderma viride*, *Stemphylium botryosum*, *Chaetomium* sp. were identified only on the

seeds with a storage period of 8 and 15 years. Other types of micromycetes were detected in all storage conditions, but on a different number of seeds (*Penicillium sp.*, *Rhizopus sp.*, *Epicoccum sp.*, *Cladosporium herbarum*, *Alternaria alternata*).

By placing the same seed samples of *Triticum aestivum* in 3 experimental conditions on blotting paper, we observed that samples were infected in a smaller proportion compared to CGA medium. The fungal pathogens *Epicoccum sp.*, *Stemphylium botryosum*, *Chaetomium sp.* identified on CGA medium, were not isolated on blotting paper.

After 8 and 18 years of storage of *Triticum aestivum* seeds at +4°C temperature, there is a strong attack of *Rhizopus sp.* and *Alternaria alternata*, being a very significant correlation between the action of these two fungal pathogens. *Epicoccum sp.* was identified on a great number of samples, but the infection degree was observed in a small number of seeds.

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IDENTIFICATION OF SOME VALUABLE GERMPLASM SOURCES IN SILVER FIR (*ABIES ALBA*) ON THE BASIS OF SEED GERMINATION CAPACITY

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Keywords: germplasm sources, technical germination, germination vigour

Abstract: The Silver Fir genetic resources conservation is a very important activity (including in our country) considered through the viewpoint of a drastic diminution of natural arboreta. The elite (*plus*) silver fir trees are the most important purveyors of high quality biological material for the creation of populations (orchards) for seed or cutting production in the process of forest genetic improvement and afforestation too. The experiments were fulfilled in order to establish the biological answer of Silver Fir seeds concerning the germinative potential in a mixture arboretum (Silver Fir and European Beech). Related to the main physiological indicators of Silver Fir seeds, the value of the germinative energy/germinative vigour, respectively 26,75%, is normal in natural conditions. The germinative capacity (technical germination) registered 32%, value corresponding to second category of seed quality.

INTRODUCTION

Silver Fir is one of the most exacting forest species concerning the stational conditions with optimum vegetation status in medium warmther climate and rainfull (4). The Silver Fir genetic resources conservation is a very important activity (including in our country) considered through the viewpoint of a drastic diminution of natural arboreta (from 10-15% in XIX century to 5-6% in present) (1).

On the other hand, the elite (*plus*) silver fir trees are the most important purveyors of high quality biological material (seeds, cuttings and grafts) for the creation of populations (orchards) for seed or cutting production in the process of forest genetic improvement and afforestation too (2,5).

The germinative energy represents a germination vigour indicator in correlation with a rapid germinations of seeds and mass rise in a short time (3).

In this respect, the present paper aims to study the seeds germination to evaluate and identify the most valuable genetic resources in natural Silver Fir arboreta.

MATERIAL AND METHODS

There were used Silver fir seeds (6,04 g/1000 seeds) harvested in 2011 in March, after the snow melting, from more biotypes of the Moldovița forest ward.

After the pre-refrigeration treatment (21 days to 4°C), the seeds were sown, in four replicates of 100 each, in Petri dishes on filter paper moistened periodically with distilled water.

The germination was carried out, according to the valid standards [SR 1634: June 1999], in a CONVIRON 4030 – G30 growth chamber, at 21°C, 95% humidity and photoperiod regime (16 hours day/8 hours night alternation). It was measured the germinative energy at 10 days and germinative capacity (technical/total germination) at 28 days.

RESULTS

The experiments were fulfilled in order to establish the biological answer of Silver Fir seeds concerning the germinative potential in a mixture arboretum, including 9 Silver Fir to 1 European Beech.

Table 1. Analysis of germination capacity

Germination	Repetition				Total	Average
	1	2	3	4		
Total germinate	36	29	33	30	128	32
Abnormal germinated	0	0	0	0	0	0

seeds						
Ungerminated seeds	15	18	20	14	67	16,75
Damaged seeds	6	1	0	3	10	1,5
Empty seeds	37	41	39	43	160	40
Parasited seeds	16	11	8	10	45	9,75
Total	100	100	100	100	400	100

Table 2. Main germinative indicators

	Repetition				Average
	1	2	3	4	
Technical germination %	36	29	33	30	32
Germinative energy (germination vigour) %	30	24	28	25	26,75

Number of germinated seeds

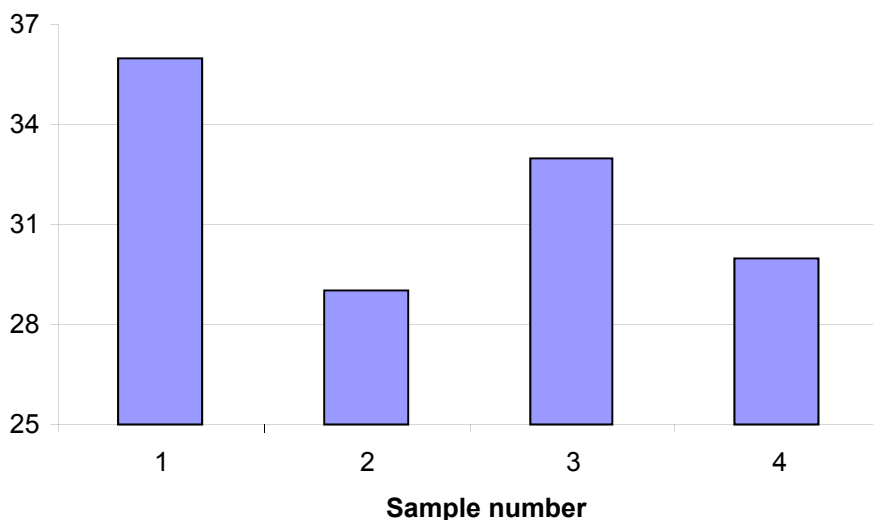


Figure 1. Number and percentage of germinated seeds

DISCUSSIONS AND CONCLUSIONS

Related to the main physiological indicators of Silver Fir seeds, in table 2 are shown values for the germinative energy/germinative vigour, respectively 26,75% which is normal in natural conditions. The geminative energy represents an indicator in correlation with a rapid germination and mass rise of Silver Fir seedlings in short time.

The germinative capacity (technical germination) registered 32%, value corresponding to second category of seed quality (fig.1). The germination capacity of Silver Fir seeds is related to the resin bags which often spoil the biological integrity of seeds and

decrease the final germination percentage. On the other hand, a fruitful year produce many seeds of a high quality.

The Silver Fir quality seed is also influenced by humidity level from previous year. For example, a droughty year increase the empty seeds number. Table 1 contain data related to the proportion between different categories of biological reproductive material after the germination experiment. In this respect we registered a high percentage of empty seeds – 40% and about 10% parasited seeds, other categories of seeds (ungerminated seeds, damaged seeds, abnormal germinated seeds) framing in normal bounds.

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THE ACIDITY INDEX EVOLUTION OF MAIZE AND SUNFLOWER CRUDE OILS UNDER STORAGE CONDITIONS

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Keywords: Acidity index, sample, crude oil, maize, sunflower.

Abstract: In this work it has searched, comparatively, the evolution of the acidity index of some maize and sunflower crude (unrefined) oil samples stored under certain conditions of temperature and light, during 60 days. The material for experiment was represented by crude oil, whose acidity index has been determined at once after obtaining, as well as at 5, 30 and 60 days of keeping at 4°C (in dark and light) and at +20°C (in dark and light). The storage of maize and sunflower crude oils at +4°C and +20°C, in dark and and light has led, comparatively with fresh samples, to increase of the acidity index after 30, and especially after 60 days. The highest values of this index have been registered after 60 days of keeping at +20°C. During storage at the two thermic thresholds, the lighting regime has not influenced the acidity index value in the both analysed oils. Comparing the evolution of the acidity index of the two crude oil types, one can say that after 5 days of storage at +20°C the values of this index have risen more in maize oil, as compared to sunflower one. After 30 days the rises have been very close in the both oil types, but after 60 days the acidity index values have registered rises much bigger in sunflower oil.

INTRODUCTION

Sunflower oils are predominantly composed of triacylglycerols (98–99%) and a small proportion of phospholipids. The so-called unsaponifiable matter contains tocopherols, sterols and waxes, among other substances (Grompone, 2011).

According to Moreau (2011), the major components of crude corn germ oil are triacylglycerols (TAG), but crude corn oil also contains other minor non-polar and polar lipid components: free fatty acids, pigments, volatiles, phospholipids, and waxes - the major undesirable components removed by several refining steps.

The lipolysis, caused by enzymes from tissues and/or produced by microorganisms, *the oxidation*, produced through microorganisms action (β -oxidation) or through oxygen from air (autooxidation), and *the thermal degradation* in the presence of oxygen are modifications which can be suffered by lipids from food raw materials (Banu et al., 2002; Georgescu et al., 2000; Leonte and Florea, 1998; Sevanian et al., 1988; Banu et al., 1997; Neamțu, 1997).

Due to the moisture and lipase enzymes from crude fats, the lipolytic transformations lead to the partial hydrolysis of glycerides up to glycerol and fatty acids (Neamțu, 1997).

In this work it has studied, comparatively, the evolution of the acidity index of some samples from maize and sunflower crude oils, stored under certain temperature and light conditions during 60 days, to see to what extent the thermal and/or lighting regime or storage period can modify the acidity index values of those samples.

MATERIALS AND METHODS

The experimental material was represented by samples of maize and sunflower crude oil, whose acidity index was determined at once after obtaining (table 1), as well as at 5, 30 and 60 days of keeping under certain conditions.

Table 1. Acidity index values of maize and sunflower fresh crude oils

Determination	Acidity index (mg KOH/g oil)	
Produce	Maize oil	Sunflower oil
Values	5,1	4,5

Some oil samples coming from the both seed species have been stored at +4°C (in dark and light) and other ones at +20°C (in dark and light).

The determination of acidity index was made through a titration method, based on measurement of volume of KOH 0,1 N solution, which neutralizes free fatty acids from one gram of oil (Beschea and Toma, 1984; Sahleanu V. and Sahlenu E., 2000).

The data of experiments (consisting in 4 replicates for each determination) were statistically processed. The analysis of variance was used to calculate differences between the results, significant differences being considered those ones at $p < 0.05$.

RESULTS AND DISCUSSIONS

In the Table 2 are reproduced the maize oil acidity index values.

Table 2. Maize oil acidity index values at certain time intervals

Thermic regime	+4°C		+20°C	
Lighting regime	Dark	Light	Dark	Light
Time*	Acidity (mg KOH/g oil)			
5 days	5.4	5,4	12.1	13.2
30 days	10.3	9,9	19.7	20.3
60 days	16.1	16,8	28.2	27.9

*Intervals of determination

As seen in the Table 2, during the storage period, the acidity index, known as lipolitic process indicator, has evidenced increases in all analyzed maize oil samples. Thus, if in crude fresh oil (blank sample) the index value was 5.1 mg KOH/g oil, after 5 days of storage at +4°C this value has become 5.4 mg KOH/g oil (in dark and light too), after 30 days the index has increased to 10.3 (in dark) and to 9.9 mg KOH/g oil (in light), that is almost 2 times compared to the blank, and after 60 days of storage the acidity index has increased to 16.1 (in dark) and to 16.8 mg KOH/g oil (in light), that is 3.1-3.3 times compared to blank sample.

Under storage conditions at temperatures of +20°C, the acidity index of maize crude oil has increased after 5 days to 12.1 mg KOH/g oil (in dark) and to 13.2 mg KOH/g oil (in light), that is 2,4-2,5 times compared to the blank sample, after 30 days to 19.7 mg KOH/g oil (in dark) and to 20.3 mg KOH/g oil (in light), that is 3.9-4 times compared to the blank, and after 60 days to 28.2 mg KOH/g oil (in dark), and to 27.9 mg KOH/g oil (in light), that is 5.5-5.4 times compared to blank sample.

The Table 3 reproduces the acidity index values of sunflower crude oil samples.

Table 3. Sunflower oil acidity index values at certain time intervals

Thermic regime	+4°C		+20°C	
Lighting regime	Dark	Light	Dark	Light
Time*	Acidity (mg KOH/g oil)			
5 days	4.5	4.5	10.5	9.8
30 days	7.5	7.7	17.8	16.6
60 days	13.3	12,8	30.3	31.7

*Intervals of determination

In the Table 3, it can see that the acidity index of sunflower oil stored 5 days at +4°C is not changed (in dark and light too), as compared to the blank (4.5 mg KOH/g oil). After 30 days of storage at +4°C, the acidity index has increased to 7.5 mg KOH/g oil (in dark) and to 7.7 mg KOH/g oil (in light), that is 1,7 times compared to the blank, and after 60 days this index has increased to 13,3 mg KOH/g oil (in dark) and to 12,8 mg KOH/g oil (in light), that is almost 3 times compared to the blank.

At temperatures of +20°C, the acidity index of sunflower crude oil has increased once with keeping period extension as well. Thus, after 5 days of storage the acidity index has become

10,5 mg KOH/g oil (in dark) and 9.8 mg KOH/g oil (in light), that is 2.2-2.3 times compared to the blank. After 30 days the acidity index was 17.8 mg KOH/g oil (in dark) and 16.6 mg KOH/g oil (in light), that is 4-3.7 times compared to the blank, and after 60 days the same index was 30.3 mg KOH/g oil (in dark) and 31.7 mg KOH/g oil (in light), that is 6.7-7 times compared to the blank.

From the two Tables (2 and 3) it can be seen that both in corn oil and sunflower oil the acidity index values have increased, compared to blanks (fresh samples) at about the same proportions, except the interval of 60 days at 20°C, where the sunflower oil acidity index has increased (compared to blank) of about 7 times beside the corn oil, where the same index has increased about 5.5 times. In the both types of analyzed oil one can observe very small differences between values obtained in the light and dark mode, so the light regime has not influenced the process of hydrolysis.

In Fig. 1 and 2 are shown the linear regressions for correlations between acidity index values and thermal regime, i.e. lighting, at the three analyzed periods (5, 30 and 60 days).

As seen from the two graphs, the positive correlations established for the samples of the both types of oil (at all three time intervals analyzed), have shown higher values of r^2 at 5 and 30 days of storage, in the case of corn oil, and at 60 days in the case of sunflower oil.

CONCLUSIONS

The study of some samples of maize and sunflower crude oils stored 60 days under various conditions (at +4°C and +20°C, in light and dark) has shown modifications of the acidity index, determined at certain time intervals, depending on temperature and storage period as well

The storage of maize and sunflower crude oils at +4°C and +20°C, in light and dark has led, comparatively with fresh samples, to increase of the acidity index after 30, and especially after 60 days. The highest values of this index have been registered after 60 days of keeping at +20°C.

During storage at the two thermic thresholds, the lighting regime has not influenced the acidity index value in the both analysed oils.

Comparing the evolution of the acidity index of the two oil types, one can say that after 5 days of storage at +20°C the values of this index have risen more in maize oil, as compared to sunflower one. After 30 days, the rises have been very close in the both oil types, but after 60 days the acidity index values have registered rises much bigger in sunflower oil.

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DETERMINANT VALUE OF THE CYTOGENETIC AND MOLECULAR IMATINIB THERAPEUTIC RESPONSE IN CHRONIC MYELOID LEUKEMIA

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Keywords: chronic myeloid leukemia, Philadelphia chromosome, BCR-ABL, Tyrosine Kinase Inhibitors, Imatinib

Abstract. The hallmark of chronic myeloid leukemia is the existence of the cytogenetic evidence of the Philadelphia chromosome (reciprocal translocation between chromosome 9 and 22, and is specifically designated t(9;22)(q34;q11). The result of the translocation is the oncogenic BCR-ABL gene fusion, located on the shorter derivative 22 chromosome. This gene encodes the Bcr-abl fusion protein the BCR-ABL tyrosine kinase - a protein that is continuously activated. The result of this unregulated activation is an unregulated neoplastic type cell division - chronic myeloid leukemia. The first targeted molecular treatment operating in cancer- inhibitor of the BCR-ABL tyrosine kinase, Imatinib mesylate, is the standard of care for chronic myeloid leukemia (CML) *Methods.* A retrospective review of patients in one department of hematology with a diagnosis of Ph/BCR-ABL positive CML and received imatinib. *Results.* From 2002 to 2012, 66 patients in CML-CP received imatinib were introduced in the study. 22 (33%) patients received imatinib as upfront therapy, the others as second or third line treatment. The cytogenetic response (CyR) achieved was major in 62% with 56% complete cytogenetic response (CCR), no CyR in 17 patients (25%). The molecular response was complete in 13 (20%) and major in 16 (24%) patients. Better cytogenetic and molecular responses were achieved by those with low and intermediary risk (Sokal) Seven patients developed under imatinib additional cytogenetic anomalies: supplemental chromosome 8 (6), duplication of Ph1 (2), trisomy 17 and 19 (1). The median of follow-up was 69 months (range 18-180) and under imatinib was 52 months (range 3-126). The Sokal score was a better predictor of survival than Hasford's. *Conclusions.* Imatinib remains the best first line treatment, but there are still a significant number of patients who did not achieve a CyR. The responses and survival were not influenced by the previous treatments but the earlier introduction of imatinib is better. The Sokal score seems to have a better prognostic role. The survival of patients with CML is evidently improved by tyrosin kinase inhibitors.

INTRODUCTION

Chronic myeloid leukemia (CML) is the first human neoplastic pathology associated with a characteristic cytogenetic abnormality - Philadelphia chromosome and fusion gene bcr-abl that could be correlated with leukemogenesis pathogenic events. BCR-ABL protein tyrosine kinase is an aberrant fusion gene transcription resulting from the Philadelphia chromosome. Appearance of tyrosine kinase inhibitor therapy - Imatinib has transformed this leukemia with a life expectancy of about 4-6 years under previous therapies (interferon) into a chronic disease with a true overall survival increasing from year to year. At present, the success of imatinib mesylate therapy in chronic phase chronic myeloid leukemia remains the best example of successful targeted therapy. This statement remains valid despite the associated toxicity and the primary and secondary resistances occurring. However, there are still worries about the long-term tolerability and efficacy of imatinib.

In our study we followed the evolution of patients treated with imatinib mesylate and second generation tyrosine kinase inhibitors for over 10 years and we have identified particularities in terms of therapeutic response, tolerance and survival. In diagnosing and monitoring patients a significant role was played by cytogenetic and molecular exam in accordance with the recommendations of European Leukemia Net.

MATERIALS AND METHODS

We retrospectively analyzed a study group consisting of 66 patients diagnosed with Ph1 positive chronic granulocytic leukemia from February 2002 to August 2012 in Sf. Spiridon Hospital Hematology Department Iasi. All patients were treated with tyrosin kinase inhibitors. Diagnosis was based on morphological and cytogenetic data. The main characteristics of our patients are presented in Table 1.

The hematologic, cytogenetic were evaluated. The molecular response was investigated in bone marrow and/or peripheral blood samples by quantitative and qualitative PCR [Mihășan et al., 2012]. Cytogenetic exam was performed in Molecular Biology Laboratory of Hospital "Sf. Spiridon" Iasi. Cytogenetic response was defined: complete cytogenetic response

(CCyR) metaphases Ph1 positive 0%; partial cytogenetic response (PCyR) metaphases Ph1 positive 1-35%; minor cytogenetic response (mCyR) metaphases Ph1 positive > 35%; no cytogenetic response metaphases Ph1 positive >95%; major cytogenetic response: response partial and complete .

Molecular response performed in the same laboratory by real time -PCR was defined: major molecular response reduction > 3 log BCR-ABL <0,1%; complete molecular response BCR-ABL transcript undetectable.

The statistic significance of variate parameters was evaluated by SPSS 14 software

Table 1. Carac theristics of study group

Parameter	Frequencies
Sex (Men/Women)	34/32
Medium age at diagnosis	41 (13-79 years)
White Blood Cells (mediane $\times 10^9/L$)	145 (10-465)
Hemoglobin value at diagnosis (mediana g/L)	9,8 (5,4-15,5)
Basophils (%)	2 (0-14)
Bone marrow blasts cells(%)	5 (1-30)
Peripheral blood blasts cells (%)	2 (1-19)
Sokal prognostic score (high/intermediate/low)	30/25/11
Hasford ^b prognostic score (high/intermediate/low)	14/30/22

RESULTS AND DISCUSSION

In our group of patients Imatinib was introduced at variable intervals in relation to diagnosis and previous treatment lines. In 22 patients (33%) Imatinib was the first line treatment, in 24 patients was second line therapy and 20 patients received imatinib as third line therapy. Time from diagnosis to initiation of Imatinib therapy ranged 0-104 months with a median of 6.5 months. Therapeutic response to Imatinib varied. Complete hematologic response was achieved in 91% of patients, complete cytogenetic response was obtained in 56% of patients (65 % of patients achieved a major cytogenetic response - partial and complete). Complete molecular response was present in 20% of patients and major molecular response in 24% of patients. Maximum cytogenetic response was achieved on average of 20 months (median 12 months) and the maximum molecular response after 19 months (median 18 months).

It was assessed the impact of different clinical and biological parameters on therapeutic response

Statistically significant impact of Sokal score was observed ($p = 0.001$) both on cytogenetic response (fig.1) to Imatinib, and on the molecular response ($p = 0.025$). Patient with low and intermediate risk calculated by Sokal score respond better at Imatinib therapy. Hasford prognostic score influenced less cytogenetic response ($p = 0.358$), but instead had a statistically significant influence on molecular response.

The line of therapy when administered Imatinib significantly influenced both cytogenetic response ($p = 0.005$) and the molecular response ($p = 0.015$) - those who received therapy in line I or II responded better than those who received treatment in the third line. Time until initiation of treatment - those receiving imatinib in less then 24 months after diagnosis responded significantly better than those who received therapy later ($p = 0.026$). Interestingly, no significant difference for those receiving Imatinib in the first 12 months from diagnosis.

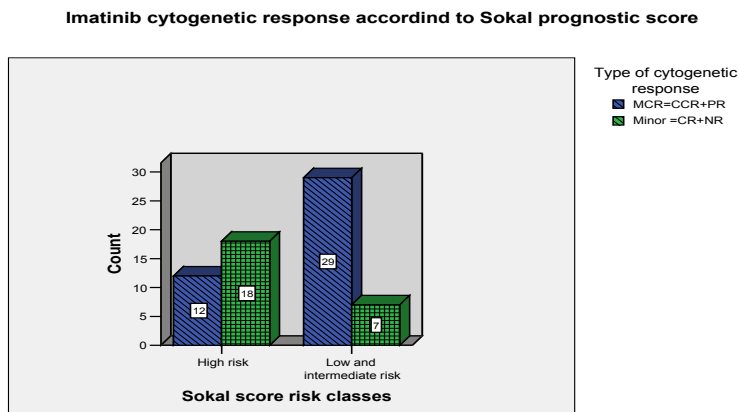


Fig.1 Imatinib cytogenetic response according to Sokal prognostic score.

Imatinib dose was increased in 26 patients, 17 patients achieved an improvement in therapeutic response. In 7 patients treatment with Imatinib was changed with second generation tyrosine kinase inhibitor: in 6 patients with Dasatinib and with Nilotinib in 1 patient. Treatment was discontinued in 17 patients due to severe cytopenias, evolution of the disease to acceleration and acute phase, lack of response or loss of response. Seven patients developed under imatinib additional chromosomal abnormalities : extra chromosome 8 (6), Ph1 duplication (2), trisomy 17 and 19 (1). During our study 13 patients progressed to accelerated phase of the disease, 6 patients to blast phase, 3 patients had second neoplasia. 10 patients died in our study, the most important cause of death was acute phase of the disease. Survival under Imatinib therapy was on average 56 months (3-126 months). The duration of overall survival was on average 76 months (18-180 months). It was evaluated the statistic influence, by Kaplan Meyer survival curves, of different parameters at diagnosis.

The only parameters that influenced survival of patients under Imatinib therapy were Sokal score (fig.2) at diagnosis ($p = 0.043$), therapeutic line receiving imatinib . Imatinib therapy in line I + II – has a positive impact on survival ($p = 0.004$), time to initiation of treatment with Imatinib less than 12 months (fig.3) or less than 24 months after diagnosis had a positive prognostic impact on survival ($p = 0.000$; $p=0.005$) . As expected cytogenetic and molecular response had a positive impact on survival under treatment in our study. .

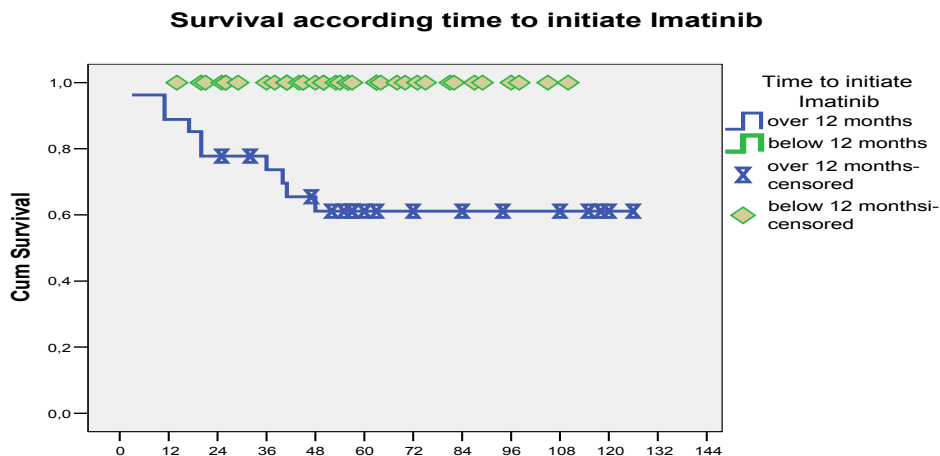


Fig. 2. Influence on survival of Sokal score.

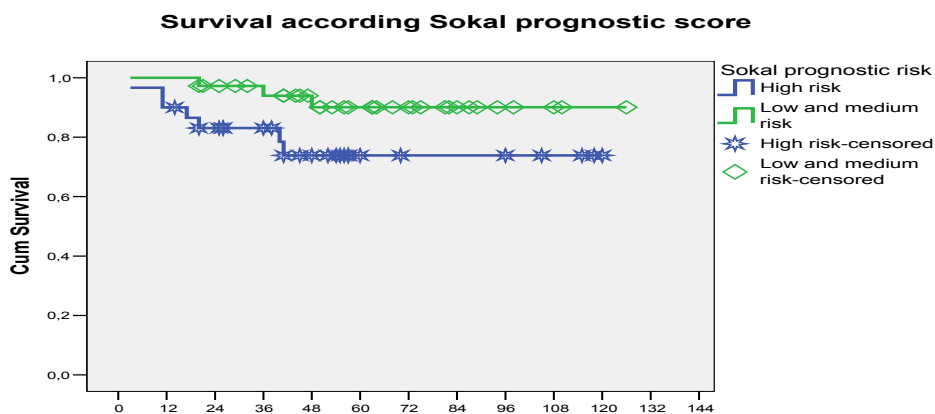


Fig. 3. Influence on survival of time to initiation of Imatinib therapy less than 12 months

We conducted a retrospective study and this type of study shows some disadvantages in data analysis. However we consider it important experience of a single center in our country in evaluation of prognostic impact of Imatinib therapy on the evolution of patients with chronic granulocytic leukemia.

Technique for diagnosis and follow-up are modern equipment required in current specialty literature - cytogenetic and molecular examinations.

The therapeutic response rate both cytogenetic and molecular in patients of our study is lower than reported in the Phase III IRIS in newly diagnosed chronic myeloid leukemia -chronic phase patients receiving Imatinib compared with interferon alfa. Cytogenetic response rate comparative with rate described in this landmark study that included 1106 patients (Deininger M et al, 2009) and 8 years of follow-up was 83 %, compared to only 56 % in our study patients.

This finding can be explained by the fact that our group of patients was not homogeneous, only 22 patients (33 %) received first-line therapy with imatinib and only 38 patients started treatment within 12 months of diagnosis. Approximately 30% of patients in our study treatment was treated with Imatinib in third line.

If compare our results with those of the phase I trial (Druker BJ et al, 2001) study with patients recruited after resistance of alfa Interferon therapy we notice that our response rate were superior - complete cytogenetic response rate was 13% in this study compared to 56 % in the our study. Our results are comparable to those of the phase II trial (Kantarjian H et al, 2002) who were recruited 454 patients in chronic phase and complete cytogenetic response rate was 41 %.

Complete hematologic response rate of 91 % obtained in our study afost comparable with both hematologic response rate obtained in the IRIS 98 % (Deininger M et al, 2009) and with the 95 % response rate obtained in Phase II trial (Kantarjian H et al, 2002).

Persistence of influence of prognostic Sokal score on therapeutic response and survival of patients with chronic myeloid leukemia after appearance of Imatinib has been discussed in several studies. (Uz B et al, 2013; Trask PC et al, 2012). Sokal score maintained impact and prognostic value on therapeutic response and on survival even Imatinib era. In our study between all analyzed parameters Sokal score at diagnosis was the one with the greatest statistics impact on the cytogenetic response, molecular response and on survival.

Time to initiation of imatinib therapy less than 12 months from diagnosis or between 12-36 months was considered with no prognostic significance in a study published by Kantarjian H et al in 2012, 368 patients with CML treated with Imatinib after failure of alfa Interferon therapy . However our study showed a statistically significant impact of these parameters on survival and on therapeutic response, possibly becuase in our study was included younger patients and 33% of patient was treated with Imatinib in first line.

Obtaining the cytogenetic and molecular response was prognostic factors with evident impact on survival in our study and in study described previously (Kantarjian H et al, 2012).

CONCLUSIONS

This study retrospectively analyzed the evolution under treatment with Imatinib of a relatively homogeneous group of patients followed in a single center. Chronic myeloid leukemia is a model of proliferation expressing a unique cytogenetic and molecular hallmark.

Given the great therapeutic impact of tyrosine kinase inhibitors in this form of cancer and because chronic myelogenous leukemia is a disease diagnosed at younger ages we need to evaluate prognostic at diagnosis and rigorously monitoring cytogenetic and molecular response to imatinib, in the light of their impact on survival. Analysis of the diagnostic parameters showed that , in our study, the response to Imatinib therapy seems to be influenced by Sokal score and the precocity of initiating therapy.

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CLINICAL PARTICULARITIES AND DIAGNOSTIC METHODS IN THE HYDATIDIFORM MOLE

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Key words: hydatidiform mole, pregnancy, hyperthyroidism

Abstract. **Aim** of this study was to establish a significant way to the prognosis improvement in trophoblastic pathology in patients with hydatidiform mole. **Objective.** A mole is a particularity of embryonic development where a pregnancy occurs paradoxically without an embryo (the mole pregnancy) and develops only its placental tissue. It is important to demonstrate that the clinical diagnostic should always be completed by a histo-pathological diagnostic and also by the description of the natural history of the disease until the time of diagnostic. **Material and method.** The cases included in the study (n=45) were hospitalized in "Elena Doamna" Clinical Hospital Iași, in the period of time between 2008 and 2013, and diagnosed with hydatidiform mole. The study was a retrospective one, case-control type. Starting from the already diagnosed illness and following the clinical and paraclinical parameters outlined in literature, the objective of the study was to establish the main risk factors that trigger the molar pregnancy. **Results.** The epidemiological characteristics show the following main risk factors for a molar pregnancy: age over 30, urban area, tobacco and alcohol consumption. The mean values of β HCG decreased significantly after the hydatidiform mole was removed, from 26,624 to 9,859 mUI/ml ($p < 0.05$). **Conclusions.** Every woman with a history of hydatidiform mole has an increased risk of developing carcinoma. After the complete or partial removal of the hydatidiform mole, it is necessary to monitor the values of β HCG twice a month until they stabilize.

INTRODUCTION

The benign and malignant proliferative lesions appeared during pregnancy in the trophoblastic layer of the veloz villi were generically grouped under the name of "gestational trophoblastic disease (GTD)", based on the concept that claims that the benign hydatidiform mole, the invasive mole and the chorio-carcinoma are successive phases of a dynamic and continuous proliferative process that interests the fetal chorion (3, 5, 9, 10, 12).

According to this concept, which was taken over by the entire modern literature regarding chorio-carcinoma, a histologically benign hydatidiform mole can be just a phase in the evolution of the disease, having the potential of persisting or relapsing as a malignant tumour (5, 8, 9).

The reality of this lineage is imposed by the fact that the hydatidiform mole invariably precedes the invasive mole and 50% of the chorio-carcinomas have a gestational origin (2, 4, 7, 8).

The fact that the concept of GTD was assimilated, contributed in a significant way to the prognosis improvement in trophoblastic pathology through a careful monitoring of the patients with "benign moles", following the same criteria as in the cases with a more aggressive histopathology in order to get an early detection of the proliferative trophoblastic sequelae (1, 13, 14).

A scientific group belonging to the World Health Organization (WHO) analysed the terminology that is sometimes confusing when referring to gestational trophoblastic diseases on the one hand, and the clinical terms, on the other hand, fighting the tendency of equating a histo-pathological term with its clinical name. WHO considers that it is useful for the invasive mole and the chorio-carcinoma to be grouped under a common clinical term, because they require a similar therapeutic conduct, even though there are important biological and prognostic differences between them (7).

AIM

The placenta is a transitory organ for gestation, and it also has an endocrine function, first of all because it produces human chorionic gonadotropin (HCG), which is a proteolipid hormone, secreted by the trophoblast. The trophoblast is the first embryo tissue which differentiates, becoming extra-embryo and taking an essential part in placenta formation.

The attempts to correlate the histologic grade of the mole with its malignant potential and thus identifying the patients who need cytostatic treatment were not satisfactory

The aim of this study was to establish a significant way to the prognosis improvement in trophoblastic pathology in patients with hydatidiform mole.

OBJECTIVE

A mole is a particularity of embryonic development where a pregnancy occurs paradoxically without an embryo (the mole pregnancy) and develops only its placental tissue. It is important to demonstrate that the clinical diagnostic should always be completed by a histo-pathological diagnostic and also by the description of the natural history of the disease until the time of diagnostic.

MATERIAL AND METHODS

The cases selected and included in the personal study (n=45) were hospitalized in "Elena Doamna" Clinical Hospital Iași, in the period of time between 2008 and 2013, and were diagnosed with hydatidiform mole. The average for the period was about 8 cases a year, with a decreasing tendency for the following period ($y = 15,8 - 2,37x$).

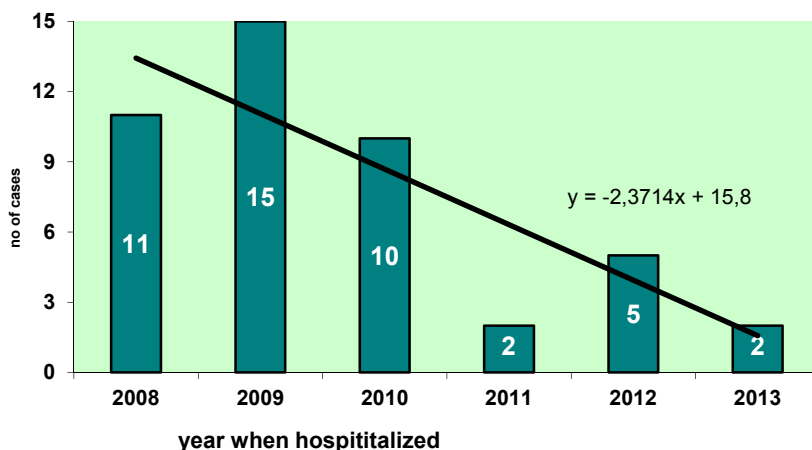


Fig. 1. The distribution of the cases with hydatidiform mole between 2008 and 2013 period

The determination of tumor markers and serum enzymes is relevant for diagnosing and monitoring the evolution of metastatic tumours.

The concentration and/or changes of the tumour markers in blood and in other fluids in the body is influenced by multiple factors: the total number of cells that produce markers, the synthesis rate and the rate of release for tumour markers, the expression of the tumour markers, the type of tumour "non-secretor", tumour blood perfusion rate, the extension of the necrosis for the tumour tissue, the presence of antibodies.

Malignancy of a hydatidiform mole is expressed by persistently increased values (in plateau) or by an increase in the titer values of β HCG during the monitoring period. In these circumstances, the best attitude is to send the patient to an oncologist immediately for a proper evaluation and treatment.

The factors associated with an increased risk of developing post-molar GTD are: age > 40 years old, uterine dimensions that are bigger than the normal pregnancy age, lutein ovarian cysts > 6 cm in diameter, values of β HCG > 100,000 mIU/ml, medical complications during pregnancy (2, 4, 6).

RESULTS AND DISCUSSIONS

The evaluation of the risk factors, through the frequency distribution and confirmatory tests of significance, shows the following profile of the patient with a molar pregnancy:

- average age of about 30 years old;
- living in the urban area is a slightly higher relative risk for the elderly patients (RR=1.43);

- the socio-occupational status did not show significant differences between the age groups, even if the frequency of unemployed people below 30 years old was about 50% ($p=0.301$);
- high educational level, high school studies, post high school and college was noticed mainly for the age group over 30 years old (65.2%), but statistically speaking the frequency distribution was not significantly higher when compared with the same level of instruction for the age group under 30 years old, where 54.5% of the patients had a high educational level ($p=0.670$);
- for older age 56.5% of the patients smoke, so the relative risk is over 4 times higher, which favours the appearing of the hydatidiform mole ($RR=4.14$);
- for the patients over 30 years old, the relative risk of getting a molar pregnancy while regularly consuming alcohol is about 3 times higher ($RR=2.87$).

Table 1. Epidemiological characteristics on age groups for the patients with hydatidiform mole

Profile	Age group ≥30 years old (n=23)		Age group <30 years old (n=22)		Significance		RR	IC95%
	n	%	n	%	X ²	p		
Urban area	15	65.2	10	45.5	1.07	0.301	1.43	0.83-2.48
Unemployed	7	30.4	11	50.0	1.07	0.301	0.61	0.29-1.28
High educational level	15	65.2	12	54.5	0.18	0.670	1.20	0.74-1.94
Smoker	13	56.5	3	13.6	7.25	0.007	4.14	1.36-9.59
Alcohol consumption	3	13.0	1	4.5	0.23	0.633	2.87	0.32-5.55

Diagnostic methods

The diagnostic of molar pregnancy suspicion before removing chorionic villi is given when there is a form of eclampsia manifested by nausea and excessive vomiting, a bigger uterine volume than expected for the pregnancy age, blood loss. The absence of the fetal heart beats and the impossibility to identify the fetal body parts through trans-abdominal palpation, ultrasound scan, amniography and, even pelvic angiography, offers presumptive elements of diagnostic (11).

The volume of the uterus can be variable, depending on blood accumulation and evacuation, its soft consistency, bundling is not perceptible (the fetus absence). Abdominal and pelvic pain is variable in intensity and also intermittent.

Generally, the symptoms are more obvious in the case of a complete mole and they are: amenorrhea, nausea, vomiting, bleeding, anemia, disproportional size of the uterus, early preeclampsia (normally, pregnancy induced hypertension occurs over 24 weeks), hyperthyroidism, luteal ovarian cysts.

Hyperthyroidism can be diagnosed in 25% of the moles, but it only manifests in 2-7% of the cases. Here are the signs of hyperthyroidism: weight body loss, tiredness, tachycardia, arrhythmia, heat intolerance, excessive sweating, and irritability. Hyperthyroidism appears because of the excessive stimulation of TSH secretion.

Signs of lung impairment are rare, only when the disease extends to the lungs, producing an acute lung failure. Sometimes, after the molar pregnancy has been removed, there can be a migration of mole fragments in the lungs, causing dyspnea, tachycardia, hypotension.

Together with hyperthyroidism and acute pulmonary failure, there have also been some other complications like cardiomyopathy and nephropathy.

Etiologic diagnosis

The first period: appeared most frequently at the age of 14 in 51.1% of the patients, followed by the age of 12 (22.2%) and 13 (17.8%). There are very few patients who had their first period at the age of 15 (2.2%) and 16 years (2.2%) and a percentage of 4.4% of the patients claim that the age of their first period was 11 years old.

46.7% of the patients had abortions, one abortion being the most frequent number (20%), but we have to mention the fact that 8.9% of the patients have more than 5 abortions in their obstetrical history, one patient declaring 14 requested abortions.

The obstetrical history of the patients showed deliveries for 31.1% of the women, 15.6% of the patients in the investigated lot having only one delivery.

17% of the patients were recorded with irregular menses, with a moderate flow in 33% of the cases.

The main *signs and symptoms* that were identified at admission were (based on the cases studied): moderate hemorrhage (51.1%); reduced hemorrhage (28.9%); hypogastric pain (42.2%) and abdominal-pelvic pain (15.6%); leucorrhea (11.1%); nausea and vomiting (11.1%).

The paraclinical diagnostic

The haemoglobin (Hb) varied from 9 to 12.5 mg/dl, the average of the lot being 11.57 ± 1.03 mg/dl.

The haematocrit (Ht) varied in the interval 21-47%, recording an average value of $39.61\% \pm 5.74$.

The individual values of leukocytes varied from 3,800 to 16,200 / mm³.

Thrombocytes varied in the interval 135,000-314,000 /mm³, with a recorded mean value of 230,364/mm³ on the cases studied, being within normal limits.

The first evaluation found the mean values of β HCG to vary from 1.5 mUI/ml to 91,300 mUI/ml, having an average value of 26,624 mUI/ml for the lot. After the removal of the hydatidiform mole, the individual values of β HCG decreased significantly, varying in the interval (142.4 - 59,100 mUI/ml), and the lot average being 9,859 mUI/ml ($p < 0.05$).

Histological examination

The hydatidiform mole (molar pregnancy) is a disease of the trophoblast that is characterized by the hydropic thickening of the chorionic villi (accumulation of liquid) and trophoblast proliferation, without myometrial and vascular invasion; chorionic villi are disintegrated and have lost blood vessels (3).

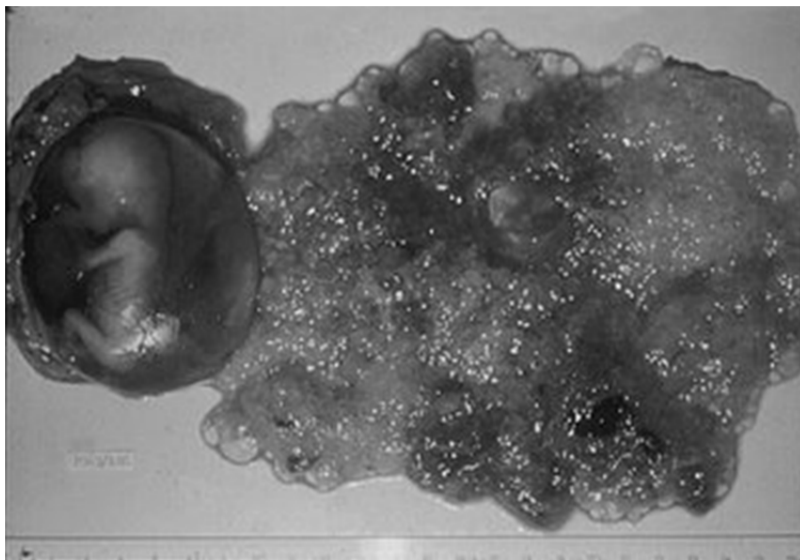


Fig. 2. The hydatidiform mole

(<http://enfermagemnossavida.blogspot.ro/2010/04/mola-hidatiforme.html>)

The invasive mole (chorioadenoma destruens) is a disease that has a local evolution, is rarely metastatic, and is characterized at microscopic level by the trophoblastic invasion of the myometrium, with identifiable villous structures. Microscopically it is characterized by cytotrophoblast hyperplasia, syncytial elements and the persistence of the villo structures (2, 8).

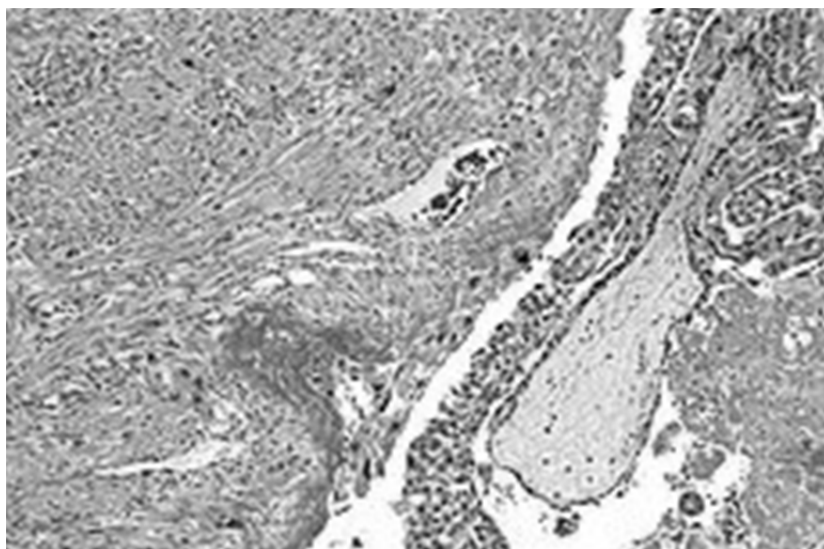
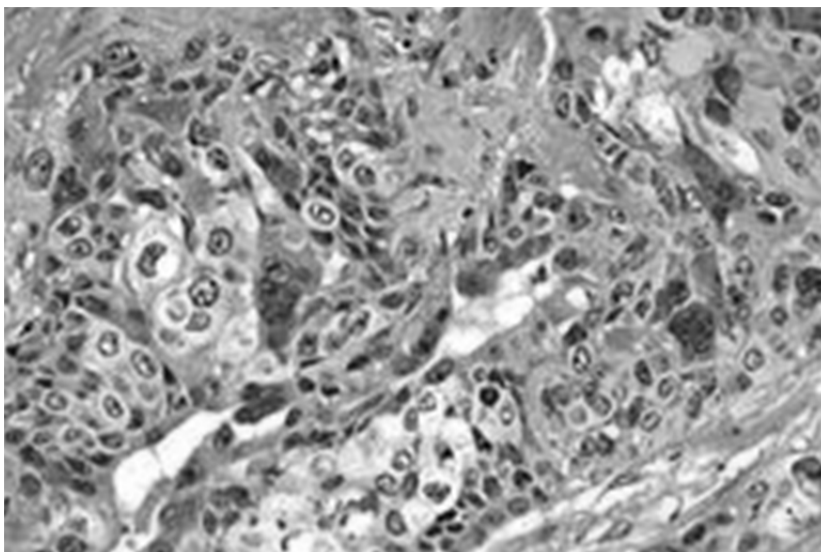


Fig. 3. The invasive hydatidiform mole

(http://en.wikipedia.org/wiki/Invasive_hydatidiform_mole)

The chorio-carcinoma is the malignant tumour of the trophoblastic epithelium. The uterine muscle and the blood vessels are invaded by areas of hemorrhage and necrosis. The groups of trophoblastic cells invade the normal tissue and disseminate in the distance in lungs, brain, liver, pelvis, vagina, spleen, intestines and kidneys (3).



Picture 4. Choriocarcinoma

(<http://en.wikipedia.org/wiki/Choriocarcinoma>)

Tumours located in the placenta are extremely rare and they come from the placental implantation premises, being derived from the cells of the intermediary trophoblast (that secretes larger quantities of human placental lactogen (HPL) than β HCG); clinically they appear as nodules in myometrium and endometrium after the removal of the mole. HPL is present in all the tumour cells, while its immuno-peroxidase is positive for β HCG only in certain cells, and the seric levels of β HCG are low. Usually these tumours appear after a non-molar abortion or a term pregnancy and only occasionally after a hydatidiform mole (8).

After the complete or partial removal of the hydatidiform mole it is necessary to monitor the values of β HCG every two weeks until they normalize (values below 5 mUI/ml), and this is proven by two consecutive measurements. After that it is recommended to have a monthly evaluation for six months, and then every 3 months up to a year. The patients will be encouraged to use contraception during this period of time (12, 15).

Every woman with a medical history of hydatidiform mole has an increased risk of developing chorio-carcinoma. That is why every subsequent pregnancy requires a histological examination of the maternal face of the placenta and the monitoring of the β HCG values for 6-8 weeks postpartum.

CONCLUSIONS

Being over 30 years old represents, through the frequency of the indicators with which this age is associated, an increased risk of having a molar pregnancy. For the older patients coming from the urban area the relative risk of having a molar pregnancy was 1.43 times higher.

Smoking, associated with age, induces a relative risk of getting a hydatidiform mole 4 times bigger. Alcohol consumption represents a risk factor about 3 times higher for the patients over 30 years old.

The obstetrical history shows mainly that: first menses appeared at the age of 14 (51.1%), 46.7% of the patients experienced abortion and only 31.1% of the women gave birth, generally to one child (15.6%). Irregular menses appear in 17% of the patients and 33% of them have a moderate flow.

The main symptoms found on admission were hemorrhage (51.1%) and hypogastric pain (42.2%).

Hematological parameters showed a slight anemia in patients with hydatidiform mole, the mean value of hemoglobin being 11.57 mg/dl, and the mean value of hematocrit being 39,61%. With few exceptions, the mean values of thrombocytes and leucocytes are kept within normal limits.

The mean values of β HCG decreased significantly after hydatidiform mole removal, from 26,624 to 9,859 mUI/ml ($p < 0.05$).

After the complete or partial removal of the hydatidiform mole, it is necessary to monitor the values of β HCG twice a month until they stabilize. The patients will be encouraged to use contraceptive methods.

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THE PROFILE OF THE PATIENT WITH PREECLAMPSIA DEPENDING ON CARDIOVASCULAR RISK

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Key words: preeclampsia, cardiovascular risk, complications

Abstract. Preeclampsia is an important problem of public health, leads among the main causes of mortality and morbidity worldwide. There are many patho-psychological abnormalities of preeclampsia, but its etiology is poorly known. **Aim.** The study intends to find out the possibilities and limitations in diagnosing and treating this disease. The **objective** of this study was to establish that the C reactive protein is an efficient marker of preeclampsia and it correlates significantly with the severity of this disease. **Material and method.** The patients were selected from the pregnant women who were hospitalized in "Cuza Vodă" Iași Clinical Hospital of Obstetrics and Gynecology (n=82), in the period of time between 2003 and 2012, with a gestational age of over 20 weeks, and who came here for a speciality consult. The study was retrospective and case-control type; it was focused on the clinical-progressive aspects of preeclampsia, by checking the correlation of inflammatory parameters with blood pressure for a lot of pregnant women with preeclampsia, in comparison with a lot of normal pregnant women. **Results.** The severe forms of preeclampsia can be found in older age (25-43 years old), with an average around 37 years old ($p=0.02$). According to the cases studied, the severity of preeclampsia was noticed for average values of blood pressure of about 155 mmHg ($p<0.001$). The average values of C reactive protein were significantly higher for women with severe preeclampsia (89.14 ± 51.31 mg/l) ($p<0.001$). **Conclusions.** The severe forms of preeclampsia represent a major cause for maternal morbidity and mortality, especially in the complicated cases with HELLP syndrome and acute pulmonary edema, cerebral hemorrhage, acute respiratory infections, cuoagulopathy.

INTRODUCTION

Prior to the year 2002, the studies could not establish a definite connection between C reactive protein and preeclampsia and this aspect was shown by Johnston C (2002); besides neither Cuanfang QUI nor Luthy DA in 2004, obtained significant correlations of these parameters (5, 8).

Kumru S. (2006) highlights the positive correlation between CRP and average blood pressure and proteinuria. This study also highlights the relations between C reactive protein and the clinical and biochemical parameters in preeclampsia; the increased values of haemoglobin, creatinine, TGO, TGP, LDH, sanguine urea and proteinuria are associated with increased values of C reactive protein (9).

With the purpose of establishing reference values of CRP for the normal pregnant women and also for those with preeclampsia, Hwang HS and collab (2007) prove the possibility of using CRP as a marker of severity in preeclampsia (7).

In recent research, Stefanovic M (2009) focuses on the endothelial dysfunction as anomaly in preeclampsia and draws the conclusion that in preeclampsia there is an increased resistance to insulin, but CRP as a marker of inflammation is not increased and is not associated with the severity of preeclampsia (12).

Carl A and collab (2008), prove in a recent study the fact that a value of over 3 mg/l is a good predictor for cardiovascular and inflammatory risk for pregnant women with a history of preeclampsia/eclampsia (4).

Also, Mihiu D. and collaborators establish CRP as a marker for the severity of preeclampsia and of the infant weight at birth (10) in a work that was published in 2008.

In his study that was reported in the year 2011, Can Murat uses mean blood pressure as a severity indicator of preeclampsia and it proves a direct association with the inflammatory reaction (3).

A prospective study, initiated by Behboudi G. and collaborators (2012) on a lot of 778 pregnant women, establishes a reference value of 4,5 mg/dl for C reactive protein (1), and Bită M (2010) studies a lot of 400 pregnant women and establishes the threshold over which you can predict eclampsia as being over 5 mg/l (2).

AIM

Hypertension is the most common medical complication during pregnancy. The study intends to find out the possibilities and limitations in diagnosing and treating preeclampsia.

OBJECTIVE

The objective of this study was to establish that the C reactive protein is an efficient marker of preeclampsia and it correlates significantly with the severity of this disease and the monitoring the level of the C reactive protein during the first trimester of pregnancy reduces the risk of getting a systemic inflammation and also the cardiovascular risk.

MATERIAL AND METHODS

The diagnostic and the evaluation of the severity of preeclampsia are based on the measurements of maternal blood pressure in the third trimester of pregnancy. If the value of the blood pressure is increased before week 20 of pregnancy, this is considered high blood pressure pre-existing the pregnancy. The measurements can be influenced by a series of factors: equipment, the resting period prior to the determination, patient posture (the right arm has to be in a strict horizontal position, at the same level with the heart – the blood pressure is lower in lateral decubitus than if the patient is sitting). The diastolic value will be determined when the sounds stop (11).

There have been two lots of study that were constituted depending on the symptomatic triad: hypertension associated with proteinuria and/or edema: Preeclampsia Group –54 patients with a gestational age over 20 weeks, with high blood pressure and proteinuria and the Control Group–28 normal pregnant women.

The 82 women in the sample, reported to the feminine population of Iași county (n=414,475*), represent 19.78 ‰ with a sample error of $\pm 10.5\%$ when compared to IC95% (Table I).

For the group of normal pregnant women, the age varied from 17 to 42, with a mean value of 29.52 ± 5.84 years old, and for the group of patients with preeclampsia, the age was situated in the interval between 19 and 45, with a mean of the lot of 29.56 ± 7.67 years old, without showing significant differences between the lots from the statistical point of view ($p=0.854$).

Depending on diastolic blood pressure increases, preeclampsia can be divided in 3 clinical forms: mild (<100 mmHg); moderate (100-110 mmHg); severe (> 110 mmHg).

Table I. Sample size by age groups

Age group	Feminine population of Iași* county	Sample		
		n	‰	% of the sample
< 19 years old	23889	3	12.56	3.66
20-24 years old	34495	20	57.98	24.39
25-29 years old	32683	26	79.55	31.71
30-34 years old	33676	14	41.57	17.07
35-39 years old	31037	10	32.22	12.20
40-44 years old	30196	8	26.49	9.76
45-49 years old	20393	1	4.90	1.22
Total	414475	82	19.78	100.00

* Source: <http://www.smarQuest.ro/ro/resources.html> and Romanian Statistical Yearbook 2011.

RESULTS AND DISCUSSIONS

The cases studied show a share of 13% patients with severe preeclampsia and 48.1% with moderate preeclampsia.

The severe forms of preeclampsia appear in older age (25-43 years old), with an average around 37 years old, while the moderate and mild forms are associated with an average of 28-29 years old ($p=0.02$).

The present study underlined the fact that when the average blood pressure is over 120 mmHg, the relative risk of having severe preeclampsia is 2.2 higher.

57.1% of the pregnant women with severe preeclampsia and 44.7% of the pregnant women with less severe forms of preeclampsia (moderate or mild) had a pathological urinalysis. However, this distribution of frequency is not statistically significant ($p=0.833$).

The cases studied did not show edema for the patients with severe preeclampsia, but they appeared for 6 patients (12.8%) with moderate or mild preeclampsia, even though the presence of the edema could not be significantly associated with the severity of preeclampsia.

Table II. Statistical differences between the forms of preeclampsia

Parameter	Preeclampsia				Statistical significance		RR	IC95%
	severe		Mild and moderate					
	(n=7)	(n=47)	χ^2	P				
	N	%	N	%				
Age \geq 30 years old	6	85.7	16	34.0	4.74	0.029	2.52	1.53÷4.15
SBP \geq 160 mmHg	7	100.0	33	70.2	1.48	0.224	1.42	1.18÷1.72
DBP \geq 100 mmHg	7	100.0	26	55.3	3.41	0.024	1.81	1.40÷2.34
Mean BP >100 mmHg	7	100.0	21	44.7	5.42	0.019	2.24	1.63÷3.08
Pathologic urinalysis	4	57.1	21	44.7	0.04	0.833	1.28	0.62÷2.62
Edema	0	-	6	12.8	0.13	0.720	-	-
Obesity	1	14.3	1	2.1	0.27	0.606	6.71	0.47÷9.58

The close relation between adiposity and CRP can be a possible explanation for the lack of predictability of CRP in the studies that do not take into account these variables. An increased CRP is a useful parameter in assessing the risk of severity of preeclampsia for the pregnant women with an increased body mass index in the third trimester of pregnancy (6, 13, 14). Our cases showed that obese patients have a 6.71 times higher risk of getting severe preeclampsia. The individual values of C reactive protein varied from 6 to 192 mg/l, for the group of patients with preeclampsia, with a very wide variation of the set of values (99%), most patients having registered values in the confidence interval (IC95%): 26.18-45.60 mg/l (42.6%), but 14.9% of the patients with preeclampsia had much increased values of this parameter registered.

For normal pregnant women, C reactive protein showed a few values that were over the reference limit (<10 mg/l), but most of them had values <6 mg/l (82.1%).

The mean values of C reactive protein were significantly higher for women with severe preeclampsia (89.14 ± 51.31 mg/l), while in the case of pregnant women with mild preeclampsia the mean value of this parameter was 22.86 ± 21.35 mg/l ($p=0.001$).

Table III. Statistical indicators of C reactive protein depending on the severity of preeclampsia

Preeclampsia	N	Mean	Std. Deviation	Std. Error	Confidence interval		Min	Max	p
					- 95%CI	+95%CI			
mild	21	22.86	21.35	4.66	13.14	32.58	6	96	0.001
moderate	26	32.08	27.10	5.31	21.13	43.02	6	96	
severe	7	89.14	51.31	19.39	41.69	136.60	48	192	
Total	54	35.89	35.57	4.84	26.18	45.60	6	192	

Based on the cases studied, the mean values of haemoglobin show a slight anaemia for the group of pregnant women with preeclampsia ($p=0.198$), without being influenced by its severity ($p=0.451$).

Table IV. Statistical indicators of haemoglobin depending on the severity of preeclampsia

Preeclampsia	N	Mean	Std. Deviation	Std. Error	Confidence interval		Mean	Max	p
					- 95%CI	+95%CI			
mild	21	10.59	2.22	0.48	9.57	11.60	6.8	15.0	0.451
moderate	26	10.93	2.36	0.46	9.98	11.88	5.8	15.8	
severe	7	11.80	1.09	0.41	10.79	12.81	10.5	13.4	
Total	54	10.91	2.18	0.30	10.31	11.50	5.8	15.8	

In the cases of severe preeclampsia, the mean values of the hematocrit were slightly lower ($33.93\% \pm 2.92$) in comparison with the ones found in the moderate forms ($34.87\% \pm 6.11$) ($p=0.847$).

Table V. Statistical indicators of the hematocrit depending on the severity of preeclampsia

Preeclampsia	N	Mean	Std. Deviation	Std. Error	Confidence interval		Min	Max	p
					- 95%CI	+95%CI			
mild	21	33.96	6.11	1.33	31.18	36.75	24.0	44.0	0.847
moderate	26	34.87	6.11	1.20	32.40	37.34	21.0	47.0	
severe	7	33.93	2.92	1.10	31.23	36.63	30.0	38.5	
Total	54	34.39	5.74	.78	32.83	35.96	21.0	47.0	

For the patients with severe preeclampsia, the main maternal and fetal impairment, that seldom has an unfavourable prognosis, is HELLP syndrome.

CONCLUSIONS

In the present study we proved that the plasmatic level of C reactive protein is correlated in a significant manner directly with the systolic/diastolic or mean blood pressure.

The high plasma level of the C reactive protein was associated with slightly increased values of haemoglobin and hematocrit.

Monitoring the level of the C reactive protein during the first trimester of pregnancy reduces the risk of getting a systemic inflammation and also the cardiovascular risk.

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