MEDICAL UNIVERSITY "PROF. PARASKEV STOYANOV"
OF VARNA
FACULTY OF PHARMACY
DEPARTMENT OF BIOLOGY

Tsonka Slavova Dimitrova

INVESTIGATIONS AND SCREENING METHODS FOR EARLY DIAGNOSIS OF PATIENTS WITH GASTROINTESTINAL DISORDERS BECAUSE OF MUSHROOM CONSUMPTION

AUTHOR'S DISSERTATION SUMMARY

for the acquisition of the educational and scientific degree of '**doctor of philosophy**' in the field of higher education No 4. Natural sciences, mathematics and informatics, professional trend No 4.3. Biological sciences and scientific speciality of medical biology

Advisers:

Prof. biol. Dobri Lazarov Ivanov, PhD
Prof. Petko Penkov Marionov, MD, PhD

Official peer reviewers:
Prof. Snezha Zlateva Zlateva, MD, PhD
Assoc. Prof. biol. Mariya Hrostova Kazakova, PhD

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The present dissertation has been elaborated in the Department of Biology at the Faculty of Pharmacy of the Medical University "Prof. Paraskev Stoyanov" of Varna.

The dissertation contains 146 type-written pages and is illustrated with 21 tables, 71 figures and one appendix. The reference list includes 219 primary sources, of which there are 13 in Cyrillic and 206 in Latin.

The dissertation has been discussed and forwarded to a public defence by the Departmental council of the Department of Biology of the Medical University "Prof. Paraskev Stoyanov" of Varna based on report by Prof. biol. Dobri Lazarov Ivanov, PhD, Head of the Department of Biology of the Medical University of Varna in the field of higher education No 4. Natural sciences, mathematics and informatics, professional trend No 4.3. Biological sciences and doctoral programme 'Medical biology', according to article No 71, paragraphs 1, 2 and 4 of the Statute-book for the Application of the Law for development of the academic staff in the Republic of Bulgaria and Decision of the Faculty Council of the Faculty of Pharmacy, record No 93/July 27, 2021.

Public defence of the dissertation will be held on October 10, starting at 9:00 a.m. in the virtual hall of Webex electronic platform of the Medical University "Prof. Paraskev Stoyanov" of Varna based on Order No R-109-334/August 8, 2021 of Prof. Valentin Lyubomirov Ignatov, MD, PhD, Rector of the Medical University "Prof. Paraskev Stoyanov" of Varna in front of a scientific jury consisting of:

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СЪДЪРЖАНИЕ

ABBREVIATIONS USED

DNA	desoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
et al.	et alierte
g	gram
kg	kilogram
L	litre
mg	milligram
mL	milliliter
RNA	ribonucleic acid

1. INTRODUCTION

Since millennia, people are aware of mushrooms. During the recent decades, the imnvestigations of the various wild mushroom species have contributed to the disclosure of a large number of their essential features. The number of the identified mushroom species which are analyzed in a versatile manner uninterruptedly increases worldwide.

The interest in mushrooms is determined by their rising importance not only for the biological variety in living nature but also for their significant usage by the population in the developing and developed countries as food and for treatment as well.

There exist edible, non-edible and toxic wild mushrooms. Sometimes, the distinction between them is very difficult. This is one of the main reasons for the cases of intoxications with mushrooms which are gathered by people in nature. These poisonings are due to a variety of toxins which the ingested mushrooms contain. The various typical peculiarities not only of the wild mushrooms but also of their toxins represent the subject of more and more intensive interdisciplinary investigations by large collectives, i.e. of ethnomycological, ethnobotanical, toxicological, morphological, molecular, genetic, etc.

The results from these investigations are of undoubted benefit for further improvement of the awareness of the population and scientists about these mushrooms and for the perfectioning of the manners of their usage in everyday practice. The elaboration of trustworthy methods for early and precise diagnosis of the intoxications with wild mushrooms as well as of effective prophylactic measures in terms of these poisonings is of rising importance, too.

In our country, there is no systematic investigation not only of the social epidemiology and early diagnosis of the poisonings with wild mushrooms as well as of the level of knowledge of different population groups about them and the usage of these mushrooms in everyday practice yet.

2. PURPOSE AND TASKS

The purpose of the present dissertation work is to analyze the poisonings with wild mushrooms among the population in the North Black Sea coast of Bulgaria, to approve easily feasible methods for their diagnosis as well as to study the degree of awareness about these mushrooms and their usage as a basis for prevention.

The tasks for accomplishment of this purpose are the following:

1. To perform a retrospective analysis of the documentation about the wild mushroom poisonings of the patients who have been hospitalized in the city of Varna between 1991 and 2015.

2. To apply Meixner's test in the patients with gastrointestinal symptoms suspected for mushroom poisoning.

3. To apply ELISA for the presence of amatoxins in the patients following toxic wild mushroom consumption.

4. To perform an inquiry investigation of the degree of awareness of the population about the wild mushrooms on the territory of the Northern Black Sea coast of Bulgaria.

5. To perform an inquiry investigation of the usage of the wild mushrooms by the population on the territory of the Northern Black Sea coast of Bulgaria.

WORKING HYPOTHESIS

The diagnostic methods approved by us could contribute to the perfectioning of the early laboratory identification and timely treatment of the poisonings with wild mushrooms and the conclusions from the inquiry investigations - to the improvement of the prevention of these socially significant intoxications.

3. MATERIAL AND METHODS

3.1. Material

We analyzed the documentation of all the patients with diagnosed A. phalloides poisoining who have been hospitalized in the Clinic for Intensive Treatment of Acute Intoxications and Toxic Allergies at Naval Hospital of Varna, Military Medical Academy, during the period between 1991 and 2015.

It deals with a total of 147 patients at a mean age of 52,54±15,27 years (range, 18 to 84 years). The males are a total of 91 at a mean age of 54,14±13,41 years (range, 18 to 86 years), and the females are a total of 56 at a mean age of 51,33±12,72 years (range, 18 to 84 years). Male:female ratio is 1,625:1.

In Fig. 1, the annual dynamics of the poisonings among all the patients during this 25-year period is illustrated.

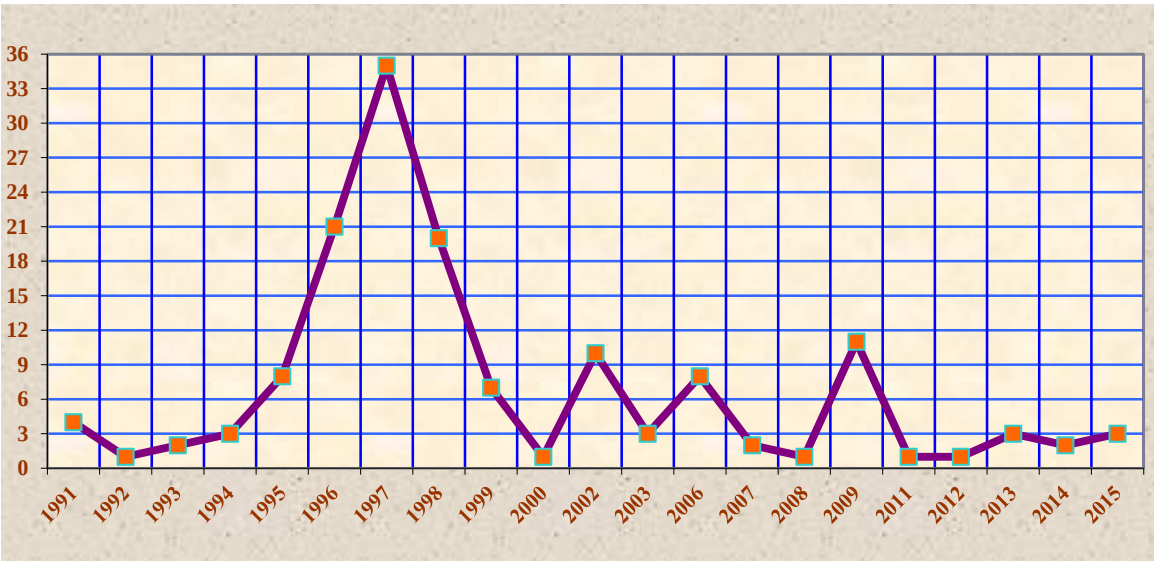


Fig. 1. Annual dynamics of the poisonings among all the patients

During the period between May 12, 2021 and July 27, 2021, in the the Clinic for Intensive Treatment of Acute Intoxications and Toxic Allergies at Naval Hospital of Varna, Military Medical Academy, 21 patients at a mean age of 54,19±17,51 years (range 21 to 80 years) have been hospitalized with gastrointestinal complaints related to wild mushroom poisoning. It deals with 15 males at a mean age of 56,47±17,68 years (range, 33 to 80 years) and six females at a mean age of 48,50±18,75 years (range 21 to 72 years). In every patient, two diagnostic examinations, Meixner's test and enzyme-linked immunosorbent assay (ELISA) after concrete methods have been performed. These analyses are based on Protocol/Decision No 102/April 22, 2021 of the Commission for Research Ethics of the Medical University "Prof. Paraskev Stoyanov" of Varna.

3.2. Methods

The intoxications with wild mushrooms in the city of Varna, registered during the period between 1991 and 2015 have been analyzed. The following parameters have been followed-up: month and year of the diagnosed intoxication, gender and age of the patients with poisoning. During the retrospective analysis of the registered intoxications with A. phalloides, the sharp augmentation of their number during the period between 1996 and 1998 stresses, indeed. There are peak values in 1997 not only among males but also among all the patients.

A. Meixner's test (1979) has been used for the identification of amatoxins in wild mushroom extracts by means of reaction with woodwool and concentrated hydrochloric acid. The positive ersult obtained consists in a changed staining from green into blue of low-quality newsprint. The extract from ingested mushroom little

parts containing amatoxin is laid on this newsprint and on the top, a drop of concentrated hydrochloric acid is placed.

During ELISA examination, urinary amanitin is measured according to an approved firm protocol with Bühlmann Amanitin ELISA Kit (Bühlmann Laboratories, Allschwil, Switzerland). BioTek apparatus, model 800 TS Absorbance Reader is made use of.

During the period between May 12, 2021 and July 27, 2021, an inquiry investigation through face-to-face interview by means of inquiry card anonymously filled-in by 200 randomly selected respondents, 100 males and 100 females, from several towns and villages in the Region of Varna has been carried out. The inquiry card includes a total of 27 items (see Appendix No 1).

The investigation has been focused on two basic characteristics such as degree of awareness of the population about edible wild mushrooms and manners of their usage.

In Fig. 2 through Fig. 4, respondents' distributions according to gender, on the one hand, and to age groups, educational level and residence, on the other hand, are presented.

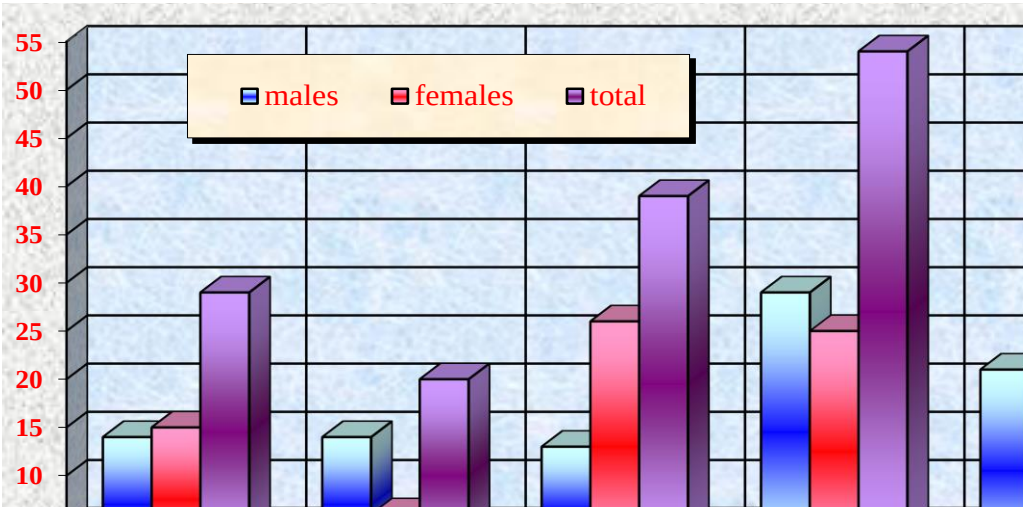


Fig. 2. Respondents' distribution according to gender and age groups

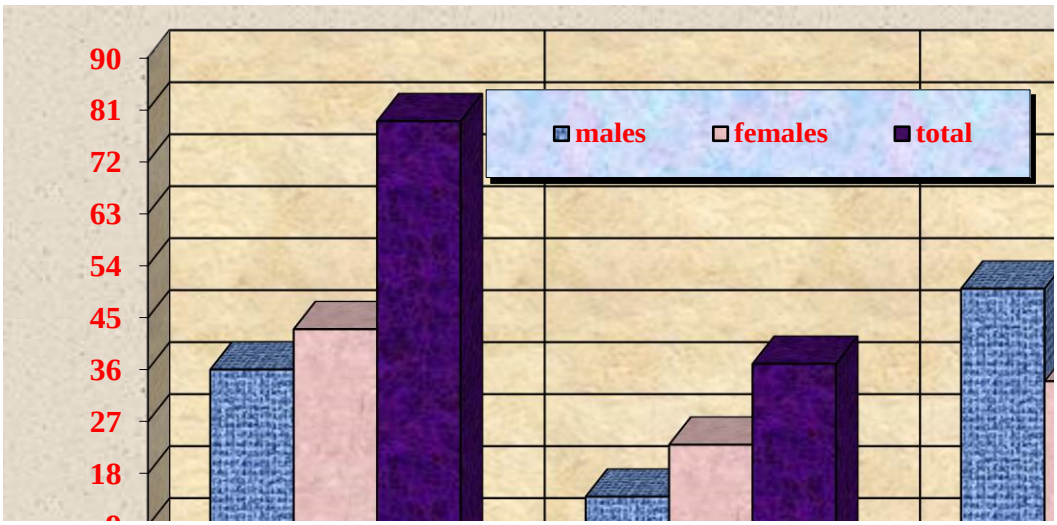


Fig. 3. Respondents' distribution according to gender and education

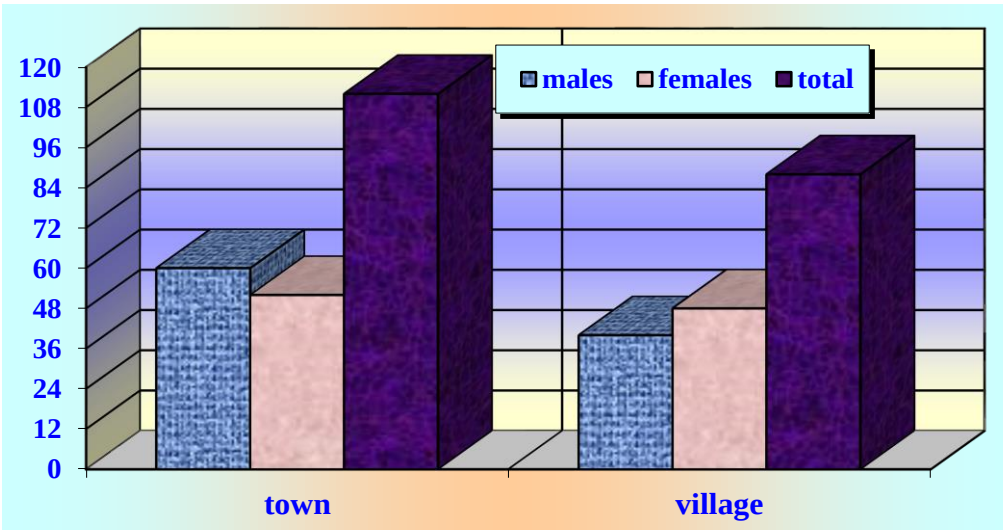


Fig. 4. Respondents' distribution according to gender and residence

The results are statistically processed by means of descriptive, variation (Student-Fisher's test and exact Student-Fisher's test) and correlation analysis (Pearson's coefficient and independent χ^2). Statistical reliability according to Student-Fisher's *t*-criterion is read at a significance level of $p<0,05$. A SPSS statistical package, version 22.0 is made use of.

4. OWN RESULTS

4.1. A retrospective analysis of wild mushroom poisonings during the period between 1991 and 2015

The annual dynamics of the intoxications with A. phalloides in all the patients who have been hospitalized in the Clinic for Intensive Treatment of Acute Intoxications and Toxic Allergies at Naval Hospital of Varna, Military Medical

Academy, during the period between 1991 and 2015, according to age groups is demonstrated in Fig. 5.

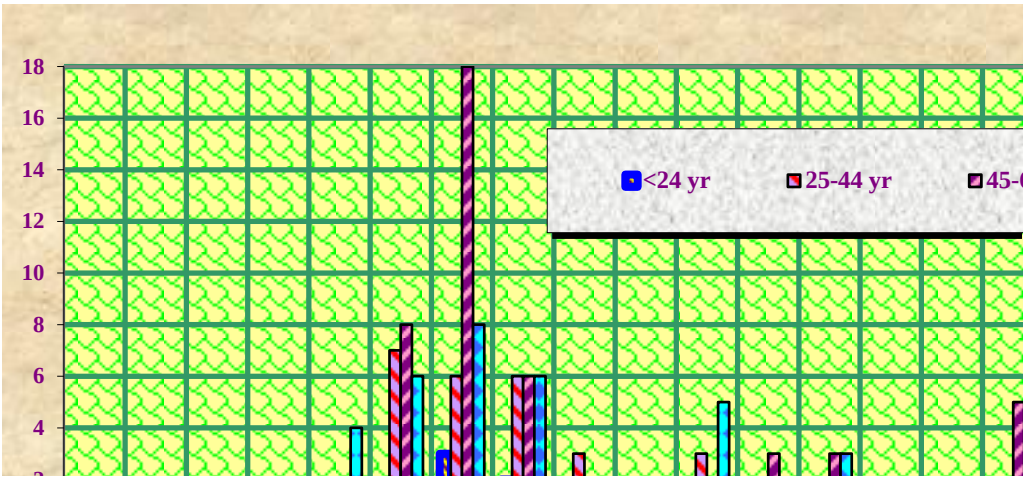


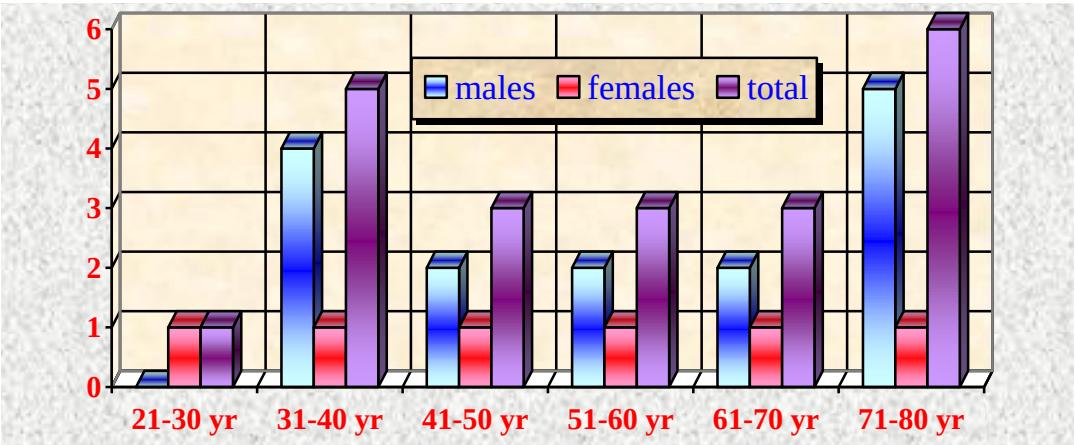
Fig. 5. Annual dynamics of the intoxications in all the patients according to age groups

The greatest number of A. phalloides intoxications among males, females and all the patients within the whole 25-year period of observation is detected in the month of October.

There exists a statistically reliable greater incidence rate of A. phalloides intoxications in the month of August among males than among females (t=2,365; p<0,02). In spitre of the treatment administered, a total of 25 patients, 13 females and 12 males, have deceased. Mortality rate of women is 23,21%, that of men is 13,19%, and overall mortality rate is 17,00%.

4.2. Examination of amatoxins using Meixner's test

The distribution of these patients according to gender and age is displayed in Fig. 6.



Фиг. 6. Patients' distribution according to gender and age

The workers and retired persons as well as the inhabitants of the city of Varna prevail among the examined subjects.

We have obtained negative results in terms of the presene of amatoxins when using Meixner's test in samples of stomach contents and gathered mushrooms from these 21 patients. This could be explained with the relatovely small volume of our excerpt. Timely application of of this test enables us to reject the initial diagnosis of an acute poisoning with A. phalloides.

The early and precise diagnosing of this intoxication plays a crucial role for the selection of the timely adequate treatment of these patients.

In Fig. 7, the finding of the negative Meixner's test for amatoxins in samples of stomach contents and gathered mushrooms can be seen.

Fig. 7. Negative Meixner's test for amatoxins (control)

4.3. ELISA examination

In Fig. 1 and Table 1, one can see some results from ELISA examination of urinary samples from our patients hospitalized in the Clinic for Intensive Treatment of Acute Intoxications and Toxic Allergies at Naval Hospital of Varna, Military Medical Academy.

The results obtained by us concerning the presence of amatoxins when making use of ELISA are negative.

Fig. 8. Urinary samples from patients examined for amanitin

Table 1. Results from the examination of urinary amanitin in patients with suspected acute poisoning with A. phalloides

Patient's number	absorption	B/B0 (%)	concentration in ng/mL
1	1,808	100	0
2	1,805	100	0
3	1,782	99	0
4	1,672	93	0
5	1,720	95,5	0
6	1,760	97,7	0
7	1,770	98,3	0
8	1,872	104	0
9	1,561	86,7	0
10	1,743	96,8	0
11	1,777	98,72	0
12	1,547	85,9	0
13	1,745	96,9	0
14	1,828	105	0
15	1,895	105	0
16	1,695	94,1	0
17	1,833	101	0
18	1,779	98,8	0
19	1,657	92	0
20	1,693	94	0
21	1,666	92,5	0
blanc sample	0,07		
B0	1,8		0
Cal A	1,6	88,8	1
Cal B	1,25	69,4	3
Cal C	0,65	36,1	10
Cal D	0,37	20,5	30
Cal E	0,1	5,55	100
low control	1,06	58,9	4
high control	0,39	21,7	29

4.4. An inquiry investigation of the awareness about wild mushrooms

A total of 122 respondents (61%), 64 males and 58 females, give positive answers to the question which edible wild mushrooms these subjects recognize. Men recognize a total of 24 and women do a total of 26 different mushrooms. The rest 78 respondents, 36 males and 42 females, can't recognize no edible wild mushrooms at all.

Their distributions according to gender and age groups are illustrated in Fig.9 and Fig. 10.

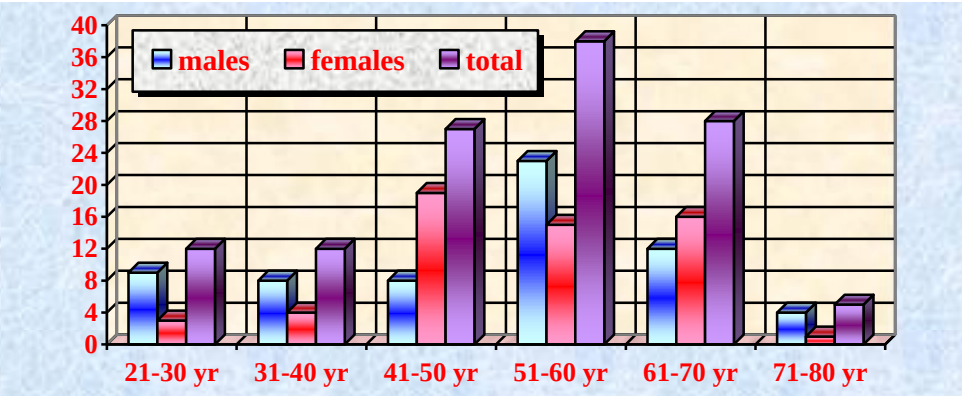


Fig. 9. Distribution of the respondents who recognize mushrooms according to gender and age groups

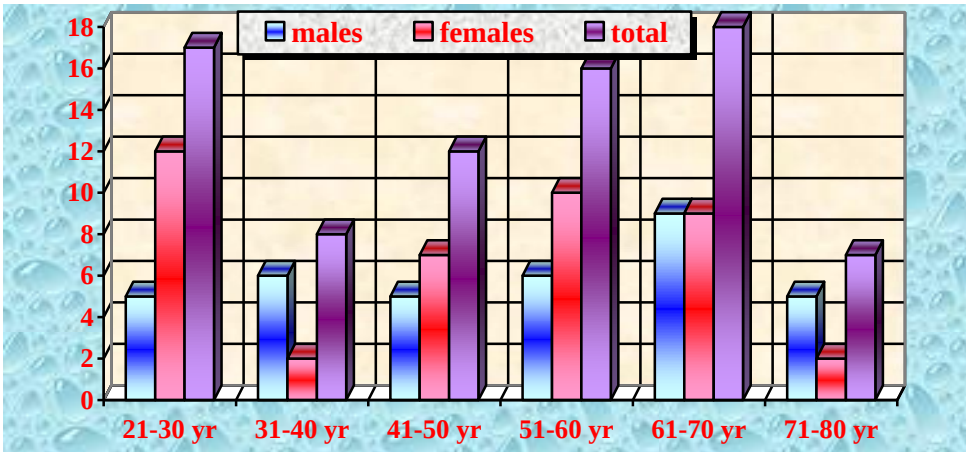


Fig. 10. Distribution of the respondents who do not recognize mushrooms according to gender and age groups

In Fig. 11, the number of males is juxtaposed to that of females according to the number of the edible wild mushrooms recognized by every single respondent.

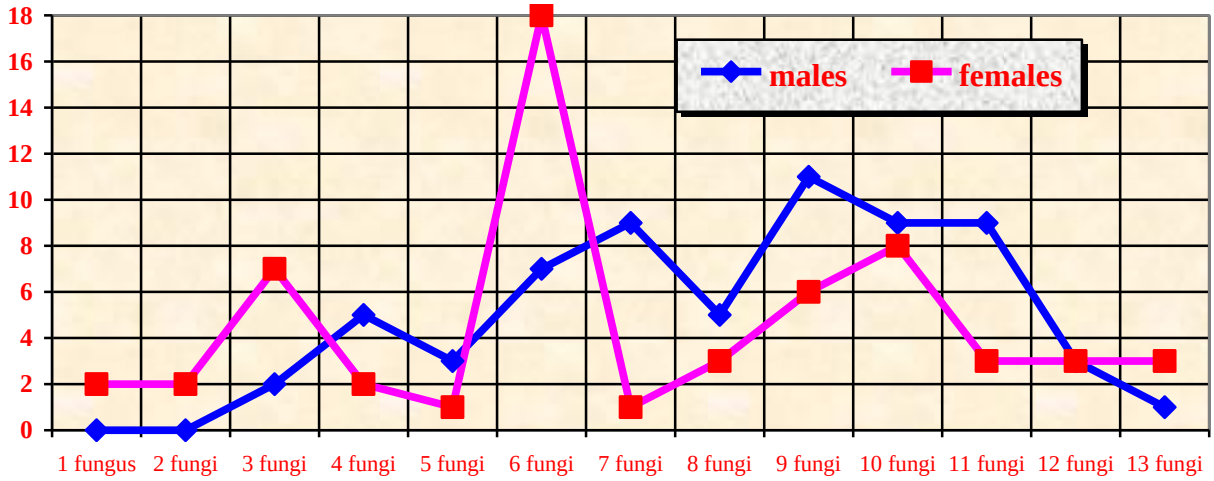


Fig. 11. Distribution of the number of respondents according to the number of edible wild mushrooms recognized by them

In Table 2, the nominations of 13 most recognized edible wild mushrooms in Bulgarian and Latin language are indicated.

Table. 2. Nominations of 13 mushrooms recognized by most respondents

No	Nomination of the mushroom		n	%
	in Bulgarian language	in Latin language		
1.	манатарка	*Boletus edulis* Bull	114	93,44
2.	сърнела	*Macrolepiota procera* S. F. Gray	107	87,70
3.	пачи крак	*Cantharellus cibarius* Fr.	92	75,41
4.	челадинка	*Marasimus oreades* Fr.	84	68,85
5.	пънчушка	*Armillariella mellea* P. Kumm	76	62,29
6.	булка	*Amanita caesarea* Pers.	75	61,47
7.	праханка	*Albatrellus ovinus* Pers.	59	48,36
8.	горска печурка	*Agaricus silvaticus* Schaeff	58	47,54
9.	масловки	*Suillus luteus* Roussel	48	39,34
10.	керино ухо	*Sarcoscypha coccinea* Labbotte	42	34,43
11.	овчи нос	*Gomphidius glutinosus* Schaeff	31	25,41
12.	брезовка	*Leccinum scabrum* Gray	27	22,13
13.	полска печурка	*Agaricus campester* L.	24	19,67

Respondents' distributions according to the more common and more seldom manners of knowledge acquisition about edible wild mushrooms according to gender are presented in Fig. 12 and Fig. 13.

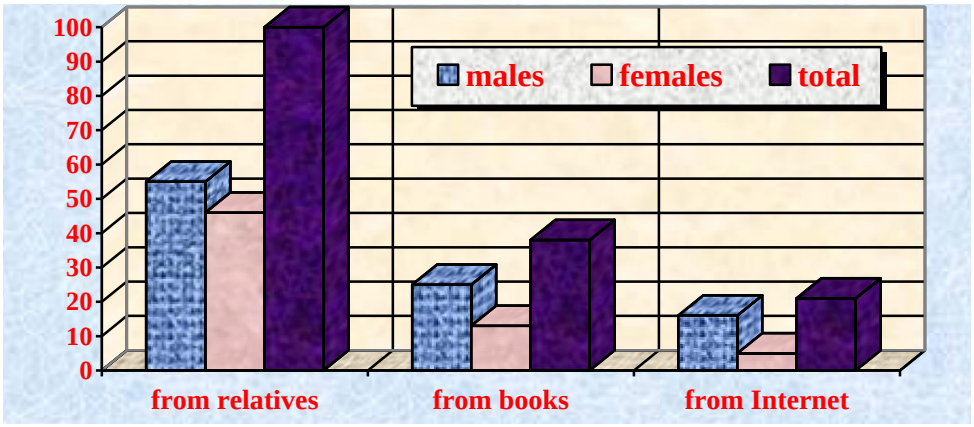


Fig. 12. Respondents' distribution according to the more common manners of knowledge acquisition about mushrooms

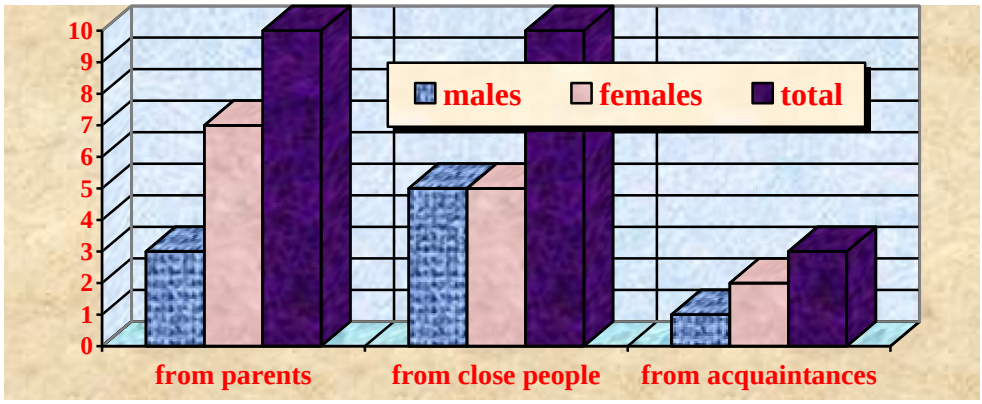


Fig. 13. Respondents' distribution according to the more seldom manners of knowledge acquisition about mushrooms

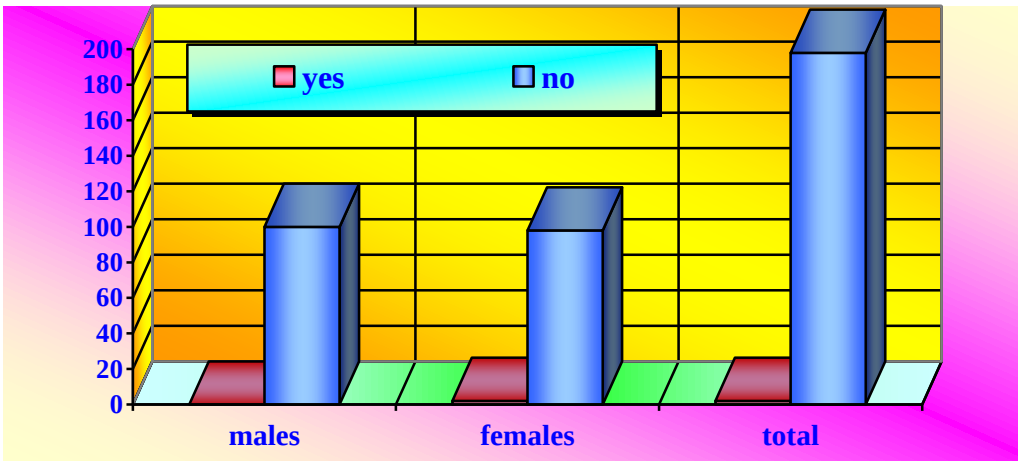


Fig. 14. Distribution of respondents who are or are not aware about education aiming at mushroom recognition according to gender

As one can see in Fig. 14, only two women have even heard about any forms of education with the aim for recognition of wild mushrooms and identification of toxic from non-toxic counterparts of the corresponding species.

Respondents' distribution according to their educational level in terms of knowledge acquisition about wild mushrooms is demonstrated in Fig. 15.

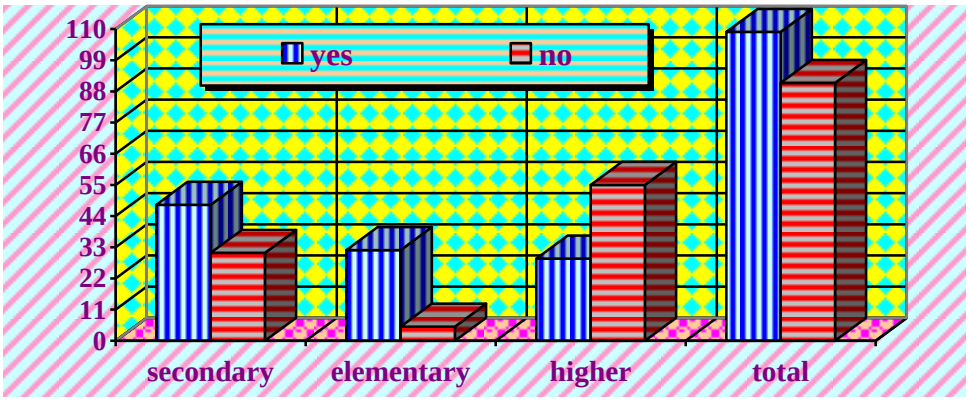


Fig. 15. Respondents' distribution according to their educational level in terms of knowledge acquisition about mushrooms

4.5. An inquiry investigation of the usage of the wild mushrooms

Respondents' distribution according to gender and annual edible wild mushroom consumption is shown in Fig. 16.

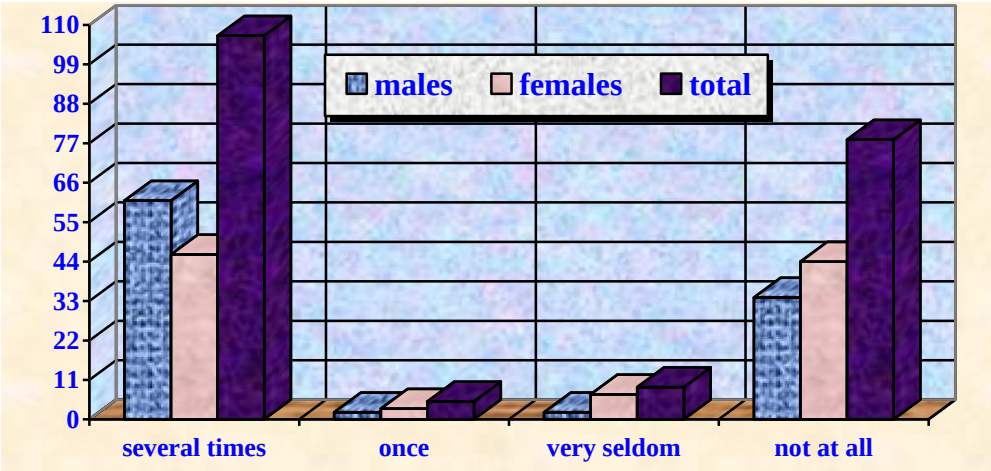


Fig. 16. Respondents' distribution according to gender and edible mushroom consumption during the year

Respondents' distributions according to gender and in terms of the manners of mushroom preparation for food such as cooking, drying, preservation and freezing are illustrated in Fig. 17 through Fig. 20.

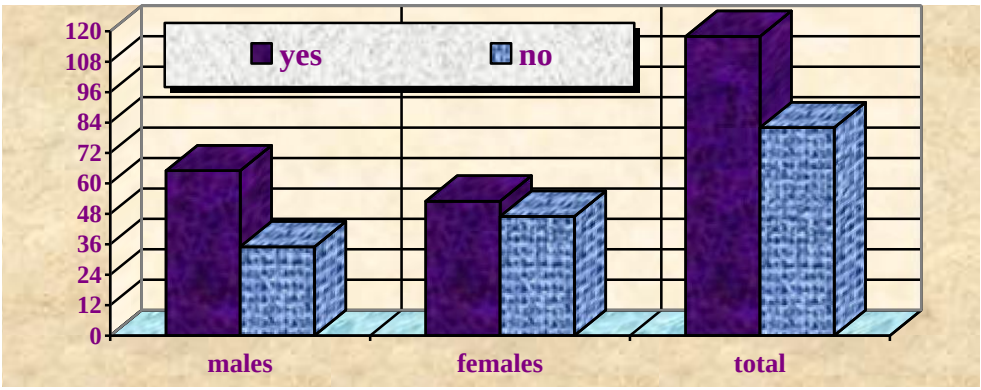


Fig. 17. Distribution of respondents who cook or do not cook mushrooms according to gender

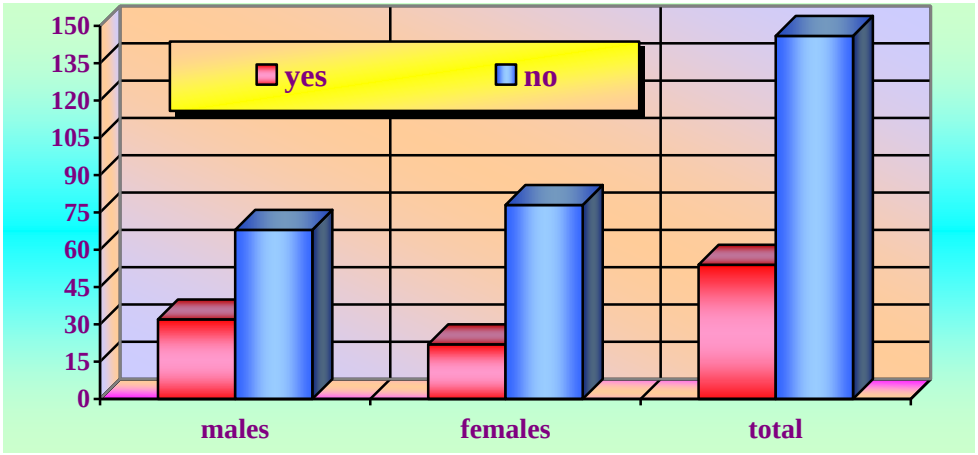


Fig. 18. Distribution of respondents who dry or not dry mushrooms according to gender

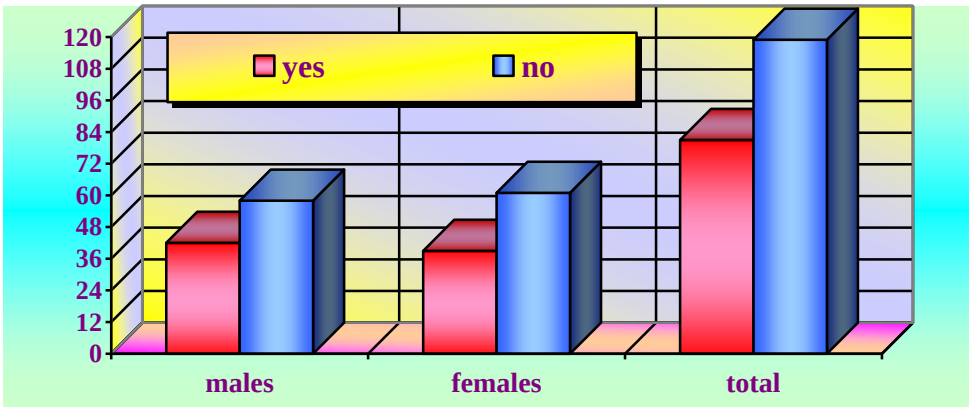


Fig. 19. Distribution of respondents who dry or not preserve mushrooms according to gender

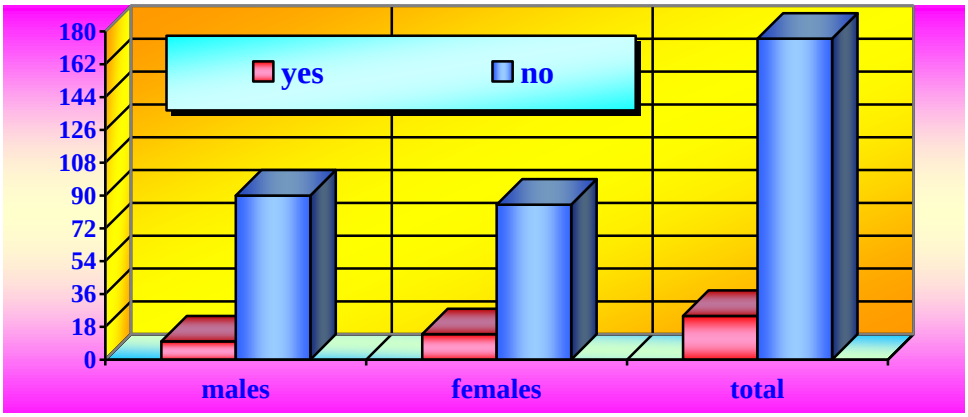


Fig. 20. Distribution of respondents who dry or not freeze mushrooms according to gender

Respondents' distribution according to the manner of mushroom acquisition and gender is generalized in Fig. 21.

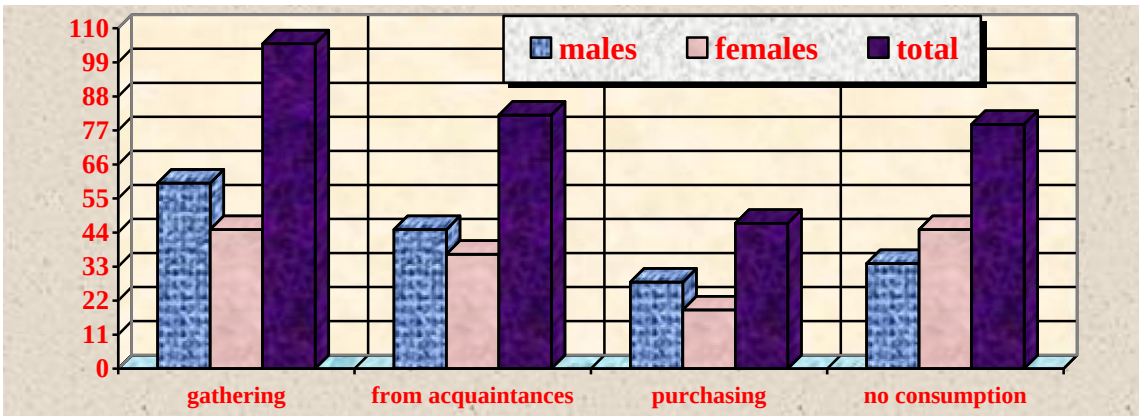


Fig. 21. Respondents' distribution according to the manner of mushroom acquisition and gender

Respondents' distributions according to education and in terms of the manners of mushroom preparation for food such as cooking, drying, preservation and freezing are shown in Fig. 22 through Fig. 25.

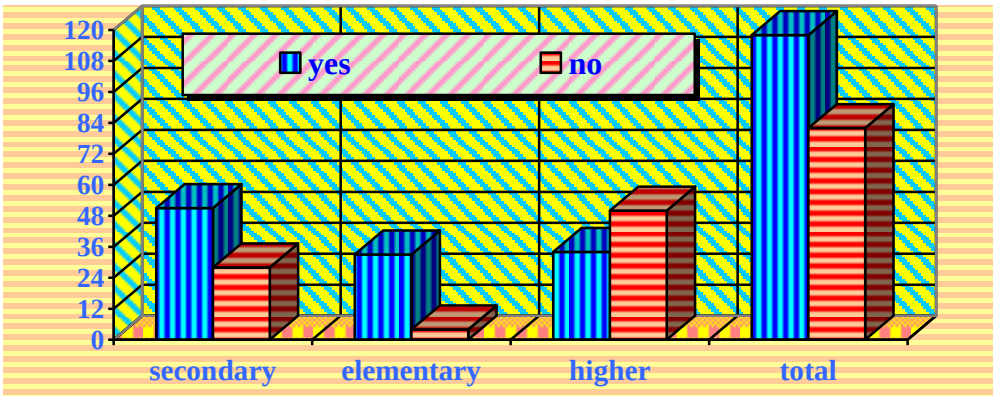


Fig. 22. Distribution of respondents who cook or do not cook mushrooms according to education

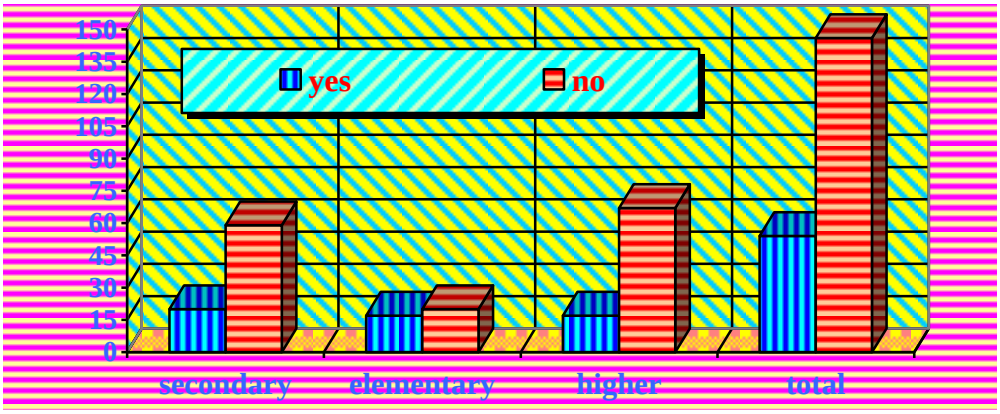


Fig. 23. Distribution of respondents who dry or do not dry mushrooms according to education

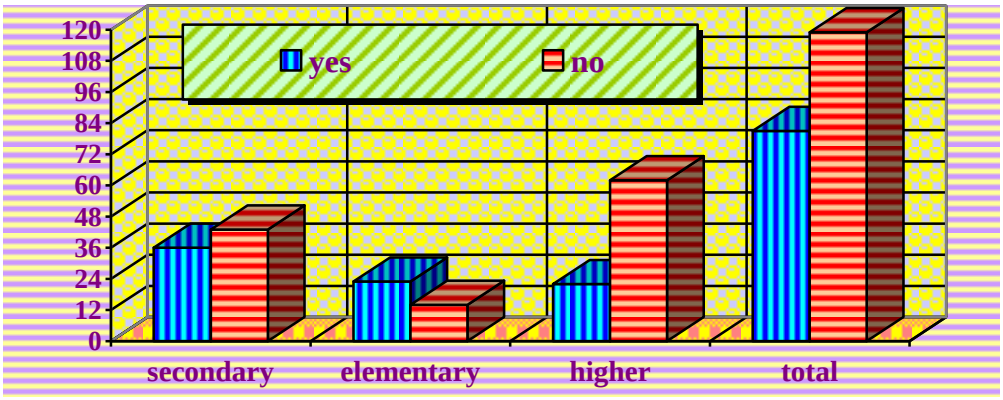


Fig. 24. Distribution of respondents who preserve or do not preserve mushrooms according to education

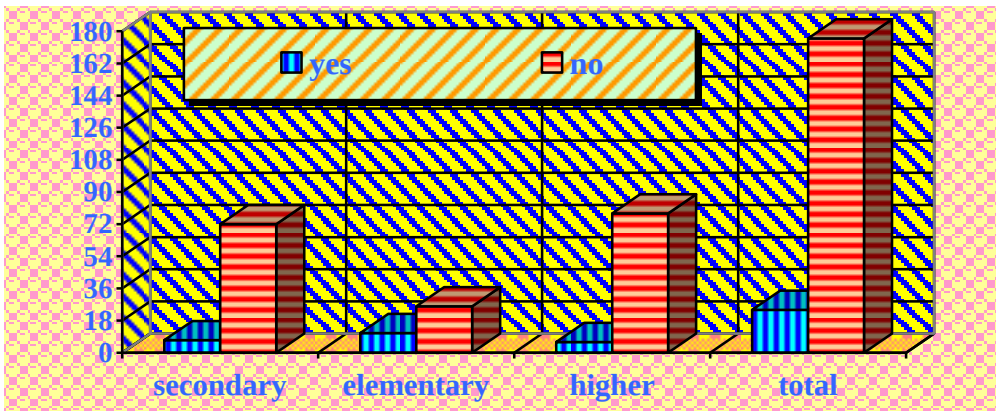


Fig. 25. Distribution of respondents who freeze or do not freeze mushrooms according to education

The distributions of the respondents with different residence in terms of the manners of mushroom preparation for food such as cooking, drying, preservation and freezing are demonstrated in Fig. 26 through Fig. 29.

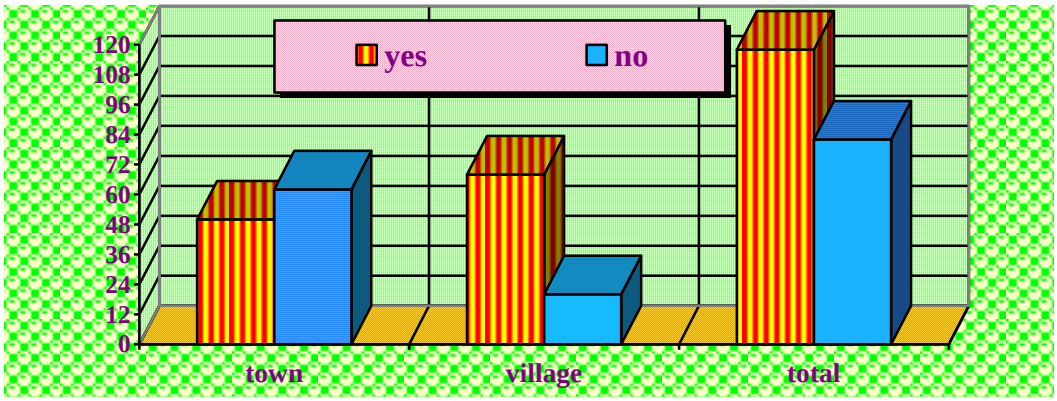


Fig. 26. Distribution of respondents who cook or do not cook mushrooms according to residence

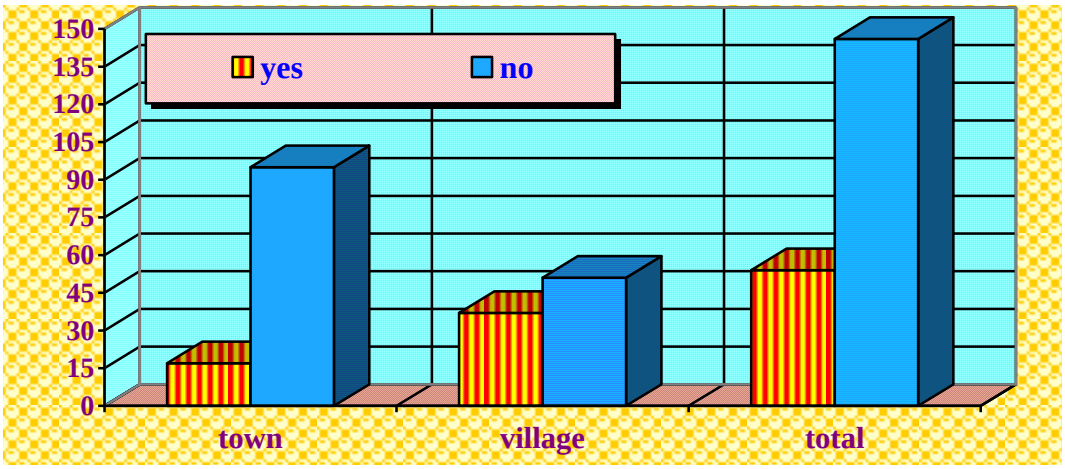


Fig. 27. Distribution of respondents who dry or do not dry mushrooms according to residence

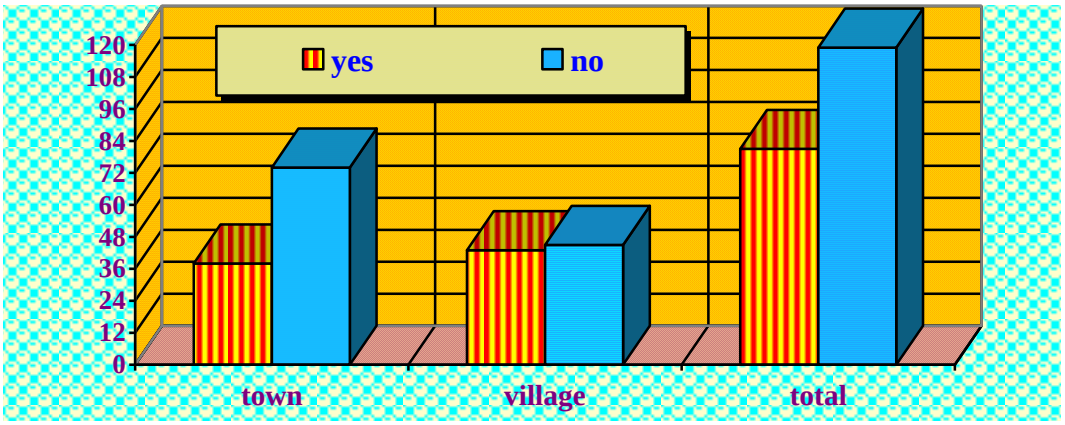


Fig. 28. Distribution of respondents who preserve or do not preserve mushrooms according to residence

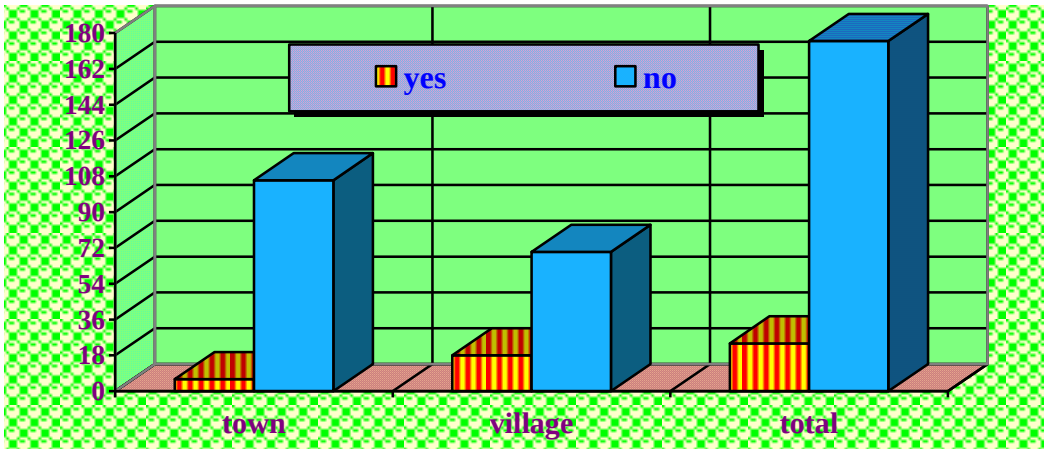


Fig. 29. Distribution of respondents who freeze or do not freeze mushrooms according to residence

Respondents' distributions in terms of mushroom usage for treatment according to gender, education and residence can be seen in Fig. 30 through Fig. 32.

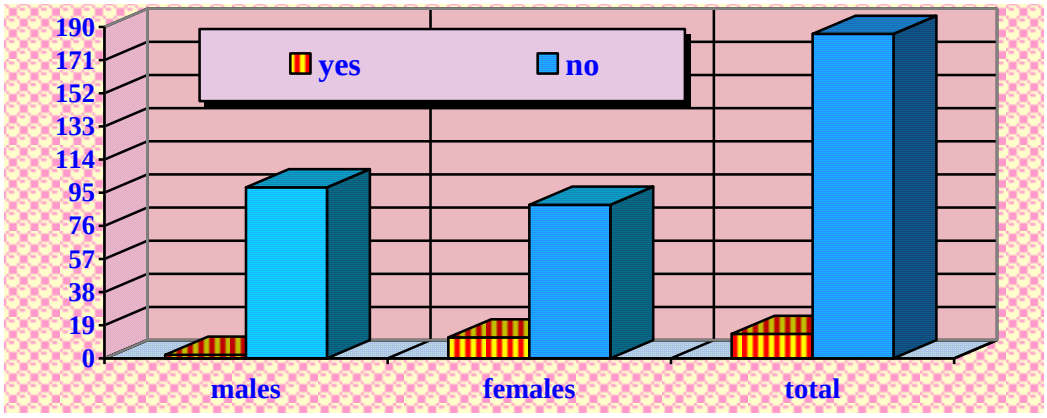


Fig. 30. Respondents' distribution in terms of mushroom usage for treatment according to gender

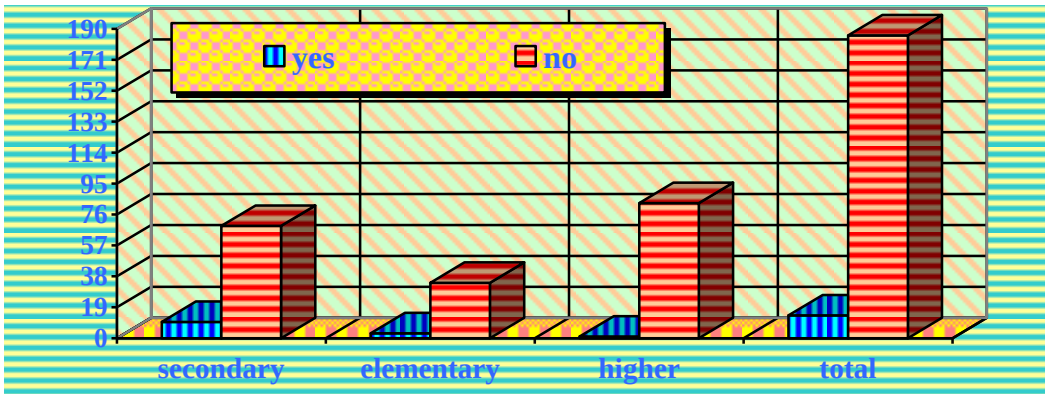


Fig. 31. Distribution of respondents who use mushrooms for treatment according to education

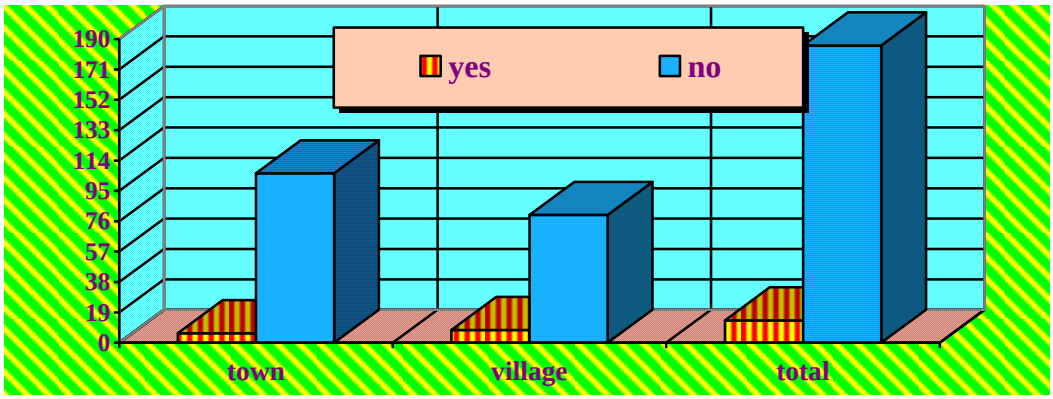


Fig. 32. Respondents' distribution in terms of mushroom usage for treatment according to residence

5. DISCUSSION

5.1. An analysis of the intoxications with A. phalloides in 1991-2015

Within the analysis of the annual dynamics of the intoxications with A. phalloides it has been established that clinically manifested poisonings have notbeen diagnosed in men during a total of eight, in women during a total of six and in all the patients during a total of four years.

The total mortality rate durintg this 25-year period is 17,00%. There is a need for an effective prevention and a precise early diagnosis of this life-threatening disease.

A total of 443 mushroom poisonings are registered in the emergency divisions of the hospitals in Parma Province, Italy, during the period betyween January 1, 1996 and December 31, 2016, predominantly in autumn (G. Cervellin *et al*., 2019). In 108 patients (in 24,38%), it deals with edible wild mushroom consumption as Boletus edulis occurs most commonly (in 63 patients or in 12,22% of the cases).

During the period between 2015 and 2018 in a hospital in Mazandara, Iran, 65 patients at a mean age of 35,68 years, 33 males and 32 females, with wild mushroom poisonings predominantly in spring are identified (I. G. Khatir *et al*., 2020). A latent period of ≤6 hours is established in 63 patients. Mean hospital length of stay amounts to 1,89 days.

In the retrospective study performed during the period between January 1, 2008 and December 31, 2018, within the National Poison Data System of the USA, a total of 8953 wild mushroom intoxications are registered, of which only 148 are included in the analysis (J. De Olano *et al*., 2021). The overall mortality rate amounts to 8,78% (13 lethal cases). The concrete toxic mushrooms are identified by mycologist in 25 patients (in 16,89% of the cases) as 20 of them are cyclopeptide-

containing. The mortality rate because of acute liver damage in both groups (treated and untreated patients) is 10% each.

The retrospective investigation during the period between 1999 and 2016 in the National Poison Data System in the USA registered a total of 133700 cases of intoxication after ingestion of wild mushrooms (on the average of 7427,78 cases annually) (W. E. Brandenburg & K. J. Ward, 2018). It deals most commonly with unintentional intoxications (in 83%; $p<0,001$) as damages caused to the organism affect predominantly children aged below six years (in 62% of the cases; $p<0,001$). There are a total of 52 fatalities (on the average of 2,9 annually) and in 68% up to 89%, they are due to cyclopeptide-producing mushrooms unintentionally ingested by older adults.

5.2. An analysis of the laboratory examinations

When applying Meixner's test in samples of stomach contents and gathered mushrooms, ELISA examination and competitive ELISA examination in urinary samples in our 21 patients hospitalized in the the Clinic for Intensive Treatment of Acute Intoxications and Toxic Allergies at Naval Hospital of Varna, Military Medical Academy, negative results concerning the presence of amatoxions are obtained. In this way, we succeed in rejecting the initial diagnosis of an acute intoxication with A. phalloides after wild mushroom consumption. Thanks to the timely application of these two methods, the treating team makes the correct decision for the performance of the necessary conservative therapy in these patients.

5.3. An analysis of the awareness about wild mushrooms

The number of the males, females and all the respondents who recognize the edible wild mushrooms accessible in the area of their residence is greater than that of the respondents who do not recognize any such mushroom at all. The edible boletus *Boletus edulis* Bull is the most commonly recognized edible wild mushroom on the territory of the Region of Varna. Next follows the parasol mushroom *Macrolepiota procera* S. F. Gray, the chanterelle *Cantharellus cibarius* Fr., and the agaric *Marasimus oreades* Fr. There is a statistically significant difference between female and male respondents in terms of the number of the subjects who recognize the edible wild mushroom forest mushroom *Agaricus silvaticus* Schaeff ($t=4,485$; $p<0,001$).

There exists a statistically significant correlation dependence between gender, on the one hand, and necessity for knowledge acquisition about the edible wild mushrooms, on the other hand, in favour of males (Pearson's coefficient $\chi^2 = 4,537$; $p=0,033$, exact Fisher's test $p=0,023$).

The relatives, books and Internet serve most commonly as a source of useful information about the edible wild mushrooms for males, females and all the respondents as a whole. Men make more frequently use of information from relatives, books and Internet than women while women do more frequently that from their parents.

The interest in the issues concerning the dangers for the existence of the edible wild mushrooms in nature under the conditions of uninterrupted environmental contamination with industrial and domestic waste is poor. The relative share of the respondents who have heard that some edible wild mushrooms become more and more difficult to be found in the environment is small.

Male and female respondents are well-informed about the established poisonings with wild mushrooms gathered on the territory of the Region of Varna. The mean number of known lethal cases in the surroundings per one respondent is 2,35±0,205 among male and 2,15±0,218 among females.

Most men and women have already seen toxic wild mushrooms. The number and relative share of these men are by 5,67 times greater while the total number and relative share of all these respondents are by 2,28 times greater than those of the respondents with a negative answer to this question.

The respondents who do not know what does ‘mycotherapy’ mean are by three times more among females, by 2,57 among all the respondents and by 2,22 times among males. A statistically insignificant correlation dependence between respondents’ knowledge about mycotherapy aas a scientific discipline, on the one hand, and their educational level, on the other hand, is observed Наблюдава се статистически недостоверна корелационна зависимост между познанията на анкетираните лица за микотерапията като научна дисциплина, от една страна, и нивото на тяхното образование, от друга страна (Pearson’s coefficient χ^2 = 2,048; p=0,359).

There exists a statistically significant correlation dependence between elementary educational level, on the one hand, and knowledge acquisition about wild mushrooms, on the other hand (Pearson’s coefficient χ^2 = 30,032; p<0,0001).

The inhabitants of the villages in the Region of Varna presenting with a positive attitude towards knowledge acquisition about these mushrooms considerably prevail versus those with a negative attitude (by 2,83 t8mes) while among the inhabitants of the towns in the Region of Varna, the negative attitude is by 1,55 more common than the positive one. A statistically significant correlation dependence between residence, on the one hand, and knowledge acquisition about wild mushrooms, on the other hand (Pearson’s coefficient χ^2 = 23,761; exact Fisher’s test p<0,0001) in favour of the village residence is established.

As a whole, the extent of awareness of the extract of the population on the territory of the Region of Varna about the the edible wild mushrooms is satisfactory.

The results from an inquiry investigation concerning ecology issues by means of 695 semi-structured interviews among respondents in 38 localities throughout the large mycophylic area of Mazovia, Poland, demonstrate that total of 35 taxa of wild mushrooms are mentioned by at least 5 respondents (M. A. Kotowski *et al*., 2021). A total of 366 respondents (52,66% of the cases) notice a steady decrease in local macrofungi abundance in this area that is due mainly to drought. Imleria badia is the only species with increased abundance as noted by fifteen independent respondents.

The review of publications about the biodiversity of 275 native medicinal species traditionally used with medical purposes in South Tyrol, Northern Italy, Southern Alps, identifies only three wild mushroom species (- Fomitopsis betulina and Fomitopsis officinalis, the family of Fomitopsidaceae belong to while a third one, Auricularia auricula-judae from the family of Auriculariaceae (J. Petelka *et al*., 2020). Although the ethnobotanical richness of this region is among the highest in Italy and throughout the Alps, recently, traditional knowledge of wild mushrooms is seriously decreased.

The dependence between 580 students' knowledge of mushrooms, on the one hand, and the place of origin, frequency of participation in mushrooming, preferred sources of information about mushrooms and self-competence in discriminating the mushrooms, on the other hand, in three university schools in Poland is determined (P. Chwaluk *et al*., 2012). [7]. Residents of large cities have to a greater extent difficulties than those from small towns and villages in distinguishing between the edible and toxic wild mushrooms. Parents are the primary source of knowledge on mushrooms for most students.

Interviews dealing with local systematics and free listings of wild edible and toxic mushrooms are performed in 133 Tsotsil people from seven communities in the highlands of Chiapas, Mexico (F. Ruan-Soto, 2018). The differences in their knowledge relating to different sociodemographic subsets such as gender, education and occupation are examined. Twenty-five edible and 15 toxic taxa are mentioned as only 62% of the respondents name toxic mushrooms, mainly *A. muscaria*, *Suillellus luridus* and *Russula emetica*. There are Some significant differences between men and women concerning the number of times toxic ethnotaxa are mentioned aer established. Education and occupation are most important for people to know more or less mushroom species.

During 13 ethnomycological expeditions and seven requests in two communities residing in La Malintzi National Park, Tlaxcala, Mexico, a total of 178 wild mushroom species are recognized as non-edible by locals inhabitants which corresponds to 103 species belonging to 45 genera (A. Ramírez-Terrazo *et al*., 2021).

Non-edible species are seen as a cosmogonic counterpart (‘twins’) of the edible species that they resemble. A total of 101 specific recognition criteria are obtained useful only when comparing paired edible versus non-edible species.

5.4. An analysis of the usage of the wild mushrooms

The positive respondents’ attitude towards the usage of the wild mushrooms prevails - in 57,50% of the cases. The difference between the positive and negative respondents’ attitude is the greatest in the age group between 41 and 50 years (by four times) followed by those in the age groups between 51 and 60 years (by 3,70 times) and between 61 and 70 years (by 2,27 times). The respondents who use the mushrooms for food several times during the year prevail. The respondents in the age group between 61 and 70 years who do not use the mushrooms for food at all, are 20 (25,32% of the cases). Only 17 respondents (8,50% of the cases), of whom, four men and 13 women report that they make use edible wild mushrooms in their everyday life.

A statistically reliable correlation dependence between male gender, on the one hand, and usage of edible wild mushrooms for food, on the other hand, се статистически достоверна корелационна зависимост между мъжкия пол, is observed (Pearson’s coefficient $\chi^2 = 13{,}261$; $p<0{,}001$).

The respondents prepare fresh mushrooms for food in four main manners such as cooking, drying, preservation and freezing. Cooking is preferred not only by males and females but also by all the respondents. Drying is practised only by 22% of females, 32% of males and 27% of all the respondents. A total of 42% of males, 39% of females and 40,50% of all the respondents report that they preserve fresh mushrooms. Only 10% of male respondents, 14% of female ones and 12% of all the respondents make use of fresh mushroom freezing.

Men gather mushrooms for selling, they purchase edible wild mushrooms from specialized trade stations and deliver the gathered mushrooms in these stations more frequently than women. For all that, mushroom delivery in the trade stations is avery seldom practice (in 13,50% of the cses only).

The gathering of the edible wild mushrooms is most intensive in spring, weaker in autumn and only episodic in summer. Men are much more active gatherer of mushrooms than women. The respondents acquire the edible wild mushrooms most commonly through independent gathering in the surroundings but more rarely through receiving from their acquaintances who have gathered the mushrooms, znd most rarely through purchasing from the trade network.

Only 31% of males, 25% of females and 28% of all the respondents share that mycotherapy finds an application in their everyday practice. The ratio between the

positive and negative answers amounts to 1:2,23 in males, 1:3 in females and 1:2,57 in all the respondents as a whole.

Only 12 women and two men (7% of all the cases) male use of the edible wild mushrooms for therapeutic purposes. There exists a statistically reliable correlation dependence between female gender, on the one hand, and usage of these mushrooms for treatment, on the other hand (Pearson's coefficient χ^2 = 7,680; p=0,006; exact Fisher's test p<0,05).

The positive attitude towards the mushrooms prevails over the negative one among the respondents with secondary on the average (in 50% versus 3%), with elementary (in 33% versus 1%), with higher education (in 32% versus 24%) and in all the respondents as a whole (in 57,50% versus 19% of the cases). The much more seldom negative attitudes towards the mushrooms among the subjects with secondary education (by 3,85 times)and particularly among those with elementary one stress, indeed.

There is a statistically reliable correlation dependence between respondents' secondary education, on the one hand, and their positive attitude towards the edible wild mushrooms, on the other hand (Pearson's coefficient χ^2 = 28,795; p<0,0001).

Cooking of fresh mushrooms is preferred by the respondents with elementary and secondary education. Drying of fresh mushrooms is used by 25,32% of the subjects with secondary, 20,24% of those with higher, 45,95% of those with elementary education and 27% of all the resoondents. Preservation of fresh mushrooms is not preferred neither by all the respondents as a whole, nor by those with secondary and, particularly, by those with higher education.Most respondent with elementary education preserve fresh mushrooms.

Freezing of the fresh edible wild mushrooms is still a more seldom practice - by only 24 of all the respondents as a whole (by 12% of the cases). Their negative answers considerably prevail over the positive ones among the subjects with elementary education (by 2,36 times), among those with secondary education (by 10,29 times) and among those with higher education (by 13 times).

We establish the following statistically significant correlations between respondents' secondary education, on the one hand, and the different manners of preparation of the edible wild mushrooms, on the other hand: cooking (Pearson's coefficient χ^2=26,864; p<0,0001), drying (Pearson's coefficient χ^2=8,800; p=0,012), preservation (Pearson's coefficient χ^2 = 15,185; p<0,001) and freezing (Pearson's coefficient χ^2 = 13,628; p=0,001).

Only 10 respondents with secondary education (12,66%), three with elementary education (8,11%), one with higher education (1,19%) and a total of 14 respondents (a total of 7% of the cases) make use of the edible wild mushrooms for therapeutic purposes. A statistically significant correlation dependence between

respondents' secondary education, on the one hand, and usage of the edible wild mushrooms for treatment, on the other hand (Pearson's coefficient χ^2 = 8,310; p=0,016) is observed.

A statistically significant correlation dependence between urban residence, on the one hand, and traditional usage of the edible wild mushrooms, on the other hand (Pearson's coefficient χ^2 = 18,554; p<0,0001) is proved.

The inhabitants of the villages who cook the fresh edible wild mushrooms are by 3,4 more than those who do not cook the mushrooms. The number of the negative answers by the respondents in the towns who dry the mushrooms is by 5,59 times smaller and that of the respondents in the villages is by 1,38 times greater than that of the positive answers. The number of the inhabitants of the towns who preserve the mushrooms is by 1,95 smaller than that of the inhabitants who preserve them, while among the inhabitants of the villages this difference is very small (43 versus 45). The negative responses prevail amomg all the respondents as a whole (in 59,50% of the cases).

We establish a total of 74 negative answers to the question about the preservation of the fresh edible wild mushrooms among the inhabitants of the towns. This number is by 1,95 times greater than that of the positive answers. The number of the negative answers to the same question is by a little bit greater than that of the positive answers (45 versus 43). The total number and relative share of the negative answers among all the respondents prevail (119 or 59,50% of the cases). Freezing of the mushrooms is used much more seldom by the the inhabitants of the towns (by 17,67 times), of the villages (by 3,89 times) and by all the respondents as a whole (by 7,33 times).

The following statistically significant correlations between urban residence, on the one hand, and the different manners of preparation of the edible wild mushrooms, on the other hand, are observed: cooking (Pearson's coefficient χ^2= 21,690; p<0,0001; exact Fisher's test p<0,0001), drying (Pearson's coefficient 18,048; p<0,0001; exact Fisher's test p<0,0001), preservation (Pearson's coefficient χ^2 = = 4,562; p=0,033; exact Fisher's test p<0,023) and freezing (Pearson's coefficient χ^2 = 10,637; p=0,001; exact Fisher's test p<0,001).

The number of the negative answers concerning the application of mycotherapy in practice considerabky prevails over that of the positive ones among the inhabitants of the villages (by three times), of the towns (by 2,94 times) and of all the respondents as a whole (by 2,57 times). The edible wild mushrooms are used very seldom for therapeutic purposes by the inhabitants of the towns (by 5,36% only), of the villages (by 9,09% only) and all the respondents as a whole (by 7% of the cases only).

Wild edible mushrooms have been collected and consumed by human groups for centuries, and today they represent a relevant source of food and income for many rural families worldwide (C. Ruiz-Almenara et al., 2019).

During the twentieth century, the Swedish urban middle class accepts mushrooms as food and is closely followed by the working-class people as the chanterelle, Cantharellus cibarius Fr., is the most popular species (I. Svanberg & H. Lindh, 2019). During the last 100 to 140 years, Sweden has changed from a mycophobic to a mycophilic society with a passionate interest in the utilization of wild mushrooms. The so-called hipster-generation, born in the 1990s, harvests mushrooms due to the interest in producing the own food and often uses social media to identify edible species.

The results from the ethnomycological study of the population in Menge District, Asossa Zone, Benshangul Gumuz Region, Ethiopia, using semi-structured interviews, focus group discussions, participant's observations and walk-in-the-woods demonstrate that 15 wild edible mushroom speciesare identified (R. Sitotaw *et al*., 2020). Their collection habits and practice are statistically significantly influenced by respondents' gender, age, and educational level ($p<0.05$).

The results from an investigation in areas regularly used or not for mushroom harvesting in the Mixteca region of Oaxaca, Mexico indicate that intensive harvesting of wild edible mushrooms does not affect the diversity and distribution of macrofungal species (C. Ruiz-Almenara *et al*., 2019).

A total 748 respondents belonging to 13 ethnic groups in the main and highest protected areas of Côte d'Ivoire are interviewed about uses of wild edible mushrooms (B. Soro *et al*., 2019). Sixty-eight useful wild fungal species belonging to 17 families and 23 genera are listed and collected. Rural people report four categories of usage such as food, medicinal, belief and recreational. Usage for food (56 species) and as medicines (16 species) prevails. These uses vary not only between sociolinguistic groups but also between visited villages. The most commonly used mushrooms are *Auricularia spp*., *Psathyrella tuberculata* and *Termitomyces spp*.

The traditional usages and relevant selling practices of edible wild mushrooms by Maya inhabitants of the municipality of San Juan Sacatepéquez in Kaqchikel area in the central highlands of Guatemala are analyzed and the diversity and composition of the mushroom assemblage offered at the market with the macrofungal diversity of woods in this area are compared by means of focused interviews with collectors and vendors (J. P. Mérida Ponce *et al*., 2019). There is a significant richness of species sold in the market, a network of commerce of purchase, sale, and resale of several species, with relatively stable prices. Selling mushrooms business in the market is an exclusive activity of women supplied by collectors or by other vendors. Fungi are

sold and bought only as food. Recently, several species of *Amanita, Cantharellus*, *Boletus*, *Lactarius*, and *Russula* are most commercialized.

During ethnobiological tours, semi-structured interviews with 37 individuals from different communities in the municipality of Villa Guerrero in Northern Jalisco, Mexico, about edible mushrooms are carried out (M. X. Haro-Luna *et al*., 2019). Traditional uses of mushrooms and related practices are assessed. There are records of 37 mushroom species with edible, medicinal, and recreational uses. Some toxic mushrooms are associated with the divine as evidenced by one case of their use as a hierophanic agent. There is a loss in practices concerning mushrooms as a result of social changes.

The uses of wild mushrooms for food and medicine in three rural areas of Sweden, Ukraine and the Russian Federation are studied by means of a total of 205 in-depth semi-structured interviews (N. Stryamets *et al*., 2015). These regions are the following: Småland (South Sweden), Roztochya (Western Ukraine) and Kortkeros (Komi Republic in North West Russia). Most respondents in Småland and all respondents in Roztochya and Kortkeros collect wild mushrooms. In Småland, some respondents make preserves from mushrooms for personal consumption during winter times. Chanterelles are frozen and consumed during winter time and funnel chanterelle are used dried for stews and sauces while most respondents collect chanterelle and funnel chanterelle once or twice per year for immediate cooking. In Ukraine and Russia, *Boletus* species are dried or marinated (pickled) and considered a delicacy. *Armillaria spp*. is mainly used marinated while *Russula spp*. is used only fresh for immediate cooking. Due to the availability of freezers in Ukraine and Russia, deep-freezing mushrooms is recently preferred over marinating as a preservation technique for winter times. In Russia, *Lactarius spp*. is most commonly salted and pickled. It is a delicacy and a part of rural cuisine for centuries. In these economically less developed rural areas of Ukraine and Russia, wild mushrooms continue to be an important part of livelihoods as a source of income and for domestic use as food and medicine while in Sweden, their collection has become mainly a recreational activity (N. Stryamets *et al*., 2015).

6. ЗАКЛЮЧЕНИЕ

В рамките на настоящата дисертационна разработка, посветена на социално-значими проблеми, ние успяхме да открoим редица интересни особености както на динамиката на социалната епидемиология на интоксикациите с диворастящите гъби на територията на Област Варна, на степента на информираност на населението за ядливите и отровни диворастящите гъби и на основните начини на употреба на ядливите

диворастящите гъби - предимно за храна. Ние апробирахме за първи път у нас използването на лесно изпълнимите диагностични методи в случаите на съмнение за отравяне с диворастящи гъби - теста на Meixner и ELISA, с които надеждно се откриват аматоксини в стомашното съдържимо и набраните гъби, респ. в урината на болните, консумирали A. phalloides. Ранното и прецизно диагностициране на тази интоксикация играе решаваща роля при избора на своевременното адекватно лечение на тези болни.

През периода между 1991 г. и 2015 г. в Клиниката за интензивно лечение на остри отравяния и токсикоалергии на ВМА-МБАЛ-Варна са хоспитализирани 147 болни на средна възраст от 52,54±15,27 г., 91 мъже и 56 жени, с диагностицирано отравяне с A. phalloides. Налице са различия между мъжете и жените по отношение на годишната динамика и сезонността на тези интоксикации. Смъртността през целия период е по-висока при жените (23,21%), отколкото при мъжете (13,19%), а общата смъртност е 17,00%.

В хода на анкетното проучване на общо 200 души, 100 мъже и 100 жени, живеещи в Област Варна, се идентифицираха редица съществени особености на познанията на населението за диворастящите гъби и за начините на употреба на тези гъби в ежедневната практика. Установиха се известни различия както между анкетираните мъже и жени в отделните възрастови групи, така и между лицата със средно, основно и висше образование и между жителите на градовете и селата.

Степента на информираност за диворастящите гъби на респондентите е задоволителна. Знанията им за микотерапията са оскъдни. Необходимо е по-нататъшно обучение на населението с цел предотвратяването на нежеланите отравяния с тези гъби. Употребата на ядливите диворастящи гъби от населението в Област Варна е разнообразна и отговаря на начина на хранене и живот. Може още да се желае по отношение на приложението на замразяването на пресните гъби и използването им за лечение.

7. ИЗВОДИ

1. В хода на ретроспективното проучване на 147 болни с отравяне с A. phalloides през периода между 1991 г. и 2015 г. се установява сравнително висока смъртност - от 17,00% (25 летални случая).

2. Апробираното от нас лабораторно изследване на стомашното съдържимо и набраните гъби с помощта на теста на Meixner допринася за бързото диагностициране на отравянето с диворастящи гъби.

3. Апробираното от нас изследване ELISA на урината подпомага бързото диагностициране на отравянето с диворастящи гъби.

4. Ранното диагностициране на тази интоксикация с помощта на двата лесно изпълними и надеждни лабораторни метода играе важна роля при избора на консервативно лечение на болните.

5. Степента на информираност на анкетираните лица от населението в Област Варна за ядливите диворастящи гъби е задоволителна и зависи от пола, нивото на образование и местоживеенето им.

6. Налице е разнообразна употреба на ядливите диворастящи гъби от населението в Област Варна, която се различава между мъжете и жените, жителите на градовете и селата и лицата със средно, основно и висше образование.

8. ПРИНОСИ НА ДИСЕРТАЦИОННИЯ ТРУД

Оригинални научно-приложни приноси

1. За първи път у нас е апробиран тестът на Meixner за откриване на аматоксини в стомашното съдържимо и набраните гъби.

2. За първи път у нас е апробирано изследването ELISA за откриване на аматоксини в урината.

3. За първи път у нас е проведено анкетно проучване върху информираността на населението за диворастящите гъби и употребата им.

Приноси с потвърдителен характер

1. Потвърдено е социално-медицинското значение на интоксикациите с диворастящи гъби.

2. Потвърдена е разнообразната употреба на ядливите диворастящи гъби от населението.

3. Потвърдена е необходимостта от ефективна профилактика на отравянията с диворастящи гъби.

9. СПИСЪК НА ПУБЛИКАЦИИТЕ, СВЪРЗАНИ С ДИСЕРТАЦИОННИЯ ТРУД

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3. Marinov P, Bonchev G, Ivanov D, Zlateva S, **Dimitrova Ts**, Georgiev K. Mushrooms intoxications. J IMAB. 2018;24(1):1887-1890.
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Приложение 1.

Анкетна карта

1. Отбележете Вашия пол
   О Мъж О Жена

2. В коя възрастова категория попадате?
   О от 21 до 30 год. О от 41 до 50 год. О от 61 до 70 год.
   О от 31 до 40 год. О от 51 до 60 год. О над 70 год.

3. Какво образование имате?
   О средно О основно О висше

4. Къде сте живели най-дълго време
   О в град О в село

5. Какво е отношението Ви към употребата на диворастящи гъби за храна?
   - не използвам
   - положително
   - отрицателно, защото са страхувам

6. Използвате ли диворастящи гъби за друго, освен за храна?

   О Да О Не О Изобщо не използвам

7. Ако отгпворът Ви е Да - за какво?

8. Доверявате ли се на хора, приготвили диворастящи гъби ?

О Да О Не

9. Колко често ядете диворастящи гъби ?
   - В годината по няколко пъти
   - Веднъж годишно
   - Много по-рядко
   - Изобщо не използвам

10. Как се сдобивате с диворастящи гъби?
    - събирам
    - мои познати събират
    - купувам
    - не консумирам
11. Застрашени ли са гъбите в местната околна среда и какви са тези заплахи?

О Да О Не

12. Има ли гъби, които все по-трудно могат да се намерят във Вашата околност през последните 10 години?

О Да О Не О Нямам мнение

13. Знаете ли как да различите гъбно отравяне?

О Да О Не

14. Чували ли сте в мястото, където живеете, за случай на отравяне с диворастящи гъби?

О Да О Не

15. За колко смъртни случаи след отравяне с гъби знаете?

16. Как приготвяте (готвите, консервирате, сушите, замразявате и др.) набраните гъби?

17. Имате ли самочувствие, че познавате диворастящите гъби?

О Да О Не

18. Кои гъби разпознавате, моля Ви, избройте ги.

19. От къде сте придобили знанията си относно диворастящите гъби?

20. Дали някой от Вашето семейство или Ваши познати са събирали диворастящи гъби с цел продажба?

О Да О Не

21. Предавали ли сте диворастящи гъби в пунктове за събирането им?

О Да О Не

22. Купували ли сте диворастящи гъби от пунктове за събирането им?

О Да О Не

23. Чували ли сте за някаква форма на обучение относно разпознаването на диворастящите гъби?

О Да О Не

Ако отговорът Ви е Да - какво:

24. Виждали ли сте отровни гъби (за които Вие смятате, че са такива) и къде?

О Да О Не

25. През коя част на годината събирате гъби?

О Пролет О Лято О Есен

26. Знаете ли какво е микотерапия ?

О Да О Не

27. Използвате ли гъби за лечение на различни заболявания и ако Да - на какви ?

О Да О Не