



*University of Plovdiv "Paisii Hilendarski"*

*Faculty of Biology*

*Department of Developmental Biology*



## **Annotation of the materials under art. 65 (1) of the Regulation for development of academic staff of University of Plovdiv "Paisii Hilendarski"**

**OF CHIEF ASSIST. PROF. PENKA LAZAROVA VASILEVA, PhD**

**Participant in the competition for the academic position "Associate professor" in the  
field of higher education 4. Natural Science, Mathematics and Informatics;  
Professional field 4.3. Biological science (Genetics – General and evolutionary genetics)  
in Department of Developmental Biology  
announced in the State Gazette, issue 98/19.11.2024**

I have been teaching at University of Plovdiv "Paisii Hilendarski" since 2010 and I am a part of the staff of the Department of Developmental Biology. I took the academic position of "Chief Assistant Professor" in 2011. I received my PhD in 2007.

For my participation in the competition for the academic position "Associate Professor" in the field of higher education 4. Natural sciences, mathematics and informatics; professional field 4.3. Biological Sciences (Genetics - general and evolutionary Genetics) in the Department of Developmental Biology of the Faculty of Biology I present:

- Habilitation thesis – monograph
- 17 scientific articles, that have not been used in the procedures for obtaining the educational and scientific degree "PhD" and for the academic position "Chief Assistant Professor", which are published in journals, referenced and indexed in world-renowned databases of scientific information (Web of Science и Scopus). One of them was published in Journal with Quartile 1, six - in journals with Quartile 3 and ten - in journals with Quartile 4. They are co-authored in English.

## I. HABILITATION THESIS

Vasileva, P., 2025. *Genetics in contribution of informed nutrition*, Rakursi Ltd Publishing house, ISBN 978-619-7599-29-9, c. 135.

**Abstract:** The scientific monograph provides up-to-date literature data, original scientific results, analyzes and discussions regarding the effect of nutritional supplements on dividing cells of a model genetic object, plant test system *Allium cepa in vivo*, subjected to the action of different concentrations of selected colorants, preservatives and sweeteners.

Due to the application of organic and synthetic additives in the production of food, medicine and cosmetics, some of which are used daily, the scientific information presented in the monograph, based on experimental work, comparative analysis and overview of results, could enrich the knowledge of a wide range of readers.

It aims to familiarize the public with one of the significant aspects of healthy eating, namely food safety, based on awareness of the usefulness and potential risk of additives.

The monograph is developed in two parts – the first covers a comprehensive literature review with data on the effects of food additives on living organisms, the second provides results of scientific experiments and a discussion on the general toxicity, cytotoxicity and genotoxicity of the colors tartrazine, sunset yellow and carmoisine, the preservative sodium benzoate and the sweetener aspartame used in food production, which improve their final appearance, facilitate storage, are necessary as protection against the possible development of harmful bacteria and other factors from the production process.

In conclusion of the research conducted and the analysis of the obtained results, a summary can be made that the studied additives applied in the production of food products have a toxic effect on the cells of the root meristem of *Allium cepa*, expressed in the suppression of root growth.

Tartrazine and sunset yellow do not affect the intensity of cell division, and carmoisine, sodium benzoate and aspartame have a cytostatic effect, as they lead to a decrease in the mitotic index. The studied food additives have a specific genotoxic effect. By anaphase method and micronucleus mutagenicity test, all tested substances were found to induce chromosomal abnormalities of the types: C-mitosis, "wandering" and lagging chromosomes, fragments and micronuclei. Sodium benzoate and aspartame provoke the formation of chromosome bridges and diagonal anaphases, and the sweetener aspartame causes the formation of pulverized chromosomes and multipolar metaphases and anaphases.

The need for placement of additives in the food industry is increasing due to the economic benefits they bring - easy processing of food products, consistent quality and longer expiration date. In this regard, food science and food technology have developed rapidly in recent decades, leading to an increasing variety of nutritional supplements. The debate about whether they are safe to use or hide negatives for the human organism is unceasing, because of the available controversies and discussions about the benefits and risks they bring to public health.

The use of nutritional supplements should not lead to an adverse effect upon consumption or regular use. The amount added should be within safe limits for humans and follow the agreed standard specifications and regulations defined in international law. Even when approved,

supplements must be constantly monitored by the expert bodies that society expects to monitor their proper use, labeling and entry into registries of permitted and safe ingredients.

Whether natural or synthetic, food additives have the same function when added to food processing.

A permanent solution to the issue of the importance of food additives is the stimulation of additional scientific research and the use of the obtained reliable evidence to update regulations and legislation related to food processing and storage, which would contribute to the objective assessment regarding the potential of unused resources for the production of natural food additives.

The scientific monograph also includes the results of the scientific research project SP 19 BF-011: "Transfer of knowledge along the axis "science - practice" through the development and promotion of model cases in genetics", led by chief assist. prof. Penka Vasileva, PhD, funded by the Scientific Research Fund of Plovdiv University "Paisiy Hilendarski".

## **II. SCIENTIFIC PUBLICATIONS IN JOURNALS, REFERENCED AND INDEXED IN WORLD-RENOWNED DATABASES OF SCIENTIFIC INFORMATION (WEB OF SCIENCE II SCOPUS).**

1. Mitkovska, V., Chassovnikarova, T., **Vasileva, P.**, Stoyanov, I., Petrov, P., Petkov, N., Ivanova, E. 2025. Sperm comet assay as a novel tool in assessing genotoxicity in high-mortality honey bee (*Apis mellifera*) populations. *Apidologie* 56, 13. Q1 (2023), SJR (2023) – 0.727, IF (2023) – 2.4.

**Abstract:** The recent increase in mortality rates amongst honey bee colonies is a cause for concern. Assessing DNA damage in reproductive cells is crucial for species survival. This study aims to evaluate the potential of the sperm comet assay as a tool for in situ assessment of the genotoxic impact on honey bee populations with established high mortality rates. Previous studies have identified the presence of pesticide residues in bees and food stocks within the hives, indicating the existence of genotoxic agents in the localities under investigation.

The current study demonstrated for the first time that the sperm comet assay could serve as a reliable novel method for assessing genotoxicity in *A. mellifera*.

The comet assay results indicate significant levels of DNA damage in spermatozoa and hemolymph in samples collected from the bee populations with registered high levels of colony losses, suggesting that environmental genotoxic agents may be responsible for impaired sperm DNA integrity. The lack of DNA repair in mature spermatozoa could lead to a permanent reduction in the reproductive potential of bee colonies. The sperm comet assay parameters are suitable and reliable biomarkers for assessing the degree of DNA damage in honey bees and could be used in a number of contexts, including colony

productivity, toxicology, biomonitoring in the ecology risk assessment and biodiversity preservation and conservation of *A. mellifera* worldwide.

2. Vasileva, P., Popova, T., Stoyanov, I., Staykova, T., Ivanova, E.N., Stoyanova, S., Velcheva, I., Yancheva, V., Georgieva, E. 2024. The mutagenic potential of pesticides Actellic, Rival, Aminopielik and polybrominated diphenyl ethers in common carp (*Cyprinus carpio* Linnaeus, 1785). *Acta Zoologica Bulgarica*, Supplement 20, 27-36. Q4 (2023), SJR(2023) 0.216, IF(2023) 0.4.

**Abstract:** The study aimed to investigate, under laboratory conditions, the potential mutagenic effects of the pesticides Actellic (pirimiphos-methyl), Rival (propamocarb hydrochloride), and Aminopielik (2,4-dichlorophenoxyacetic acid), as well as the organic contaminants polybrominated diphenyl ethers (PBDEs), in common carp, *Cyprinus carpio*, which was selected as a test organism. The fish were exposed to two selected concentrations of the studied chemicals, as follows: Actellic (10 µg/l, 60 µg/l), Rival (40 µg/l, 80 µg/l), and Aminopielik (50 µg/l, 100 µg/l), and PBDEs according to their maximum allowable concentrations (MAC) of 0.14 µg/l in water and 0.0085 µg/kg in biota. The micronucleus assay was performed to evaluate the genomic DNA damage in the fish erythrocytes. The results showed micronuclei formation and other nuclear abnormalities in the fish erythrocytes, such as lobbed, blebbed, and notched nuclei in the exposed fish compared to the control group. The highest percentages of erythrocytes with DNA damage were observed after treatment with Rival (80 µg/l) and Aminopielik (100 µg/l). The exposure to Actellic (10 µg/l) and PBDEs also revealed mutagenic effects but at lower values.

The common carp, a species of significant economic importance in Bulgaria, is also a bioaccumulative species that feeds on benthic organisms. Therefore, it should continue to be used in additional studies employing other toxicological approaches.

3. Popova, T., Georgieva, D., **Vasileva, P.**, Stoyanov, I., Staykova, T., Ivanova, E.N. 2024. Concerning the potential toxic effect of Coumaphos and Oxalic acid applied in beekeeping. *Acta Zoologica Bulgarica*, Supplement 20, 79-86. Q4 (2023), SJR (2023) - 0.216, IF (2023) - 0.4.

**Abstract:** The potential mutagenic and cytotoxic effects of the beekeeping insecticide Coumaphos and oxalic acid in *Allium cepa* test object were investigated. The results showed that Coumaphos in the concentration recommended for agricultural practice has a cytotoxic effect and acts as a suppressor and blocker of seed germination.

The insecticide Coumaphos, used in beekeeping against *Varroa*, in concentration 0.06%, stopped *Allium cepa* seed germination and a 10 times lower concentration of the chemical significantly reduced the germination process. Consequently, Coumaphos possess a clear cytotoxic effect. The lower concentrations (10 and 20 times lower) have mutagenic effects on *Allium cepa* apical root meristem and cause aberrations as micronuclei, anaphase/telophase bridges, and acentric fragments/chromosomes. Oxalic acid, an alternative organic method against varroaosis, significantly reduced *Allium cepa* seed

germination when applied in the concentration used in beekeeping. The lower tested concentrations reveal a mitostatic effect and a low mutagenic effect expressed as micronuclei formation. Given that Coumaphos residues have been found in samples of food stocks in some of the studied bee colonies, investigations of its toxicity should continue in situ as well.

4. Stoyanov, I., **Vasileva, P.**, Staykova, T., Popova, T., Ivanova, E.N. 2023. Cytogenetic effects of the neonicotinoid insecticides Nuprid 200 SL and Calypso 480 SC on plant model system. OBM Genetics, 7 (4), 1-11. ISSN 2577-5790. Q4, SJR - 0.16.

**Abstract:** Data from the study indicate that the insecticides Nuprid 200 SL (with the active substance imidacloprid) and Calypso 480 SC (with the active substance thiacloprid) have a cytostatic effect by inhibiting cell division from the root apical meristem of *Allium cepa*. The comparative analysis of the mutagenic action of the studied pesticides shows a higher genotoxic potential of Calypso 480 SC. Stock solutions of both pesticides induced chromosomal abnormalities in *Allium cepa* cells with a much higher frequency than the control, but the chromosomal aberration index for Calypso 480 SC ( $1.07 \pm 0.38\%$ ) exceeded by value the index for Nuprid 200 SL ( $0.70 \pm 0.41\%$ ). The tested solutions of pesticides cause a wide range of anomalies associated with disorders in forming the cell's division apparatus and the integrity of chromosomes.

The direct toxicity of neonicotinoids to environmental organisms requires an assessment of their long-term effects on ecosystems. The mitosodepressant effect we have reported, the appearance of a large number of chromosomal aberrations and mitotic abnormalities in *Allium cepa* cells when treated with the neonicotinoid insecticides Nuprid 200 SL and Calypso 480 SC is evidence of their high toxic potential and the significant risk posed by their use in agriculture from environmental pollution.

5. Stoyanov I., **Vasileva P.**, Staykova T., Popova T., Ivanova E. 2022. Concerning the genotoxic potential of pesticides applied in agriculture. Journal of IMAB, vol. 28, Supplement 12 SEEK & 32 IMAB, 65-68. ISSN: 1312 773X. Q4, JCI – 0.05.

**Abstract:** Pesticides, which are widely used in the cultivation of agricultural crops, cause adverse effects on non-target organisms, including human health. These compounds are easily absorbed by different routes and due to their high stability they can be metabolized and stored in different organs. The *Allium test* enables the study of the mutagenic effect of pesticides, based on microscopic recording of chromosomal aberrations and mitotic disorders in onion root meristem cells.

This investigation aims to evaluate the mutagenic effect of the pesticides Nuprid, Calypso, Chlorpyrifos, Aktara, Actellic, Rival, Verita and Raundup on the of *Allium cepa* L. root meristem cells as a model system. Material and methods: The mutagenic effect of the pesticides has been investigated by the usage of anaphase and a micronucleus tests. Analysis of the data from the present study shows that the tested pesticides Nuprid, Calypso, Chlorpyrifos, Aktara, Actellic, Rival, Verita and Raundup have a pronounced genotoxic

effect on the root meristem of *Allium cepa*, as evidenced by the significantly higher frequency of chromosomal aberrations, reported in the experimental variants compared to the control. A positive correlation was found between the concentration of the studied pesticides and the frequency of chromosomal disorders.

The presence of a large number of chromosomal aberrations and mitotic abnormalities found in the *Allium cepa* model system after treating with the studied pesticides is a clear evidence for their high genotoxic potential, for the significant risk of environmental pollution after their applying in the agriculture as well as for the human health.

6. Mitkovska, V., Dzhoglov, S., Boyadzhiev, D., Staykova, T., Popova, T., **Vasileva, P.**, Stoyanov, I., Ivanova, E.N. 2022. Spermatozoa DNA damage in men with reproductive problems. J of IMAB, 28(2): 4511-4516. Q4, JCI – 0.05.

**Abstract:** Male infertility is a serious emerging problem of public health, with high social significance due to its direct connection to negative demographic trends in Bulgaria and in different regions of the world. The targeted and multifaceted studies in this field are contradictory and incomplete. The present study analyzes the degree of spermatozoa DNA damages in men with reproductive problems. The aim of the study was to investigate the spermatozoa DNA damage in men with reproductive problems by complex approach combining comet assay and conventional sperm analysis. Totally 80 individuals have been studied by the usage of the conventional sperm analysis and the comet assay. The impact of environmental and lifestyle factors was analyzed based on completing the participants' voluntary questionnaire. About 21% of the participants among the studied group were undergoing occupational hazards, 22% were smokers, 42% – alcohol consumers, 13% – drug users, 7% taking anabolic steroids, 31% – taking medications and 5% work or live under stress. The results obtained show that about 55% of the subjects studied were with normozoospermia, 19% – with asthenozoospermia, 13% – with oligoasthenozoospermia, 10% – with oligoasthenoteratozoospermia and 3% – with asthenoteratozoospermia. Elevated levels of mean values of comet parameters were observed when the concentration, motility and percentage of cells with normal morphology were decreased ( $p < 0.05$ ).

The statistically significant relationships between the analyzed characteristics could be success fully used for prognostic purposes in characterizing male re productive health. The results of our study also confirmed that some environmental factors affecting negatively sperm structure and function should continue to be a significant public health concern nowadays.

7. Mitkovska, V., Dzhoglov, S., Boyadzhiev, D., **Vasileva, P.**, Stoyanov, I., Popova, T., Staykova, T., Ivanova E.N. 2022. Study on the relationship between male infertility and DNA integrity in spermatozoa nuclei. Comptes Rendus de L'Academie Bulgare des Sciences, 75 (1), 121–128. ISSN: 1310–1331 (Print), 2367–5535 (Online). Q3, SJR 0.182, IF 0.378

**Abstract:** The purpose of the study is to assess the potential relationships between the diagnoses for male infertility based on conventional sperm analysis and the levels of sperm nuclei with damaged DNA in men with reproductive problems using the acridine orange fluorescent test. Totally 87 men with reproductive problems have been investigated. Excellent and good fertility potential was established for 58.6% of the studied individuals, and fair and poor potential of male fertility– for 41.4%. Different diagnoses have been found for the men included in the study– normozoospermia (58.6%), asthenozoospermia (18.4%), oligoasthenozoospermia (11.5%), oligoasthenoteratozoospermia (9.2%) and asthenoteratozoospermia (2.3%). Statistically significant dependencies ( $P < 0001$ ) have been observed between the studied sperm quality parameters, as well as for infertility diagnoses done and the level of spermatozoa nuclei with DNA damage. The present study suggests that the acridine orange fluorescence test is sufficiently informative to determine the quality of the fertility potential.

The obtained results could be used as a basis for future detailed research on male reproductive health and the potential risks for its deterioration.

8. Popova, T., **Vasileva, P.**, Stoyanov, I., Ilieva, I., Staykova, T., Ivanova, E.N. 2021. Cytotoxic and mutagenic effects of pesticides Verita WG and Actara 25 WG on sweet pepper (*Capsicum annuum* L.) and onion (*Allium cepa* L.) Bulgarian Journal of Agricultural Science, 27(3): 569-574. Q3, SJR – 0.25, IF- 0.79.

**Abstract:** The aim of the study is to investigate the potential mutagenic and cytotoxic effects of insecticide Actara and fungi cide Verita in the root meristems of onion (*Allium cepa* L.) and sweet pepper (*Capsicum annuum* L.). Three concentrations of each of these pesticides were investigated (stock solutions used in agricultural practices, and 10- and 20- times lower concentrations). The results showed that the onion and sweet pepper have different sensitivity towards Actara and Verita. The Verita 0.15% solution (recommended for agricultural usage), significantly reduces the pepper seeds germination. Similar effects in pepper seeds were found after treatment with other concentrations of the tested pesticides. In contrast, the treated onion seeds showed overall increased germination. Mitosis-depressive action of both pesticides was found (strongly expressed in onion after Verita 0.15% treatment and pepper after Verita-lowest concentration and Actara 0.02%). Different chromosomal aberrations were observed in the cells: anaphase/telophase bridges, lagging chromosomes and/or chromosome fragments, and micronuclei. Both studied pesticides have a genotoxic effect in the concentrations used in practice as well as in concentration lower than recommended. This indicates that even minimal residual amounts of them, accumulated in the soil, can damage plant chromosomes and could have a real mutagenic effect.

9. Stoyanov, I., **Vasileva, P.**, Staykova, T., Ivanova, V., Popova, T., Ivanova, E. 2020. Mitosis inhibitory and clastogenic effects of waters from anthropogenically affected areas. Journal of Central European Agriculture, 21(3): 667-675. ISSN 1332-9049, Q4, SJR – 0.207, IF – 0.7.

**Abstract:** The study aims to analyse the effect of waters, anthropogenically influenced by various pollutants, on the mitotic division and chromosomal apparatus of cells by establishing their potential mitoinhibitory and clastogenic effect. The cytotoxic and mutagenic effect of contaminated water was examined by the application of the *Allium cepa* test system. Mitotic depression has been established for samples with available anthropogenic contamination. Microscopic analysis showed an increased incidence of chromosomal aberrations in the test samples compared to the control, resulting from the genotoxic effect available. Chromosomal abnormalities of the type of lagging and ‘vagrant’ chromosomes, chromosomal fragments, anaphase and telophase bridges, micronuclei, as well as deviations from normal cell division such as C-mitoses and asynchronous mitoses have been observed. The analysis of the spectrum of chromosomal aberrations shows some differences in the frequency of occurrence of the different types of disorders, which reflects the specificity of the genotoxic effect of the water samples from the surveyed areas.

10. Mitkovska, M., Dzhoglov, S., Stoyanov, I., Popova, T., **Vasileva, P.**, Staykova, T., Ivanova, E. N. 2019. Genomic and chromosome mutations in complex with environmental and lifestyle factors as reasons for azoospermia and oligoasthenoteratozoospermia. *Ecologia Balkanica*, 11(2): 73–77. Q4, SJR – 0.137.

**Abstract.** The aim of the study was to investigate and characterize the manifestation of genomic and chromosomal mutations in complex with the environmental and lifestyle factors as reasons for azoospermia and oligoasthenoteratozoospermia by applying a classical cytogenetic assay and questionnaire survey. 1540 men were included in the survey. By conventional sperm analysis, 183 of them were diagnosed with azoospermia and oligoasthenoteratozoospermia. Based on the cytogenetic analysis, it was concluded that trisomies, in particular Klinefelter syndrome, and structural chromosome aberrations such as translocations and chromatid fragmentation, are directly related to male infertility. Together with harmful habits such as smoking and alcohol use, they are among the major causes of azoospermia and oligoasthenoteratozoospermia. The results obtained could be successfully used in the implementation of a system of activities for the prophylaxis of male reproductive health.

11. Staykova, T., Tzenov, P., Vasileva, Y., Azis, S., Ivanova, E. N., Stoyanov, I., Vasileva, P., Popova, T. 2020. Genetic characterization of silkworm (*Bombyx mori* L.) strains (*lepidoptera: Bombycidae*) with different geographical origin on the basis of isozyme markers. *Acta Scientiarum - Biological Sciences*, 42, Article number e47970: 1-9. Q4, SJR 0.193, IF – 0.674.

**Abstract:** The aim of the study was to assess genetics variations within and among strains with different geographical origin, belonging to Bulgarian germplasm bank of mulberry silkworm (*Bombyx mori* L.) and to establish their relationships using isoenzyme markers. Polyacrilamide gel electrophoresis (PAGE) was used to study the isoenzymes of nonspecific esterases (EST), malate dehydrogenase (MDH) and acid phosphatase (ACP) from



haemolymph, phosphoglucomutase (PGM) and hexokinase (HK) from silk glands and alkaline phosphatase (ALP) from midgut of mulberry silkworm (*Bombyx mori* L.). Variability was evident in all of these enzyme systems among twelve strains from Bulgarian germplasm resources of *B. mori*. Total of nine loci were detected. All of them (100%) were polymorphic. “Null” alleles in four loci were determined. Intra- and inter-strain polymorphism was obtained. The degree of polymorphism ranged from 0% to 77.8%. Low levels of observed heterozygosity in comparison with the expected one have been calculated in almost all of strains as well as deviations from Hardy-Weinberg equilibrium in almost all analyzed loci, resulting of excess of homozygotes. The value of  $F_{ST}$  was 0.4903. The dendrogram constructed with the values of genetic distance manifests that Romanian strain Cislau Tokay is formed one main clade while the rest strains studied (from Bulgaria, Japan, China, Vietnam, Spain, Syria and Egypt) are formed the other clade with subclades. The genetic data of the tested strains from different geographical regions, would be used for identifying suitable parents for breeding programs with a view to yield improvement in this species with economic significance.

12. Ivanova, E. N., Vulchev, I., Staykova, T., Antov, M., Popova, T., **Vasileva, P.**, Stoyanov, I. 2020. Ontogenetic and Caste Differentiation in the Expression of Water-soluble Proteins and Some Isozymes in *Reticulitermes lucifugus* (Rossi, 1792) (Isoptera: Rhinotermitidae). Acta Zoologica Bulgarica, Supplement 15: 3-10. Q3, SJR-0.213, IF – 0.448.

**Abstract:** Ontogenetic and caste differentiation in the expression of water-soluble proteins, non-specific esterases, malic enzymes, lactate dehydrogenases and superoxide dismutase in the species *Reticulitermes lucifugus*, spread out in Bulgaria has been investigated. A total of 41 loci have been analysed. Stage and caste specific protein and isoenzyme expressions have been established as follows: 11 of the protein loci analysed have been characterized by a permanent activity without varying of the product expression in all of the castes; for seven of the protein loci, a constant action has been found in ontogenesis but with a caste specific variability in their expression; for four of the protein loci analysed differential gene regulation, expressed in gene activity in some of the castes and inactivation in the others has been established; eight of the detected protein loci have demonstrated a ontogenetic and caste-specific expression; four of the sixth esterase loci have been characterized by a permanent action in the course of the ontogenesis; one of the two malic enzyme loci has been expressed only in reproductive castes (neotenics and queen) and the other one has been expressed in all stages of individual development; both lactate dehydrogenase loci have been expressed specifically in ontogenesis, depending on caste differentiation; superoxide dismutase locus has been found in all of the castes and stages of ontogenesis in *R. lucifugus*.

13. Staykova, T., Tzenov, P., Vasileva, Y., Takova, N., Ivanova, E. N., Stoyanov, I., **Vasileva, P.**, Popova, T., Antov, M. 2020. Isoenzyme Polymorphism of Silkworm *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae) Breeds from Germplasm Resources of Bulgaria. Acta Zoologica Bulgarica, Supplement 15: 11-19. Q3, SJR-0.213, IF – 0.448.

**Abstract:** Polyacrilamide gel electrophoresis (PAGE) was used to study the isoenzyme polymorphism of nonspecific esterases (EST), malate dehydrogenase (MDH) and acid phosphatase (ACP) from haemolymph, phosphoglucosmutase (PGM) and hexokinase (HK) from silk glands and alkaline phosphatase (ALP) from midgut of mulberry silkworm (*Bombyx mori* L.). Genetic variations within and among five breeds kept in Bulgaria were found out. A total of seven polymorphic loci with 19 alleles were established. For the AES 1-zebra breed we found variability in all loci, whereas in Hebar 2 only at one of them. The observed polymorphism demonstrated breed specificity. The Bes A0 allele was specific to the AES 1-zebra gene pool, the Bes D3 and Bes D0 alleles were specific for Sh 4 and Mdh A3 was specific only for AES 1-zebra. We also observed inter-breed differences regarding the degree of polymorphism and heterozygosity. The lowest values for both parameters were found in the Bulgarian breed Hebar 2 and the highest for the Spanish breed AES 1-zebra. The breeds were found to form two distinct clusters indicating the presence of distinct genetic diversity among them. Hebar 2 is formed one main cluster, while the rest breeds studied are formed the other one. Inter-breeds differences regarding the degree of polymorphism and heterozygosity were established, too. The degree of polymorphism ranged from 11.1% to 77.8%. Low levels of observed heterozygosity and deviations from Hardy-Weinberg equilibrium in the most of analysed loci were found. The dendrogram constructed with the values of genetic distance showed that Hebar 2 formed one main cluster, while the rest breeds studied formed the other one. The results of the present study complement the data on the genetic heterogeneity of breeds from the silkworm genetic resources of Bulgaria and could help the breeders in the process to identify suitable parents for the breeding programs for yield improvement.

14. Stoyanov, I., **Vasileva, P.**, Popova, T., Slavova, B. 2018. The effects of lead and cadmium on cell division and chromosomal structure in *Allium cepa* test system in vivo. *Ecologia Balkanica*, 10 (2): 73-81. Q4, SJR-0.137.

**Abstract:** The study focuses on the study of the potential mitoinhibiting and genotoxic effect of lead and cadmium in the root meristem of *Allium cepa* L. *in vivo*. A cytotoxic effect of lead and cadmium was registered, as evidenced by the lower mitotic index values in all test concentrations compared to the control. The analysis of the microscopic preparations for the experimental variants of the two heavy metals showed a significant genotoxic effect. All analyzed samples of lead and cadmium demonstrate an increased frequency of chromosomal aberrations compared to the control, as a positive dependence was established between the frequencies reported and the concentration of the studied metals. Lead and cadmium solutions cause a wide range of chromosomal aberrations, varying with the dose of the metal. The most common observed disorders are: acentric fragments, lagging and “vagrant” chromosomes, micronuclei, chromosome bridges, and asynchronous mitosis, demonstrating the genotoxic potential of the studied heavy metals. For both metals a maximum frequency of aberrations for the concentration limit is established. By comparing the two heavy metals, it has been found that lead has a greater cytostatic potential than cadmium by more effectively inhibiting cell division. The results obtained regarding the

frequency of chromosomal aberrations show a higher genotoxic effect of cadmium compared to lead.

15. Stoyanov, I., Staykova, T., **Vasileva, P.**, Ivanova, E. N. 2017. Genetic variability in populations of *Messor barbarus* (Hymenoptera, Formicidae) from Bulgaria based on isoenzyme analysis. Acta Zoologica Bulgarica Supplement 8, 2017: 31-35. Q3, SJR-0.213, IF – 0.369.

**Abstract:** Genetic heterogeneity in 13 populations of *Messor barbarus* (Linnaeus, 1767) (Formicidae) from Bulgaria has been studied using analysis of seven enzyme and protein systems, which have been found to be appropriate genetic markers for characterization of genetic variability within and between populations. Totally, 49 allelic variants were found for the studied loci. A comparative analysis of gene pool and genotypic structure of the tested populations have been carried out. Moderate to high percentage of polymorphism (59.1–86.4) and low levels of heterozygosity have been calculated (0.030–0.066). Deviations from Hardy-Weinberg equilibrium in almost all analyzed loci, in favor of homozygotes, have been recorded. The mean value of inbreeding coefficient (FIS) has been found to be high (0.8212), demonstrating a high level of inbreeding in the studied populations.

Specificity in allelic composition of the tested populations and differences in allele frequencies were identified. A comparative analysis of genotypic structure of the studied populations has been carried out and intraspecific phylogenetic relationships have been studied. The comparative analysis of allele frequencies in the tested populations of *Messor barbarus* revealed differences in their values, thus demonstrating dependence on the territorial location of populations. This can be explained with colonization of close geographical locations and the founder effect.

The obtained data of the genetic variability provide new information concerning polymorphism and phylogenetic relations between the studied populations.

16. **Vasileva, P.**, Hubenova, T., Zaikov, A., Stoyanov I. 2017. Morphometric variability, allometric growth and sexual dimorphism in narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823 (Crustacea: Decapoda) during the ontogenesis. Acta zool bulg. Suppl. 8: 99-106. Q3, SJR-0.213, IF – 0.369.

**Abstract:** A study on the variability of morphometric characteristics of 927 one-summer-old, one-year-old and two summer-old narrow-clawed crayfish, cultivated at the Institute of Fisheries and Aquaculture in Plovdiv was carried out. The weight-length relationships of the two sexes at different age groups were determined using graphic equations. In the equations expressing the relationship between the weight and the length of the body, the exponent  $b$  had values ranging from 2.85 to 3.34, which demonstrate that the crayfish of all age groups have allometric growth. In all age-groups, male narrow-clawed crayfish reach significantly higher average body weight compared to females. Eleven morphometric characters demonstrate the sexual dimorphism of the one-summer-old, one year-old and

two-summer-old narrow-clawed crayfish. The characters with higher values in males are the length and the width of the carapace; the length, the width and the corpulence of the chelae; the length of the dactyl; the length of the chelae from its carpal join to its tip and the length of the palm. The characters with higher values in females are the length and the width of the abdomen and the length and the width of the telson.

17. Stoyanov, I., Staykova, T., Stojanova, A., **Vasileva, P.**, Ivanova, E. N. 2015. Isoenzymic genetic variability in populations of *Messor structor*, (Hymenoptera, Formicidae) from Bulgaria. Acta Zoologica Bulgarica 67 (3), 2015: 337-344. Q4, SJR – 0.237, IF- 0.310.

**Abstract:** Genetic variability in 36 populations of *Messor structor* (Latreille, 1798) from Bulgaria was studied using analysis of two enzyme systems corresponding to four loci (Sod-1, Sod-2, Sod-3 and Me-1). Three of the studied loci were found to be polymorphic. One of them was found to be monomorphic (Sod-3). One to three alleles were detected for the different isozyme systems: one allele at Sod-3 locus (Sod-3<sup>100</sup>), two alleles at Sod-1 (Sod-1<sup>100</sup> and Sod-1<sup>95</sup>) and Sod-2 (Sod-2<sup>100</sup> and Sod-2<sup>97</sup>) loci and three alleles at Me-1 locus (Me-1<sup>100</sup>, Me-1<sup>98</sup> and Me-1<sup>96</sup>). The observed and expected heterozygosities (Ho and He) ranged from 0.0 to 0.111 and from 0.168 to 0.372, respectively. Allele frequencies of all loci were used to estimate Nei's (1972) genetic distance, which was found to range from 0.001 (between Boliarino and Lubenova mahala) to 0.462 (between Chirpan and Merichleri). The estimated mean FIS and FST values from isozyme data were 0.8738 and 0.1432, respectively. The Neighbour-joining method and Unweighted Pair Group Method with Arithmetic Mean phylogenetic trees were obtained using genetic distance matrix methods. Two of the studied populations (Merichleri and Belozem), were grouped separately in dendrograms as compared to all other populations which formed a large cluster consisting of three sub-clusters.

This study provides new information on the genetic variability of harvester ant *Messor structor* from Bulgaria on the basis of isoenzymic analysis.

The isozyme genetic markers indicated in this study could be used appropriately for comparisons, discrimination and characterization of Bulgarian and European *Messor structor* populations.

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