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ORAL PRESENTATIONS

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Energy States and Binding Energies of Hydrogenic Impurity

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Abstract

In this study, the energy states and binding energies of hydrogenic impurity in spherical quantum dot with finite and infinite confinement potential are calculated. Quantum Genetic Algorithm (QGA) and Hartree Fock Roothaan (HFR) method are employed for calculation of energy states and wave functions. We present our results as a function of dot radius and for a several values of Al concentration ratio. The impurity charge, dot radius and potential barrier strongly affect the energy states and binding energy of quantum dot. As dot radius decreases, binding energy increases for the finite confinement potential. After a peak value, it decreases to a limiting value corresponding radius, in which there are no bound states in the well. In large dot radii, impurity energies approach the corresponding energies of a free space hydrogen atom.

Key words: Spherical Quantum dot, binding energy, electronic structure, QGA and HFR methods.

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Effect of Different Animal and Plant Oil Additives on Physicochemical, Mechanical, Antimicrobial and Antioxidant Properties of Chitosan Films

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Abstract

Protective chemicals and synthetic polymers have been used for food packaging with the increase of industrial food consumption. However, the degradation of synthetic polymers is difficult in the nature. Also, non-biodegradable polymers are harmful to human health and causes environmental pollution. So, many researchers have been developing bio-based edible materials such as films and bio-plastics which can be alternatives to the synthetic polymers. Bio-based edible films can be extend the shelf life of the foods without any harmful effects to the human health and can increase the nutritive of the foods. Edible films have been obtained from biological polymers such as gelatin, alginate, cellulose, cutin and chitosan. Among these biopolymers, chitosan which is the second abundant and economic biopolymer in the nature is widely used for film production. Chitosan films protect food from fungal degradation and extend the shelf life of food as they can form an excellent oxygen barrier. Chitosan films have long life and very good mechanical properties. However, when prepared alone, it is not water resistant. For this reason, hydrophobic materials such as essential oils can be added to the chitosan films to increase the water resistant. Antimicrobial, antioxidant activity, physiochemical and mechanical properties of the chitosan films have been significantly changed with the incorporation of the essential oil. However, essential oils obtained from the plants are expensive and its process is difficult. In the present study, to obtain low cost composite films, animal fat, butter, olive oil, corn oil and sunflower oil were used as chitosan film additives for the first time. All the produced films were characterized by FT-IR, DSC, TGA, SEM, mechanical properties, contact angle and optical transmittance. Chitosan blended films were investigated by means of antimicrobial properties against common nine food-borne pathogens by disc diffusion test. The results demonstrated that chitosan edible films contained different fat content could inhibit microorganism's growth significantly in the range of 14.56-29.84 mm. Chitosan films incorporated fat component promise as an effective method for the inhibition of bacteria in foods and which can be used further as the novel non-reactive and green technological antimicrobial packaging materials.

Keywords: chitosan, daily used oil, film, characterization, antimicrobial

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Fauna And Trophic Relationships Of Leaf-Mining Nepticulidae (Insecta: Lepidoptera) Of Middle And South America

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Abstract

Larvae of Nepticulidae are miners of green tissues of plants. Being very small and leading hidden lifestyle, these insects remain insufficiently investigated in many regions. The goal of our study was a taxonomic and trophic assessment of the fauna of Middle and South America in the light of the latest taxonomic and trophic data. The current research possesses a very high degree of novelty. We identified new host-plant data for 114 taxa (including undescribed species) and discovered about 146 previously unknown species. Drawing on newly established diagnostic characters, we revised taxonomic status of a few genera and designated many species groups. Though many Nepticulidae taxa are characterized by high morphological differentiation, we identified complexes of low morphological differentiation. After analyzing trophic relationships, we established that 96% of Nepticulidae of the region under study are trophically associated with Asterids (41.5%) and Rosids (54.8%). Among the specialized Asterid miners, about 57% taxa are associated with Campanulids and 38%, with Lamiids, whereas among the Rosids miners, the majority of taxa (74%) are associated with Fabids and 23%, with Malvids. In total, the Nepticulidae taxa of the Middle and South America are trophically associated with 24 families of host-plants belonging to 16 plant clades. Trophic specialization of the Nepticulidae of Middle and South America exhibits characteristics different from the Holarctic fauna. Nearly 30 Nepticulidae species were discovered at the altitude of more than 3700 m above the sea level and represent the world's highest occurring Nepticulidae fauna (the altitudinal record belongs to *Stigmella nivea*, which was collected in Peru at the altitude of 4700 m above the sea level). Though we identified five groups of relative mining abundance of Nepticulidae in Middle and South America, about 46% of discovered species are characterized by moderately abundant mining and 34%, not abundant mining. Some of the identified species are relevant from the economical point of view as potential pests of cultivated plants, also berry or aromatic plants.

Keywords: fauna, Middle America, Nepticulidae, South America, trophic relationships.

ISSN

Effects of Pesticide Production Waste on Survival, Growth and Fertility of the Great Pond Snail *Lymnaea Stagnalis* L.

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Abstract

To date, a number of aspects of the impact of man-made wastes on aquatic ecosystems have not been adequately studied, while the use of hydrobionts in biotesting practice is expedient and promising. One of the adequate test systems for laboratory biotesting is the freshwater gastropod mollusk – the great pond snail (*Lymnaea stagnalis* L.) This paper presents the results of the assessment of the ecotoxicity of wastes generated at the treatment facilities for the production of plant protection products by LLC "Frondees" in the treatment of chemically contaminated sewage for individuals *L. stagnalis* reared under laboratory conditions using such key indices as survival, growth and total fecundity.

The experiment was carried out under standardized laboratory conditions. The duration of the exposure was 6 months. The age of the mollusks at the beginning and the end of the experiment was 2 and 8 months, respectively, and there were 20 individuals for each of the set waste concentrations. The concentrations of the sample of the test waste in the aqueous solution were: 0.0001, 0.001, 0.0275 and 0.11 mg/ml. The individuals of the control group were incubated in the tap water.

In the course of the exposure period, the mollusks were fed with fresh lettuce leaves ad libitum three times a week. The egg masses were collected weekly as well as the aquatic environment was refreshed, the wet weight of the mollusks and the number of surviving individuals were estimated. The total fecundity was estimated as the ratio of the total number of embryonic capsules produced by the mollusks of the experimental group to the number of individuals of this experimental group during the experiment. Student t-tests were conducted to evaluate the differences between the control and the experiment by the criteria we had chosen using the "Statistica 6.0" program.

As a result of chronic exposure to pesticide production waste at the highest concentrations (0.0275 mg/ml and 0.11 mg/ml), statistically significant growth inhibition and decreased fertility in *L. stagnalis* individuals have been revealed. The obtained results testify to the high sensitivity of the test system used to waste products of pesticide production effects, as well as the prospects of using *L. stagnalis* for laboratory biotesting based on the use of such indicators of ecotoxicity as reduced growth rates and inhibition of the reproductive function.

Keywords: gastropod mollusks, great pond snail (*Lymnaea stagnalis*), pesticide production waste, ecotoxicity, growth, total fecundity, egg masses, embryonic capsules

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Antibiotics and Antibiotic Resistance of Soil Bacteria in Ecosystems

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Abstract

Antibiotics are extensively used for animal farming and agricultural purposes. Residues from human activity and farms may contain antibiotics and antibiotic resistance genes that can contaminate natural environments.

The purpose of this study was to detect the antibiotic resistance soil bacteria in different ecosystems: natural ecosystem (primeval beech forest) and agroecosystems.

A total of 184 isolates from soil of primeval forest and rhizosphere of medicinal plants: M*entha piperita, Inula helenium, Thymus serpillum, Rosa odorata* and *Calendula officinalis* were examined for resistance to 9 antibiotics. Two bacteria *Serratia marcescens* and *Yersinia enterocolitica* isolated from soil with cultivated *Thymus serpillum* have displayed the moderate resistance to most of tested antibiotics. From the rizosphere of *Inula helenium* was isolated one of the most antibiotics resistant bacteria among of others tested - *Pantoea agglomerans*. *Serratia odorifera biogroup 1* was isolated from agroecosystem of *Mentha piperita* and used for tests. Strain was resistant to vancomycin, lincomycin, ampicillin and moderately resistant to cefepime. From agroecosystems of *Calendula officinalis and Rosa odorata,* where used organic manure, high level of antibiotic resistance was detected for *Enterococcus faecalis* and *Bacillus cereus*. The study of soil samples from the primeval beech forests have showed that the microbial community characterized by a low content of antibiotic-resistant microorganisms. Only two strains, *Bacillus cereus* and *Pantoea agglomerans,* among 78 isolated bacteria have demonstrated a high level of resistance to antibiotics.

Conclusion: differences between the levels of antibiotic resistance in soil microbial community of natural ecosystems and agroecosystems have been observed. The populations of soil microorganisms in agroecosystems recognized to be more antibiotic resistant. Two species of antibiotic-resistant bacteria *Bacillus cereus* and *Pantoea agglomerans* were common to both ecosystems. The effect of antibiotics on the biosphere is wide and can impact on the structure, diversity and activity of environmental microbiota.

Keywords: ecosystem, bacteria, antibiotic resistance, soil.

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In Vitro Morphogenetic Studies On Swertia Chirata Buch. Ham Ex Wall.-An Endangered Medicinal Plant

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Abstract

The Indian Himalayan region nestles a plethora of valuable medicinal plants. *Swertia chirata* is one such multipurpose medicinal herb native to temperate Himalaya. Effective use of *S. chirata* has been reported in the indigenous systems of medicines viz Ayurveda, Unani, British and American Pharmacopeias. Widely known to be used in treatment of chronic fever, asthma, stomach and liver disorders, malaria and several other ailments, the plant enjoys an escalating demand in the world market. However, problems in conventional methods of propagation aided by unscientific harvesting practices and lack of commercial plantations have led to the plant being categorized as critically endangered as per IUCN criteria. The problems propel an urgent need for serious studies on establishment and propagation of *S. chirata*.

A study was designed to develop alternate propagation pathways for large scale propagation of the plant. Young shoots of *S. chirata* growing in natural habitat in Uttarakhand state of India were collected. Surface disinfected nodal segments containing axillary buds were cultured aseptically and axillary bud break was obtained on MS medium containing Benzylaminopurine (BAP), Indole acetic acid (IAA) and Gibberellic acid (GA_3). Best results of *in vitro* shoot multiplication were obtained on MS medium with BAP + IAA and adjuvant Adenine sulphate (Ads) with 11.80 fold shoot multiplication in 4 weeks. *In vitro* rooting was achieved on auxin supplemented medium wherein Indole butyric acid (IBA) proved to be most effective in inducing healthy roots (mean number of 35.30 roots in 4 weeks). *In vitro* roots of axenic shoots were further used to initiate callogenesis. MS medium supplemented with BAP and 2, 4-dichlorophenoxyacetic acid exhibited 100% callus development in root segments and subsequent shoot induction in 5 weeks. Adventitious shoots so developed were multiplied on medium supplemented with BAP+ IAA + Ads giving an average of 10.70 shoots in 4 weeks. Individual elongated shoots were rooted on 1/2X MS medium. Maximum rooting (average 14.40 roots in 4 weeks) was obtained on medium containing IBA. Regenerated plantlets were hardened *in vitro* followed by transfer to a rooting mixture of soil: sand: manure (1:1:1) in pots.Well developed plants were subsequently shifted to field conditions and over 85% survival rate was recorded.

Keywords: *Swertia chirata*, organogenesis, axillary bud proliferation, callus, direct organogenesis, *in vitro* rooting, hardening, transplantation

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The Response of Perennial Plants to Seed Stress: Overwiev of Long-term Observations

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In the recent years, the response of plants to seed treatment with electromagnetic field (EMF) or low temperature plasma (cold plasma, CP) was extensively studied and numerous studies reported that improved seed germination and seedling growth can be achieved for a large variety of annual plants. We chose perennial species for investigation with the intention to perform longer-term observations. These are important for several reasons: 1) evaluation of the early effects on plant development, 2) estimation of the sustainability and dynamics of the observed effects, and 3) evaluation of the impact of treatments on resistance to common diseases or quantities of secondary metabolites. We report the results of long-term observations of experiments performed on 3 perennial woody species (black mulberry, *Morus nigra* L.; Smirnov's rhododendron, *Rhododendron smirnowii* Trautv.; Norway spruce, *Picea abies*) and 2 perennial medicinal plants (purple coneflower, *Echinacea purpurea* (L.) Moench; red clover, *Trifolium pratense L.*).

The results indicate that response of perennial plants to seed treatment with physical stressors is dependent on a variety of factors (plant species, seed dormancy state, and the duration of seed storage) but the resulting effects on seedling development can be several fold stronger in comparison to that reported for annual plants. Longer-term observations revealed that the effects persisted for more than a year for all studied plant species. The treatment-induced pattern of seedling growth changes varied with time. For all three studied wooden plant species plants grown from seeds, characterized by the negative effects of EMF or CP treatments on germination and early growth, in end of the second vegetation season performed substantially better – developed more leaves and branches, had greater total leaf surface area, larger height than the control plants (e.g., the mean total leaf surface area per plant for *R. smirnowii* was up to 16-fold larger; Norway spuce seedlings grown from CP treated seeds, had 50-60% larger height and 40-50% increased branching in comparison to the control seedlings).

EMF and CP treatments improved germination and early growth of perennial medicinal plants. The germination rate of *E. purpurea* was increased by all treatments; CP but not EMF treatments stimulated germination of two cultivars of *Trifolium pratense*. We report treatment induced changes in morphometric traits of both medicinal plants studied (height, branching, leaf number, root weight). However, the most novel our finding was that in both medicinal plants pre-sowing seed treatment with EMF and CP induces significant changes in pharmaceutically important secondary metabolite content in plant leaves. The amount of cichoric acid per plant of *E. purpurea* was increased up to 3.8-fold, and amount of vitamin C – up to 1.9 fold. Similar finding was obtained with *Trifolium pratense*: the amount and ratio of izoflavones formononetine and biochanine A in leaf extracts was substantially changed by pre-sowing seed treatment with CP.

These results suggest that commonly used estimates of stressor effects, such as germination rate or seedling morphology, are not sufficient to define the stress response, at least for perennials.

Keywords: Cold plasma, Electromagnetic field, Perennial plants, Plant stress response, Pre-sowing seed treatment

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Embryology Of Some Species Of *Rosaceae* From Eastern Carpathians

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Abstract

Significant part of *Rosaceae* taxons in Eastern Carpathians is represented by polyploid series and by polymorphic hardly identifiable microspecies, reproductive process of which is associated with sexual reproduction and apomixis.

Our work is aimed to investigate the embryology of the species, which were not studied enough in this respect: *Potentilla aurea* L., *Crataegus oxyacantha* L., *C. monogyna* Jacq., *Alchemilla glabra* Neyg. and species that have not yet been studied, the *Fragaria viridis* Duch., *Potentilla obscura* Willd., *Cotoneaster horizontalis* Decaisne, *Crataegus corallina* hort., *Alchemilla flabellata* Bus.

Microsporogenesis in species used in our studies occurs with profound abnormalities, resulting into production of non-viable pollen grains or into total degeneration of microsporangium, with exception of *F. viridis* and sex individuals in populations of *P. aurea*.

Multicellular female archesporium is a source of both, the eusporic embryonic sacs, which develop from megaspores as *Polygonum* type, and the diploid apomictic sacs formed mitotically from sporogenous cells (diplosporia). In the same species apomictic embryonic sacs develop not only from sporogenic, but also from somatic cells of the nucellus (aposporia).

Two or three eusporic embryonic sacs are reached complete differentiation in *F. viridis*. The formation of both haploid and diploid apomictic embryonic sacs (diplosporia, occasionally aposporia) was observed for *P. aurea* and *P. obscura.* The *P. aurea* revealed both: sexual reproduction and pseudogamy. The complete differentiation of one to three apomictic embryonic sacs was found in *P. obscura.* The induced parthenogenesis is a characteristic of this species.

The *C. horizontalis, Crataegus* and *Alchemilla* species preserved the reproductive structures associated with sexual reproduction, but they are functionally depressed and are replaced by developing apomictic embryonic sacs of aposporic origin. Pseudogamy was found in C. *horizontalis* and *Crataegus* species.

In sexually reproducing species and facultative apomicts the endosperm is formed as a result of triple fusion and precedes the development of the embryo. In autonomous apomicts, the initial stages of embryo development precede the development of endosperm. The embryo of *Asterad* var. *Geum.* type develops.

Conclusion: these species are sexual by way of seed reproduction (*F. viridis*) and apomictic (agamic). It is necessary to note among them the species with various forms of apomixis: faculatively apomictic, which form agamic-sex complexes in local populations (*P. aurea*); pseudogamic apomictic with induced parthenogenesis (*P. obscura*); autonomous apomictic species (*C. horizontalis*, *C. oxyacantha*, *C. monogyna*, *C. corallina*, *A. flabellata*, *A. glabra*).

Keywords: archesporium, embryo sac, apomixis

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Plant Cryopreservation: Results, Problems And Perspectives

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Abstract

The genetic resources are exposed to pests, diseases and other natural hazards such as drought, weather damage, human error and ctr. Field genebanks require considerable inputs in the form of labor, land, management and materials, and, in addition, their capacity to ensure the maintenance of much diversity is limited. Cryopreservation in liquid nitrogen (LN, −196 °C) is the only technique currently available to ensure the safe and cost-efficient long-term conservation of the germplasm of problem species, including non-orthodox seed species and vegetatively propagated plants. All cellular divisions and metabolic processes are stopped at this temperature. The plant material can thus be stored without alteration or modification for a theoretically unlimited period of time. Different types of tissues and organs can be cryopreserved, including cell suspensions, pollen, embryogenic cultures, somatic and zygotic embryos, seeds, shoot tips and dormant buds. For vegetatively propagated species, the organs most commonly used in cryopreservation, are shoot tips, excised from apical or axillary buds of in vitro-grown shoot cultures. Over the last 20 years, cryopreservation protocols have been established for several hundreds of plant species, including numerous fruit tree species. In particular, the vitrification and encapsulation-dehydration methods have been continuously improved and are thus the most frequently employed for cryopreservation of clonally propagated plants. Using appropriate protocols survival of plants after cryopreservation extends 60 percent. However, storage protocols for some recalcitrant species and genotypes still to be improved. Recently established Laboratory of Cryobiology of LRCAF deals with cryopreservation of orchard plants – apple, pear, sweet, sour cherry, strawberry and ornamental plants (orchids). Problems connected with cryopreservation: plant cold hardening, cell damage, oxidative stress, genetic and epigenetic polymorphism, age, virus eradication analyzing there.

Keywords: cold hardening, dehydration, orchard plants, polymorphism, stress vitrification.

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Cytotoxic Properties of Chitosan Solutions are Concentration-dependent

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Abstract

Chitosan is a naturally occurring polymer obtained from the N-deacetylation of chitin, which is the earth's second most abundant carbohydrate polymer (after cellulose). Ready availability and abundance of chitin offer usage for industrial and commercial applications maybe because of lack of information about its properties and mechanisms of action on different cell types and molecules/atoms. To investigate cytotoxic properties of chitosan solutions some experimental investigations were performed. Chitosan solutions from *Hilobius abietis* in range of concentrations between 0.01 and 10 gL^{-1} were cast onto polystyrene Petri dishes for film formation. Two types of cell lines were investigated: tumor cells (mouse hepatoma MH-22A) and Chinese hamster ovary (CHO) cells. To measure plating efficiency (cloning efficiency), cells were plated at low densities (400 cells per dish) and allowed to grow for 9 days. To see the colonies clearly, they were stained with crystal violet (Merck, Germany). Significant differences in growth and proliferation between normal and cancerous cells in dependence of chitin concentration were not revealed. Obtained results indicated that cells fully or partly did not attach to the surfaces of Petri dishes, coated with chitosan solutions with concentrations ranging between 10 and 2 gL^{-1} ($p<0.05$ in comparison with control cell cultures at ordinary conditions of growth). In these dishes on the next day after seeding cell debris was observed on the top of culture media, that is chitosan films not always have surfaces with adhesive for mammalian cells properties. During cultivation on chitosan films cells had no characteristics of apoptosis, they proliferated and migrated on the surfaces of Petri dishes. At lower concentrations (between 0.2 and 1 gL^{-1}) cells formed both normal and sparse colonies, which were differ from normal colonies. Approximately all plated cells had plating or cloning efficiency that was more than 70 % and could form colonies on the surfaces of Petri dishes coated with chitosan concentrations ranging between 1 and 0.01 gL^{-1}. Consequently, in this study concentration-dependent cytotoxicity of chitosan films on both normal and cancerous cells has been revealed. Low concentrations of chitosan in films on Petri dishes did not significantly effect on mammalian cell cultures.

Keywords: chitin, chitosan, cytotoxic properties.

ISSN

Production And Characterization Of Chitosan Based Edible Films From *Berberis Crataegina*'s Fruit Extract And Seed Oil

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Abstract

Chitosan based edible films were prepared by supplementing *Berberis crataegina* DC.'s seed oil and fruit extract into chitosan matrix. The produced films were characterized both physiochemically (SEM, DSC, FT-IR, UV-vis, contact angle and mechanical analysis) and biologically (anti-quorum sensing, antimicrobial and antioxidant). Chitosan-fruit extract film revealed higher thermal stability, antioxidant, antimicrobial and anti-quorum sensing activity compared to other films. Addition of B. crataegina's seed oil and fruit extract into the chitosan film notably decreased the UV-vis transmittance but ameliorate the tensile strength values. Hydrophobicity of the chitosan-seed oil film was observed to be higher (92.64±4.17) than chitosan-control film (84.67±1.50) while chitosan-fruit extract film exhibited slightly lower hydrophobicity (73.82±7.42) than chitosan film. The overall high thermal stability, antioxidant and antimicrobial activity of chitosan-fruit extract film revealed that *B. crataegina*'s fruit extract can be used as an effective ingredient for production of edible film with enhanced physicochemical and biological properties.

Keywords: biodegradable film; *Berberis crataegina*; antimicrobial film; antioxidant activity

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Research of Influence of Contrasting Trophic Conditions of Vernalization on the Allelic State of VRN Genes and the Development Rates of *Triticum aestivum L.*

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A complex of different external and internal factors are important for the successful passing of vernalization. Among them, the most important are trophic factors [2]. The need for metabolites during the vernalization at the sprouts phase is ensured by the reserve of plastic substances of the endosperm. Mainly, carbohydrates act as trophic factors that also act as signal molecules - universal regulators of expression of genes of determining growth processes, development and floral morphogenesis of plants [3]. It is known that the genetic control of the need for vernalization of soft wheat plants is carried out by the system of VRN genes (3-5 genes) [1]. The VRN genes expression under the influence of vernalization causes the transition of winter wheat plants to earing [5]. The support of trophic factors during the vernalization, probably, determines the genetic and epigenetic control of the vernalization genes expression. The aim of the work was to investigate the influence of contrasting trophic conditions of vernalization on the allelic state of VRN genes and the rates of development of two varieties of winter wheat Mironovskaya 808 and Olviya. The different trophic conditions were created by vernalization on integral seeds (control) and isolated buds (experiment) adding water and 3-% solution of saccharose during 45 days at temperature 4±1°C. The molecular genetic analyses were carried out on the sprouts at different phases of vernalization - 15, 30, 45 days. DNA was isolated using a set of reagents "Diatom Prep 100" according to the producer methods. The allele-specific primers (Grain Gene Mass Wheat) were used for studying of the allelic state of Vrn-A1, Vrn-B1 and Vrn-D1 genes. The PCR was conducted according to a standard program for amplification of the primers. The distribution of PCR products was carried out by electrophoresis during 90-120 minutes in a 1% agarose gel containing ethidium bromide. At the end of vernalization, the vernalized sprouts were planted in vegetation vessels and cultivated under conditions of the vegetative experiment. The phenological observations were made, noting the duration of the period of transition to the earing. According to the results of research, it was established that the recessive state of the genes Vrn-A1, Vrn-V1 and Vrn-D1 on the 15th and 30th day of vernalization was unchanged in all variants of the two winter wheat varieties. After 45 days of vernalization, at the locus of the Vrn-B1 gene, the recessive and dominant alleles in sprouts, vernalized under normal trophic conditions and adding 3% solution of saccharose, were detected. All variants of wheat plants, grown from vernalized sprouts in contrasting trophic conditions, transferred to the generative phase, but at different times. It could indicate an epigenetic regulation of the vernalization process [4]. It was established that the different trophic conditions during the vernalization effected on the changes of the allelic state of the Vrn-B1 gene and determined the speed of the phenol phases passing and transition to generative development of winter wheat.

Keywords: soft wheat, vernalization, VRN genes, trophic factors, PCR, development rates.

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ISSN

Man And Technosphere

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Abstract

This article is devoted to a problem of interaction of the person with production and to creation of optimum conditions for dwelling in a technosphere. The **technosphere** — is the site of the biosphere transformed by people by means of direct or indirect influence of technical means for the best compliance to material and social requirements. Forming a technosphere, human sought for increase of comfort of habitat and at the same time for ensuring protection against negative impacts from the nature.The sharp increase in anthropogenous pressure upon the nature led to ecological disruption and caused degradation not only habitats, but also human health. Relevance of the studied question consists in intensive development of an industrial complex and is connected with the intensive growth of the cities. The purpose of studying of the matter is acquaintance with major factors of the production environment

Keywords: Production environment, human, technosphere, health, danger, harmful production factor, dangerous production factor, working conditions, safe working conditions.

ISSN

Assesing DNA Damage Induced By Ionising Radiation Using Comet Assay

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Abstract

Ionizing radiation can cause different kinds of DNA damage - single- and double-strand breaks, alkali-labile sites, base damage, and crosslinks. Comet assay (an alkaline version of the single cell gel electrophoresis assay) lets measure DNA damage at the level of individual cell and in this study was used to evaluate CHO cell line DNA damage induced by ionising radiation.

CHO cells were irradiated with different doses (2-10Gy) of 6MV energy x-ray radiation, within a 2 hour span embedded in agarose slides, lysed, in alkaline solution exposed to an electric field(~0.74 V/cm, 300mA), painted with ethidium bromide and visualized using fluorescence microscope. In parallel with the comet assay clonogenic cell viability assay was performed.

During electrophoresis damaged DNA extends towards anode and form so called comet tail. The amount of DNA migrated outside of cell boundaries to agarose is proportional to the DNA damage caused by ionising radiation. DNA damage was evaluated using open source OpenComet software by measuring the relative intensity of comet head and tail fluorescence.

Comet assay results show a linear relationship between radiation dose and DNA damage. This relationship agrees with other studies[1].

After cell viability assay was performed cell survival curve showed higher cell viability compared with a typical cell survival curve[2], dose was ~40% lower. After closer inspection of irradiation setup it was deduced that the air cavity in the Petri dish caused sharp drop in the dose distribution, therefore cell were irradiated with lower dose.

Keyword: Comet assay, DNA damage, ionising radiation

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ISSN

Patterns of Dwarfing of the European Yew (*Taxus baccata*) at the Subalpine Treeline Ecotone with Limestone Ground in the Greater Caucasus (Georgia, the Caucasus)

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Abstract

Genus *Taxus* L. (Taxaceae) from the Northern Hemisphere is a relict conifer of the Tertiary (Pre-Miocene origin). On the base of literature data *T. baccata*in Georgia occurs from sea-level up to 2,050 m. Grows as a small stands or individual trees, seldomly forms pure stands (E. Georgia, Batsara Reserve, 900-1,400m a.s.l., 237ha).

On the base of personal observation *T. baccata*, prefers calcareous substrate of the Greater Caucasus. As low trees (8-10 m tall) it founds in maximum elevation about 1,800 m (e.g., on Mingaria Mts, Samegrelo region). In upper altitude, yew encompasses subalpine zone.On Khvamli massif (Lechkhumi region),a marked ecotone (krumholz). Mount topped 2002 m enriched with calcicolous relict endemics (e.g., *Woronowia speciosa*). Side by side of karstic cavities occur a few gentle areas (mini-plateau). One of the latter ecotone sites, with plenty of sun, is niche of the homogenous population of semi-prostrate (1-1.5m height) patch of *T. baccata* with a rare small straight-trunks (~5-6 m height) specimens. Soils of gentle areas are deeper and more nutrient-rich. Therefore, yew shrubland appears as dominating arboreal vegetation, with high competitiveability. Such remarkable dense, sub-prostrate stand is quite unusual (maybe single) growth of yew in the Caucasus. It seems that native clonal population of yew descend from a single founders via vegetative propagation.

Another limestone outcrops site in Okhachkue (Samegrelo), about 2,200m a.s.l. provide a home of solitary, crook-stem forms of yew (~1 m height). The latter shrub tends to grow on the shaded habitat (which is usual for shade-tolerant yew) dominated by the elfin stands of *Rhododendron caucasicum*. Observation reveals that yew in Okhachkue located at the top edge of the altitudinal distribution in the Caucasus. The both dwarfing events of *T. baccata* holds great scientific interest by means of investigation adaptation strategy in the harsh environments, trend of migration in high elevation, reproduction and other strategy to survive coinciding to global climate oscillations.

ISSN

Microbiota of Seeds and Seedlings' Rhizosphere of *Rhododendron smirnowii* Grown from Seeds Treated with Cold Plasma, Vacuum and Electromagnetic Field

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Abstract

We have demonstrated recently [1] that pre-sowing treatment of *Rhododendron smirnowii* Trautv. seeds with electromagnetic field (EMF), vacuum and cold plasma (CP) strongly affects germination kinetics and seedling growth. Seed treatment with EMF and vacuum increased germination percentage up to 70% but CP treatments negatively affected germination. However, these methods were not estimated to have a forceful effect on microbiological cleanliness of the seeds. We succeeded to isolate 11 species of fungi, where 6 were identified as plant pathogens (*Sporocadus* sp., *Fusarium avenaceum* , *Alternaria alternata*, *Cladosporium cladosporioides*, *Botrytis cinerea*) and other 5 were assigned to *Penicilllium* genus. Differences in *composition* of endophytic *mycobiota in seeds were statistically not significant.*

Long term observations revealed that seedlings grown from CP treated seeds, characterized by negative effects on germination (decreased germination percent by 23%), 13 months after sowing had considerably larger (by 69%) height, more branches and better developed roots, substantially larger (7.4-fold) total leaf surface area per plant in comparison to control plants. We have tested the hypothesis that plants grown from the treated seeds induce changes in soil microorganism community promoting plant growth. The obtained results showed that physical treatments affected the amount (colony forming units (CFU)) of rhizospheric bacteria in roots: control samples had less CFU than after treatment either with EML, CP or vacuum. 104 bacteria isolates were collected from roots and 40 isolates from soil. DNA sequencing results showed that the dominant bacterial groups were *Agrobacterium* sp., *Bacillus* sp., *Burkholderia* sp., *Paenibacillus* sp., *Pseudomonas* sp., *Rhizobium* sp.

Keywords: Cold plasma, Electromagnetic field, *Rhododendron smirnowii*, Plant stress response, Pre-sowing seed treatment, Seed contamination, Soil and rhizosphere microbiota

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Facile and Green Synthesis of Paramagnetic $MnFe_2O_4$ Using *Spinacia Oleracea* Extract

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Abstract

The topic of nanotechnology is one of the best active areas in superior material science and nanoparticles (NPs) demonstrate entirely developed features based on special properties such as dimension, morphology and dispersion. Green nanotechnology for nanoparticle synthesis (GS) using plants is becoming increasingly importance among investigators as an eco-friendly alternative to conventional chemical and physical process as this perspective remove the use of toxic components. Due to the excellent electrical and magnetic properties, high magnetic permeability and electrical resistance, spinel ferrites are used in the field of microwave, and electronic devices. Among the soft magnetic ferrite materials, $MnFe_2O_4$ is important due to its applications in the gas sensors, absorbent material for hot gases and also as a potential candidate of contrast agents in magnetic resonance imaging (MRI). The superparamagnetic $MnFe_2O_4$ nanoparticles were found to have a very high magnetization and large relaxivity owing to their large magnetic spin magnitude. Several ferrite thin films have been reported in the literature in order to look at mainly the structural aspects. However, to the best of our knowledge, there has been no previous work on the synthesis and physical properties of $MnFe_2O_4$-NPs by using spinach extract. In this study, we are the first to report the eco-friendly green synthesis of $MnFe_2O_4$-NPs by using aqueous spinach (*Spinacia oleracea*) extract. In this study, we synthesised $MnFe_2O_4$ nanoparticles (NPs) produced using was produced using $Mn(NO_3)_2.4H_2O$ and $Fe(NO_3)_3.9H_2O$ solution in aqueous spinach. The ZnO-NPs were examined as morphological, optical and electrical by XRD, FE-SEM, four probe dc system and Uv/vis spectrophotometer. Also, its DNA cleavage properties were investigated. All diffraction peaks matched with the standard pattern of bulk Jacobsite $MnFe_2O_4$ (JCPDS 10-0319) and it confirms the cubic spinel structures. The average crystallite size calculated using Scherrer formula is 26 nm. No impurity and other phase related peaks are present in XRD pattern, which indicates the purity of the prepared sample. The FE-SEM micrographs revealed the formation of plate-like structures and the average particle sizes were found to be 25–50 nm. The UV-vis measurements showed that the average optical transparency is over 70 % in the visible range. We demonstrated $MnFe_2O_4$ interaction with plasmid DNA.

Keywords: Green synthesis method; *Spinacia oleracea*; nano-particles; plasmid DNA (pBR322).

ISSN

Distribution and Spreading of Invasive Slugs *Arion lusitanicus* Mabille, 1868 (Mollusca, Pulmonata) in Lithuania

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Arion lusitanicus is one of the most harmful invasive slugs' species for agriculture, horticulture and natural ecosystems. These slugs are spreading from Western Europe and the first finding place in Lithuania was gardens on the valley of the river Veršva in Kaunas near Linkuva mansion in 2008.

For data selection informative campaign with announcements in newspapers and internet sites was used. In total 176 reports from 38 regions by inhabitants from various localities of Lithuania were received during 2016. 53 reports were verified: samples of slugs were collected, transported alive to Vilnius University, colded in freezer and preserved in 70% ethanol for farther identification using reproductive system characters. Also biotopes and abundance of slugs have been evaluated in verified places.

Totally 49 finding places of *A. lusitanicus* were proved for 13 regions of Lithuania. In some places (in 8 reported places), instead of *A. lusitanicus*, two other species of big slugs were observed: *Arion rufus* (Linnaeus, 1758) or *Limax maximus* Linnaeus, 1758. Mostly only some streets of the cities or gardens have been invaded by *A. lusitanicus* yet, for example in Panevėžys, Marijampolė, Trakai, Priekulė, Mažeikiai, Šakiai, Prienai, Birštonas and Kaišiadorys. But in some places slugs became superfluous and invaded almost all Kaunas (15 finding places) and Vilnius (14 finding places).

Most of finding places of *A. lusitanicus* were urbophytocenoses near water. In settlements slugs are frequent in abandoned homesteads with high grass and in shady zones of yard. In suitable places density of *A. lusitanicus* reached up to 23 individuals in one square meter.

Summarizing data of *A. lusitanicus* spreading, we can conclude that new places in Lithuania occur randomly and are associated with passive, indirect type of distribution. Obvious, that only systemic integration of various control measures could stop further distribution of these slugs in Lithuania.

Keywords: slugs, *Arion lusitanicus*, invasive species, distribution.

ISSN

In vitro Potential of *Paenibacillus alvei* DZ-3 as Biocontrol Agent Against Several Phytopathogenic Fungi

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Abstract

Fungal phytopathogens cause significant losses in many economically important crops and vegetables. One way to control these devastating pathogens is by using higher doses of fungicides which not only increase the cost of production but also cause significant damage to the environment. Consequently, there is an increasing demand from consumers and officials to reduce the use of chemical pesticides. In this context, biological control through the use of natural antagonistic microorganisms has emerged as a promising alternative.

The goal of this paper is to evaluate environmentally friendly treatment for *in vitro* control of some fungal phytopathogens.

In the present study, the bacterial strain DZ-3, which shows strong antifungal activity, is isolated from the rotten apples compost samples from the composting plant in Resen, Macedonia and identified as *Paenibacillus alvei* according to morphological and taxonomic characteristics and 16S rRNA gene sequence analysis.

As test microorganisms we used *Botrytis cinerea* FNS- FCC 23, *Fusarium oxysporum* FNS- FCC 103, *Plasmopara viticola* FNS- FCC 65, *Alternaria alternata* FNS- FCC 624, but also *Aspergilus ochraceus* FNS- FCC 46, *Aspergilus niger* FNS- FCC 142 and *Penicillium commune* FNS- FCC 864.

The effect of *Paenibacillus alvei* DZ-3 on growth of tested fungi was evaluated by dual culture technique and disk diffusion method. A clear inhibition zones were observed in all test microorganisms.

The above-described results indicate that *Paenibacillus alvei* DZ-3 may have the potential as biocontrol agent to control various phytopathogenic fungi.

Keywords: phytopathogen, *Paenibacillus alvei,* antifungal activity, compost

ISSN

Diversity of Biologically Valuable Fauna in Organic Plant-based Agriculture Farming

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Abstract

Organic farming is certainly determined as the tool to increase natural biodiversity in agricultural ecosystems. However, researches argue about real value of organic farming and different management regimes are questioned. One of most uncertain questions in recent literature and this research also: 'could plant-based organic farming significantly increase biodiversity and maintain right yield?' This study researches differently prepared green manure, used as fertilizers in organic farm. Experiments were held in Dotnuva and Joniškėlis. Four green manure preparation techniques were used: direct aboveground red clover (*Trifolium pratense* L.) mass plowing, legume biomass ensiling, legume and grains biomass ensiling, legume and grains biomass composting. For comparison, no feritilaser and cattle manure were used too. All of them had different compounds ratio (C/N), degradation time, mineralization environment.

Microorganisms, earthworms and insects were observed as indicators of biodiversity content. Ecological groups, species, quontity, quality, activity were identified three times in agricultural vegetation. Ecological environment was suitable for microorganisms activity increase. Inserted green manure influenced different structure of cultures, according to manure preperation technicue. Better earthworms activity was measured everythere processed manure were inserted. Ensiled legume fertiliser led to bigest amount of earthworms burrows (225 units/m^2). Most found earthworm species: red earthworm (*Lumbricus rubellus* Hofm.) and field worm (*Allolobophora calignosa* Sav.). Significant increase in earthworm amount were observed using ensiled legume biomass feritilaser. Earthworms quantity enlarged 4 times comaring April and July data and succeded 225 units/m^2. Surprisingly, insects quantity was lowest there cattle manure feritilaser was applied, all green manure showed good insects activity. Biggest amount traced there ensiled legume and grains biomass was applied. *Carabidae* family was most abundant. To summit up, results staits to consider wider ensilage practise usage for better biological activity in organic plant-based ecosystems.

Keywords: green manure, agriculture ecosystem, biodiversity, red clover

ISSN

Molecular detection of vector-borne pathogens in Lithuania

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Abstract

Vector-borne diseases constitute a major health problem in many parts of the world. In the past three decades, many vector-borne pathogens have emerged, creating new challenges for public and animal health in Europe. With the progress in molecular diagnosis, new species, strains, and genetic variants of microorganisms are being detected in ticks and other vectors worldwide. The Baltic countries are an endemic area for a number of tick-borne diseases. The advances in molecular biology during the last two decades and using of molecular diagnostic techniques have allowed researchers to better diagnose, trace and genetically characterize the causative agents of important zoonotic tick-borne diseases such as Lyme borreliosis, tick-borne encephalitis, human granulocytic anaplasmosis, and have led to the discovery of new emerging vector-borne pathogenic organisms in Lithuania.

Keywords: Vector-borne pathogens, molecular diagnosis, Lithuania

POSTER PRESENTATIONS

Design of a Chitosan Based Bio-Scaffolding for Regenerative Medicine: Compatibility Analysis for Cartilage Regeneration

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Background: Evaluation of any material that is utilised for clinical applications is essential in order to ensure that it acquires the physical, mechanical and biocompatible properties required for its proposed function. Understanding of the conditions that regulate the mechanical and physical characteristics of a material particularly biopolymers is crucial in the field of cell and tissue engineering. The aim of this work is to assemble some polymers and crosslink with each other using β-Glycerophosphate to produce scaffolding which is suitable for *in vitro* tissue regeneration.

Materials and methods: The experimental procedures in this project involved the culturing of cells in different suspensions containing; control, CSG hydrogel, fibronectin, and hydrogel with fibronectin. The experiment carried out was aimed at finding the cell viability of the CSG hydrogel, and to study how the cells interaction with the CSG hydrogel to find out whether they can grow and survive in the CSG hydrogel for a period of 24 hours, and to identify if the CSG hydrogel is cytotoxic and whether it could possibly be used for the in vivo growth of cells in a sight of damaged articular cartilage.

Results and conclusions: According the results obtained from series of tests proved that CSG3 has the most optimum properties, as it presented the highest viscosity at 37^{o}C, highest stability during temperature changes and an enhanced elastic behaviour at physiological temperature.

Keywords: Chitosan, Hydrogel, β-Glycerophosphate, Crosslinking, Cell culture.

ISSN

The Comparative Enzyme And Histological Analysis Of The *Barbus Peleponnesius* From The Aquaculture And Natural Population In The Vardar River In The Republic Of Macedonia

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Abstract

Enzymatic biomarkers are sensitive to environmental changes and they respond by changing their activity. Barbus peloponnesius is considered as a potential bio indicator to changes that can be caused in an environment by various pollutants. The results from the organs of the experimental group of fishes from the aquaculture, that were treated with a sub lethal dose of insecticides (1mg/L), showed a significant increase of the kinetic activity of the enzyme EROD and B(a)PMO, compared to the group control. Whereas the group of fishes treated with the same dose of herbicides did not show a significant increase, however there was a kinetic activity alteration in these enzymes. A significant increase of the enzymatic activity (EROD and B(a)PMO) was also seen in the fishes from the polluted River Vardar. The fishes treated with sub lethal concentrations (2µg/L) of insecticides and herbicides, in the hepatic parenchyma, kidney tissue and the ovaries showed haemolysis, coagulation, degeneration and cytoplasmic vacuolization of the hepatocytes, dilatation of the sinusoids, degeneration of the glomeruli and renal tubules, widening of the space between the basal membranes and the epithelial cells of the renal tubules, fracture and degeneration of the ovoplasm and disruption of the follicular epithelium as well.

ISSN

Optimizing the Condition of Sterilization and *In Vitro* Establishment of Vegetative Buds from Ancient and Old Value Trees of Ukraine

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Today, in Ukraine and many countries in all over the world one of the main questions is how to protect and preserve ancient trees as a centuries-old, historical monuments of nature. Due to the natural aging condition this centuries-old trees are characterized by high risk of extinction as a result of biotic, abiotic and anthropogenic factors. Many of these tree species are being threatened and are endangered because of age, as result of biotic, abiotic and anthropogenic factors, that cannot keep up with natural changes in ecosystem [1].

Most of Ancient trees are a national treasure whose value is difficult to overestimate for cultural and spiritual development.

The development of biotechnological methods can solve the problem of maintaining the unique gene pool of woody plants, study their ecosystem role and development of ornamental horticulture [2].

As the source of explants were used ancient trees from natural conditions such as: Linden of P. Mogulu, (Kyiv) age over 400 years, Linden of T. Shevchenko, (Chernihiv region) age over 600 years, Oak of T. Shevchenko, (Kyiv) age over 300 years, Oak of M. Zalizniak, (Cherkasy region) age over 1000 years.

In researches, optimal explants for introduction to the culture in vitro of centuries-old trees were as winter shoots and awakening shoots had been getting from deferred shoot in control laboratory condition.

As a result, for winter shoots the most effective is sterilization with using shoots (3.0–5.0 cm) which have washed under soapy water 20 min, and then immersed in 75% (v/v) alcohol for 30 s, before surface sterilization in 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 8–10 min. On another way for shoots of centuries-old trees, which have been awakening were used sterilization solution of 25% (w/v) perhydrol (H_2O_2) for 7-10 min. After being washed three times with sterile distilled water (5 min) all shoot tips (0.5–1.5 cm) and nodal segments (0.5–1.0 cm) were excised and implanted to MS medium [3] for culture initiation. All nutrition medium have contained 30 $g \cdot l^{-1}$ sucrose, 1 $g \cdot l^{-1}$ glycation and 6.5 $g \cdot l^{-1}$ agar. The pH of medium was adjusted to 5.7-5.8. All cultures have maintained in a growth chamber at 24–25 °C under cool, white fluorescent lamps with 16 h photoperiod.

After 3-4 weeks have recieved 70-80% of aseptic explants developed into 2.0–3.0 cm shoots, which were used for the following studies.

Keywords: Ancient trees, explant, regeneration, *in vitro*.

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ISSN

New Methods of Parasitology Diagnostic with Using Of Anti-Freeze and Auto Cool Liquid

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Authors for the keeping of coprology material proposed to use as the conserving medium of anti-freeze (Innovation invention patent of Kazakhstan Republic №30082 from 15.07.2015) and auto cool liquid (Innovation invention patent of Kazakhstan Republic №30081 from 15.07.2015). Conserving and keeping of excrements and other biologic substrates in these liquids prevent the development of propagative stages of parasites, reproduction of micro-organisms.

Then on the ground of these technique liquids the next media for the conserving and flotation exploration of excrements were proposed.

1) Anti-freeze with the addition of 25-30 mass per cents sodium chloride – with sediment on the bottom of glass (application for invention rights №2015/1143.1 from 1.10.2015).

2) Anti-freeze with the addition of 40% of sugar (application for invention rights №2015/1144.1 from 1.10.2015).

3) Anti-freeze with the addition of 20-25% sodium chloride and 20-25% of sugar (application for invention rights №2015/1142.1 from 1.10.2015).

4) Auto cool liquid with the addition of 25-30% sodium chloride (application for invention rights №2015/1148 from 1.10.2015).

5) Auto cool liquid with the addition of 40% sugar (application for invention rights №2015/1146.1 from 1.10.2015).

6) Auto cool liquid with the addition of 20-25% sodium chloride and 20-25% sugar (application for invention rights № 2015/1147.1 from 1.10.2015).

All of solutions had the hardness from 1,18 until 1,24, that were higher than saturated in the room temperature solution of sodium chloride [1]. However the main diagnostic value of elaborated media determined by the low adhesion of ethylene glycol with exterior protein capsules of helminthes' eggs and coccidian ova cysts. Badly moistened small subjects are pushed out by solution onto the surface.

Modification of twist method [2.] proposed by L.T.Bulekbayeva, N.E.Tarassovskaya, R.Takhirov, improves the diagnostic possibility of this method. Excrement probes are put to the glass vessel, flooded by the sale anti-freeze or auto cool liquid in the volume proportion between material and conserving solution 1:3 – 1:5 and kept to examination procedure (several weeks or months). In exploration process the liquid (homogenate of excrements with anti-freeze) is intensively mixed by the glass stick during 20-30 seconds, then the stick is quickly extracted from the solution and stayed on it's end liquid drop put to the subject glass for the exploration (application for useful model rights №№ 2015/0980 and 2015/0979 from 27.08.2015).

Keywords: conserving of excrements, flotation, anti-freeze, cool liquid, twist method.

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ISSN

Alterations In Sphingomyelin:Phosphatidylcholine Ratio In Chromatin Preparations From Rat Liver And Thymus Cells After The Cisplatin *In Vivo* Action

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It is well known, that chromatin lipids are implicated in various processes such as DNA replication, transcription, chromatin assembly, acetylation and methylation of histones. The regulatory effects of chromatin lipids exhibit concentration depending disposition.

The results of our earlier investigations were revealed the dramatically changes in absolute quantities of all phospholipid fractions of chromatin preparations from rat liver and thymus cells after the antitumor drug cisplatin *in vivo* action. Along with decrease in quantities of other phospholipid fractions, a special interest was rendered to interdependent alterations of two choline inclusive lipids, namely sphingomyelin and phosphatidylcholine. Comparative analysis of alterations in absolute quantities and ratio of sphingomyelin and phosphatidylcholine in chromatin preparations from rat liver and thymus cells after the 24h. in *vivo* action of cisplatin was carried out.

We showed that the sphingomyelin:phosphatidylcholine ratio in rat liver and thymus chromatin preparations was equal to 0.47 and 0.36 respectively. The cisplatin action leads to multidirectional alterations in choline inclusive lipids absolute content and in sphingomyelin: phosphatidylcholine ratio in the investigated chromatin preparations. Thus, in liver chromatin the value of this ratio decreased up to 0.30, which is equal to 36% of diminition. On the contrary, in case of rat thymus chromatin the cisplatin caused increase in sphingomyelin: phosphatidylcholine ratio up to 0.53 (increase by about 49%).

These multidirectional alterations of absolute content and the ratio of chromatin choline inclusive lipids in rat liver and thymus may be explained by difference of metabolic status of these tissues as well as by differences in sensitivity to cisplatin treatment of enzymes, that catalyze the degradation of lipids in rat liver and thymus nuclei.

Taking into consideration the regulatory role of chromatin phospholipids as well as the crucial importance of sphingomyelin:phosphatidylcholine crosstalk in cell fate, one can assume that these cisplatin caused alterations may be connected with the antitumor effects of the drug.

ISSN

"Hairy" Root Cultures As A Source Of Biologically Active Substances

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Abstract

Using of genetic engineering approaches makes it possible to biofortificate plants for the purpose of increase of medical compounds accumulation and obtain new samples synthesized non-relevant for the plant species recombinant biologically active compounds (BAC). *Agrobacterium rhizogenes*-mediated transformation is a useful tool of the plants improvement. Transgenic roots can produce both natural and recombinant BAC. We established "hairy" root cultures of *Artemisia tilesii* Ledeb, *A.dracunculus* L., *A.annua* L., *A.vulgaris* L., *A. ludoviciana* L., *A. absinthium* L., *Bidens pilosa* L., *Althaea officinalis* L., *Tragopogon porrifolius* L., *Lactuca sativa* L., *Cichorium intybus* L., *Ruta graveolens* L. carried human interferon-α2b gene or agrobacterial genes. These "hairy" root cultures were used for study of accumulation of sugars, artemisinin, interferon, analysis of antioxidant and antiviral activity.

The results obtained testify that artemisinin accumulated in *Artemisia* "hairy" roots in amount greater in some lines than in the control (up to 1,02 mg/g DW, *A.vulgaris*). The transformation did not effect significantly on the antioxidant activity of the root aqueous extracts. The analysis of sugars content in transgenic root lines exhibited variations. For example five times increase of fructose content and threefold increase of sucrose content in *A.vulgaris* "hairy" root lines was found. Ethanolic and DMSO *R. graveolens* "hairy" root extracts demonstrated antimicrobial activity. The diameter of zone of inhibition of bacterial growth was up to 45 mm (*Micrococcus luteus*).

"Hairy" roots carrying *ifn-α2b* gene synthesized interferon-like compound. Its highest content was up to 2766 pg/g (*A. officinalis* "hairy" root culture). Antiviral activity of the extracts from transgenic roots (*B.pilosa, A.tilesii, A.officinalis, L.sativa, C. intybus*) carried *ifn-α2b* gene significantly differed in our study. The high antiviral activity was indentified in PBS extracts of *A.officinalis* (40760 IU/g) and *A. tilesii* (98 437 IU/g).

Our studies demonstrated that "hairy" roots of medicinal plants can synthesize both natural BAC (sugars, secondary metabolites) and recombinant protein (human interferon-α2b). So the "hairy" root cultures from the collection can be the used for natural and recombinant BAC synthesis.

Keywords: medicinal plants, "hairy" root culture, artemisinin, sugars, antiviral activity.

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Density of Erythrocytes in Suspension under the Effect of Millimeter Electromagnetic Waves

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Abstract

According to the new division radiofrequency waves in the range of 30-300 GHz are considered to be extremely high frequency electromagnetic waves (EMW EHF). Their natural background is not very high on the Earth, but due to the increasing of number of the artificial sources of EMW EHF their irradiation intensity is permanently increasing [1]. Living organisms are continuously exposed to this effect [2]. In the presented work change of suspension density of rat blood erythrocytes with different concentrations after the irradiation by different exposure and 50.3 GHz frequency (resonant for water) was studied. It was shown that the irradiation leads to decreasing of the suspension absorption. That is why at 670 nm light dispersion in suspension of erythrocytes indicate the part of whole undamaged erythrocytes, at all rest one-type conditions (density, concentration, etc.) the absorption fall indicates the occurrence of sedimentation of erythrocytes and part of preserved whole erythrocytes is reduced. By the virtue of the measurement of absorption was carried out at 670 nm, it may be assumed that EMW EHF irradiation is a factor accelerating the sedimentation of erythrocytes. On the other hand, it can be connected to the fact that aggregation of erythrocytes takes place. Suspension density of erythrocytes depending on suspension concentration was also studied in 293 K temperature. Obtained data indicate that in control suspension the density of erythrocytes enhances with concentration increasing. In irradiated suspension, vice versa, with concentration decreasing the density enhances. The same regularity is observed at 303 K temperature, but in this case at the same absorptions the suspension density is lower, which indicates the enhancement of suspension volume of erythrocytes due to temperature increasing. Thus, the irradiation results in decreasing of suspension absorption, which, apparently, is connected to aggregation and distortion of part of erythrocytes. On the other hand, if in control suspension with concentration increasing of erythrocytes a respective increasing of density is obtained, in irradiated suspension, vice versa, density decreases. Absorption change value induced by irradiation is in direct dependence on suspension density, the higher is density the less is absorption value of irradiated suspension.

Keywords: Millimeter range electromagnetic waves, erythrocytes in suspension, density.

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Whole Body Extremely High Frequency Electromagnetic Irradiation Exposure Effect on Lipid Peroxidation in Rats

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Abstract

Extremely high frequencies (EHF) electromagnetic irradiation (EMI) (a range from 30 GHz to 300 GHz), or millimeter waves with low intensity induces significant changes in living organisms of a different level of ogranization. It has been shown that EMI leads to changes of membrane properties: to acceleration or suppression in active ions transport, to changes in biological membranes permeability due to proteins conformation changies and by means of membrane lipid peroxidation [1].

The aim of this study is evaluation if the influence of the reapeated (20 min per day for a duration of 5 days, day after day) exposure to EMI EHF of 42,2 GHz and 50,3 GHz (power dencity, 64μWt/cm (2)) frequencies on tissue lipid peroxidation (LPO) of rats. The concentration of malondialdehyde (MDA) as the final-product of the LPO was estimated in brain, liver, heart und skeletal muscle of animals. Non pedigreed rats with 80-100g body weight were used for this study. Animals were divided into two groups: sham-exposed (control) and experimental (10 animals each). As a source of monocromatic EMI EHF generator G4-141 type with working intervale of 37.50-53.57 GHz (State Scientific-Production Enterprise "Istok", Russia) was used. Exposure took place in a ventilated plexiglas cage, where rats could move inside given space, under a horn antenna at a 15cm distance. After completion of each exposure period, rats were sacrifised and organs were isolated to assess MDA-rate. Experiments were performed in a blind manner and repeated.

Our observations indicate a significant increase ($P<0,05$) in level of MDA in brain of EMI- exposed group of animals as compared to sham-exposed, depending on EMI frequency used and exposure duration. The effects were more expressed with 50,3 GHz. At the same time, we recorded slightly elevated MDA-rate in experimental animals liver in comparision with the control group. The MDA values concerning heart and sceletal muscles remained at the same levels in the EMI- exposed rats and did not differ significantly for all used EMI frequencies.

The obtained results suggest that multiple exposure to the EMI EHF slightly increase tissue lipid peroxidation in some tissues, especially in brain of rats, so the nervous system is particulalry vulnerable to EMI-induced oxidative stress.

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Effect of Millimeter Range Electromagnetic Waves on Erythrocyte Suspension

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Abstract

Millimeter range electromagnetic waves (MM EMW) permanently impact on living objects, though this effect is revealed on any level of organization of living material. In the present work the study of MM EMW effect on electrokinetic potential values of erythrocyte suspension has been carried out taking into the account the fact that blood and its constituents are one of the important organ-systems in living organisms. For this aim the values of the electrokinetic potentials of erythrocytes in suspension were determined after the suspension irradiation during 1 hour by 41.8, 50.3 and 51.8 GHz frequencies. The results show that at the irradiation by 41.8 GHz frequency an enhancement of electrokinetic potential absolute value occurs as compared to control. Thus, if in control sample the value of the non-irradiated erythrocyte electrokinetic potential is equal to -14.5 mV, in the irradiated sample by 41.8 GHz it is equal to -17 mV. The fact is interesting that the frequency 41.8 GHz is not resonant for water, but its biological response is high. Furthermore, it was mentioned in literature that frequencies in the range of 41.8-42.2 GHz have a biological activity, which, most apparently, is connected to direct influence of the irradiation energy on biological targets [1,2]. On the other hand, the experiments have been carried out in the case of water resonant frequencies – 50.3 and 51.8 GHz [3]. In this case a decreasing of the electrokinetic potential absolute values of erythrocytes was observed compared to control and the values were equal to -8.06 and -8.7 mV respectively. In the case of the irradiation with resonant frequencies the irradiation energy transits to erythrocytes being mediated by water [3]. Therefore, MM EMW irradiation results in significant changes in the electrokinetic potential values of erythrocytes. Moreover, at different frequencies various changes are observed which indicate the differing from each other mechanisms of the effect of these waves on erythrocytes in the suspension.

Keywords: Millimeter range electromagnetic waves, erythrocytes in suspension, water resonant and non-resonant frequencies, elektrokinetic potential.

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Microscopic Diagnostics Of Species (*Nepeta* Genus) Based On The Leaf Epidermis Structure

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Abstract

Currently, widely used in botany, the method of microscopic analysis makes it possible to give an objective assessment of the authenticity of medicinal plant raw materials, except for the simple enumeration of anatomical and diagnostic features, their sizes are normalized. Some closely related plants have similar anatomical and diagnostic features, differing in frequency and size. In this regard, the aim of the work was to identify diagnostic features in the epidermal structure of the leaf in *Nepeta Cataria, Nepeta Grandiflora,* which can be used for diagnosis at a species level.

Microscopic studies were conducted on freshly collected and fixed biomaterial. The surface, compressed preparations and cross sections were produced by hand using a dangerous blade. When describing the anatomical structure, the conventional terminology proposed by K. Ezau was used [1, p. 218]. The description of external signs was carried out in accordance with the requirements included in the State Pharmacopoeia [2, p. 252-257].

It is established that in the studied species, the outlines of the epidermal cells are sinuous, the projection of the epidermal cell area is planar in plan, and the corners in the adjacent boundaries are rounded and pointed. The epidermis of the top leaf of *Nepeta grandiflora L* is represented by thin-walled sinuous cells with a diameter of 40-50 μm, the epidermis of the upper side of the *Nepeta cataria L.* leaf is represented by large, tortuous-walled epidermal cells (35-40 μm) tightly closed with each other. There is abundant pubescence of simple hairs. The length of simple hairs in *N. Cataria L.* is 100-120 microns, in *N. Grandiflora* it is 90-100 microns. The stomata of *Nepeta grandiflora L.* are very small, located on both sides of the leaf. Stem *Nepeta cataria* L. small, numerous, rounded, located on both sides of the leaf chaotically. The size of stomata is on average 35 μm in diameter. The type of stomatal apparatus is diatomic. Essential oil glands *Nepeta* grandiflora L. are large, rounded. In. the essential oil glands are monocyclic, large, round in shape. Formed by *Nepeta cataria L* 4 secretory cells. No outlets.

The results of the work are a theoretical basis for establishing the authenticity of medicinal plant material, they make a great contribution to the taxonomy. The established anatomical and morphological structural criteria of identification of plant raw materials will allow to determine the possibility of their use in medicine.

Key words: microscopic diagnostics, stomatal apparatus, essential oil glands, medicinal raw materials, *Nepeta*

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Screening Of Immunomodulatory Agents for Protection of Plants of the Tomato during Fuzarium Wilt

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Abstract

Fusariosis of agricultural plants occupies one of the leading positions of distribution and harmfulness at a present time. Microorganisms, being sources of fuzarium wilt belong to soil pathogens also well protected from influence of external factors in a saprophytic phase of the development in the soil, on crop residues and also in vascular system of plants during vegetation. It explains difficulty in protection against this group of pathogens. Depending on environmental conditions (temperature, soil and air humidity) fusarium wilt develops differently: high developed withering and then drying up still of green plants, the disease gains long or chronic character. For the first case, death of plants happen because of sharp violation of vital signs owing to obstruction of conductive vessels a mycelium, second of all because of toxicant effect emitted by *Fusarium sp.* The results which have been received recently on tomato plants at laboratory of applied biophysics and biochemistry showed that the first type of fuzarium wilt is observed at high temperature of cultivation (38-40ºC) but the second – in normal condition.

There are a numerous protection system against of plant diseases caused by fungi, including the resistant varieties of cereals and numerous of chemical and biological methods of protection.

In the last decade, several researches on inductor discovery of plant resistance on basis of immune answer metabolites have been carried out intensively. As a distinct of fungicides, immunomodulators do not cause development of high virulence of pathogen strains and are ideal for the majority of plant disease prophylaxis.

Screening of immunomodulatory agents at early stages of development of the tomato plant «Tamara» variety was held. Plants have been grown up in soil culture in a climate chamber at a temperature of 24 $^{\circ C}$ and illuminating intensity of 100 μE/m^{2}s with 14 hours photoperiod and after some time have been transferred to tap water. Plants processed the salicylic acid (SA), β-aminobutyric acid (BABA), β-1,3-glucan (Sigma-Aldrich) by a spraying method, control sample − distilled water. Infection with *Fusarium oxysporum* pathogen (Sacc.), causing fuzarium wilt of a tomato, carried out in 48 h after processing by immunomodulators, by making suspension culture of a fungi through roots in an aqueous medium. Leaves were analyzed in 72 h after an inoculation of plants by pathogen. The content of photosynthetic pigments was determined by the HPLC method [Rodriguez-Amaya D. B., 2004], photochemical activity of PS 2 by RAM-fluorimetry [Krause G.H., Weis E., 1991], content of hydrogen peroxide by [J.G. Mohanty et al., 1997] and activity of lipid peroxidation (LPO) by [Heath R.L., Packer L., 1968].

As a results of pathogen attack on tomato plants, 32% increasing of hydrogen peroxide content was achieved. It demonstrates the development of an oxidative stress in tomato plants. At the same time detected double increasing of content of LPO stable products – a malondialdehyde indicated activation of destructive processes in lipid bilayer of membranes. Damage of tomato plants by pathogen was caused by decreasing of chlorophyll *(a+b)* and carotenoids content, calculated per unit of leaf area and destruction of primary photosynthetic reactions. Pretreatment of plants using BABA, SA and β-1,3-glucan had a protective action on pigment apparatus of tomato chloroplasts during fusarium wilt and in BABA-treated plants also were observed of recovery of photochemical reaction activity of PS 2. Action of immunomodulators on hydrogen peroxide content in the infected leaves was not so uniquely: BABA did not change this parameter, SA reduced it to a control level, and β-1,3-glucan caused the substantial increase of H_2O_2 amount. At the same time, all immunomodulators exerted pronounced protective effect on a condition of membrane lipids that was shown in decreasing of a malondialdehyde quantity in the infected tomato leaves to a control level. The received results demonstrated stabilizing action of the studied immunomodulators on a structural and functional state of photosynthetic apparatus and membrane lipids which these mechanisms demand the further studying.

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ISSN

Investigation of introduction, growth tendencies and biologically active compounds accumulation in showy tick-trefoil (*Desmodium canadense* (L.) DC.)

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The aim of this study - to perform phenology‘s observations of showy tick – trefoil, investigate a viability of seeds in both field and laboratory conditions, to prepare raw of pharmacy and measure biologically active compounds of showy tick – trefoil.

The results of this research showed that seeds of showy tick – trefoil are better germinated in both field and laboratory, when under stratification; the viability of stratificated seeds in laboratory was 16 %, not stratificated 5 %, the viability of stratificated seeds in field was 5 %, whereas not stratificated seeds failed to germinate. It was also found that showy tick - trefoil contains many compounds, such as volatile compounds, flavonoids and phenolic compounds. In addition, this plant distinguish itself with its antioxidant activity. Using the gas chromatography - mass spectrometry method it was found that the biggest amount of votalite compounds between organs were in leaves (13,128 x 10^{6} s. v), followed by the stem blossoms, then stems and, finally, seeds. Moreover, it was established that the biggest variety of volatile compounds are found and identified in the blossoms – founded 16 different compounds (identified - 11 compounds). Furthermore, the biggest amount of volatile coumpounds, between the vegetation phases, were found at the end of flowering phase (9,621 x 10^{6} s. v), whereas, the biggest variety of these volatile compounds were found and identified during the intensive growth phase of the plant. Spectrophotometric analysis showed that the biggest amount of phenolic compound (16,05 RE mg/g) and flavanoid, as well as, antioxidant activity (13,8 RE mg/g) were found between the organs of showy tick-trefoil, particularly, in the parennial plant's leaves. Additionally, the biggest amount of phenolic compound between the phases of vegetation were found in butonisation (30,55 RE mg/g) and at the beggining of flowering phases (30,25 RE mg/g); the biggest amount of flavonoids were found only at the beggining of flowering phase; and the biggest antioxidant activity was established in butonisation phase (27,29 RE mg/g), however, a very similar amount was noted at the beginning of flowering phase as well (27,25 RE mg/g). Using the liquid chromatography (HPLC) method, it was found that the biggest amount of phenolic compounds, between showy tick-trefoil's organs, were in the leaves. It was also established that cumarine predominates leaves, fisetin – blossoms. The research also showed that the biggest amount of phenolic compounds were found at the beginning of flowering, where coumarine dominates.

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Results of Clinical Trials of "Kombucha" Drink In the Treatment of Coronary Heart Disease

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Abstract

Drinking Kombucha in combination with standard therapy leads to a decrease in the activity of peroxidation processes in the blood, which was reflected in a positive effect on biochemical parameters of blood at patients with ischemic heart disease and arterial hypertension, in particular, on lipid metabolism and indicators of the lipid peroxidation processes. The authors note a tendency to of total cholesterol decrease in blood serum. The total blood cholesterol before beverage reception was at the level of 6.6±0.4 μmol/L and after 2 weeks of observing its content fell to 6.1±0.3 μmol/L. Kombucha drinking slightly reduced the level of transaminases: AST - to 0.38±0.04 μmol/L, ALT up to 0,41±0,02 μmol/L. Glucose level did not change after intake of Kombucha. Most patients after intake of the beverage noticed sleep normalization, reduced medication, blood pressure stabilization, improved emotional mood. The article is prepared in the framework of MES RK project "Technology Work Out and Commercial Production of Healthy-Prophylactic Beverage Based on Biologically Active Microorganisms Association of Tea Fungus - Kombucha", 2017-2019".

Keywords: Kombucha, ischemic heart disease, arterial hypertension, cholesterol, lipid metabolism

ISSN

Investigation of Developed "Full Viral" Vector Based On GVA Genome for Reporter Gene Expression

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Abstract

Currently, "full viral" vectors and "deconstructed viral" vectors are used for production of heterologous proteins in plants. The development of new vectors and techniques for creating vectors based on plant viruses does not cease for the goal of creation a more efficient vector with high expression of target proteins. For creation of this viral vector we used Grapevine virus A (GVA). GVA is responsible for considerable crop losses. GVA (a Vitivirus) is associated with the Kober Stem Grooving disease. Viral genome is positive RNA with 5 open reading frames (ORF).

PCASS vector carrying the complete genome of the grapevine virus A (pCASSgva) was used for creation a viral vector based on GVA. The GVA genome was modified by insertion of a cassette carrying reporter gene (eGFP) flanked by 2A self-cleaving peptides between ORF 4(CP) and ORF 5(P10). Stop-codons of CP and eGFP were deleted by PCR mutagenesis. eGFP and ORF5 were fused in-frame to CP. Subsequently the modified GVA genome was subcloned into a pCambia binary vector. The expression of the eGFP in agroinfiltrated *N.benthamiana* leaves at 2-4 dpi was confirmed by using fluorescent microscopy and CP expression was verified by Western blotting. CP was cleaved from eGFP and P10 proteins. But any symptoms of infection at 10-20 dpi on surface of upper leaves were not observed. It will be investigated using the viral vector for the expression of target proteins by magnifection method.

Keywords: GVA, vector, eGFP.

ISSN

Mutations in the *GIGYF2* Gene in Familial Parkinson Disease in Kazakh Population

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Abstract

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. The high prevalence of primary parkinsonism, the presence of phenotypic and genetic heterogeneity of its various forms, the increase in the number of cases of diseases with early onset determine the urgency and complexity of this problem in modern neurology. To date, seven different GIGYF2 missense mutations have been identified in patients with familial PD of European descent. The 7 coding exons of the *GIGYF2* gene were sequenced in Kazakh family with early-onset familial PD. None of the reported GIGYF2 mutations or digenic mutations were detected. In summary, we found no evidence for PD-associated roles of GIGYF2 mutations. Our data suggest that GIGYF2 is unlikely to play a major role in PD in Kazakh patients, similar to other populations.

Keywords: Parkinson's disease, GIGYF2, Kazakh Population.

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Biological Control of Leaf Disease of Barley with *Bacillus* Strain

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Abstract

In recent years much attention has been paid to the use of biological agents for plant disease control. *Bacillus* strains have been used successfully for crop protection and numerous products are currently commercialized throughout the world. However, not all strains of *Bacillus* spp. are capable of becoming active ingredients in biocontrol products for commercial use. The main constraint to large-scale application of *Bacillus*-based biofungicides is their inconsistent performance in the field, from site to site and from year to year.

Here we describe the effects of *B. amyloliquefaciens* subsp. *plantarum* strain IMV B-7404 on spot blotch of barley caused by *Bipolaris sorokiniana* when applied to leaves as cell suspension or metabolites. The strain was selected as a potential biological control agent by *in-vitro* agar plate assay. The strain strongly inhibited the growth of 12 important plant pathogenic fungi.

In growth chamber assays the severity of spot blotch of barley decreased when culture filtrate was applied on leaves or introduced into the plant growth medium before fungal inoculation. Apparently, the metabolites of strain IMV B-7404 play a role in disease prevention, which indicates that the strain can stimulate induced systemic resistance (ISR). When cell suspension was sprayed onto leaves the disease severity depends on environmental and probably some other conditions.

We suggest that effective biological control by *Bacillus*-based products depends on spraying leaves with cell suspension to co-ordinate with the process of plant infection by the pathogen. The ecological behavior and biology of both the antagonist and the target pathogen is the key element in enhancing the efficacy of biocontrol products. It is necessary to know the stage of infection that is most vulnerable. This information needs to be determined individually for every pathosystem and in relation to environmental conditions.

Keywords: *Bacillus*, biological control, direct antagonism, induced systemic resistance, barley, *Bipolaris sorokiniana*

ISSN

Interaction of Heterocyclic Compounds with Efflux Pumps in *Salmonella Enterica*

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Antibiotics are powerful medicines that fight diseases caused by bacterial infections. They either kill bacteria or keep them from reproducing. But there is another side of such treatment using antibiotics – too frequent usage of it for human and farm, water animal therapy for the growth control. Such usage of antibiotics resulted in resistance of pathogenic bacteria to multiple drugs because many of commonly used antibiotics are substrates of efflux pumps. Inhibitors of efflux pumps have a great potential as pharmacological agents that restore the drug susceptibility of multidrug resistant pathogens.

It is very important to discover compounds inhibiting the efflux pumps and enhancing the efficiency of antibiotics as well as to investigate the mechanisms of efflux pumps modulation.

Heterocyclic compounds such as carbazole and phenothiazine could be considered as potential efflux pump inhibitors and substrates. Phenothiazine is heterocyclic compound with two benzene rings linked in a tricyclic system through nitrogen and sulfur atoms. For many years phenoathiazine has been used in veterinary medicine as an antihelminic drug. Such compounds bearing amino alkyl side chain connected to the nitrogen atom are important in medicinal chemistry. The investigation of substituted 10*H*-phenothiazines has strong growth during the last years because of a wide range of applications. Such derivatives are widely employed as antibacterial, antiviral, anti-inflammatory, anticancer, sedatives or tranquilizers agents. 9*H*-Carbazole and its derivatives are an important type of nitrogen-containing aromatic heterocyclic compounds, it possess desirable electronic and charge-transport properties, large π-conjugated system. The various functional groups could be easily introduced into the structurally rigid carbazolyl ring. These characteristics result in the large potential applications of carbazole-based derivatives in medicinal chemistry. It could be used as antitumor, antimicrobial, antihistaminic, antioxidative, anti-inflammatory, psychotropic agents. Carbazole rings are present in a variety of naturally occurring medicinally active substances.

The aim of our work was to evaluate interaction of phenothiazine and carbazole-based compounds with efflux pumps of the cells. Studies of heterocyclic compounds using ethidium bromide agar cartwheel method and effects of the compounds on accumulation of the efflux indicator tetraphenylphosphonium will be presented.

Keywords: antibiotics, efflux pumps, multidrug resistance, inhibitors, substrates, heterocycles.

ISSN

Dipole Polarizability of Hydrogenic Impurity

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Abstract

In this study, we have investigated the static dipole polarizabilty of shallow donor in GaAs quantum dot with infinite potential barrier as a function of dot radius. Also we have performed oscillator strength for the 1s-2p and 2p-3d dipole transitions. The results present that the dot radius has a great effect on static dipole polarizability. As the dot radius increases, static dipole polarizability increases monotonically, and then goes to a constant value corresponding value of a free space hydrogen atom. Polarizability values calculated from Buckingham's formula are in good agreement with the hydrogen atom in large dot radii.

Keywords: Spherical quantum dot, oscillator strength, static dipole polarizability.

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Morphometric Analysis of Foramen Magnum in Raccoon Dogs and Red Foxes

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Abstract

The aim of the study: to measure foramen magnum and to evaluate the sex and species differences.

Material and Methods. Sixty four skulls were examined including 32 skulls of raccoon dogs (16 males and 16 females) and 32 skulls of foxes (16 males and 16 females). Using the method suggested by A. von den Driesch (1976), the height and breadth of foramen magnum was measured. The index of foramen magnum was calculated using V. Onar (1997) formula and the area of foramen magnum was calculated by D. Jain (2014) formulae (Routal and Teixeira). The obtained data were processed using the descriptive statistics (n, X, σ, min, and max). The statistical significance of the differences was evaluated by Student‘s t-test. The data were considered significant when $p<0.05$.

Results. Comparison of the measurements, index and area of foramen magnum in males and females of raccoon dogs showed no statistically significant differences. Statistically significant differences neither were observed between the foramen magnum of males and females of red foxes.

Meanwhile, comparison of species showed significant differences. All investigated measurements of foramen magnum (height, width, index, and area) of red fox males were bigger than those of male raccoon dogs; the differences were discriminate, i.e. highly significant ($P<0.001$). Yet the difference of the index of foramen magnum was of low significance ($P<0.05$). Comparable results were obtained by comparison of the data obtained on females: the breadth, height and area of foramen magnum in red female foxes were bigger than in female raccoon dogs ($P<0.001$) yet the difference of the index of foramen magnum was statistically insignificant ($P>0.05$).

Conclusions:

1. The size and form of foramen magnum in males and females of raccoon dogs and red foxes showed no differences.
2. Foramen magnum of red foxes is bigger than that of raccoon dogs yet the form differences are negligible.

Keywords: foramen magnum, raccoon dog, red fox, skull

ISSN

Characterization of a Spring Viraemia of Carp Virus Isolated in East Ukraine

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Abstract

During a fish health inspection in carp farms in the East region of Ukraine, a Spring Viraemia of Carp Virus was isolated from yearlings of common carp *Cyprinus carpio*. Preliminary examination of infected fish revealed a range of lesions particularly in spleen and kidney tissues. The virus grew in the fish cell lines of FHM and EPC with infectious titer of $10^{6,2-6,5}$ and $10^{5,5-5,8}$ $TCID_{50}$/ml respectively. The morphological changes, such as vacuole enlargements and cells rounding, were caused by virus in appropriate cell lines. Investigation by electron microscopy demonstrated that isolated virus was ultrastructurally similar to rhabdoviruses. Virions were non-enveloped with typical bullet profile, and approximately 80 to 180 nm in length and 60 to 90 nm in diameter. In addition, the nucleotide sequences of glycoprotein gene G fragments in size of 606 base pairs were analysed. The phylogenetic analysis of the glycoprotein gene G based on the comparison of Ukrainian isolates of SVCV with other available sequences revealed a close relationship of Ukrainian isolates of SVCV with sequences that represent the Fijan strain.

Keywords: SVCV, common carp, cell culture, molecular identification.

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ISSN

Inflammatory Bowel Diseases and Application of Serum Hepcidin Quantification

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Abstract.

Inflammatory bowel diseases (IBD) includes different intestinal pathologies, most common among them are Colitis Ulcerosa (CU) and Crohn's Disease (CD). Pathogenesis of IBD is still unclear; however they are multifactor diseases, with genetic and autoimmune compounds, in combination of environmental factors. One of IBD symptoms is iron deficiency anemia.

Aims. We aimed to search for connection between serum hepcidin quantification and anemia in IBD.

Methods. We included 24 patients with IBD - 11 with Colitis Ulcerosa (CU), and 13 with Crohn's Disease (CD). Their results were compared to age and gender matched healthy controls. Laboratory assessments were analyzed for included groups – iron, ferritin, CRP, IL-6 and hepcidin. Ferrozine, nephelometric, ELISA and statistical methods were used during analyzes and obtained results interpretation.

Results. 14 from our patients had with iron deficiency anemia (IDA) and low hepcidin concentrations (6.2 ± 1.0 μg/L) compared to control group (20.4 ± 2.2 μg/L); $P<0.001$. 10 of included cases had combination of IDA and anemia of chronic disease (ACD). Their hepcidin levels were increased (57.7 ± 6.6 μg/L) in comparison to healthy controls (20.4 ± 2.2 μg/L); $P<0.001$. In patients with ACD/IDA, quantified serum hepcidin correlates positively to increased IL-6 ($r=0.774$, $P<0.005$) and CRP concentrations ($r=0.801$, $P<0.001$).

Conclusion. Quantification of serum hepcidin levels in IBD patients might be a key element in diagnosis and treatment of anemia in these patients. Serum hepcidin levels are useful marker for differential diagnosis between iron deficiency anemia and combination iron deficiency anemia/ anemia of chronic disease.

Keywords: hepcidin, inflammation, IBD, iron deficiency, Chron's disease, colitis ulcerosa, anemia

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ISSN

Seed Treatment with Cold Plasma and Electromagnetic Field Improves Germination, Plant Growth and Increases the Amount of Phytoestrogens in the Leaves of Red Clover (*Trifolium pratense*)

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Red clover (*Trifolium pratense L*) is widely used as a fodder plant as well as a dietary supplement marketed for use in alleviation of menopausal symptoms or treatment of prostatic diseases. Commercial extracts of red clover contain estrogenic isoflavones, with formononetin (F) and biochanin A (BA) found in the largest amounts. We studied the effects of pre-sowing seed treatment, using radio-frequency electromagnetic field (EMF, 10 and 15 min) and cold plasma (CP, 5 and 7 min), on germination, growth and biochemical traits of seedlings of two cultivars of red clover – 'Vyčiai' and 'Sadūnai'. The impact of treatments on morphological traits, nutritive value (crude protein content, digestibility, etc. measured by near-infrared spectroscopy) and concentration of isoflavones F and BA (by HPLC analysis) was estimated in leaves of plants grown in the experimental plots for 5 months.

The germination tests *in vitro* revealed that CP treatments increased germination rate, and this effect was more pronounced for 'Vyčiai' (germination rate increased up to 24%). EMF treatments did not affect germination indices, only EMF (15 min) reduced germination percentage of 'Sadūnai' by 12%.

Treatments induced changes in plant morphometric parameters were dependent on cultivar of red clover. The average number of stems per one plant for 'Vyčiai' was increased from 26 to 40% in all treated groups, in comparison to control, whereas the number of stems of 'Sadūnai' cultivar was not affected by seed treatments. Dry weight of 'Vyčiai' plants in CP (5 min) and EMF (15 min) groups was by 37 and 43% larger, dry weight of stems was by 36 and 42%, dry weight of leaves – by 49 and 34% larger in comparison to control, respectively. Treatment induced positive morphometric changes for 'Sadūnai' were less pronounced and EMF (10 min) induced decrease in dry weight (-20%) of plants and weight of leaves (-14%).

Seed treatments did not change the nutritive value of 'Sadūnai' cultivar leaf and stem biomass, but CP (5 min) treatment increased protein content (by 14%) and digestibility (by 8%) of 'Vyčiai' cultivar.

We report for the first time, that seed treatment with CP induced substantial changes of the amount of isoflavones in leaves of red clover: the content of F was decreased (up to 34%), and that of BA – increased (up to 58%), so that F/BA ratio in 'Sadūnai' increased from 0.84 to 1.48 (CP 5 min) and 1.0 (CP 7 min), in 'Vyčiai' – from 0.59 to 1.34 (CP 5 min). EMF treatments decreased the amount of both F and BA (by 15-20%). The amount of BA in one plant of CP (5 min) group is 2.35 fold, and amount of F – 1.35 fold larger in comparison to the average amount of these isoflavones in one plant from the control group.

The obtained data indicate that short time pre-sowing treatment of red clover seeds with CP and EMF induces multiple stress response and leads to modulation of economically important plant traits – improved germination, increased biomass, nutritive value, and amount of secondary metabolites.

Keywords: Cold plasma, Electromagnetic field, Germination, Plant stress response, Pre-sowing seed treatment, Phytoestrogens, Red clover

ISSN

Stimulation of Norway spruce seedling growth and branching induced by pre-sowing Seed Treatment with Cold Plasma and Electromagnetic Field

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Norway spruce (*Picea abies* (L.) Karst.) is one of the most important forest trees in Europe both due to economic and ecological aspects. In Lithuania it covers 23.4% of total forest lands and is considered as one of the most productive trees. Norway spruce is subject to increasing concerns about forest decline and that underlies the need to develop novel technologies that could lead to improved seedling performance and resistance.

Plant seed treatment by electromagnetic fields (EMF) or cold plasma (CP) is recognized as an innovative tool for seed germination enhancement and early seedling growth. The aim of our study was to estimate the effects of pre-sowing treatment of seeds by radio-frequency CP and EMF treatments on Norway spruce germination and seedling growth, and to elucidate possible involvement of reactive oxygen species (ROS) in the molecular mechanism of the induced stress response and signal transduction.

The obtained results indicate that pre-sowing treatment of Norway spruce seeds with CP, vacuum, and EMF can effectively improve seed germination and seedling growth. Treatment with CP (7 min) and EMF (5 min) significantly increased (by 8-10%) the germination yield, whereas the effect of CP (5 min) was negative. The majority of treatments accelerated the germination rate significantly, with CP (2 min) treatment being the most effective. Only the CP (7 min) treatment affected the germination rate negatively. Long-term observations revealed that the treatment-induced pattern of seedling growth changes varied with time. In the end of the second vegetation season (17 months from sowing) seedlings grown from CP5 and CP7 treated seeds, characterized by the negative effects on either germination rate or yield, had 50-60% larger height and 40-50% increased branching in comparison to the control seedlings. In contrast, the EMF treatments that positively affected germination, had no effect on seedling growth on a longer time scale.

Seed treatments with CP and EMF increase the number of paramagnetic centers in dry seeds, however changes in EPR signal do not correlate with changes in the germination kinetics. The obtained results indicate that an increase in organic radical (and, likely, ROS) production is involved in the early stages of the response to CP and EMF treatments in dry seeds. Our study provides the first evidence that ROS (H_2O_2) release from germinating seeds of Norway spruce displays periodic variations and the level of H_2O_2 release is modulated by the pre-sowing seed treatment with CP and EMF. We hypothesize, that effects of CP and EMF on seed germination at least partially may be explained by the induced changes of H_2O_2 amount in germinating seeds.

The revealed facts indicate not only the persistency but also the complexity of the plant seed response to stress induced by short time seed treatment with CP and EMF, leading to the long lasting and continuously developing changes in the traits of growing perennial plant.

Keywords: Cold plasma, Electromagnetic field, Germination, Norway spruce, Plant stress response, Pre-sowing seed treatment

ISSN

Investigation of Antimicrobial Properties of Different Fruits

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Abstract

Fruits are a source of natural compounds that could be widely used for different purposes due to their antimicrobial and antioxidative properties. The food industry needs organic raw that is stable to various industrial processes and prolong the shelf-life of food products. Fruits of European cranberry (*Vaccinium oxycoccos),* highbush blueberry (*Vaccinium corymbosum),* and snowball tree (*Viburnum opulus)* are much appreciated in folk medicine also because of large amounts of biologically active substances. Different cultivars of European cranberry, highbush blueberry, and snowball tree were selected for investigations of antimicrobial properties. The antimicrobial activity of fruit ethanol extracts were determined by the agar well diffusion method. Determination of the antibacterial properties showed that fruit extracts inhibited the growth of wide range of human pathogenic bacteria, both gram positive and gram-negative. Investigation of the antimicrobial properties showed that *V. oxycoccos* extracts inhibited the growth of wide range of human pathogenic bacteria, both gram-negative *(Escherichia coli and Salmonella typhimurium)* and gram-positive *(Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, and Bacillus subtilis).* Examination of *V. corymbosum* fruit ethanol extracts by the diffusion to agar method showed that among gram-negative test cultures *Citrobacter freundii* was the most sensitive. The most effective antibacterial activity was exhibited by the *Viburnum opulus* fruit extracts against *Salmonella typhimurium, Salmonella agona*, and *Lysteria monocytogenes*. *Staphylococcus epidermidis* and *Micrococcus luteus* displayed the highest resistance. In contrast, the growth of the yeast cultures exhibited little or no sensitivity to the fruit ethanol extracts of all species investigated. Our study indicates that fruits of *V. oxycoccos, V. corymbosum, and Viburnum opulus* may act as antimicrobials which control a wide range of pathogens.

Keywords: bacterium, cultivar, test culture, pathogen.

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Effects of elevated temperature and acidity to copper toxicity to *Lemna minor*

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Abstract

This study examines effects of elevated temperature and water acidity to Cu toxicity to *Lemna minor*. The plants were treated with 1-1000 ml/l concentrations of Cu in their growth medium under current temperature (24 °C), elevated temperature (26 °C) and decreased water pH (5.5). *Lemna minor* had a strong phytotoxicity response to Cu application (inhibition of growth, biomass and increased content of malondialdehyde). Temperature and water pH decrease had high impact on Cu toxicity on *Lemna minor*. The highest decrease in growth rate of Lemna minor was observed under higher temperature. Some changes in *Lemna minor* biomass and lipid peroxidation response to Cu was also observed in case of higher temperature and water acidity.

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Production of Recombinant Fibronectin Type III 9-10 Domain in Bacterial Expression System

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Abstract

Fibronectin (Fn) is a glycoprotein that plays important roles in cell adhesion, growth, differentiation and migration by mediating a wide variety of cellular interactions with the extracellular matrix (ECM). Fn usually exists as a dimer composed of two nearly identical 220~250 kDa subunits where each monomer is composed of homologous repeats of three prototypical domains: type I, type II and type III. Fn interacts with many integrins such as α3β1, α5β1, α8β1, αvβ1, αIIββ3, αvβ3, αvβ5, and αvβ6. In previous studies, the specific integrin-recognition sequences involved in cell adhesion have been identified. The best known of these – Arg-Gly-Asp (RGD) sequence – is located in the central cell-binding domain - $FnIII_{10.}$ It is the most important recognition site that can interact with about half of all known integrins. Another important sequence which acts in synergy with the RGD site is - Pro-His-Ser-Arg-Asn (the 'synergy site' PHSRN) found in Fn repeat $III_{9,}$ that promotes specific α5β1 integrin binding.

Because of the ECM binding properties Fn is a perfect protein for nanofabrication and integrin-mediated immobilization of cells into synthetic scaffolds. Heterologous expressions of large eucaryotic proteins, such as Fn, in bacterial expression system is complicated. Therefore the aim of this study was to establish a recombinant protein production system for the "cell-binding domain" of Fn protein - including $FnIII_{9-10}$ fragment and to assess the effect of His-tag position on the recombinant peptide purification efficiency. For this purpose, a sequence of the $FnIII_{9-10}$ fragment was cloned to pLATE bacterial expression vector using a ligation independent cloning system. This vector includes bacteriophage T7 promoter that ensures high yields of expressed proteins. Two constructs including either amino- or carboxy- terminal 6xHis-tag were developed. The constructs were transformed into *E.coli* strain BL21 (DE3) containing the T7 RNA polymerase gene under the control of the IPTG - inducible lacUV5 promoter. Cell growth and protein induction conditions were optimized to ensure maximum yields of the recombinant peptides. The peptides were purified using Ni-NTA column. Both constructs eluted at low imidazole concentrations, suggesting that binding of the His-tag was hindered by interactions with structure of the Fn peptide, and the position of the tag had no effect on this interaction. Cross-reactivity of the recombinant peptides with monoclonal antibody specific to the "cell-binding domain" of Fn was assessed by using immunoblot analysis.

Keywords: fibronectin, type III module, cell-binding domain, recombinant protein production, ligation independent cloning, His-tag.

ISSN

Comparison Of Natural And Synthetic Chitin Films

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Abstract

Natural and synthetic chitin films obtained from the same source were produced and some properties were examined comparatively. Firstly natural chitin film was obtained from elytra of an insect (*Oryctes nasicornis* L.) and purity of the obtained chitin film (degree of acetylation: 79 ± 2%) was proved by ^{13}C nuclear magnetic resonance (^{13}C NMR). And then synthetic film was produced by dissolving of natural chitin film in LiCl–DMAc. The obtained natural and synthetic films were characterized by AFM, TGA, DSC, FTIR, mechanical properties, light transmission and contact angle. The analyses results demonstrated that natural chitin film lost very important properties such as high thermal stability, transparency, nanofibrous nature, tensile strength, Young's modulus and hydrophobicity after transforming the synthetic film.

Keywords: insect; elytra; chitin; natural film; synthetic film

ISSN

Growth Activity Of Hydrocarbon Oxidizing Microorganisms Of The Caspian Region On The Environment Of Oil-Containing And Petroleum Products

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Abstract

Pollution of the environment as a result of man's technogenic activity impairs the ecological situation in quite large areas. The most typical anthropogenic factors of environmental pollution are oil and oil products, which have a negative impact on living organisms. Studying of microorganisms living in the oil-polluted environment is important because of their participation in the utilization of hydrocarbons. Therefore, it is of considerable interest to systematically study the degradation of oil and oil products by native strains of microorganisms isolated from contaminated territories. From the accumulative cultures of hydrocarbon oxidizing microorganisms obtained by using samples of water, soil, that taken from the area of oil and gas fields of Karazhanbas and Kalamkas, more than 400 isolates, were isolated 57 of which were capable of stable growth in liquid and solid mineral medium of Voroshilova-Dianova. In the presence of 1% of the oil of these fields. 7 isolates that identified as strains of *Ochrobactrum sp. skar4, Rhizobium sp.skar7, Achromobacter sp.skar8, Rhodococcus fascians skar21, Roseomonas mucosa wkal24, Stenotrophomonas sp.wkal52, Sphingobacterium sp.wkar54* characterized as high-biomass growth (4 times or more) that determined gravimetrically and also characterized by a high percentage of degradation of oil components (50- 91%), determined by the fluorimetric method [1,2]. These strains were selected for cultivation on a medium containing petroleum products. In the presence of toluene in the nutrient medium, as the only source of carbon and energy, the growth of all selected strains of microorganisms was insignificant. During the cultivation on a diesel fuel medium for 3 cultures of 7 - *Ochrobactrum sp. skar4, Achromobacter sp.skar8, Rhodococcus fascians skar21* , there was a significant increase in biomass, which for 6 days of cultivation of microorganisms exceeded initial values in 6 or more times. During the cultivation in a medium with gasoline good growth activity demonstrated *Ochrobactrum sp. skar4, Rhodococcus fascians skar21, Sphingobacterium sp.wkar54* (*biomass increase in* 3 times or more after 6 days of culture). The remaining strains, that characterized by a good growth in local oil deposits, showed a slight increase in biomass during cultivation on the stadied petroleum products studied.

Thus, strains of *Ochrobactrum sp. skar4, Rhodococcus fascians skar21, Achromobacter sp.skar, Sphingobacterium sp.*wkar54 have a good growth activity on oil, diesel fuel gasoline, have high potential degradation of petroleum hydrocarbons, and can be used for creation of consortiums on their basis for further usage in bioremediation.

Keywords: Bioremediation, oil degradation, hydrocarbon oxidizing microorganisms, growth activity.

ISSN

Biostratigraphy and Paleoenvironment of the Sarmatian (S.L.) Deposits from the Eastern Part of Moldavian Platform (Comarna Borehole) Based On Foraminifera

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Abstract

Due to the marine salinity conditions change with a brackish regime during Lower Sarmatian from Paratethys, the Moldavian Platform deposits of this time period are characterized by stenohaline and euryhaline microfaunal forms. *Cibicides lobatulus*, *Quinqueloculina fluviata* and *Quinqueloculina karreri ovata* foraminifera species mark the beginning of Sarmatian in the Moldavian Platform and the presence of other genera, like *Nonion, Elphidium, Articulina, Bulimina, Bolivina* indicate that a part of the Upper Badenian specific microfauna has adapted to lower salinity water of Paratethys. *Cibicides* genus, having small size, marks the sudden change of the environmental conditions and the installation of a brackish regime. The predominance of benthic foraminifera forms indicates a normal oxygen content at the substrate surface and the abundance of foraminifera species with calcareous tests indicates that the sedimentation of the Lower Sarmatian deposits was made in the warm sea conditions.

Keywords: foraminifera, biostratigraphy, paleoecology, Sarmatian (s.l.), Miocene, Moldavian Platform

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ISSN

Effect of Growth Regulators on *Miscanthus x giganteus* Greef et Deuter Direct Regeneration *in vitro*

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Miscanthus x giganteus Greef et Deuter is a one of the plants most widely considered as a species useful for sustainable agriculture, firstly has been widely used as a landscape ornamental plant and latter has received attention as a biomass feedstock of its great biomass production and low-maintenance requirements. However, *Miscanthus*×*giganteus* Greef et Deuter is a triploid hybrid (2n=57) that cannot produce viable seeds, and it has traditionally been propagated through rhizome division, which is slow, low throughput and labor-intensive. On the other hand, the sterility of *Miscanthus*×*giganteus* Greef et Deuter prevents putative invasion by this species. Nevertheless, the principal limitations of *M. giganteus* Greef et Deuter growing are its high establishment cost, its poor overwintering and the insufficient water supplies. Micropropagation is a practical method for rapid and large-scale production of many plant species. Although protocols exist for *Miscanthus*×*giganteus* Greef et Deuter micropropagation, the multiplication rate and plant quality need to be improved to meet commercial demands. The objective of the present investigation was to evaluate the effect of growth regulators on direct regeneration of *Miscanthus x giganteus* Greef et Deuter. Research was carried out in Institute of Biology and Plant Biotechnology of Aleksandras Stulginskis University and Laboratory of Agrobiotechnology of Join Research Center in 2016-2017. Segments of *Miscanthus x giganteus* Greef et Deuter rhizomes were cultivated on MS nutrient medium supplemented with different concentrations of BAP, NAA and TDZ. Our results showed that rhizomes segments cultured on MS media without growth regulators did not show any response. On the medium supplemented with growth regulators shoot regeneration frequency varied depending on used growth regulators combinations and concentrations. Combination of 2.0 mg l^{-1} TDZ + 0.05 mg l^{-1} NAA in culture medium promoted the highest shoots regenerations frequency with a highest number of shoots per explant. Developed regeneration procedure can be employed in micropropagation and in strategies for further genetic enhancement and improvement of commercial *Miscanthus* × *giganteus* Greef et Deuter cultivars.

Keywords: *Miscanthus* × *giganteus* Greef et Deuter, direct regeneration, growth regulators.

ISSN

Bartonella Infection in Red Squirrel (*Sciurus vulgaris*) and Their Ectoparasites

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Abstract

Bartonella are vector-transmitted, intracellular, gram-negative bacteria that is leading to blood-borne infections. Prevention of diseases is becoming important not only of pathologies caused by bacteria but also there is the risk of infection, because wild and domestic mammals are the main reservoirs of bacteria. Rodents are associated with vector-borne (tick, flea, mite, lice) pathogens that is causing infectious diseases worldwide. The aim of this study was to identify infection of *Bartonella* species in Red Squirrels (*Sciurus vulgaris*) and their ectoparasites using molecular tools. A total 32 samples of squirrels were analysed from different regions of Lithuania. Squirrels were infested with *Ixodes ricinus* tiks (154) and *Ceratophyllus sciurorum* fleas (31). *Bartonella* DNA in samples was detected using a nested-PCR of the 16S-23S rRNA ITS region gene. *Bartonella* DNA was detected in 28% squirrels, 22.6% fleas and 0.6% ticks. Sequence analysis of 16S-23S rRNA ITS region showed that sequences were identical or similar to *Bartonella washoensis*. The results of this study suggest that fleas may be substantial vector for transmitting of *Bartonella washoensis*.

Keywords: *Bartonella*, squirrel, tick, flea, Lithuania.

ISSN

The Response Of Photosynthetic Parameters Of Different Growth stage Winter Wheat And Pea To Different Levels Of Salinity Stress

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Abstract

The aim of this work was to investigate the response of physiological parameters of winter wheat (*Triticum aestivum* L. cv. ʾAdaʾ) and pea (*Pisum sativum* L. cv. Early onward) to different levels of salinity stress and evaluate their resistance. The main experiment was divided into two separate experiments. The first group of plants was treated with 100 mM and 200 mM salinity concentrations 2 days after seedling (germination stage according to BBCH scale) in the first experiment. The second group of plants was exposed to an identical salinity treatment when developed the second true leaf (leaf development stage according to BBCH scale). After salinity treatments, which lasted 2 weeks each, the response of physiological parameters as chlorophylls fluorescence ratio (*Fv/Fm*), photosynthetic and transpiration rate, water use efficiency, chlorophyll and carotenoid concentration were measured.

The greatest effect of salinity was identified in net photosynthetic rate, transpiration rate and chlorophyll *a/b* ratio in pea plants. Photosynthetic and transpiration rate significantly decreased, while chlorophyll *a/b* ratio increased. At leaf development stage, investigated parameters of pea plants were more sensitive to salinity than wheat. The physiological parameters similarly to the germination stage, transpiration and photosynthetic rate decreased mostly and showed the greatest percentage reduction over control. The results of the experiment have shown that both plants were sensitive to salinity stress. According to all parameters investigated, wheat plants were more resistant to increasing salinity stress than pea plants.

Keywords: salinity stress, growth stages, photosynthesis, transpiration, chlorophylls, chlorophyll fluorescence.

ISSN

The response of morphological and physiological parameters of *Hordeum sativum* L. and *Panicum miliaceum* L. under the effect of drought stress at different fertilization

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Abstract

Drought is considered as one of most often occurring consequences of global climate change as well as one of the biggest threats to ecology and agricultural systems. The stress of drought may be harmful to various plant functions; however, the most visible impact is reduced carbon fixation which decreases photosynthesis and results in reduction of harvest productivity. Thus, it is very important to find measures which would help to increase plant resistance and preserve the productiveness of the harvest.

Thus, this research is aiming to investigate the impact of different fertilizers on spring barley (*Hordeum sativum* L.) and proso millet (*Panicum miliaceum* L.) resistance under the impact of drought. Seeds have been planted into pots with already prepared sand and neutralized peat substrate. The organic („DCM“) and mineral („YaraMila complex“) fertilizers have been added for the first time during the planting stage and the second time after 19 days. The first spraying with leave fertilizer („Aton AZ“) was executed when plants had two real leaves – after 15 days, the second – four days after the first spraying, the third – 8 days after the last spraying. The stress of drought has lasted for 10 days. At the end of the experiment morphometric and physiological parameters have been measured.

The fertilizer impact on morphometric indicators of barley and millet has been stronger than the one of drought. However, drought had much stronger effect on photosynthesis indicators. The results show that drought has reduced the rate of photosynthesis in barley; however, a low and insignificant reduction of photosynthetic rate and only after applying organic fertilizer has been detected in millet. The transpiration rate and intercellular CO_2 concentration have also significantly lowered in plants at drought impact. The lowest transpiration rate caused by drought has been detected using leave spray fertilizer, and the lowest intercellular CO_2 concentration using mineral fertilizers. Water use efficiency of barley and millet increased at drought conditions. Mineral fertilizer has had most effective impact on barley with regard to this factor at drought, and organic fertilizer has been most effective on millet.

Keywords: fertilization, drought stress, photosynthesis, transpiration, water use efficiency, chlorophyll fluorescence.

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Rickettsia species in ticks and mites collected from small rodents in Lithuania

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Abstract

Rickettsia species are obligate intracellular gram-negative bacteria responsible for human infections in many countries around the world. Vectors of these pathogens include arthropods such as ticks and mites, which feed on very different species of small rodents. Nevertheless, there are only few studies focusing on investigation of rickettsial pathogens in rodent ectoparasites. The aim of this study was to characterize the species of ticks and mites found on small rodents in Lithuania and to examine collected ectoparasites for the presence of *Rickettsia* pathogens. A total of 238 small rodents of six different species were collected in Curonian Spit (Lithuania) during 2013-2014. Rodents were found to be infested with *Ixodes ricinus* ticks (n=578) and 5 species of parasitic mites (n=570) from Laelapidae family (*Laelaps agilis*, *Hyperlaelaps microti*, *Haemogamassus nidi*, *Eulaelaps stabularis*, *Myonyssus gigas*). *Rickettsia* DNA was detected in 16.5% *I. ricinus* ticks and 7.4% Laelapidae mites. Infected ectoparasites were found on *Apodemus flavicollis*, *Micromys minutus*, *Myodes glareolus*, *Microtus oeconomus* and *Microtus arvalis* rodents. PCR and sequence analysis of partial *gltA* and *17kDa* genes revealed the presence of *R. helvetica*, in *I. ricinus* ticks and *L. agilis* mites, and *R. felis* in *H. microti* mites. The results of the study demonstrate the presence of spotted fever group rickettsiae in ticks and mites in Lithuania. This study is the first reports of *Rickettsia* species in ticks and mites in Lithuania, and the first detection of *R. felis* in Gamasida mites.

Keywords: *Rickettsia*, ticks, mites, rodents, Lithuania.

ISSN

Antioxidant and Antimicrobial Activity of Oak (*Quercus* spp.) Acorn Collected in Lithuania

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Abstract

Acorns, also called as oak nuts, are fruits of oak tree (*Quercus* spp.). Acorn coffee is the most popular form of usage of acorns in Lithuania. However, studies show wider applications of acorns as functional food or food ingredient [1-3]. The antiproliferative and apoptosis-inducing activities [4], antioxidant and antibacterial activities [3] of acorn extracts are also studied and described. The studies reveal that all activities of plants strongly depend on their species, geographic origin and growing conditions. The method of sample preparation also has a high impact on the various activities of the plants.

The aim of this study was to compare antioxidant and antimicrobial activities of acorn extracts prepared using different approach. Acorns were collected in three places in Lithuania. The grinded fresh acorns were extracted with methanol using different conditions, i.e. in room temperature, using exposure to ultrasound, applying ultrasound with heating, using only heating. Antimicrobial activity of prepared extracts was tested against some bacteria (*Micrococcus luteus*, *Enterococcus faecalis*, and *Bacillus cereus*) and fungi (*Alternaria alternate* and *Penicillium funiculosum*) using well diffusion assay.

Not all extracts inhibited growth of all tested bacteria, the inhibition level was strain and extract preparation method depended. Similarly not all extracts showed antifungal activity. Spectrophotometric tests were used to evaluate the antioxidant activity and total flavonoid content in the extracts. Both antioxidant activity and flavonoid content varied between differently prepared extracts. Antioxidant and antibacterial activity in the tested samples also were depended on the acorn collection place, what might be related with oak tree genotype or phenotype. To our knowledge such study of acorns collected in Lithuania had not been performed before.

Keywords: *Quercus* spp, acorns extract, antioxidant activity, antibacterial activity, antifungal activity.

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ISSN

Molecular Detection Of Vector-borne Agents in Edible Dormouse (*Glis glis*) In Slovakia

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Abstract

Edible dormouse (*Glis glis*) serves as a competent reservoir host for some *Borrelia* spirochetes in nature [1]. Because of its protected status there is scarce data about the prevalence of other vector-borne pathogens in their populations. For this purpose, 217 animals of edible dormouse (*Glis glis*) were captured in two sylvatic regions in Slovakia (Rozhanovce game reserve and Slovak Karst) during the years 2013 to 2016. Dormice were found from June to September during the inspection of nest boxes, which are frequently used by dormice to hibernate and rear their offspring [2]. At the time of capture ticks were not recorded on dormice ears. Extracted DNA from ear biopsies was screened for the presence of *Borrelia burgdorferi* sensu lato, *Rickettsia* spp., *Anaplasma phagocytophilum* and *Borrelia miyamotoi* using genus/species specific PCR based methods followed by sequencing analysis. The highest prevalence was found for *B. burgdorferi* s.l. spirochetes reaching 6.9 %. Multilocus sequence typing analysis revealed only the presence of rodent-associated pathogenic *Borrelia afzelii*. *Rickettsia* spp. was found in 4 individuals (1.8 %), while *B. miyamotoi* DNA was amplified in 2 samples (0.9 %). All samples showed negative result for the presence of *A. phagocytophilum*. Our results point that despite their arboreal life style dormice represent important blood source for sheep ticks (*Ixodes ricinus*), which serve as main vectors for studied zoonotic agents. These microorganisms can persist for some time in dormice, possibly increasing the chance for infection of questing ticks.

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Keywords: dormouse, *Glis glis*, bacteria, *Borrelia*.

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ISSN

Climate Warming Determined Changes of Introduced Conifer Species Bud Swelling Phenophase, Considering the Aspect of Hardiness Zones in their Natural Habitats

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Abstract

Many plant species across the globe had shifted their ranges or changed behaviours such as advanced bud burst, leaf unfolding, flowering, a delay in leaf colouring and fall, and consequently the extended duration of vegetation period due to changing climatic conditions. Changing climate may determine better adaptation of introduced species especially in boreal climate zones.

Long term phenological observations (data for some species available starting since the 1956) of introduced species to Lithuania were carried out by dendrologists of Kaunas Botanical Garden of Vytautas Magnus University. Sequences of the start date of bud swelling phenophase of 11 introduced conifer species (*Abies veitchii*, *Larix decidua*, *L. kaempferi*, *L. laricina*, *L. x marschlinsii*, *Larix sibirica*, *Larix sukaczewii*, *Picea mariana*, *Pinus mugo*, *Taxus baccata* and *Thuja occidentalis*) were analyzed.

Longest data sequences (1956–2016) were available for *Larix decidua*, *L. kaempferi*, *L. laricina*, *L. sibirica*, *Pinus mugo* and *Taxus baccata*. The start of bud swelling phenophase advanced on average from 10 (*L. kaempferi*) to 35 days (*T. baccata*) in 1956–2016, while the speed of changes was respectively from -0.17 to -0.59 days/year. Data sequences since 1980 were available for all 11 conifer species. In comparison to longer period (1956–2016), results from shorter period (1980–2016) show that changes of bud swelling phenophase were faster: the speed of changes for the same species in shorter period was -0.52 days/year (*L. kaempferi*) and -0.75 days/year (*T. baccata*). Most rapid changes in 1980–2016 period were estimated for *Abies veitchii* and *Picea mariana* (the speed of changes respectively was -0.98 and -0.92 days/year) and slowest – for *Larix sibirica* and *L. sukaczewii* (-0.10 and +0.12 days/year).

The advance of bud swelling phenophase start date of introduced conifer species was most influenced by March temperature in Lithuania: data of 9 species correlated statistically significant with this temperature (*r* ranged from -0.40 for *Picea mariana* till -0.71 for *Larix kaempferi*).

The connection between the observed conifer species hardiness zone of natural habitat and the reaction of bud swelling phenophase to the climate warming was not clearly expressed if all species from different genuses were compared. Cluster analysis showed two major clusters – first included all *Larix* species, *Taxus baccata* and *Thuja occidentalis* (their habitats belong to hardiness zones ranging from 1 till 8), and second – *Abies veitchii*, *Picea mariana* and *Pinus mugo* (hardiness zones 2–7). If species from one genus were compared (6 different species of *Larix*), the connection with hardiness zone was expressed more clearly: the reaction of bud swelling of species introduced from colder (1–5) hardiness zones was slower (the speed of bud swelling phenophase changes was -0.10 days/year for *Larix sibirica* and +0.12 days/year for *L. sukaczewii*) to climate warming, than of species introduced from warmer (5–7) hardiness zones (-0.42 days/year for *Larix kaempferi* and -0.52 days/year for *L. x marschlinsii*).

The obtained result data indicate the advance of bud swelling phenophase of 10 introduced to Lithuania conifer species (the speed of changes varied from -0.10 till -0.98 days/year in 1980–2016) and the delay of *Larix sukaczewii* (respectively, +0.12 days/ year) bud swelling phenophase, mainly influenced by March temperature, depending also on the hardiness zone of species natural habitat within the range of one genus.

Keywords: Bud swelling phenophase, Climate warming, Conifer species, Hardiness zone

ISSN

Transgenerational Stress Memory in *E. purpurea*: Seed Treatment with Cold Plasma and Electromagnetic Field induces changes in Germination, Seedling Growth and Phytohormone Pattern in the Seeds of Second Generation

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Abstract

Purple coneflower, *Echinacea purpurea* (L.) Moench, is widely used as medicinal plant and dietary supplement. We investigated the effects of pre-sowing seed treatment with physical stressors – cold plasma (CP, 2-7 min) and electromagnetic field (EMF, 5-15 min) on seed germination and seedling performance. We reported earlier [1] that all applied seed treatments with CP and EMF not only stimulated germination and plant growth in the first year of vegetation but also induced increase in the amounts of biologically active constituents (cichoric acid, vitamin C) and antioxidant activity in leaves of plants (up to 3-fold). In this study, we report also results obtained on the persistency of treatment effects in the second year of plant vegetation, estimated by changes in the morphometric traits of plants and also some inherited traits in the second generation.

We observed that in the second year of vegetation the most sustainable positive effects were in short CP treatment (2 min) and EMF treatments (5, 10 and 15 min) groups. Plants grown from CP (2 min) treated seeds had larger number of branches, flowers, and leaves (by 45, 32 and 26%, respectively); larger fresh weight of stems, flowers, and leaves (by 31, 30 and 29%, respectively) in comparison to control. The only effect in plants from all EMF treated groups was larger stem height (from 14 to 26%) and stem weight (from 38 to 49%).

The seeds from plants grown from CP- and EMF-treated seeds were collected (second generation seeds, non-treated) and tested *in vitro* for the germination parameters, early seedling growth and amount of phytohormones in seedlings grown from these seeds. The germination tests *in vitro* revealed that short time CP and EMF (2 and 5 min, respectively) treatments induced increase in germination rate in the seeds of second generation as compared to control. However, the decrease in germination rate (from 10 to 20%) of longer treatment groups was observed.

The seedlings grown from seeds of the second generation in all CP and EMF treated groups had considerably larger seedling length (up to 17%) and weight (up to 25%), except for EML (5 min) group. Seedlings were subjected for extraction of plant hormones in order to investigate possible involvement of phytohormones in the stress "memory" mechanism and to find out correlations between hormone amount and seedling growth. In this study, four types of plant hormones were quantified by HPLC in the extracts of seedlings: auxins (indole-3-acetic acid, IAA), indole-3-butyric acid (IBA), cytokinins (zeatin, Z), gibberellins (gibberellic acid, GA3 and gibberellin A7, GA7), abscisic acid (ABA), and salicylic acid (SA). Considerably lower ABA/GA7 (from 1.2 to 8.5-fold), IAA/Z (from 29 to 80%) ratios and IAA+IBB/Z values (from 1.5 to 4.2-fold) were obtained for seedlings of second generation of all treated groups as compared to the control, whereas ABA/GA3 ratio decreased only in seedlings from CP (7 min) group, and did not change in CP (2 min) and EMF (15 min) groups.

Keywords: *Echinacea purpurea*, Cold plasma, Electromagnetic field, Phytohormones, Seed stress response, Stress memory.

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Microbiological activity of the optimized sod-podzolic soil as an important element of stability of agroecosystems

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Abstract

The main direction of modern land use, in accordance with the principles of ecological imperative, is the agroecological concept, which aims to achieve optimal functioning of the agroecosystem. Sustainable development of the agroecosystem is achieved by optimizing its structure and all components.

Biological activity is one of the most sensitive indicators, reflecting the ecological condition and level of soil fertility. Synthesis of the basic mass of organic matter is carried out mainly by plants. The main activity of soil microorganisms is the mineralization of organic matter. In the process of mineralization exempt nutrients, which determines to a considerable extent on natural soil fertility.

On vital activity of microorganisms is influenced by different factors: soil temperature, humidity, types of soil-forming processes, grow crops etc.

A major role in the decomposition of organic compounds of different soil bacteria play. Destruction of cellulose on (82-94%) is carried out by bacteria and only 18-16% by mold fungi.

In the experimental base «Budagovo» (Smolevichi region, Belarus) from 1978 to 1990, was conducted a field experiment on optimization of sod-podzolic waterlogged soils (luvisols and albeluvisols for the WRB) by applying absolutely dry peat in doses of 100, 200, 300 and 400 t / ha (tons per hectare). The aim was to create an artificial arable horizon with an organic matter content of 7-8%, including 4-5% humus.

More than 20 years to prove the effectiveness of the method optimization the experimental plots were taken soil samples for various tests, including microbiological activity.

For the microbiological analysis of the soil samples were taken from a depth of 10-50 cm from four field replications.

The intensity of decomposition of fiber was taken into account by the gravimetric method. The difference between the weight of the original and remaining after extraction from the soil tissue was judged by the intensity of the process of destruction of cellulose.

The intensity of decomposition of fiber in the optimized waterlogged soil (%of initial fabric weight) were as follows: background – 16 %, background+100 t/ha – 20%; background+200 t/ha – 22 %; background+300 t/ha – 25% background+400 t/ha – 26%.

On a mineral optimized soil, microbiological processes proceed more slowly 2.2 times under perennial grasses than under a tilled crop.

Microorganisms are unevenly distributed in the profile. The upper half of the application of cotton fabric, which was located approximately up to 30 cm, was subjected to greater decomposition. With the depth the number of microorganisms in the soil is reduced.

By the method of applications of cotton fabric, a direct correlation of microbiological intensity is established from the doses of introduced organogenic additives.

Keywords: microbiological activity, soils, agroecology, optimization of soil.

ISSN

Comparison of Chitin Surface Structure in Order *Blattodea*

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Abstract

Chitin is widely used in various areas and it is regarded as one of the most important polysaccharide after cellulose. It has advantages such as thermal stability, biodegradability, non-toxicity and biocompatibility, which makes this biopolymer very attractive research object for biomedical, pharmaceutical, environmental, agriculture and food industries. Also, chitin is present in nature: in the cell walls of fungi and yeast, exoskeleton of Arthropods, Hydrozoa, Bryozoa, Porifera and some Anthozoan species. Within the exoskeleton, the various concentrations of water and chitin determines and defines the mechanical properties of the insects and crustaceans' exoskeleton. Moreover, the organization of chitin fibers and pores also contributes surface and physical properties of the exoskeleton. The tropical genus of *Blaberus* - *Blaberus giganteus* is known as one of the largest cockroaches. In the present study, we preferred this organism because of the following reasons: very high survival level, easy culturing, big size, not fastidious diet, transparent wings and dorsal pronotum. A chemical method was followed for the chitin isolation from the cockroach wings and dorsal pronotum, which includes four main steps: demineralization, deproteinization, depigmentation and oil removal. This study reports production of natural chitin from the wing and the dorsal pronotum of cockroach. The physical properties of chitin were identified.

Keywords: Chitin, surface, *Blattodea*

ISSN

Stress Regulating Properties Of Two *Bacillus* Sp. Bacterial Endophytes In Apple (*Malus* ×*Domestica*borkh.) Culture *In Vitro*

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Abstract

Vegetative propagation methods are commonly used for horticultural plants of *Rosaceae*family, such as apple, pear, cherry, peach and strawberry. Development of *in vitro*micropropagation is important for plant biotechnology research and agricultural applications. *In vitro*environment imposes unfavorable conditions that lead to imbalance of plant physiological equilibrium and induction of oxidative stress, which occurs via production and accumulation of reactive oxygen (ROS) species. ROS cause lipid peroxidation leading to membrane injury, enzyme inactivation and DNA damage. It has been demonstrated that endophytic microorganisms play important role in plant acclimation and stress response. However, capacityof endophytes to modulate plant stress responce under *in vitro* conditions is largerly unexplored. Therefore the aim of this study was to assess effect of two endophytic *Bacillus*sp. bacteria strains (Da4 and Oa4) on ROS production and gene expression in apple cell suspension and stress reducing properties in shoot culture *in vitro*. Our study revealed that the two endophytic bacteria strains associated to apple cv. Gala cells in suspension (Fig. 1), and demonstrated different intracellular ROS production regulating properties (Fig. 2). Protein expression analysis using DIGE revealed that incubation of the cells with ROS production inhibiting strain Oa4 resulted in highly increased abundance of 29 proteoforms involved in defense responce, metabolic and oxidation –reduction processes (Fig. 3). Meanwhile 12 and 25 proteins of increased or decrased abundance, respectively, were detected after incubation with either of the endophytic strains. In apple cv. Gala shoot culture, ROS production was mostly detectable in leaf and injured tissues (Fig. 4). Lipid peroxidation analysis established that oxidative damage was the most prominent during first week after replanting and during onset of senescence of the culture (Fig. 5). Both endophytic *Bacillus*sp. strains reduced the shoot lipid injury level approx. 2.2 fold after one week of co-cultivation. Further, contrasting effect on the apple shoot morphologicaltraits was observed afterthree weeksof co-cultivation. As compared to control, shoots inoculated with the strain Da4 formed 2.2 and 1.8 fold larger biomass and adventitious shoot number, respectively. Meanwhile, strainOa4 had inhibitory effect. The results suggested that the two endophytic strains exploited different interactions with plant cells and had different plant oxidative stress regulating properties under *in vitro*conditions.

Keywords: endophytic bacteria, domestic apple, plant stress, gene expression.

ISSN

Stimulation of Norway spruce seedling growth and branching induced by pre-sowing Seed Treatment with Cold Plasma and Electromagnetic Field

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Abstract

Norway spruce (*Picea abies* (L.) Karst.) is one of the most important forest trees in Europe both due to economic and ecological aspects. In Lithuania it covers 23.4% of total forest lands and is considered as one of the most productive trees. Norway spruce is subject to increasing concerns about forest decline and that underlies the need to develop novel technologies that could lead to improved seedling performance and resistance.

Plant seed treatment by electromagnetic fields (EMF) or cold plasma (CP) is recognized as an innovative tool for seed germination enhancement and early seedling growth. The aim of our study was to estimate the effects of pre-sowing treatment of seeds by radio-frequency CP and EMF treatments on Norway spruce germination and seedling growth, and to elucidate possible involvement of reactive oxygen species (ROS) in the molecular mechanism of the induced stress response and signal transduction.

The obtained results indicate that pre-sowing treatment of Norway spruce seeds with CP, vacuum, and EMF can effectively improve seed germination and seedling growth. Treatment with CP (7 min) and EMF (5 min) significantly increased (by 8-10%) the germination yield, whereas the effect of CP (5 min) was negative. The majority of treatments accelerated the germination rate significantly, with CP (2 min) treatment being the most effective. Only the CP (7 min) treatment affected the germination rate negatively. Long-term observations revealed that the treatment-induced pattern of seedling growth changes varied with time. In the end of the second vegetation season (17 months from sowing) seedlings grown from CP5 and CP7 treated seeds, characterized by the negative effects on either germination rate or yield, had 50-60% larger height and 40-50% increased branching in comparison to the control seedlings. In contrast, the EMF treatments that positively affected germination, had no effect on seedling growth on a longer time scale.

Seed treatments with CP and EMF increase the number of paramagnetic centers in dry seeds, however changes in EPR signal do not correlate with changes in the germination kinetics. The obtained results indicate that an increase in organic radical (and, likely, ROS) production is involved in the early stages of the response to CP and EMF treatments in dry seeds. Our study provides the first evidence that ROS (H_2O_2) release from germinating seeds of Norway spruce displays periodic variations and the level of H_2O_2 release is modulated by the pre-sowing seed treatment with CP and EMF. We hypothesize, that effects of CP and EMF on seed germination at least partially may be explained by the induced changes of H_2O_2 amount in germinating seeds.

The revealed facts indicate not only the persistency but also the complexity of the plant seed response to stress induced by short time seed treatment with CP and EMF, leading to the long lasting and continuously developing changes in the traits of growing perennial plant.

Keywords: Cold plasma, Electromagnetic field, Germination, Norway spruce, Plant stress response, Pre-sowing seed treatment

ISSN

Quantitative analysis of flavonides and vitamine C in wild plants of flora of Kazakhstan

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Abstract

To develop effective pharmacological drugs it is necessary to select a medicinal plant raw material containing biologically active components with a broad-spectrum action. Such biologically active components can be represented by flavonoids and vitamin C, which are an ideal combination that possesses different kinds of pharmacological activity, including capillary-strengthening, anti-inflammatory, antibacterial, antioxidant. The purpose of this study is to determine the concentration of flavonoids and vitamin C in samples which were extracted from different parts of wild plants using two solvents with different polarity. The objects of study were wild plants of flora of Kazakhstan, belonging to different families*: Rumex confertus, Solidago virgaurea, Vicia subvillosa, Urtica urens, Peganum harmala, Onobrychis arenaria, Chenopodium botrys, Mentha arvensis, Conium maculatum, Artemisia absinthium, Paeonia intermedia, Salvia deserta, Astragalus lanuginosus, Platycladus orientalis, Veronica incana, Vexibia alopecuroide, Genista tinctoria, Caragana camilli, Chelidonium majus, Sanguisorba officinalis, Linum pallescens, Allium schubertii, Astragal sieversianus*. Extracts were obtained by two-stage maceration. The amount of flavonoids and vitamin C was determined photometrically using aluminum chloride, ferric chloride and potassium ferricyanide, respectively[1,2].

The results of studies have shown that the content of flavonoids in extracts ranged from 4,2 to 128,8 mg/g. The maximum content of flavonoids was typical for extracts *Rumex confertus* (roots, dichloromethane) = 128,8 mg/g, *Vexibia alopecuroides* (roots, dichloromethane) = 76,4 mg/g, *Vexibia alopecuroides* (roots, ethanol) = 48,9 mg/g, *Salvia deserta* (roots, dichloromethane) = 35,6 mg/g, *Veronica incana* (aerial part, ethanol) = 30,3 mg/g, *Paeonia intermedia* (aerial part, dichloromethane) = 30,1 mg/g, *Veronica incana* (aerial part, dichloromethane) = 30,0 mg/g. The content of vitamin C ranged from 0,8 to 74,7 mg/g. The maximum content of vitamin C was typical for extracts *Mentha arvensis* (whole plant, dichloromethane) = 74,7 mg/g, *Paeonia intermedia* (roots, ethanol) = 42,8 mg/g, *Paeonia intermedia* (aerial part, ethanol) = 42,2 mg/g, *Paeonia intermedia* (aerial part, dichloromethane) = 37,4 mg/g, *Urtica urens* (aerial part, ethanol) = 35,0 mg/g, *Veronica incana* (aerial part, ethanol) = 30,2 mg/g, *Platycladus orientalis* (aerial part, ethanol) = 30,0 mg/g, *Vexibia alopecuroides* (roots, dichloromethane) = 28,7 mg/g, *Salvia deserta* (roots, dichloromethane) = 28,5 mg/g. The high content of both flavonoids and vitamin C differed extracts of Vexibia alopecuroides (roots, dichloromethane), Paeonia intermedia (aerial part, dichloromethane), Salvia deserta (roots, dichloromethane), Veronica incana (aerial part, ethanol).

Probably, researched herbal extracts with a high content of flavonoids and vitamin C can be further investigated in order to develop drugs with broad spectrum of action on a plant basis.

Keywords: Plants, herbal extracts, flavonides, vitamine C

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Conversion of Waste Parasite Insect (*Hylobius Abietis* L.) into Antioxidative, Antimicrobial, Biodegradable and Edible Films

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Abstract

Hylobius abietis is a plant parasitic insect belonging to the order Coleoptera and causes severe damages to coniferous forests in Northern and Eastern Europe. This current study aimed to provide a new viewpoint to waste of this insect by producing chitosan. Dry insect corpses consisted of 27.9% chitin, and 86.2% of chitin was converted into chitosan. FT-IR spectra analyses confirmed the purity and deacetylation degree of the produced chitosan (molecular weight of chitosan; 7.3 kDa). This chitosan exhibited antimicrobial activity against 18 bacterial strains. Further, biodegradable chitosan composite films with β-carotene were produced. Antioxidant activity of chitosan films were found to be higher than chitosan gels; and β-carotene incorporation further increased the antioxidative properties of the chitosan films. This study demonstrated that the waste of parasitic insect like H. abietis can be evaluated as a source for production of biodegradable and edible chitosan-based films for applications in food coating.

Keywords: Hylobius abietis; biowaste, chitin; chitosan.

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Formulation and Biopharmaceutical Evaluation of Lipid Nanoparticle Formulation Containing Dexpanthenol

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Abstract

Dexpanthenol (DXP) is an alcoholic analogue of pantothenic acid with reported ant-inflammatory and regenerative properties. Topical DXP acts like a moisturizer, improving *stratum corneum* hydration, reducing trans epidermal water loss and maintaining skin softness and elasticity. However, DXP lacks the ability to penetrate into deeper layers of the skin, which reduces its potential applications. Studies over the last decades showed possible application of lipid vesicles as a tool to improve drug topical delivery.

The aim of this study was to prepare an optimized lipid nanoparticle formulation (LNF) containing 1 % of DXP for dermal delivery. Soy phosphatidylcholine (PC) (>95 % purity, Lipoid GmbH, Switzerland), cholesterol (CHOL) (Sigma Aldrich, Germany), Tween 80 (T) (Sigma Aldrich, Germany) and deionized water were selected as main components forming the LNF. Optimization plan was prepared using experimental design software Design Expert® 7 (Stat-Ease). It was based on LNFs responses of particle size, polydispersity index (PDI), ζ-potential, pH, entrapment efficiency (EE) and *in vitro* release. The optimization factor was the amount of PC, while the selected amounts of CHOL and T were 3 % and 8 % of PC mass, respectively. The preparation procedure of LNs was the following: the components were stirred for 2 hours and left for "swelling of lipids" overnight followed by direct sonication as homogenization method. The LNs were analyzed 24 hours after preparation.

The response optimization of experiments was the liposome formulation containing 797,0 mg of PC per 10 mL of preparation. The experimental results of characterization of optimal liposome formulation were in good agreement with those predicted by the optimization software. The characteristics of obtained optimal formulation were: particle size 62,75± 0.97nm, polydispersity index was 0,224±0.006, pH 7,2±0.08, EE 51.41 ± 5.09%. ζ-potential observed with lipid preparations showed that the negative surface charge ranged from −9 to −14 mV. The *in vitro* release study performed for 6 hours showed prolonged release of DXP. Optimized LNF showed no significant differences in their particle size, PDI, pH and ζ-potential after more than 2 weeks under the room temperature.

The optimization procedure allowed formulation of an optimal and stable LNF based on their size, PDI, ζ-potential, pH, EE and *in vitro* release. Prepared formulations should be tested for skin penetration for further evaluation of their suitability for their application.

Keywords: Dexpanthenol, lipid nanoparticles, optimization, biopharmaceutical evaluation

ISSN

Babesia Infection in Red Squirrel (*Sciurus vulgaris*) and Their Ectoparasites

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Abstract

Babesia spp. are tick-borne protozoan parasite that have been reported in many countries. This pathogen infected domestic and wild mammalian hosts. The aim of this study was to identify infection of *Babesia* spp. pathogen in squirrels (*Sciurus vulgaris*) and their ectoparasites using molecular tools. A total 33 samples of squirrels were analysed from different regions of Lithuania. Squirrels were infested with *Ixodes ricinus* nymphs (125) and larvae (32), and *Ceratophyllus sciurorum* fleas (40). *Babesia* DNA in samples was detected using a semi-nested PCR of the 18S rRNR gene. *Bartonella* DNA was detected in 57.6% squirrels, 13.4% ticks and 2.5% fleas. Sequence analysis of 18S rRNR gene showed that sequences were similar to *Babesia microti*.

Keywords: *Babesia*, squirrel, tick, flea, Lithuania.

ISSN

Detection of *Oeciacus hirundinis* in Nests of *Delichon urbica* L. in Lithuania

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Abstract

Swallows are common bird in Lithuania, lives in human environments. Nests mold in shelters, under house roof, balcony and house's wall junctions. Along with the birds settle and their parasites. Cimicidae are a family of blood-sucking ectoparasites. The swallow bug *Oeciacus hirundinis* L. primarily parasitizes swallows and martins (Hirundinidae). Every year when the birds return to the same nest, they increases the probability of survival of the parasite population and abundance. Ectoparasites actively proliferate, or on the host's death lack of food, are looking for new hosts. Nests in people's living environment is a threat because the parasites can enter the premises and parasiteze pets. The aim of this study was to determine the prevalence of parasitic bugs in swallow nests. Non - invasive testing method were selected for study, when the parasites collected from the nests after Swallows migration. In study time identidied 6363 *Oeciacus hirundinis* species parasitic bug - 2502 larvae, 1858 males and 2003 females. Most *O. hirundinis* widespread in *Delichon urbica* – 65.5%, and *Hirundo rustica* - 18.8%.

Keywords: *Oeciacus hirundinis*, Cimicidae, parasite, House martin.

ISSN

Identification of *Wolbachia* Endosymbiont in Fleas (Siphonaptera) by Molecular Methods

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Abstract

Wolbachia pipientis is a vertically transmitted, gram-negative obligate intracellular symbiont of arthropods. The bacterium is best known for its ability to manipulate host reproductive biology where it can induce cytoplasmic incompatibility, parthenogenesis, feminization and male-killing. The aim of this study was to carry out molecular identification of *Wolbachia* species and determine their prevalence in fleas. Fleas were collected from small mammals in 2013-2014 from different regions of Lithuania. Ten flea species were collected: *Megabothris walkeri, M. turbidus, Ctenophthalmus agyrtes, Ct. uncinatus, Ct. assimilis, Hystrichopsylla talpae, H. orientalis, Nosopsyllus fasciatus, Peromyscopsylla bidentata, Paleopsylla soricis*. A total 152 fleas was screened for *Wolbachia* using wsp81F and wsp691R primers which amplified 590-632 bp fragment of the *wsp* gene. *Wolbachia* DNA was detected in 21,7% (33/152) fleas.

Keywords: *Wolbachia*, symbiont, fleas, Lithuania.

ISSN

Simultaneous Occurrence Of *Borrelia miyamotoi* And *Borrelia burgdorferi* Sensu Lato In *Ixodes ricinus* Ticks In Urban And Sylvatic Foci In Slovakia

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Abstract

Ixodes ricinus is involved in the transmission of a large variety of pathogens of medical and veterinary importance including *Borrelia burgdorferi* sensu lato (s.l.) and the newly described emerging pathogen *Borrelia miyamotoi*. *B. burgdorferi* s.l. complex is a diverse group of worldwide distributed bacteria that includes about 20 genospecies. In Europe, several genospecies can cause Lyme disease. *B. miyamotoi,* a relapsing fever-related spirochete transmitted by Ixodes ticks has been recently shown to be a human pathogen. This pathogen has been detected in Slovakia for the first time in 2012. The aim of this work was to find out the infection prevalence of by *B. burgdorferi* s.l. and *B. miyamotoi* in urban and sylvatic foci in Slovakia.

Ticks were obtained from multiple locations by flagging during 2011-2013. The research was situated in eight different sites in Western (urban parks in Bratislava City - Železná studienka and Horský park, urban park in Malacky City, SAS campus and the National Nature Reservation (NNR) Jurský Šúr), Northern (Martinské hole mountains) and Eastern Slovakia (Košice City and Drienovská mokraď Wetland) to determine the B. burgdorferi s.l. genospecies diversity and the prevalence of B. miyamotoi in questing ticks.

The total of 2197 *Ixodes ricinus* ticks collected in all locations were examined for the presence of *B. burgdorferi* s.l. and B. miyamotoi. 15.8 % of all tested ticks were found to be positive by touch-down PCR with *B. burgdorferi* s.l.-specific primers. The lowest prevalence of infected ticks was found in the SAS campus (5.6 %), followed by Železná studienka (11.9 %), Horský park (14.5 %), Martinské hole mountains (14.7 %), NNR Šúr (16.4 %), Drienovská mokraď Wetland (19.3 %), Malacky City (20.3 %) and Košice City (26.8 %). Six genospecies in positive samples were determined by RFLP method. The most frequent of all genospecies was *B. afzelii* (56.8 %), *B. garinii* (21.6 %) and *B. valaisiana* (9.2 %). *B. miyamotoi* infection was detected in ticks by real-time PCR at six study sites, with infection prevalence 1.1 %. Horský park had a low incidence rate, only 0.6 %, followed by Železná studienka (0.6 %), Martinské hole mountains (1.3 %), Košice City (1.4 %), Malacky City (1.6 %) and Drienovská mokraď Wetland (2.1 %).

Keywords: *Borrelia miyamotoi*, *B. burgdorferi* sensu lato, urban foci, sylvatic foci

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Identification of *Wolbachia* Endosymbiont Bacteria in Filarial Nematodes

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Abstract

Wolbachia is intracellular endosymbiont bacteria of filarial nematodes and related with the pathogenesis of filarial diseases, in particular in heavy *Dirofilaria* spp. infections. The aim of this study was to examine *Wolbachia* sp. in *Dirofilaria* infected dogs in Lithuania. Blood samples were collected from dogs presented in veterinary clinics of Kaunas. Out of the 183 dog blood samples, 30 samples were positive for *Dirofilaria repens*. *Wolbachia* DNA was detected by PCR of 16S rRNA gene using primers wolbF and wolbR. The obtained specific products of 1018 base pairs were considered as a positive result. *Wolbachia* DNA was detected in 76.6% (23/30) of *Dirofilaria* positive samples. The two positive samples were sequenced. Sequence analysis of 16S rRNA gene showed 99% similarity with *Wolbachia* sp. endosymbionts of nematodes.

Keywords: *Wolbachia*, endosymbiont, *Dirofilaria repens*, nematodes, Lithuania.

ISSN

Quantitative analysis of flavonides and vitamine C in wild plants of flora of Kazakhstan

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Abstract

To develop effective pharmacological drugs it is necessary to select a medicinal plant raw material containing biologically active components with a broad-spectrum action. Such biologically active components can be represented by flavonoids and vitamin C, which are an ideal combination that possesses different kinds of pharmacological activity, including capillary-strengthening, anti-inflammatory, antibacterial, antioxidant. The purpose of this study is to determine the concentration of flavonoids and vitamin C in samples which were extracted from different parts of wild plants using two solvents with different polarity. The objects of study were wild plants of flora of Kazakhstan, belonging to different families*: Rumex confertus, Solidago virgaurea, Vicia subvillosa, Urtica urens, Peganum harmala, Onobrychis arenaria, Chenopodium botrys, Mentha arvensis, Conium maculatum, Artemisia absinthium, Paeonia intermedia, Salvia deserta, Astragalus lanuginosus, Platycladus orientalis, Veronica incana, Vexibia alopecuroide, Genista tinctoria, Caragana camilli, Chelidonium majus, Sanguisorba officinalis, Linum pallescens, Allium schubertii, Astragal sieversianus*. Extracts were obtained by two-stage maceration. The amount of flavonoids and vitamin C was determined photometrically using aluminum chloride, ferric chloride and potassium ferricyanide, respectively[1,2].

The results of studies have shown that the content of flavonoids in extracts ranged from 4,2 to 128,8 mg/g. The maximum content of flavonoids was typical for extracts *Rumex confertus* (roots, dichloromethane) = 128,8 mg/g, *Vexibia alopecuroides* (roots, dichloromethane) = 76,4 mg/g, *Vexibia alopecuroides* (roots, ethanol) = 48,9 mg/g, *Salvia deserta* (roots, dichloromethane) = 35,6 mg/g, *Veronica incana* (aerial part, ethanol) = 30,3 mg/g, *Paeonia intermedia* (aerial part, dichloromethane) = 30,1 mg/g, *Veronica incana* (aerial part, dichloromethane) = 30,0 mg/g. The content of vitamin C ranged from 0,8 to 74,7 mg/g. The maximum content of vitamin C was typical for extracts *Mentha arvensis* (whole plant, dichloromethane) = 74,7 mg/g, *Paeonia intermedia* (roots, ethanol) = 42,8 mg/g, *Paeonia intermedia* (aerial part, ethanol) = 42,2 mg/g, *Paeonia intermedia* (aerial part, dichloromethane) = 37,4 mg/g, *Urtica urens* (aerial part, ethanol) = 35,0 mg/g, *Veronica incana* (aerial part, ethanol) = 30,2 mg/g, *Platycladus orientalis* (aerial part, ethanol) = 30,0 mg/g, *Vexibia alopecuroides* (roots, dichloromethane) = 28,7 mg/g, *Salvia deserta* (roots, dichloromethane) = 28,5 mg/g. The high content of both flavonoids and vitamin C differed extracts of Vexibia alopecuroides (roots, dichloromethane), Paeonia intermedia (aerial part, dichloromethane), Salvia deserta (roots, dichloromethane), Veronica incana (aerial part, ethanol).

Probably, researched herbal extracts with a high content of flavonoids and vitamin C can be further investigated in order to develop drugs with broad spectrum of action on a plant basis.

Keywords: Plants, herbal extracts, flavonides, vitamine C

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Validation and Optimization of MTX Efficacy Prediction Model in Patients with Rheumatoid Artrithis in Kazakh Population

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Abstract

Methotrexate (MTX) is the most commonly prescribed currently using disease modifying anti-rheumatic drug (DMARD) for the treatment of RA. Polyglutamated metabolites of MTX (PGM-MTX), accumulated in erythrocytes, were found to be associated with clinical response to MTX, suggesting that measurement of erythrocytal PGM-MTX concentrations, e.g. by high-performance liquid chromatography, might be useful for dose individualization in patients with RA. Also it was shown that genetic factors contribute significantly into RA occurrence and establishing. In the current study, we intended to validate and optimize the model for MTX efficacy consisting of sex, rheumatoid factor and smoking status, the DAS, and 4 polymorphisms in the AMPD1, ATIC, ITPA, and MTHFD1 genes in our population.

Materials and Methods. All participants in this project were included in the study only after the signing of a written agreement, approved by the Ethics Committee of the KazNMU. The diagnosis of RA and its gradation was based on the criteria of the American College of Rheumatology and the European Anti-Rheumatic League, as described.

DNA was isolated from peripheral blood lymphocytes by using QIAmp DNA Blood mini kit (Qiagen) according to the manufacturer's protocol. All samples were genotyped for AMPD (rs17602729), ATIC (rs2372536), ITPA (rs1127354), and MTHFD (rs17850560) gene polymorphisms using real time PCR with TaqMan probes.

MTX-PGs were extracted from deproteinized (with perchloric acid) erythrocyte lysate of all samples. The detection of concentration of MTX-PGs was performed by LC-MS TOF (Agilent, USA).

Results. As a result, we obtained different combinations of haplotypes of the above genes, which, in combination with the determination of the level of MTX-PGs in erythrocytes using HPLC-MS, were used by us to validate the prediction model for MTX efficacy. MTX-PGs were not detected in erythrocytes of control group. In 50% of the patients the concentration of various MTX-PGs residues was in the same range, 18% had a low metabolite content and the remaining MTX-PGs concentration was above the average.

There was approved an association with the polymorphisms and time of excretion of MTX. There was observed an increase of time of excretion of MTX in samples which were homozygous for minor alleles of those polymorphisms.

We suppose that further research of this model is necessary in order to introduce it into clinical practice. Because randomized controlled trials should establish whether the prediction models can realize individualized MTX therapy to obtain maximal efficacy with minimal toxicity in order to improve disease outcome.

Keywords: MTX, MTX-PGs, AMPD (rs17602729), ATIC (rs2372536), ITPA (rs1127354), MTHFD (rs17850560), HPLC-MS.

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Modification of *Pseudomonas aeruginosa* Respiration by Derivative of 4-(1-Adamantyl)phenol

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Background: Derivatives of 4-(1-adamantyl)phenol are the promising class of antimicrobials, which produce structural and functional damage to the bacterial cell membrane. It's well-known that most antibiotics may perturb bacterial cellular metabolism and respiration. The aim of this work was to study the functioning of *Pseudomonas aeruginosa* respiratory chain and related system of oxidation phosphorylation before and after derivative of 4-(1-adamantyl)phenol administration.
Materials and methods: *P. aeruginosa* 2094 was cultured in tryptic soy broth (TSB). The changes in oxygen consumption induced by succinate and glutamate additions to cell were studied at 35 ºC using Clark electrode. Quantitation of total protein was measured according to Lowry. Derivative of 4-(1-adamantyl)phenol (compound KVM-97) was tested at 0.5× and 1.0× minimum inhibitory concentration (MIC). Specific inhibitors of the respiratory chain complexes such as rotenone (0.05 mmol) and malonate (2.0 mmol) were used. All reactions were stimulated by addition of ADP (0.2 mmol). We calculated the substrate oxidation, state 3, and state 4 respiration rates, and respiratory control index (RCI).
Results: Respiratory activity (without substrates) appeared to be decreased from 20.0±1.1 to 16.0±0.97 nmol O_2/min/mg protein ($P < 0.05$) following KVM-97 at subinhibitory concentration (0.5× MIC). Addition of KVM-97 at concentration 1.0× MIC resulted in full suppression of cellular respiration.
In the presence of glutamate addition of test-compound (0.5× MIC) led to reduce the state 3 and state 4 respiration rates by 36.5 % and 25 %, respectively, with no substantial changes by higher concentration. Uncoupling of the respiratory chain was manifested in RCI decrease from 2.37±0.19 to 1.45±0.15 ($P < 0.05$).

In the presence of succinate and KVM-97 (0.5× MIC) state 3 respiration rate decreased on 25 % whereas state 4 respiration rate increased slightly (up to 11 %). RCI decrease from 2.65±0.18 to 1.80±0.30 ($P < 0.05$). The high KVM-97 concentration (1.0× MIC) dramatically inhibited *P. aeruginosa* respiration.
Conclusion: The data obtained allow to suggest that 4-(1-adamantyl)phenol derivatives can inhibit the respiratory processes in *P. aeruginosa* cells. It was found that KVM-97 negative effect, which was more pronounced with succinate oxidation (complex II) rather than glutamate oxidation (complex I), was dose-dependent. Compound-induced blockade the respiratory chain led to inhibition the ADP phosphorylation system.

Keywords: *Pseudomonas aeruginosa*, adamantane derivative, anti-bacterial agents, respiratory rate.

ISSN

Effect of calli types on lectin activity in common bean

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Abstract

Cell and tissue cultures *in vitro* are widely used in modern biological experiments, particularly for production of natural biologically active food supplements and protein substances. Legumes are characterized by high level of proteins and protein compounds such as lectins [1]. Studying different patterns of hormonal regulation of lectins *in vitro* allows understand physiological mechanisms of growth and development in common bean. In this regard selecting the source of explants and optimizing concentration of minerals and hormones in culture media has helped us to obtain morphogenic and non-morphogenic calli. Presence of 2 mg/l of 2.4-D *и* indolyl acetic acid (IAA) in a medium resulted in formation of 87% and 15 % of morphogenic calluses respectively. Increased concentration of 2.4-D caused a degradation of morphogenetic calli, whereas in presence of 8 mg/l of 2.4-D necrosis was detected. It was supposed that high 2.4-D concentrations increased the rate of ethylene formation and simultaneously decreased the speed of cell extention. Probably high auxin concentrations influence on suppression of growth for dicotyledonous plants to be associated with the ethylene synthesis [2]. Non-morphogenic calli are characterized by low lectin concentration (about 18.4- 25.2 mg/100 g of wet weight) in all samples under investigation. It was assumed that these differences in lectin concentration might be related to hormones in the media, because morphogenic type was formed on culture media with IAA but low concentrations of 2.4-D. According to the literature the synthesis of lectins is regulated by abscisic acid (ABA) and high concentrations of 2.4-D which is known to decrease the content of ABA [3-5]. Biological activity of lectins, extracted from different types of calli, was determined visually by intensity of hemagglutination and related tityes. The level of lectins is controlled by concentration of auxins and cytokinins in media. All morphogenic samples of calli demonstrated higher activity comparing to non-morphogenic agglomeration. Maximal dozes' of lectins were observed at 2.4-D concentration of 1 mg/l and 0.5 mg/l of kinetin. The highest lectin activity was indicated in two representatives of morphogenic type of calli, ″Aktatti″ and ″Juravushka″. Experiments demonstrated that agglutinative activity in plants and the level of lectins in calli may show fluctuations in lectin activity across cells and tissues. The data might be used for study the hormonal processes during differentiation, proliferation and early development of common bean and improving current methods of lectin extraction.

Keywords: Phaseolus *vulgaris*, lectins, callus culture, activity, hormonal dependence.

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Factors Affecting *Zantedeschia* Spreng. Dedifferentiation *In Vitro*

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Abstract

Zantedeschia Spreng. is an economically important ornamental plant species. Improvement of the plant's aesthetic parameters and creation of novel variation of decorative plants are important economic goals for the commercial ornamental industry. The effect of growth regulators combination and explant type on dediferentation induction from *Zantedeschia aethiopica* and *Zantedeschia elliottianna* was investigated. Research was carried out in Institute of Biology and Plant Biotechnology of Aleksandras Stulginskis University and Laboratory of Agrobiotechnology of Join Research Center in 2016–2017. Explants (leaf discs, spathe discs and petiole segments) of were cultured on MS medium supplemented with different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 6-benzylamino purine (BAP), 30.0 g l^{-1} sucrose and 8.0 g l^{-1} agar. In the medium without growth regulators leaf disc and spathe discs did not show any response, while 16.3 % (*Zantedeschia aethiopica*) and 24.3 % (*Zantedeschia elliottianna*) of isolated petiole segments formed callus. Our results showed that appropriate growth regulators combination for callus induction varied dependent on genetic background and explant type. It was documented that isolated petiole segments of the arum lily and calla lily induced statistically reliable more callus in a medium supplemented with combination BAP + IAA, while combination BAP + IBA promoted callus formation from spathe discs tissues. Petiole segments manifested the highest dedifferentiation capability among the tested explant types. The results of the study showed that somatic tissues of arum lily and calla lily ability to induce dedifferentiation seems to be a valuable material for improvement of ornamental values in this plants.

Keywords: *Zantedeschia* Spreng., explant type, callus induction, growth regulators.

ISSN

The interdependence of calcein electroextraction and DNA electrotransfer when processes are performed simultaneously

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Abstract

Electroporation, as a cell membrane permeabilization phenomenon to various molecules, occur when cells are affected with electric field. Induced molecule diffusion through electroporated membrane is termed electrotransfer. These induced processes are employed for molecule extraction from cells as well as DNA electrotranfer to the cells. However it is not much known how molecule electroextraction is affected with DNA electrotransfer when performed simultaneously.

To test this we have used chinese hamster ovary (CHO) cell line as an object. For electroextraction we used calcein-AM. Once freely diffused inside the cells, calcein-AM is metabolized into calcein. Due to its hydrophilic properties calcein cannot diffuse back to the medium through cell membrane. However calcein extraction is observed after temporary compromising cell membrane with electroporation. DNA electrotransfer was performed using PGL-4 Luciferase coding plasmid. Calcein extraction was measured with flow cytometer (BD Acura-C6). Luciferase protein relative concentration was measured with microplate reader (GENios Tecan). Electroporation was performed with one HV (1400 V/cm 100 μs) pulse.

Obtained results indicated an inverse dependency between DNA transfection and calcein electroextraction. When calcein extraction was measured a highly statisticaly significant increase ($P<0.001$) of extracted calcein was observed when there was 100 μg/ml plasmid in medium compared with electroextraction when there is no plasmid inside the medium. When DNA transfection efficiency was measured using same experiment setup an inverse results were obtained. With plasmid concentration of 100 μg/ml in cell suspension a statistical significant decrease of transfection efficiency was observed compared with electrotransfection when there is no calcein inside the cell. When plasmid concentration inside the medium was decreased to 10 μg/ml similar tendency of electrotransfection remained, yet no significance was observed. Cell viability in all performed experiments was measured using colonogenic assay. Results indicate around 20 percent of viability decrease when electroporation was performed. However DNA transfection or calcein electroextraction did not had any negative effect to cell viability.

In this work we present an inverse dependency of DNA electrotransfection and calcein electroextraction when performed simultaneously. The increase of electroextraction or decrease of electrotransfection was observed with no effect to cell viability.

Keywords: Calcein, Electroporation, DNA transfection, electroextraction

ISSN

The Effect Of Hyperthermic Chemotherapy Treatment To Human Gastric Cancer Cells

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Abstract

Gastric cancer is a form of cancer, which requires many deaths in Lithuania and around the world. Every year in Lithuania this desease kills about 700 patients. New therapies are being investigated in order to help patients get well or even slightly increase their life expectancy. Hyperthermic intraperitoneal chemotherapy (HIPEC) is a method of treatment, which is quite recently applied in Lithuania. Most of the science publications (total found 700) are about clinical effectiveness of HIPEC, but there is a lack of fundamental knowledge about the effect of different types of cancer cells, including gastric cancer.

The aim of the study was to determine hyperthermic chemotherapy effect on human gastric cancer cells.

The effect of hyperthermic chemotherapy was investigated using MTT cell viability determination method, flow cytometry for apoptosis measurement and analysis of cells morphology using microscopy.

The results demonstrated that the effect of temperature 41°C - 45°C, AGS cell viability decreased by approximately 32%, compared to the control cells (AGS cells affected by *in vitro* incubation at 37°C). The maximum effect of chemotherapeutic drug cisplatin to decrease AGS cells vitality occurred, when drug concentration was increased to 200 uM. The combinational effect of hyperthermia and chemotherapy to maximally decrease viability of AGS cells was observed at 45°C temperature and cysplatin concentration of 200 uM, compared with the control group.

Keywords: Gastric cancer, HIPEC, cancer cells, cisplatin.

ISSN

Influence of environmental conditions on *Lactococcus lactis* infection with c2 and sk1 phages

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Abstract

Lactococcus lactis is one of the most commonly used in the dairy industry lactic acid bacteria [1]. Virus infection of these bacteria inhibits lactose conversion to lactic acid. Therefore, quality of the product changes, in some cases the production is stopped. Usually *L. lactis* is infected by phages belonging to the *Siphoviridae* family. The aim of this study was to determine the influence of phage c2 and sk1 infection on energetical metabolism of *L. lactis* cells.

It is usual in the dairy industry to inactivate bacteriophages by heat [2], but higher the temperature is, more taste and presentation of the final product is affected. In order to explore the phage inactivation by heat, phage c2 and sk1 suspensions were incubated at 63°C and 90°C for various time periods. The heating considerably affected the courses of infections: after 30 min incubation at 63 °C phage sk1 remained infective, but after 30 min at 90 °C this phage was completely inactivated. The infectivity of phage c2 decreases more than 7 times after 15 min of incubation at 90 °C, and after 60 min the phage c2 was completely inactivated. Also our results shows that these phages do not infect heated *L. lactis* cells.

It is known [3] that the supplement of a medium with divalent cations, such as Ca2+, Mg2+, Mn2+ or Sr2+, is rather often required for the productive infection. Our results indicated that the reproduction of phages c2 and sk1 also does not occur if the growth medium is not supplemented with these ions. Also our results have shown that these phages weakly adsorb on *L. lactis* cells without Ca2+ supplement.

Examination of the respiration process have shown that if cells are uninfected, they actively respire and use all the oxygen from the medium. Once the phages c2 or sk1 begins to disrupt cells, the dissolved oxygen concentration in medium gradually increases.

Usually *L. lactis* starter cultures are freeze-dried or spray-dried. We dried *L. lactis* culture and bacteriophage c2 at 23°C at atmospheric pressure. According to our results, in one year c2 phage infectivity decreased 7 times, while *L. lactis* cells was completely inactivated.

Keywords: *Lactococcus lactis*, lactic acid bacteria, bacteriophages.

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Dynamics Of Cell Adhesion After Electroporation

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Abstract

Cell is a semi open system which is constantly exchanging various molecules with it's surrounding, however not all molecules enter or leave the cell. Cell electroporation (EP) is a process, when cells in suspension, monolayer or in a tissue are temporary permeabilized for various exogenous molecules by means of electric pulses, which are believed to create transient electropores in cells plasma membrane. Phenomena of EP facilitates ability to transfer molecules of interest that can vary in size of tens of kDa (electrotransfer, electrotransfection) into cells, or to remove from (electroextraction). Method of electroporation is already applicable in veterinary and clinics together for treatment of various oncological deseases (electrochemotherapy, electrogenetransfer).

Furthermore, it was shown that EP cause delayed cell death, even though cells after reversible EP fully recover their plasma membrane. Dealayed cell death is assumed to appear due to extraction of vital biocompounds from cells (ions, RNA, DNA, proteins) during or post-electric fields while electropores are present. Intensity of letal changes in the cells caused by EP mainly depend on intensity, duration and number of pulses. Despite that, one of cells response as adhesion after EP is poorly invetigated.

In this work we present methodology and results of CHO cells adhesion to growing dish dynamics after EP by means of intensity and number of electric pulses.

ISSN

Evaluation of Polymorphism of Samples of the Collection of Leguminous Crops

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Abstract

The lupine working collection of the Belarusian State University's (BSU) Plant Genetics Sector includes more than 800 samples of yellow lupine (*Lupinus luteus* L.), narrow-leafed (blue) lupine (*L. angustifolius* L.), white lupine (*L. albus* L.), and other species. The collection samples' morphological, biochemical, and molecular genetic parameters should be described to use them rationally for various breeding purposes. Based on our studies, core collections of yellow lupine and narrow-leafed lupine have been formed, and the complex of morphological, biochemical, and molecular genetic traits of these samples have been described.

All the available samples in the collection of various types of lupine are evaluated for their resistance to pathogens in field and laboratory conditions. The collection samples of different types of lupine were also assessed in respect of the parameters of plant productivity, and in accordance with gamete selection methods in laboratory conditions.

New genotypes with the anthracnose resistance allele were obtained by hybridizing the narrow-leafed lupine's parental forms.

The samples with a determinate type of branching were distinguished among the mutant populations of white lupine.

The samples of different types of lupine are evaluated for alkaloid content in their seeds and the herbage of plants. The samples with consistently high (Borre, BSHA 892, Gjulzowski, Weiko et al.) and low alkaloid content (Fakel, Cyt, Niemchinowski 97 et al.) were distinguished. The intervarietal hybridization allowed identifying the genotypes, which are complementary by the alkaloidness trait, whose crossing restores the alkaloid biosynthesis. The samples of yellow lupine containing indole alkaloid gramine were identified (Cyt, Yantar, et al.). Identifying the genotypes of various types of lupine in accordance with the spectra of storage proteins allowed revealing marker biochemical indices and determining phylogenetic relationships of their genomes. Phylogenetic trees of the core collections of yellow lupine and narrow-leafed lupine have been built.

All the samples of narrow-leafed lupine are evaluated for the genes of economically valuable traits (anthracnose resistance, pods shattering resistance, undemanding vernalization, and low alkaloid content) using the markers recommended for use in marker-assisted selection programmes. Evaluation of the diversity of samples in the yellow lupine collection is carried out based on the primers associated with the biochemical processes in the cell.

Studies have begun of the morphological, biochemical, and molecular genetic properties of the chickpea and field bean samples, newly introduced in Belarus. The obtained data can be used to form sample passports, develop the models of varieties of different uses, and develop a new selection strategy for biochemical and molecular genetic testing of genotypes.

These studies are conducted with the financial support in the framework of the Belarusian State Programmes on the Cultivated Plants' Genetic Pool.

Keywords: lupine, chickpea, alkaloid, storage protein, phylogeny, molecular marker

ISSN

Hyperthermic chemotherapy effect on glutamate dehydrogenase activity in human ovarian, gastric and pancreatic cancer cells

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Abstract

Cancer is one of the leading causes of increasing morbidity and mortality worldwide. Tumours are characterized by high heterogeneity and transformation of metabolism to dysregulated Warburg-like glucose metabolism, dependence on glutaminolysis and fatty acid synthesis providing resistance for cancer treatments [1]. Cisplatin is clinically proven as mainline treatment of various cancers. To overcome the resistance cancer cells to cisplatin and reducing toxic side effects the hyperthermic intraperitoneal chemotherapy (HIPEC) is used [2].

Mitochondria in tumour cells perform the role of biosynthetic organelles and are to great extent dependent on activity glutamate dehydrogenase (GDH) that controls the supply of α- ketoglutarate from glutamine. In this study we investigated effects of mild hyperthermia 40^{o}C and 43^{o}C, cisplatin or combination of both treatments on GDH activity in three cancer cell lines (Ovcar-3, AGS and T3M4). The response to treatments and recovery of cell viability and GDH activity was strongly dependent on cell line. After hyperthermic treatment GDH activity in Ovcar-3 cell lines increased by 31% after heating 1 h at 40^{o}C, and twice – at 43^{o}C; in cell line T3M4 – by 31% after heating at 43^{o}C; whereas GDH activity in AGS cell line was resistant to hyperthermia. During recovery GDH first undergoes inhibition (after 24h) and then is activated by 20-30% (after 48h) in all heated cells as compared to the control. Treatment with cisplatin alone strongly inhibited (by 40%) GDH activity only in Ovcar-3 cell line after 48h recovery, but did not affect GDH activity in T3M4 cell line or even stimulated GDH (by 20%) in AGS cell line. Hyperthermic chemotherapy inhibited GDH activity stronger in comparison to the cisplatin or hyperthermia as applied separately in all cancer cell lines, however the extent of inhibition different. The cell line Ovcar-3 was the most sensitive to the combinatory treatment. After 48h recovery GDH activity in Ovcar-3 line decreased by 99%, whereas in T3M4 and AGS lines only 20% of inhibition was achieved.

The comparison the obtained results on thermal sensitivity of GDH with those obtained for the normal tissues and other tumour cell lines derived from mouse leads to the hypothesis about strong variation in expression of GDH isoforms characterized by different thermal sensitivity in tumour cell lines of different origin (species, tissues). Activation of GDH has a positive effect on cell viability.

Keywords: Cancer cells, Cisplatin, Hyperthermia, Combinatory treatment, Glutamate dehydrogenase.

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ISSN

Borrelia burgdorferi s.l. in *Ixodes ricinus* Ticks in the Urban Parks of Ukraine

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Abstract

Lyme borreliosis is one of the most known arthropod-borne disease in temperate regions of the Northern hemisphere. The geographic distribution overlaps with the occurrence of principal tick vector – *Ixodes ricinus*. The etiologic agents of borreliosis are spirochetes of *Borrelia burgdorferi* sensu lato complex. Currently, this complex is represented by 21 species. In Europe, several genospecies are pathogenic for humans, namely *B. afzelii, B. garinii, B. burgdorferi* sensu stricto, *B. bavariensis* and *B. spielmanii*.

In Ukraine, more than 1.600 clinical cases of human borreliosis have been recorded in 2011 and 1200 – in 2012. The largest group of patients (80%) consists of urban residents.

The aim of this study was to study the prevalence and genetic diversity of *Borrelia* species in questing *I. ricinus* ticks collected in the Ukrainian urban parks.

During 2014 - 2015, altogether 754 *I. ricinus* ticks were collected by flagging the vegetation in 5 Ukrainian big cities: Uzhorod, L'viv, Zhytomyr, Kyiv and Poltava. In total, 20% of examined ticks (24% of adults and 18% of nymphs) were infected with spirochetes from *B. burgdorferi* s.l. complex. Prevalence of *B. burgdorferi* s.l. infection was 17% in L'viv and Poltava, 11% in Zhytomyr and 23% in Kyiv urban parks. Ticks collected in Uzhorod urban parks were free for *B. burgdorferi* pathogens. The most prevalent genospecies identified in ticks was *B. afzelii* 89%. *B. garinii* was found in 7% of infected *I. ricinus* ticks, *B. burgdorferi* s.s. – 1.25%, *B. valaisiana* – 1.25%, *B. bavariensis* – 0.50%, *B. spielmanii* – 0.50% and *B. lusitaniae* – 0.50%.

This study is the first molecular investigation of questing *I. ricinus* ticks for infection with *B. burgdorferi* s.l. from urban parks in Ukraine.

Keywords: *Borrelia burgdorferi, Ixodes ricinus,* urban park, Ukraine

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Roman Snail (*Helix pomatia*) Chitin Extraction, Physical and Chemical Characteristics

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Abstract

It is long known that mollusks body parts contain chitin, but there were no profound research about it, this is partly because chitin is not the dominant feature of mollusks organisms. The purpose of this research is to investigate one of the dominant mollusk species in Lithuania – Roman snail (*Helix pomatia*) and to determine the type of chitin. The chitin was extracted from periostracum, uncalcified part of the shell, jaw and radula of gastropod *Helix pomatia*. Chitin was chemically extracted, Fourier transform infrared (FTIR) spectroscopy was applied to determine the structure of chitin and scanning electron microscope (SEM) was employed to establish structural patterns. The same principle of chemical chitin extraction was used for all specimen, however certain modifications were introduced due to impurities and differences of chitin mass. Radula, jaw and periostracum was extracted in its original shape, therefore preserving initial structure and appearance. The scientific novelty of this study is that chitin of α form was detected in *Helix pomatia* specie.

Keywords: Chitin, Roman snail (*Helix pomatia*)

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Importance of Biodiversity in a Red Clover Breeding Program

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Abstract

Red clover (*Trifolium pratense* L.) is one of the most important perennial forage grasses which are valued for protein, carbohydrates, minerals and biologically active substances quantity. *Trifolium pratense* is valued by ability to fix atmospheric nitrogen and transfer it into the soil. At present use of mineral nitrogen has become issue in organic farms. National and international law has limited use of mineral nitrogen fertilizer in organic agriculture, therefore the use of red clover in a crop rotation may be a solution to that. Consequently, use of red clover as a plough down has become an important practice on many farms in nowadays. Unfortunately, red clover is short-lived perennial legume and yield is used only for two or three years. The yield and resistance to diseases is decreasing in every next year of harvest. Moreover, changing environmental conditions are unfavourable for foreign and old native varieties. These varieties became a less tolerance to winter conditions in Lithuania and can be damaged easily by pests and diseases. In response, we need to find a new gene pool which possess not only economically beneficial properties, but also can be resistance to adverse factors. Most promising genetic material is founding *in situ* where a high biodiversity is prevalent. Compared to cultivars wild populations have exceptional morphological characteristics and are naturally adapted for biotic and abiotic stress. Therefore, a new breeding material is very important to complement the gene pool of red clover which is declining for years. Our research shows an overview of the yield of native and foreign varieties and breeding lines in the experimental fields of the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry in 2013-2014. Further, seeds were collected of twenty wild ecotypes from different geographical areas in Lithuania in 2016. Ecotype, which has shown highest weight of hundred seeds, can be used in subsequent stages of the selection.

Keywords: Gene pool, Breeding line, *Trifolium pratense*, *in situ*

ISSN

Generation of Hydrogen Peroxide by High-Voltage Pulses in Cell-Free Media

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Abstract

Electroporation offers a number of applications in biology, oncology, immunology, and biotechnology. However, when a high-voltage is applied to the electrolyte solution, besides membrane permeabilization, various electrolysis reactions occur at the electrode-solution interfaces. One of the results of these electrochemical reactions is generation of reactive oxygen species (ROS). Although ROS formation following electroporation with long (μs–ms) electric pulses or nsPEF exposure has been reported earlier, no detailed analysis of this process has been carried out yet. In this study, generation of hydrogen peroxide as a result of the exposure of a cell-free media by high-voltage pulses has been studied.

Generation of hydrogen peroxide in hightly buffered solution HB1, Dulbecco's Modified Eagle's medium (DMEM), Phosphate buffered saline (PBS), and distilled water was studied. The cuvette with stainless-steel electrodes was used. Hydrogen peroxide was detected with AmplexRed® (10- Acetyl-3,7-dihydroxyphenoxazine), which has a great specificity, stability, and selectivity. AmplexRed is nonfluorescent until, in the presence of horseradish peroxidase, it reacts with hydrogen peroxide to produce highly fluorescent resorufin. 50 μM of the AmplexRed dye were added to the medium and treated with high-voltage pulses. Fluorescence intensity in a solution was measured using TECA7 GeniosPro spectrophotometer (Tecan Group, Männedorf, Switzerland).

Treatment of HB1 medium by a single square-wave electric pulse with the duration of 0.5–2 ms and the amplitude of 100– 400 V (0.5-2.0 kV/cm) significantly increased dye emission. The AmplexRed fluorescence intensity was also dependent on the number of pulses, the conductivity, and composition of the medium (PBS, H2O, DMEM, HB1).

Conclusion: During high–voltage electric pulses, hydrogen peroxide is generated in cell–free media. Pulses of micro– millisecond duration increased fluorescence of hydrogen peroxide indicator AmplexRed depending on the duration, voltage, and/or number pulses. The conductivity and composition of the medium were also important.

Keywords: reactive oxygen species, Amplex Red, electrochemical reactions, stainless steel.

ISSN

Changes in Properties and Contamination with Heavy Metals in Soils at Outdoor Shooting Ranges

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Abstract

The aim of the reasearch was to investigate the physical properties and contamination of heavy metals of two outdoor shooting ranges. Soil samples were taken from two open 50-meter outdoor shooting ranges located in Alytus and Kaunas. Both ranges are open, with no roofing and with two shooting lines at different distances (25 and 50 meters). These structurally similar shooting ranges differ in their activity.

The results showed that soil was more contaminated with lead and copper in more active shooting range (Alytus): the maximum concentration of lead was 8272 mg/kg, copper – 10.11 mg/kg. In less active shooting range (Kaunas) the maximum concentration of lead was 6758 mg/kg, copper – 7.79 mg/kg. Heavy metal concentrations in both shooting ranges tended to increase with the distance from the start of the fire line ($p<0.05$).

The acidity of shooting ranges soils was typical to alkaline reaction (pH 6.2 – 7.4). Both shooting ranges soil density varied between 1.03 to 1.54 g/cm^3. The highest density of the shooting ranges was at 50 meters distance from the fire line (Alytus – 1.23 g/cm^3, Kaunas – 1.54 g/cm^3). It was observed that the organic matter content and porosity of the soil significantly decreased with increasing distance from the start of the firing line ($p<0.05$). Density of the soil significantly increased with increasing distance from the start of the firing line ($p<0.05$).

Keywords: contamination, heavy metals, soil, shooting range.

ISSN

Reason of Multidrug Resistance in MX-1 Cell Cultures

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Background. Multidrug resistance (MDR) is cancer cells simultaneous resistance to many drugs used for chemotherapy. MDR efflux pumps is one of the main molecular mechanisms responsible for this type of cell resistance [1]. In this study we explored activity of the efflux pumps in wild-type and our derived mutant cell sublines.

The aim: to assay the efficiency of MDR pumps in MX-1 cells.

Methods. The levels of MX-1 cell resistance to doxorubicin (DOX), ethidium (Et^+), monensin (Mon) and nigericin (Nig) were estimated using MTT viability test. The stability of MX- cell resistance to TPP^+ were estimated also using MTT viability test.

Results. In this study a subline of tetraphenylphosphonium (TPP^+)-resistant MX-1 cells was developed. These cells were more resistant also to DOX about 500, ethidium bromide - 16, Mon - 130 and Nig – 170 times. The resistance to TPP^+ remained after three days cultivation without TPP^+.

Conclusion. Cell of MX-1^{TPP+} subline was more resistant to DOX, Et^+, Mon and Nig. Moreover the resistance to TPP^+ was detected after three days cultivation without TPP^+.

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The Response of *Helianthus annuus* to Pre-sowing Seed Treatment with Stressors: induced Changes in Seed Proteome, Germination and Seedling Growth

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Abstract

Seed treatment with cold plasma (CP) or electromagnetic field (EF) is a modern eco-agricultural technology for stimulation of plant germination and performance. Numerous studies demonstrated the effectiveness of such treatments for enhancing germination and growth of a large variety of crops. The molecular determinants of seed response to treatments are not established and no genomic studies of plant seed response to CP and EMF treatments have been reported. In order to gain an insight into the molecular mechanisms underlying effect of stressors on plant seeds, we estimated changes induced in differential protein expression of the model plant – sunflower (*Helianthus annuus*). We studied the effects of pre-sowing seed treatment, using vacuum (7 min), radio-frequency EF (5-15 min) and CP (2-7 min), on germination and growth of new confectionary cultivar 'Nyķrségi fekete' (donated by University of Debrecen IAREF, Hungary) *of H. annuus* harwested in 2016. The germination tests were performed both in *vitro* and in casettes and the obtained results indicated that pre-sowing seed treatments with CP and EMF did not change the final germination percentage, but CP (7 min) treatment and EMF (10 and 15 min) treatments increased both germination rate (by 20-24%) and uniformity of germination (up to 130%). Morphometric parameters of seedlings were estimated 7 days after sowing and the results showed that CP (7 min) treatment had negative effect on seedling length and stem weight, whereas EMF (15 min) treatment increased the weight of leaves (by 14%).

Differential protein expression in control and affected seeds was assessed using gel based proteome analysis. Despite the minor effects of CP and EMF treatments observed on germination and early growth of seedlings, the 2D DIGE analysis of sunflower seed samples revealed 44 proteoforms differentially expressed in seeds after applied treatments. The EMF and CP treatment resulted in significantly lower abundance of 4 and 8 protein spots, meanwhile higher abundance of 12 proteins spots was detected for both CP and EMF treatments. Only two proteoforms of the upregulated expression were responsive to both treatments. Proteins involved in biological processes of response to stress and metabolism were identified.

Keywords: Cold plasma, Electromagnetic field, *Helianthus annuus*, Proteome, Seed stress response.

ISSN

The Effect of Protein Corona of Zinc Oxide Nanoparticles on CHO Cells Plasma Membrane Permeability and Mitochondrial Membrane Potential

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Abstract

Nowadays nanoparticles (NPs) are exciting object for researchers due to their properties and wide application. Nanoscale materials are used in cosmetic products, sun protection, food area, paint production, electronics, medicine etc. As the use of nanomaterials increase worldwide, the risk on human health and environmental increases. It is, therefore, necessary to assess the potential adverse effects of exposure and their mechanisms in cells. Futhermore, the nanoparticles are coated by proteins and other biomolecules when entering living organism and these surface modifications may have enormous influence to NPs fate and its bioeffects. In this study ZnO NPs coating with protein, also known as protein corona, was formed from model protein bovine serum albumin (BSA). The aim of this study was to evaluate the effect of ZnO NPs coating with albumin (ZnO-BSA NPs) on Chinese hamster ovary (CHO) cell viability, proliferation, plasma membrane permeability and mitochondrial membrane potential.

We have demonstrated that in the concentration range 0.1-10 μg/ml ZnO NPs have stimulating effect on CHO cells viability (after 3h incubation) determined by MTT assay and proliferation (after 24h incubation) determined by staining with crystal violet. At higher concentrations (25-200 μg/ml) cell viability decreased in a concentration-dependent manner and reached minimal viability of 55% at 150 μg/ml as compared to control. The effect of ZnO NPs on cell proliferation followed the same trend except that decrease was more pronounced and reached 60% already at 25 μg/ml.

Plasma membrane permeability induction may play an important role in NPs-induced cell death. We have applied lactate dehydrogenase (LDH) leakage test for plasma membrane permeability evaluation. LDH activity in the cell medium increased linearly increasing NPs concentration and reached 5-fold higher level as compared to control. ZnO-BSA NPs induced higher permeability as compared to plain ZnO NPs.

NPs ability to increase plasma membrane permeability may have similar effect to the membranes of intracellular organelles. The increased permeability of mitochondrial inner membrane may result in depletion of mitochondrial membrane potential. We have evaluated the impact of NPs on mitochondrial membrane potential using JC-1 dye. Lipophilic JC-1 dye accumulates in mitochondria as red-fluorescent J-aggregates when mitochondrial potential is high but if the inner membrane has some injuries the potential gets lower and JC-1 forms green-fluorescent monomers in cytosol. This red/green fluorescence ratio indicates mitochondrial inner membrane potential and possible damage of membrane or inhibition of potential generating system. ZnO NPs (100 μg/ml) decreased mitochondrial membrane potential by 15% as compared to control, ZnO-BSA NPs had no effect. It can be concluded that protein corona enhance ZnO NPs-induced plasma membrane but not mitochondrial membrane permeabilization.

Keywords: nanoparticles, zinc oxide, membrane permeability, mitochondrial membrane potential

ISSN

Genetic variability of *Borrelia burgdorferi* Sensu Lato in Europe

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Abstract

B. burgdorferi sensu lato, the causative agents of Lyme borreliosis, is a complex of spirochetes, consisting of 20 known genospecies. Genetic variability within and between the genospecies is linked to different clinical symptomatics as well as to their different association to reservoir hosts.

The aim of our study was to compare the prevalence, genospecies distribution and genetic variability of *B. burgdorferi* sensu lato between different geographical regions in Europe using Multilocus sequence typing (Slovakia, Finland, Croatia).

Questing ticks were collected in Slovakia, Finland and Croatia and were tested for the prevalence of *B. burgdorferi* s.l. by the amplification of 222-255bp fragment of 5S-23S rRNA intergenic spacer. Positive samples were further typed to Borrelial genospecies by Restriction Fragment Length Polymorphism (RFLP). Phylogenetic relationships between representative samples were further analysed by amplication of 5-7 houskeeping genes that are part of MLST typing scheme. PCR products were purified using commercial DNA-purifying kit and sent for sequencing. Sequences were analysed by SeqMan pro (DNASTAR). Phylogenetic trees were constructed in MEGA using Maximum likelihood.

Prevalence of *B.burgdorferi* s.l. ranged from 6.8% in Slovakia to 21.1% in Croatia. Samples from Croatia showed higher heterogeneity than samples from Slovakia and Finland, using MLST. By comparing the three *B. afzelii* samples from Finland to *B. afzelii* sequences from MLST database, we have found it´s relation to other samples from Europe. *B. garinii* from Finland clustered with *B. garinii* isolates from Central Europe. Common southern European genospecies *B. lusitaniae* from Slovak mountains are genetically more related to Serbian isolates, than to type genospecies from Portugal, based on the analysis of concatenated sequences of 7 genes, and represent isolated genotype. We have found new allelic profiles for *B.afzelii, B.garinii, B.lusitaniae* and *B.spielmanii* and recorded for the first time *B. bavariensis* and *B.spielmanii* in ticks from Croatia.

The study was realized with the financial support of projects APVV 0274-14, VEGA 2/0119/17 and DAAD project Molecular epidemiology of Lyme borreliosis spirochetes in Europe and with the help of Heiki Henttonen and Tarja Sironen

Keywords: Borrelia, Multilocus sequence typing, Europe, sheep tick

ISSN

Single And Combined Impact Of Elevated Tropospheric Ozone And Simulated Warmed Climate Conditions On Summer Rape (*Brassica Napus* L.) And Wild Mustard (*Sinapis Arvensis* L.)

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Abstract

A future climate with increased air temperature and concentrations of atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) will likely alter crops productivity, as well as prosperity of weeds. In this study there were examined the impact of simulated warmed climate conditions (25/18 ºC and 800 ppm *vs.* 21/14 ºC and 400 ppm, air temperature day/night and CO_2) and elevated concentration of tropospheric ozone (90 ppb *vs.* 0 ppb) on summer rape (*Brassica napus* L.) and wild mustard (*Sinapis arvensis* L.) and if simulated warmed climate conditions interacted with elevated concentration of tropospheric ozone in combined treatment. The physiological response and growth parameters of summer rape and wild mustard were in different manner. Summer rape grown under simulated warmed climate conditions showed significantly increased photosynthetic rate, leaf area, shoot and root dry weight, while in wild mustard all these parameters were not changed significantly. Under ambient climate conditions elevated O_3 decreased gas exchange parameters and leaf area in both species with more significant changes in wild mustard and significantly decreased their shoot and root dry weight. In contrast, no changes were observed in shoot and root dry weight of summer rape. Despite the fact that photosynthetic rate and growth of wild mustard were not stimulated significantly under simulated warmed climate conditions, the negative impact of elevated concentration of tropospheric ozone was mitigated by simulated warmed climate conditions for both species and, what is more interestingly, for wild mustard even more with significant increase in their shoot dry weight under combined impact of elevated tropospheric ozone and simulated warmed climate conditions. By contrary, shoot dry weight of summer rape under combined impact of elevated tropospheric ozone and simulated warmed climate conditions did not differ significantly from plants grown under ambient climate conditions without O_3 treatment, although they maintained significantly higher leaf area and root dry weight. The results of this study highlight the importance of studying the combined impact of climate change factors for plant performance and showed that responses of weeds that use different physiological strategies than crops to combined treatment with climate factors can be far away from that of single treatments. As tropospheric ozone concentration is predicted to increase together with warming climate, reliable predictions of plant responses to future climate can only be made if changes in air temperature and concentrations of atmospheric CO_2 and tropospheric O_3 are studied in parallel.

Keywords: elevated tropospheric ozone, warmed climate, *Brassica napus*, *Sinapis arvensis*, physiological response, growht

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ISSN

The Effect of Cytokonins on *Stevia Rebaudiana* Bertoni Organogenesis *in Vitro*

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Abstract

Stevia rebaudiana Bertoni is a important medicinal plant which is mostly know as a sugar substitute. The sweeting components (glycosides) are 300 times sweeter than sucrose. Steviol glycosides are applied as a natural sweetener, because SGs does not affect blood sugar level. Stevia propagation by *in vitro* techniques could be attractive alternative for production sufficient amount of genetically homogeneous plants in a short period of time. Research was carried out in Institute of Biology and Plant Biotechnology of Aleksandras Stulginskis University and Laboratory of Agrobiotechnology of Join Research Center in 2016-2017. Stem segments cultured on MS medium supplemented with BAP, TDZ, KIN (1.0 μM; 2.0 μM; 3.0 μM; 4.0 μM; 5.0 μM; 6.0 μM), 30.0 g l^{-1} sucrose and 8.0 g l^{-1} agar. Our results showed that on MS media without growth regulators shoot formation frequency from stem segments was an average 7.78 %. Incorporation of cytokinin into culture medium significantly improved organogenesis process in comparison with control; however positive effect was strongly affected by cytokinin type and concentration. The highest (76.67%) shoot formation frequency as well as number of shoots per explant has been obtained on the medium supplemented with 3.0 μM TDZ. On the medium supplemented with BAP and KIN shoot regeneration was significantly lower as compare with TDZ. Results of this study reveal a potential procedure for *Stevia rebaudiana* Bertoni stevia direct regeneration from stem segment tissues.

Keywords: *Stevia rebaudiana* Bertoni, growth regulator, *in vitro*, shoots formation frequency

ISSN

Zinc Oxide Nanoparticles Cytotoxicity and Uptake Mechanisms in Different Cells Lines

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Abstract

The wide scale use of zinc oxide nanoparticles (ZnO NPs) in the world consumer market makes human beings more prone to the exposure to ZnO nanoparticles and its adverse effects. Zinc oxide nanomaterials are of particular concern because dissolution leads to release of the toxic divalent zinc ions.

In this study, the potential cytotoxicity of ZnO NPs (20 nm) at various concentrations has been investigated using three different cell lines: Chinese hamster ovary (CHO) cells, human breast carcinoma (MX-1) cells and rabbit myogenic stem (Fr-1) cells. The cytotoxicity of ZnO NPs was evaluated using the MTT assay. The aim of this study was to compare the cytotoxicity of plain ZnO NPs and ZnO NPs coated with bovine serum albumin (ZnO-BSA NPs) and to find out the impact of NPs processing and internalization pathways on NPs cytotoxicity using various inhibitors. Furthermore, the potential cytotoxic effect of divalent zinc ions was evaluated using $ZnSO_4$ solutions at zinc ion concentrations equivalent to ZnO NPs concentrations at full dissolution.

The results showed that ZnO NPs at concentrations varying from 10 to 250 μg/ml had negative concentration-dependent effect on CHO cells. At concentrations higher than 100 μg/ml, ZnO NPs decreased the viability of the tested cells down to 70%. However, the same study with MX-1 and Fr-1 cells led to different results; ZnO NPs increased MX-1 cells viability at all concentrations with the highest increase (35%) increase at 150 μg/ml. Results with FR-1 cells showed concentration-dependant decrease of cell viability with down to 72% viability at 150 μg/ml ZnO NPs, however no cytotoxic effects were observed with the highest ZnO NPs concentrations (200 and 250 μg/ml). Albumin coating decreased ZnO NP cytotoxicity to all three cell types.

Zinc ions had stimulating effect for all cell lines at low concentrations: 25 and 50 μg/ml Zn^{2+} increased viability by 15% and 6% respectively in MX-1 cells, 10 μg/ml Zn^{2+} increased viability of CHO and Fr-1 by 5% and 14% respectively. Higher Zn^{2+} concentrations decreased MX-1 cells viability down to 45%, and CHO and Fr-1 cells viability down to 60%.

Investigation of ZnO NPs internalization pathways has shown that: 1) None of the investigated uptake mechanisms are specific to cancer (MX-1) cells; 2) Macropinocytosis might be the main ZnO NPs uptake mechanism in CHO cells; 3) Inhibition of endosomes trafficking has a positive impact on CHO cells; 4) Inhibition of endosomes acidification has a positive impact on FR-1 cells; 5) Macropinocytosis and clathrin-mediated endocytosis might be secondary NP uptake mechanisms in FR-1 cells.

Keywords: Cytotoxicity; CHO cells; MX-1 cells; FR-1 cells; ZnO

ISSN

Biofilm of Human Pathogens Formation on Chitin

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Abstract

Biofilms are known as bacteria formed colonies which can attach on the various surfaces and play a major role in the contamination of medicine instruments and food products. Bacteria form a biofilm in response to many factors, but the most important is the resistance to various classes of antibiotics (e.g. *Salmonella spp., Acinetobacter baumannii*). This can lead to serious health problems in humans. For example, *A. baumannii* bacterium causes serious infections including bacteremia, pneumonia, meningitis, urinary tract infection and wound infection in patients with low immune system. *Shigella sonnei* is a primary bacterium which causes a global human health complication – shigellosis, which is a big problem concerning underdeveloped countries, because it can be spread through feco-oral transmission. *Vibrio parahaemolyticus* bacterium is naturally found in marine waters and commonly causes severe gastroenteritis. Usually, *Escherichia coli* bacterium is not harmful to humans, but there are some strains, e.g. *E. coli O157:H7* which releases toxins and causes diarrhea, abdominal pain or even kidney damage. For these reasons, it is important to find and produce bio-based antimicrobial coating materials, which might be able to inhibit bacterial biofilm formation. Chitin is one of the most versatile polysaccharide in the world due to its large set of applications in various fields and properties such as non-toxicity, biodegradability, biocompatibility and antibacterial activity. This study demonstrates that chitin has advantages to decrease the possibility of contamination and can be used as surfaces and coating materials in medicine and food industries.

Keywords: Antibacterial activity, chitin, pathogens, biofilm

ISSN

Release of Milk Thistle (*Silybum Marianum* L.) Extract Compounents from Semisolid Formulations and their Penetration into Human Skin

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Abstract

The purposeful development of semisolid formulations should provide the efficient release of biologically active compounds and their transdermal delivery into human skin. Silymarin is the dry extract produced from the dried fruits of milk thistle (*Silybum marianum L.*) and containing flavonoid taxifolin and flavonolignans such as silychristin, silydianin, silybin (A and B) and isosilybin (A and B). This extract demonstrates antioxidant, anti-inflammatory activities [1]. The aim of this study was to evaluate the release of milk thistle extract active compounds (taxifolin, silychristin, silibin A and B) from experimental semisolid formulations and their penetration into the human skin layers *ex vivo*. Emulsion creams (w/o), poloxomer based gelified cream and hydrogel, each of them containing silymarin in 2-hydroxypropyl-β-cyclodextrin 4% aqueous solution, and poloxomer based gelified cream containing silymarin in labrasol 30% aqueous solution. All w/o creams and gelified creams contained 10% of oleic acid as penetration enhancer. Formulations were characterized by pH and viscosity. *In vitro* release studies were performed at 32±0.5°C using modified Franz type diffusion cells. *Ex vivo* skin permeation studies were conducted using Bronaugh type flow-through diffusion cells with full-thickness human skin. The samples were analysed by validated HPLC method. The pH of the semisolid formulations was within the range 4.67–6.65. The viscosity of formulations at 32°C temperature was within the range from 27.9±1.1 to 554.0±8.7 Pa·s. The release kinetics of silymarin compounds conformed to zero order model (r^2=0.9713–0.9966). The highest amount of silymarin compounds (120.0±8.6 μg/cm²) in 5 hours was released from hydrogel containing 25% of poloxamer 407. The results of penetration study showed poor skin penetration of silymarin compounds. Only 0.80±0.46 μg/cm² of silychistin penetrated into derma after application of gelified cream containing silymarin in 2-hydroxypropyl-β-cyclodextrin 4% aqueous solution, and 1.4 fold less silychistin was determined in derma after application of gelified cream containing silymarin in labrasol 30% aqueous solution. Only traces of silibin B was detected in derma. The results of biopharmaceutical studies demonstrated low penetration of biologically active compounds of milk thistle extract into human skin *ex vivo* after application of experimental semisolid formulations thus indicating insufficient efficacy of oleic acid as penetration enhancer.

Keywords: Semisolid formulation, Silymarin, *In vitro* release, Skin penetration *ex vivo*.

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In Vitro Release of Propolis Phenolic Compounds from Poloxamer 407-Based Hydrogels

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Abstract

Hydrogels are three-dimensional hydrophilic polymeric networks that are able to absorb large amounts of water. Poloxamer-based hydrogels have advantages such as reverse thermal gelation, prolonged release of the active substances, biocompatibility, low toxicity [1]. The natural material propolis is provided antioxidant, anti-inflammatory and antibacterial activities by biologically active compounds [2]. The objective of this study was to investigate the release of propolis phenolic acids (vanilic acid, caffeic acid, *p*-coumaric acid, ferulic acid) and vanillin from poloxamer 407-based hydrogels *in vitro*. The formulations containing 20–30% (w/w) of poloxamer 407 and an equal amount of aqueous propolis extract. Semisolid formulations were characterized by pH, viscosity and its dependence on temperature. *In vitro* release experiments were performed at 32 ± 0,5˚C temperature using the modified Franz type diffusion cells. The samples were analysed by HPLC. The pH of the hydrogels was within the range of 4,86–5,20, which are suitable to be applied on the skin. The viscosity of these formulations increased from 0,22 ± 0,02 to 492,35 ± 28,33 Pa·s increasing the poloxamer 407 concentration. Hydrogel containing 20% of poloxamer showed to be the most sensitive system for the changes of temperature. The release kinetics of propolis phenolic compounds conformed to zero order model (r^2=0.988). The release of investigated phenolic compounds decreased increasing the concentration of poloxamer 407 from 20% to 30%. During 4 hours the largest amount (129,1 ± 8,1 μg/cm^2) of these compounds was released from hydrogel, containing 20% of poloxamer. The released amount was 1,8–2,3 times higher as compared with the released amounts of phenolic compounds from 25% and 30% of hydrogels.The results of physico-chemical and biopharmaceutical studies revealed that hydrogel containing 25% of poloxamer 407 is the most suitable to be applied on the human skin in case of disorders.

Keywords: Poloxamer 407, In vitro release, Propolis, Phenolic compounds.

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Mycobiota of Woody Debris Components of *Betula pendula* Roth. In Ukrainian Polissya

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Abstract

Research of biodiversity of microbiota is an important value for forest ecosystems and the general state of forest stands. The large availability number of species of microbiota plays an important role in the destruction of woody debris. Fungi are implement decomposition of organic matter (lignin and cellulose) dead wood pieces, in that way, they create favorable conditions for mineral nutrition of the next generation of vegetation.

As a result of mycological analysis of samples of woody debris of *Betula pendula* Roth. was isolated and identified 95 fungal species from 62 genera. The largest number of fungi (79 species) belonged to Division of *Ascomycota*, but Department of *Zygomycota* was represented by species *Micromycetes* random (8).

Most fungi were isolated from samples of woody debris, which according to the literature are an active destroyers of cellulose and potential producers of biologically active substances with which, they keep successfully competition in woody debris with other species of microorganisms. These include the genera *Micromycetes*: *Aspergillus* (*A. niger*, *A. foenicis*, *A. ficuum*, *A. sulphureus*, *A. tamarii*), *Penicillium* (*P. variabiie*, *P. verukozum*, *P. canescens*, *P. notatum*, *P. chrysogenum*, *P. spinulosum*), *Fusarium* (*F. solani*, *F. gibbosum*, *F. sambuzila*, *F. semitectum*, *F. sambucinus*), *Trichoderma* (*T. harzianum*, *T. viride*), *Alternaria (A. alternata, A. longipes, A. tunuissima, A. consortiale, A. chartarum)*, *Chaetomium* (*Ch. cochlioides, Ch. globosum*, *Ch. dolichotruchum*).

The regularity of increasing occurrence of species of microbiota on components of woody detbris was observed between the snags and logs (K_{SC} = 0,68), the lowest – between snags and branches (K_{SC} = 0,41), and between classes of destruction of woody debris the greatest similarity is established: between II -III (K_{SC} = 0,78), and the lowest – between I and V (K_{SC} = 0,43).

Using the algorithm, k-means method, was implemented clustering of mycobiota species to certain components of woody debris and degradation classes with division into 6 groups. In the first three clusters were only by one species. These types of microbiota are characteriied by high total frequency of occurrence and are highly specialized. As for Fourth cluster consists of 76 species of microbiota, their frequency detection is low. Fifth cluster formed by representatives of microbiota that are most common on logs and snags of III-IV degradation classes. The sixth cluster formed by *Aspergillus niger*, *Trichoderma harzianun*, which occur most on logs I-III and dead wood I-III classes destruction.

Found that the most favorable conditions for development of Micromycetes is logs III and V classes of destruction and snags of I-III classes of degradation.

Keywords: mycobiota, mortmass, logs, snags, coarse woody debris, class of destruction, *Betula pendula* Roth.

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Determination of Geoecological Factors Influence on the Water Quality of the Ishim River by Meaning of Geoinformation Systems

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Abstract

On the basis of a set of research methods it was conducted: retrospective analysis of available materials, monitoring studies, instrumental studies, and it was defined the boundaries of the catchment area of the Ishim river through the use of geoinformation systems. It is revealed that the basin of the Ishim River in the territory of the North Kazakhstan region is located to a greater extent on the southern margin of the West Siberian Plain, where the surface markings do not exceed 200 m above sea level, and belongs to the ancient Neocene plateaus occupying large spaces between the valleys of the Tobol and Irtysh rivers.

MapInfo Professional is one of the most common in our country instrumental GIS desktop type. Using the Mapinfo Professional program, we calculated the catchment area of the transboundary Ishim river flowing through Kazakhstan and Russia, which is the main waterway and the centralized source of fresh drinking water for the North Kazakhstan region. The Ishim refers to the type of rivers with exceptionally snowy food, it has a pronounced spring flood, the beginning of which usually occurs at the beginning of April, and the peak – for the third decade of April. The quality of surface water in the North Kazakhstan region is related to the seasonal change, which provides physicochemical processes in surface and underground waters that cause pollution of the water environment of the region, which in turn affects the quality of drinking water. However, the anthropogenic factor is the most important in recent years. Also, an additional study is required to determine the chemical contaminants that come from the watershed during the washout process.

Keywords: Geoecological factors, watershed, water quality, anthropogenic factor, geoinformation technologies.

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Fungal Spores as a Biological Air Pollutants in the Ambient Air of Vinnytsia City, Central Ukraine

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Background: Fungal spores are often underestimated as a biological component of the atmosphere. The number of spores in the cubic meter of the air can rise up to dozens of thousands per day. Due to spores' worldwide spread, pathogenic and allergenic properties fungal spore content should be controlled in a frame of hay fever prevention and plant pathogens control.

Methods: Spores collection from 2009 to 2014 used volumetric methods employing a Burkard trap placed at a height of 25 meters above the ground on the roof of a Vinnytsia Medical University, Ukraine. Samples taken from March 1 until October 31 were analyzed by mean of three horizontal transects in years 2009-2011 and by mean of twelve vertical transects at a bi-hourly mode in years 2012-2014 under the light microscope with x400 and x1000 magnifications.

Results: Up to 30 fungal spore categories were determined in the atmosphere of Vinnytsia city. They included such known human pathogens as *Alternaria, Epicoccum, Aspergillus* and plant pathogens as *Botrytis, Puccinia, Fusarium, Ustilago, Pleospora*. The high rates of *Cladosporium* and Ascomycota spores were seen in the air too.

The highest annual concentrations were determined for Cladosporium and Ascospores. They reached 16,500 and 3,700 spores per cubic meter respectively. Spores of *Didymella* which were counted separately reached 2750 spores/m^3 peak during the study period. The highest noted concentrations of allergenic *Alternaria* spores reached up to 3,700 spores per cubic meter. This case was reported in 2010.

Concentrations of *Curvularia* (peak by 26 spores per cubic meter of air), Erysiphales, *Peronospora* (peak by 86 spores per cubic meter of air), *Chaetomium*, (maximum 87 spores per cubic meter), *Torula* (maximum 94 spores per cubic meter), *Puccinia* (maximum 105 spores per cubic meter), Basidiospores (maximum 107 spores per cubic meter), Pithomyces and *Pleospora* (maximum 112 spores per cubic meter each), *Helminthosporium* (maximum 135 spores per cubic meter), *Aspergillus/Penicillum* (maximum 145 spores per cubic meter), were the lowest.

The highest spores concentrations were seen during July and August. High rates of sporulation remained in autumn. The highest concentrations of allergenic *Alternaria* were recorded in July. They remained high to the end of September. *Cladosporium*, which predominated in the spore rain, was the most active in June and July.

Conclusion: Fungal spores season and concentration control is important tool to control both seasonal allergy and plant pathogens in the atmosphere of particular region.

Keywords: fungal spores, seasonal allergy, plant pathogenes

ISSN

Redox Processes Intensity In Bulls' Semen Due To The Action Of L-carnitine

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In sperm, there is a process of oxidation, which is used substrates for ATP synthesis and processes of destruction of proteins, lipids and other compounds that are essential to life. These processes are accompanied by the absorption and release of Oxygen. An important role in cell energy processes performs L-carnitine, which regulates the intensity of energy metabolism in mitochondria. L-carnitine is found in high concentrations in the epididymis, where it plays an important role in sperm maturation, affects their mobility and serves as an antioxidant.

The aim of this study was to investigate the impact of L-carnitine on the redox processes intensity in semen, identifying links between biochemical and physiological parameters of ejaculates. The study was done in the Institute of Animal Biology NAAS, Ukraine. The material for research was semen. Diluted 1:1 semen was divided into two parts - one control and three experimental. L-carnitine was added to the diluent «Bioexel» in an amount of 10 mg/100 ml in the part II, 30 mg/100 ml - in III-th and 60 mg/100 ml - in IV-th and dissolve the sperm with this liquid 3:1. In the diluted semen was investigated the intensity of oxygen consumption (ng-atom O/min×0.1 ml of semen), the activity of enzymes superoxidedismutase, catalase and glutathioneperoxidase, the survival of spermatozoa (h).

L-carnitine, which is added to the diluent of semen 10 and 30 mg/100 ml, stimulates aerobic glycolysis by 5,9 and 11,9 % and decreases to 5,6 and 11,4 % oxidative processes that are not related to the synthesis of ATP, which increases the survival of bulls' spermatozoa. The results of the current studings show that carnitine promotes the increasing of the activity of catalase and glutathioneperoxidase, and the activity of superoxidedismutase decreases.

We established that the L-carnitine impacts on reducing the overall consumption of Oxygen by increasing the share of mitochondrial Oxygen consumption and aerobic glycolysis along with the free radical oxidation decreasing. In this case, antioxidant enzymes are transforming active forms of Oxygen in semen with the formation of endogenous Oxygen that as a result provides the maintenance of Oxygen homeostasis of spermatozoa.

The results confirm that the biochemical effect of the investigated substance depends on its concentration in sperm and, thus, L-carnitine served as the effector (activator or inhibitor) of the enzymes. The dose of L-carnitine 30 mg/100 ml inhibits intensity of production of reactive Oxygen bulls' sperm, normalizes the activity of antioxidant enzymes and promotes the growth of the metabolic activity of gametes, which increases the spermatozoa survival.

Keywords: L-carnitine, enzymes, bulls' semen, sperm

ISSN

Geranium Robertianum L. Introduction, Biologically Active Compounds In Overground Part During Plant Vegetation

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Abstract

The aim of these chemical analyses is to determinate total content of phenolic compounds, total content of flavonoid compounds and antioxidant activity of *Geranium robertianum L*. methanolic extracts in the different vegetation periods: intense growth, flower buds, start blossoming, massive blossoming, end of blossoming. The object of these analyses is *G. robertianum L*. It is a annual, sometimes biennial herbaceous plant of *Geraniaceae* family can be found widely in Europe, with the exception of the far north, in temperate parts of Asia, North Africa, Atlantic area of North America, and temperate parts of South America (Allen & Hatfield, 2004; Gruenwald et al., 2000). In Lithuania grows in humid deciduous or mixed forests, bushes, ash, roadsides. The extracts studied come from Sector of Medicinal Plants, Kaunas Botanical Garden of Vytautas Magnus University were harvested during the year 2016. According to the literature, *G. robertianum* L. has been used for a long time in the folk medicine of several countries in different preparations, for a multitude of therapeutic purposes. Its anti-inflammatory, haemostatic, antidiabetic, antibacterial, antidiarrhoeic, antiallergic, anti-cancer, antihepatotoxic, diuretic and tonic properties, as well as its suitability for the treatment of digestive system ailments has made this species very appreciated in herbal medicine (Harborne &Williams, 2002). In this study, the amount of non-volatiles compounds was performed using spectrophotometry methods. The amount of phenolic compounds was evaluated using Folin–Ciocalteu reagent spectrophotometry method. Colorimetric aluminium chloride method was carried out to determinate the total content of flavonoid compounds. Lastly, the antioxidant activity was estimated by DPPH (2,2-diphenyl-1-picrylhydrazyl).

Keywords: *Geranium robertianum L.*, phenolic, flavonoid, antioxidant activity, spectrophotometry methods.

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New Finding Place of Rare Species *Trachysphaera cf. lobata* (Diplopoda, Glomerida, Glomeridae) and other Diplopods Species Found in Some Regions of Lithuania

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Abstract

The arthropods class Diplopoda, the millipedes contain approximately 12,000 described species, which are widespread in the world. Although diplopods play an important ecological role in most terrestrial ecosystems, little is known about the group's diversity and biology compared with other arthropod groups. Millipedes are well covered in foreign scholar literature, as well as Lithuanian neighbouring countries. According to Fauna European (2013) Estonia, Latvia, Belarus, Kaliningrad, and especially Poland, stand out as countries with the biggest number of millipedes species.

For this reason the main aim of the research is to update and complement studies of millipedes in Lithuania. The research started in 2016. The diplopods were collected by hand viewing shelters mainly in Kaunas region and at random in Šilute and Šakiai districts. From 2016 to the present 17 diplopods species were identified which belong to four families: *Polyzoniidae, Blaninlidae, Julidae, Paradoxosomatidae.* This research resulted a successful finding of species *Trachysphaera cf. lobata* (Diplopoda, Glomerida, Glomeridae) which is very rare in Europe and found only in a couple of countries. Because of their tiny size and stone resembling appearance these millipedes are not frequently encountered by an unskilled observer. This might explain why this specie has not been known in Lithuania and its neighbour countries. As result of this study we present a list of found diplopods in some regions of Lithuania with short remarks on their distribution.

Keywords: millipedes, Diplopoda, Glomeridae, diversity, Lithuania.

ISSN

The Use of Molecular Markers for *Juniperus communis* Research

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Abstract

Juniperus communis has the widest distribution area of the conifers in western hemisphere, but species is suffering from high fragmentation in a lot of regions. One of the most valuable method to estimate Jc population diversity is the use of molecular markers. Molecular genetic research is characterized as highly accurate and gives more reasonable results than the use of phenotypic traits. For Jc research the most used molecular markers are based on Polymerase Chain Reaction (PCR) such as Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR) and Inter Simple Sequence Repeat (ISSR), but there are also a few research that used alloenzyme and Single Nucleotide Polymorphism (SNP). These all markers have some benefits and limitations. The earliest research for Jc is based on RAPD to estimate systematic relationships of *Juniperus* spp. But RAPD markers have some limitations such as they are all practically dominant. The later studies of population genetics used AFLP markers, but these are also dominant. Later on five nuclear microsatellite markers (Jc 016, Jc 031, Jc 032, Jc 035, Jc 037) were developed, they are all co-dominat and there are huge increase in studies that evaluate the genetic resources of Jc. The overall knowledge of Jc given from the use of molecular markers is very valuable for understanding of the habitant fragmentation influence to Jc and for presumptions of future adaptability of the species to environmental change.

Keywords: *Juniperus communis*, Molecular marker, Genetic diversity, Populations.

ISSN

Mechanism of Oxidation of Thiamine and its Phosphate Esters in Pseudoperoxidase Reaction Catalyzed by Metmyoglobin in the Presence of Paracetamol and Tyrosine

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Using HPLC, absorption and fluorescence spectroscopy methods it was shown that incubation of thiamine in aqueous solution with myoglobin and hydrogen peroxide resulted in formation of thiochrome, oxodihydrothiochrome, and thiaminedisulfide. If tyrosine or paracetamol are present in the incubation mixture besides metmyoglobin, hydrogen peroxide, and thiamine we observed significant growth of thiochrome yield and formation of oxodihydrothiochrome and thiaminedisulfide decreased. At high excesses of paracetamol or tyrosine the production of oxodihydrothiochrome and thiamine disulfide were inhibited and thiochrome was the only product of thiamine oxidation.

In comparison to thiamine, its phosphate esters (thiamine monophosphate and diphosphate) are stable in presence of metmyoglobin and hydrogen peroxide and even prolonged times of incubation did not lead to their oxidative transformations. However, addition of monophenol compounds to the incubation mixture leads to oxidation of thiamine phosphate esters to the corresponding thiochrome phosphates.

From the other side, formation of tyrosine dimers as well as paracetamol dimers and oligomers is lowered down in presence of thiamine and its phosphate esters. Mechanism of the coupled oxidation of thiamine and its phosphate esters with oxidation of monophenols in peroxidase reaction catalyzed by metmyoglobin and hydrogen peroxide is discussed. The obtained results indicate that at oxidative stress conditions presence of paracetamol leads to increased oxidation of thiamine and its phosphate esters producing thiochrome and thiochrome phosphates. Thus, toxicity of paracetamol in organism may be related not only to formation of toxic NAPQI by microsomal monooxygenases of liver but also to a decreased level of thiamine diphosphate which leads to inhibition of thiamine-dependent enzymes activity and contributes in development of patophysiological processes at oxidative stress. The obtained results suggest increase of thiamine administration at pathologies related to oxygen stress conditions and particularly when treatment with paracetamol is performed.

Keywords: metmyoglobin, oxoferryl forms of hemoproteins, phenoxyl radicals, thiamine, thiochrome, oxidative stress

ISSN

Uncharged derivatives of Thioflavin T do not exhibit viscosity-dependent fluorescence

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Aggregation of proteins into insoluble ordered filamentous structures – amyloid fibrils – is known to be related to range of neurodegenerative disorders such as Alzheimer's, Parkinson's diseases. Amyloid fibrils detection and studies *in vitro* rely mainly on the use of Thioflavin T (ThT) as a staining reagent. Mechanism of optical properties alteration for ThT upon binding to amyloid fibrils is well understood: this probe behaves as a fluorescent molecular rotor, i.e. it has viscosity-dependent fluorescence, and dramatic enhancement of its emission intensity takes place upon binding to fibrils due to suppression of non-radiative deactivation channel coupled to twisted internal charge transfer (TICT) process.

Fluorescent probe ThT is very convenient as a contrast agent for amyloid fibrils detection since its fluorescence quantum yield changes by ~3 orders of magnitude upon embedding into amyloid fibrils and emissions of the free dye or ThT bound to native or unfolded proteins are weak and do not interfere much.

ThT is charged at physiological pH (cationic form with Z=+1) and does not cross blood-brain barrier readily, thus, it has little use as *in vivo* imaging agent for amyloid fibrils detection. Therefore, uncharged lipophylic derivatives of ThT were proposed as promising candidates for *in vivo* imaging.

Several neutral ThT derivatives (BTA-0, BTA-1, BTA-2) were studied in this work and we showed that, in contrast to ThT, fluorescence intensity of the neutral ThT derivatives does not depend on viscosity/rigidity of the microenvironment, i.e the non-charged molecules do not demonstrate properties of fluorescent molecular rotors and behave as ordinary lipophilic fluorophores with high fluorescence quantum yield.

Only cationic forms of ThT derivatives exhibited viscosity-dependent fluorescence intensity.

Keywords: twisted internal charge transfer, fluorescent molecular rotor, amyloid fibrils

ISSN

Spectral properties of Th-C7 – a new Thioflavin T derivative

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Fluorescent probes are widely used for biophysical investigations and in medical diagnostics. The probes which fluorescent properties are sensitive to polarity and viscosity of microenvironment play significant role in biomedical research. Specific group of fluorescent probes with viscosity-dependent emission are referred to as fluorescent molecular rotors (FMR). In low-viscous solutions FMR practically do not fluoresce, however, in highly viscous media or upon binding to macromolecules quantum yield of their emission can increase by 2-3 orders of magnitude. Thioflavin T (ThT) is an example of FMR that is used for detection of amyloid fibrils - filamentous aggregates of proteins, related to several neurodegenerative disorders. Despite excellent sensitivity of this probe to presence of amyloid fibrils, spectral properties of ThT (absorbance and fluorescence spectra are located within wavelength range 400-550 nm) are not optimal for investigations in biological tissues. Therefore FMR with absorbance and fluorescence spectra at longer wavelengths (red and near-IR ranges) are considered as promising candidates for *in vivo* imaging of amyloid fibrils.

In this work we studied fluorescence properties of monocarbostyryl-benzothiazole (Th-C7), derivative of ThT with carboxyl group linker. When bound to amyloid fibrils the dye has absorbance with maximum at 550 nm and fluorescence at 600 nm. Its fluorescence properties are highly sensitive to viscosity and polarity of solvent. Growth of solvent viscosity by 3 orders of magnitude (from aqueous to glycerol solution) results in fluorescence quantum yield increase by ~500 times. It was established that twisted internal charge transfer (TICT) represents the main non-radiative decay process in the excited state of the dye. TICT rate depends on ratio of temperature and viscosity of solvent (T/η). In viscous medium TICT rate is significantly decreased which leads to growth of fluorescence intensity of Th-C7.

Binding of Th-C7 to amyloid fibrils leads to practically complete restriction of torsional motion of the dye fragments and fluorescence quantum yield is increased by more than 2 orders of magnitude. It is noteworthy that presence of proteins in native or partially aggregated forms practically did not affect fluorescent properties of the dye.

We showed that Th-C7, a new derivative of ThT, having absorbance and fluorescence in red spectral range, exhibits properties of fluorescent molecular rotors and can be used as fluorescent probe sensitive to solvent viscosity and presence of amyloid fibrils.

Keywords: twisted internal charge transfer, fluorescent molecular rotor, amyloid fibrils

ISSN

Some Features of Invasive Plants Stipulating Active Spread within Last Decades

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Abstract

Biological invasions are among the greatest threats to biodiversity of natural ecosystems. The majority of alien plant species were intensively spreading at the end of 19th and beginning of the 20th century. Most of invasive species of temperate climate zone are escapers from the warmer regions of the world. Due to ongoing climate change alien species are extending their invasive range of distribution towards more northern areas. Climate warming also has an impact to ecosystems leading to invasion process intensification. One of nowadays flourishing alien species in more southern Baltic States is wild cucumber (*Echinocystis lobata* (Michx.) Torr. et A. Gray). It is an annual vine, native in North America, brought to Europe as ornamental and medicinal plant which later escaped from cultivation and became invasive. According to Ellenberg indicatory values of plants in Central Europe, wild cucumber is highly thermophilic species. When rainfall is high enough, rise of ambient temperatures which are less than optimal for plant growth might have the positive impact to the plant development. Within decades of ongoing millennium wild cucumber has intensively spread along river banks of Lithuania. Such fact coincides with the 2011-2015 climate data assuming these years as the warmest period. For this interval annual mean temperature of Lithuania was 7.7 °C and mean temperature of vegetation period 13.3 °C. Therefore the aim of this study was to evaluate patterns of the plant dynamics. Study was funded by Lithuanian Research Council, Grant number No. SIT-02/2015. Wild cucumber was sampled in ruderal places and riverbanks. Morphological and gravimetric parameters highlighted huge potential of aboveground expansion for this species. Gravimetric and morphometric parameters per individual were much higher compared to what we may expect from the literature: maximum morphological parameters observed were as follows: stem length up to 242 m, aboveground mass up to 1300 g and the number of seeds was close to 1000 seeds. Obtained results suggest that among useful strategical features might be establishment of the huge aboveground part of the plant which allows to produce big numbers of the seeds and scatter them far away from the point where germination of the invader took part.

Keywords: climate, growth, morphometric parameters, biomass

ISSN

Characterization of a Spring Viraemia of Carp Virus Isolated In East Ukraine

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Abstract

During a fish health inspection in carp farms in the East region of Ukraine, a Spring Viraemia of Carp Virus was isolated from yearlings of common carp *Cyprinus carpio*. Preliminary examination of infected fish revealed a range of lesions particularly in spleen and kidney tissues. The virus grew in the fish cell lines of FHM and EPC with infectious titer of $10^{6,2\text{-}6,5}$ and $10^{5,5\text{-}5,8}$ $TCID_{50}$/ml respectively. The morphological changes, such as vacuole enlargements and cells rounding, were caused by virus in appropriate cell lines. Investigation by electron microscopy demonstrated that isolated virus was ultrastructurally similar to rhabdoviruses. Virions were non-enveloped with typical bullet profile, and approximately 80 to 180 nm in length and 60 to 90 nm in diameter. In addition, the nucleotide sequences of glycoprotein gene G fragments in size of 606 base pairs were analysed. The phylogenetic analysis of the glycoprotein gene G based on the comparison of Ukrainian isolates of SVCV with other available sequences revealed a close relationship of Ukrainian isolates of SVCV with sequences that represent the Fijan strain.

Keywords: SVCV, common carp, cell culture, molecular identification.

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ISSN

Influence of NMDA and GABA synaptic dysfunction on the evoked gamma oscillations in a computational model of schizophrenia

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Abstract

Schizophrenia is a psychiatric disorder which is characterized by delusions, hallucinations, and affects thoughts, behavior and emotions. Major neuronal degeneration is not observed in schizophrenic patients, but abnormalities in cortical circuits are present. These abnormalities are reflected in impaired EEG gamma frequency (30-80Hz), being crucial for many processes including sensation, perception, working memory and attention. NMDA and GABA synaptic dysfunction are proposed as one of the possible mechanisms underlying the decreased gamma frequency oscillations in schizophrenia.

We use a computational modeling approach to investigate the influence of NMDA and GABA synaptic dysfunction on the evoked gamma oscillations in cortex. We employ a model of a spiking neural network composed of 800 pyramidal neurons (PCs), 150 regular-spiking interneurons (RSIs) and 50 fast-spiking interneurons (FSIs) [1]. All cells are randomly interconnected. The background activity in the cortex is represented by an independent Poisson noise input to network neurons at 4 Hz. Sensory drive stimulus is modeled as excitatory stimulation of the network neurons at 40Hz. GABA receptor-gated channel time constant of FSIs is increased, and NMDA receptor-gated channel weight of FSIs is decreased to represent synaptic dysfunction in schizophrenia.

Prolonged decay time of GABA receptor-gated channels and decreased weight of NMDA receptor-gated channels onto FSIs lead to the impaired network activity: oscillation power is decreased at 40Hz and increased at 20Hz in PCs. The effect of synaptic GABA impairment is more profound

NMDA and GABA synaptic dysfunction leads to the decreased gamma frequency oscillations in a spiking neural network of cortex. Computational modeling approach is a powerful tool to understand complex non-linear dynamical systems and intrinsic mechanisms of neuronal network activity in healthy and diseased brain.

Keywords: schizophrenia, spiking-neural network, gamma oscillations, NMDA receptor, GABA receptor

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Event-Related Synchronization / Desynchronization in Terms of Switch of Manual Motor Programs in Men

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Abstract

In everyday life of each human there are rare situations of deadlock which is not accompanied by the subsequent switching to an alternative answer. Therefore, the scientific community is paying some increasing attention to the characteristics of brain processes providing switch of manual motor programs. However, the specific details of these processes in the cerebral cortex are still very limited.

32 men aged 18-23 years, right-handed participated in the experiment. They do not have neuropsychiatric disorders and traumatic brain injury in their anamnesis. EEG registration was performed monopolar, on the international system 10/20, with closed eyes during manual reaction in Go-Stop-Change paradigm. In case of the appearance of low tone (sound of 600 Hz, 70 %) men need to press and release the left button of the console (go-response) quickly with the help of the right index finger. The emergence of high tone (1600 Hz sound, 30 %) required rapid pressing and releasing with the help of middle finger the right button of the console (stop-change-response). Event-related desynchronization (ERD) and event-related synchronization (ERS) of spectral power (SP) of EEG frequency (7 Hz to 23 Hz) were estimated in frontal, central and parietal lobes. Calculation of ERD / ERS maps was conducted in Matlab environment (Pfurtscheller & Lopes da Silva, 1999).

Significant ERS response is established at a frequency of 6 Hz in symmetrical frontal, central and parietal parts, at 9 Hz frequency - in the left parietal area, 14-15 Hz - in frontal, central and parietal areas of the right cortex, at the frequency of 22 Hz - in the right frontal lead. Instead, at the frequency of 7-8 Hz in the frontal and central areas of both hemispheres EEG desynchronization is recorded.

The most sensitive to the manual movement stop, followed by switching to an alternative event is associated with synchronization of electrical cortical activity, which showed higher values at a frequency of 6 Hz and 14-15 Hz in frontal and central allocations and was lower at frequency of 16-17 Hz in both parietal leads than that during the Go-response.

Keywords: Go/Stop-Change paradigm, finger movements, spectral power, electroencephalogram, frequency spectrum.

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Comparison of *Impatiens parviflora* DC. Biomass Growing in Two Contrasting Environments

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Abstract

Small Balsam (*Impatiens parviflora* DC.) is one of the most widespread invasive plants in Europe. This annual species was introduced from Central Asia and since 19th century was increasingly spreading in our continent. The objective of our study was to analyse and compare biomass of *Impatiens parviflora* DC. growing in different habitats. Two contrasting environments in Kaunas city for the assessment in each area 20 plots of $1m^2$ size were chosen. Study was carried out during May-October of 2016. Each month 12 plants (among them the highest four, prevailing high four and the smallest high four) were sampled for gravimetric analysis. Dry mass of the roots, steams, leaves, flowers and seeds were studied. Our research revealed that *Impatiens parviflora* DC. differs in productivity depending on habitat type. Vegetation interval was longer for *Impatiens parviflora* DC. when compared to *Impatiens glandulifera* Royle and naturally growing *Impatiens noli-tangere* L. It shows intermediate position according to adaption of *Impatiens parviflora* DC. to Lithuania climate conditions and might be among variables causing wider spread of *Impatiens parviflora* DC. than *Impatiens glandulifera* Royle.

Keywords: Small Balsam, *Balsaminaceae*, invasive plant

ISSN

Genetic Diversity of Reed Canary Grass (*Phalaris arundinacea* L.) populations

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Abstract

Reed canary grass (*Phalaris arundinacea* L.) is perennial herbaceous plant belonging to *Poaceae* family. This species is widely spread in Europe and Asia. *Phalaris arundinacea* is valuable component of wetlands and might be applied as animal feed, ornamental plant or biofuel. It is riverside and lakeside species very common for Lithuania. Molecular studies of this plant have been carried out in North America and West Europe using inter-simple sequence repeats or microsatellite (SSR) markers. Genetic diversity of reed canary grass populations has never been assessed in the Baltic States. Study was funded by Lithuanian Research Council, Grant number No. SIT-02/2015. The aim of present study was to evaluate molecular diversity of riparian populations of *Phalaris arundinacea* growing in Southern part of Lithuania. For assessment 14 SSR markers (csm045, csm049, csm071, csm074, csm075, csm090, csm101, csm104, csm106, csm111, csm122, phi071, umc2185, umc2779) were selected. Genomic DNA was extracted using modified CTAB method. The length of polymorphic DNA fragments ranged from 90 to 163 bp. Fourteen SSR primer pairs have generated 95 alleles. The number of alleles per population ranged between 29 and 61. Our examinations revealed that the level of polymorphism at SSR loci for populations ranged between 30.5 % and 64.2 %. Molecular variance of populations of the lower reaches was higher when compared to the sites of the higher reaches.

Keywords: *Poaceae*, microsatellite markers, SSR, molecular markers, molecular variance.

ISSN

Phenological Characteristics of Wild Cucumber (*Echinocystis lobata* (Michx.) Torr. et A. Gray.) in Nemunas coastal Populations

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Abstract

Wild cucumber – herbaceous, annual plant, usually growing in coastal habitats like banks of the rivers, around the lakes and other over moistured sites also wastelands. This plant is native in North America. It can be cultivated as an ornamental plant for its flowers and spiny, ornamental fruits. Wild cucumber (*Echinocystis lobata*) in Lithuania and in other parts of Europe is recognized as invasive species, and considered to be one of the most dangerous according to intensity of the spread along continent part with the temperate climate. The objective of present study was to perform comparative phenology of the wild cucumber (*Echinocystis lobata*) populations and to collect plant material. Population detection and sampling took place in April–September, 2015. For observations of the plant development during vegetation season 4 out of 25 riparian populations of the Nemunas basin were selected – below Kaunas hydroelectric power station (Kaunas, Panemunė district), before tributary Nevėžis flows into the Nemunas (Kaunas, Lampėdžiai district), and sites upper and lower than Jurbarkas city. Morphometric analyses were monthly performed at selected sites. Our analysis revealed that main and lateral shoot length (cm), averaged per plant has expanded from 62 cm in June to 1152 cm in August. The average length of the main stem (cm) in June–August has augment from 62 cm to 471 cm. The avarage number of plant tendrils augment from 5 to 69 during growing season. In the course of the growing season plant stem diameter has increased from 3.9 mm to 6.2 mm. Within vegetation average number of the leaves (longer than 1 cm) per plant increased from 6 to 71 during the growing season. The length of the longest plant leaflet ranged from 5.8 cm to 12.5 cm. The biggest width of the leaflet (mean per plant) ranged from 6.6 cm to 16.7 cm. Comparison of 4 different sites with *Echinocystis lobata* revealed the maximum diameter of the stem, the utmost number of leaves as well as the largest maximum leaflet length and a maximum leaflet width along the Nemunas riverbanks in population below Jurbarkas.

Keywords: *Cucurbitaceae*, *Echinocystis lobata*, populations, phenology.

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Development of Capillary Electrophoresis Equipment for Salt Removing from Protein Solutions

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Abstract

Protein precipitation by salts is often used method for protein purification. One of must often used salt for protein precipitation is ammonium sulfate. After protein precipitation procedure, protein solution requires method to remove salts from solution. The aim of this study was to develop on capillary electrophoresis principles based equipment to remove salts from protein solution. Cheap and easy to access parts were chosen to construct apparatus. Lysozyme solution after purification by protein precipitation procedure was used as sample to test effectiveness of apparatus. Salt removing conditions were: 0,5 M, pH=2.58 acetic acid was used as background electrolyte; capillary length – 12 centimeters; applied voltage – 2,7 kV; sample volume up to 1 ml. After salt removing procedure, sample were analyzed using capillary zone electrophoresis. Analysis conditions were; 0,5 M acetic acid was used as background electrolyte, applied voltage was 14 kV. Injection 50mbar*30s; analysis time 40 minutes. Detector type – contactless conductivity detector

Keywords: Capillary electrophoresis, Lysozyme, Purification by protein precipitation, Contactless conductivity detector.

ISSN

Silver Nanoparticle Antibacterial Effect: Negligible Particle Specific Antibacterial Activity and Silver Ions Migration into *E. Coli* Bacteria Cells

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Abstract

Antimicrobial effects of silver salts and silver nanoparticles are well known and are used in wide range of applications, but its antimicrobial mechanism(s) is not yet fully understood. In this publication we compare silver ions (Ag^+) and silver nanoparticles (collargol & silver proteinate) antimicrobial activity by measuring Ag^+ concentration in nanoparticle suspensions and their impact on membrane potential of *E. Coli* KMY as a model of gram-negative bacteria via tetraphenylphosphonium (TPP^+) selective electrodes. In our experiments we conclude that silver nanoparticles antibacterial effects mainly are, if not only, a result of Ag^+ released by silver nanoparticles and thus are not particle specific. Furthermore, by measuring Ag^+ migration into *E. Coli* KMY bacteria with selective Ag^+ electrode, we show that silver ions enter cells in a manner similar to facilitated diffusion.

Keywords: silver ions, silver nanoparticles, antimicrobial mechanism, gram-negative bacteria, *Escherichia Coli*.

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Growth of Different Aspen Genotypes *in Vitro* under Water Deficit Stress

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Abstract

Six aspen genotypes – R-38 (*Populus tremula* L.), 117-3 (*Populus tremuloides* × *P. tremula*), 64-3 (*Populus tremuloides* × *P. tremula*), Hb8 (*Populus tremuloides* Michx. × *P. tremula*), 64-15(15B) (new hybrid aspen) and Gig-27 (new hybrid aspen) – were compared in order to assess their behaviour under water deficit stress. Aspen explants (10 – 12 mm apical shoot segments) were cultured in 150 × 20 mm glass tubes sealed with parafilm. Woody Plant Medium was used as a basal culture medium and water deficit was induced with addition of polyethylene glycol (PEG-6000) to the medium. PEG-6000 was added at the concentrations of 50, 100, and 150 g/l. The result analysis included such parameters as plant height, leaf, shoot and root number per plant, root length, green and dry plant mass, cellulose, chlorophyll content and leaf stomata number.

Some of the studied aspen genotypes were found to be more resistant to the inhibitory effect of water deficit on shoot growth than others. At 50 g/l PEG, hybrid aspen 117-3 explants showed the least shoot height difference from the control group (although this same genotype had the most reduced shoots at 150 g/l PEG). Meanwhile, at 100 g/l PEG, the shoot height of aspen R-38 explants was least different from the control group. Interestingly, R-38 was one of the two genotypes (another one – hybrid aspen 64-3) that developed more adventitious shoots at 100 g/l PEG than under control conditions.

Root number was most reduced by PEG in the genotypes 117-3 and 64-3, while the genotypes Gig-27 and R-38 were least affected (at 100 g/l PEG).

The highest plant dry mass under water deficit stress accumulated, at 50 g/l PEG, in the genotype 117-3 and, at 100 g/l PEG, in the genotypes 64-15 and Hb8. Higher leaf stomata numbers in stressed, in comparison to control, explants were found in the genotypes 117-3 (at 50 g/l PEG) and R-38 (at 100 g/l PEG).

Water-deficit stress induced very different changes in the total amount of chlorophylls: several-fold reductions were found in the genotypes Hb8, Gig-27 and 64-15, in contrast to up to two times chlorophyll increases in the genotypes 64-3 and 117-3 (at 50 and 100 g/l PEG).

In summary, it can be suggested that the genotypes 117-3 and R-38 would grow relatively well under the conditions of water deficit, while the genotypes 64-3 and 64-15 might be also fit to survive a short-term water deficit stress.

Keywords: aspen, PEG, water deficit stress

ISSN

Constructing, Optimising and Using Miniaturised Colorimetric Equipment with Integrated RGB LED

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Abstract

Spectrophotometers and colorimeters used for chemical analysis are expensive and hardly repaired. The priority of this work was to construct cheap, open-source colorimetric equipment that can detect total content of flavonoids, phenolic compounds and to measure radical scavenging activity. Colorimeters and spectrophotometers used in laboratories are big and it is sometimes a problem to transport them to the place where the samples are so mobility and size was the second priority.

Simple, cheap, small but reliable colorimeter was a vision of this work. When all tests and optimisations were completed miniaturised colorimeter was determined perfect for measuring absorption of total content of flavonoids, total content of phenolic compounds and radical scavenging activity. Miniaturised colorimeter consists only four main parts: body which was printed with 3D printer "PrintrBot", light emitting diode, light detector and Arduino Nano microcontroller. Constructed colorimeter was designed to connect to PC via Micro-USB connection and collect information equal to one point per second. Special program "Universal Acquisition 2.21" was designed for miniaturised colorimeter for collecting, processing and averaging data.

Optimised miniaturised colorimeter was able to detect total content of flavonoids, total amount of phenolic compounds and measure radical scavenging activity using three different wavelengths containing light emitting diode.

Keywords: miniaturised, colorimeter, radical scavenging, flavonoids, phenolic compounds, universal acquisition, LED.

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The Effects of Mineral Fertilzers on Growth and Reproduction of *Eisenia fetida*

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Abstract

Soil organisms were widely used as index of biological quality of soil. Worms are an important component of soil system, especially due to their effect on soil structure and functions. The aim of the study was to assess the effects of mineral fertilizers as ammonium nitrate and urea on earthworm *Eisenia fetida* growth and reproduction. To achieve this, experiment was conducted using OECD artificial soil spiked with NH_4NO_3 (0, 1, 2, 3 g NH_4NO_3 kg^{-1}) and $CO(NH_2)_2$ (0, 1, 2, 3 g $CO(NH_2)_2$ kg^{-1}). Growth was assessed at day 14 and 28, cocoon production - at day 56. Weight of worms was significantly affected by both fertilizers, analysis showed that NH_4NO_3 and also $CO(NH_2)_2$ concentrations have statistically significant influence on weight of worms ($p < 0,05$). The cocoon production was significantly affected by increased $CO(NH_2)_2$ substance but no significantly affected by increased NH_4NO_3. It is concluded that mineral fertilizers positively effects lifecycle of earthworms growth and reproduction.

Keywords: mineral fertilizers, earthworms, *Eisenia fetida,* growth, reproduction, oxidative stress

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ISSN

Biotic Environment of *Hypericum maculatum* Crantz. Populations in Lithuania

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Abstract

In Lithuania *Hypericum maculatum* Crantz. is the second widely spread species of *Hypericum* L. genera. This species has a tendency to produce big amounts of essential oils and flavonoids which might be of wide application in pharmacology. There is a relationship between some environmental conditions and the essential oil composition due to both biotic and abiotic stresses which have the impact for the formation of secondary metabolites.

Present study is aimed at evaluation of environment of *H. maculatum* according to associated plant species. In Lithuania, eight different sites with *H. maculatum* were selected and neighbouring herbaceous plants were recorded and the abundance of each species was evaluated. Abiotic environment characteristics were obtained using Ellenberg's indicator values (EIV) of the neighbouring species. According to Ellenberg's indicator values of plants in Central Europe environmental indexes for *H.maculatum* were: 8 for light, 3 for continentality, 6 for soil moisture, 3 for soil reaction and 2 for soil nitrogen, the species were indifferent for the temperature. For populations growing in Lithuania climate is colder and more humid compared to Germany.

For eight selected sites 133 plant species as 28 family representatives were registered. In most cases species coverage data allowed to group sites according to their habitats. Abiotic environment EIV of *H. maculatum* sites characterized by the Ellenberg's indicator values of associated plant species ranged rather wide: 6.21–7.14 EIV for the light light, 5.36–5.80 EIV for the temperature, 4.93–6.13 EIV for the soil moisture, 5.54–6.86 EIV for the soil reaction and 2.71–5.21 EIV for the soil nitrogen. The biggest differences in the EIV between populations were for soil properties like soil nitrogen (1.92 times), soil moisture (1.24 times) and soil reaction (1.24 times) and the minimum difference between population were for climate properties. In conclusion, the average indexes of EIV of *H.maculatum* growing in Lithuania for the soil reaction and the soil nitrogen were higher and for the light, the temperature and the soil moisture were lower than in Central Europe, Germany.

Keywords: medicinal plants, herbaceous plant, *Hypericaceae,* Imperforate St. John's-wort, population, Ellenberg's indicatory values, EIV

ISSN

The effects of phenoxy herbicide MCPA on epiphytic lichen (*Ramalina fraxinea* (L.) Arch.)) and their sensivity

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Abstract

There are a lot of various types of pesticides, which are applied to plants, weeds and soils. Due to good selection effect, phenoxy acetic herbicide is often used for broad-leaved plants to protect grass and grain. Representatives of this group, that are met most often, are 2-methyl-4-chlorophenoxyacetic acid (MCPA). The aim of this study was to examine negative effect of MCPA to lichen Ramalina fraxinea physiological and biochemical parameters. Lichen has been exposed by different MCPA concentrations (0, 20, 50, 100 mg/l). Toxic effects were found after 48 hours when lichen change color. Our results showed that different concentrations of MCPA caused decrease in chlorophyll content, induced membrane lipid peroxidation and cell membrane damage in *Ramalina fraxinea*. It is concluded that MCPA had negatively affected physiological and biochemical parameters and caused oxidative stress.

Keywords: phenxy herbicide, MCPA, lichen, *Ramalina fraxinea, toxic effects, oxidative stress*

ISSN

The Determination Of Lipopolysaccharides' Concentration In *Escherichia Coli* Suspension

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Abstract

Lipopolysaccharide (LPS) is a glycolipid, which is a part of the outer membrane of gram-negative bacteria. The structure of LPS consists of polisaccharydes and the toxic part called lipid A. The imunne system of human has a strong response to LPS molecules and can raise a septik shock in human's organism. The object of this research is to evaluate the effect of divalent metal Zn^{2+}, Mg^{2+} and Fe^{2+} ions or antibiotic Colistin (or Polymyxin E) on the change of the concentration of lipopolysaccharides *E. coli* KMY-1 bacteria. The lowest concentration of LPS – was determined when *E. coli* bacteria were affected by 0,035 mg/ml of Colistin solution. Optical density of bacteria suspension was equal to 1 in every experiment. It was investigated that divalent metal ions influenced the growth of the *E. coli* bacteria and the release of the LPS. The most effective ion was Zn^{2+}, which had decreased the LPS concentration comparing with Mg^{2+} or Fe^{2+}.

Keywords: lipopolysaccharides, *E. coli*, colistin, divalent, metal, ions.

ISSN

Cytotoxicity of vitamins C, K3 and their mixture for melanoma B16-F1 and hepatoma MH-22A cells in vitro

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Abstract

Melanoma – is one of the most prevalent skin cancer form, which is formed often by the sun's ultraviolet (UV) light and it damages DNA or do injuries for the skin cells. This cancer is one of the most diagnosed tumor in the USA. According to the Lithuanian Cancer Registry data, in 2012 the male and female skin cancer was one of the most abundant of the malignant oncological diseases locations (National Cancer Society, 2015). Concerning mortality, liver carcinoma is in the third place in the world. We need to find effective, non-invasive treatment that may cause cancer cells death except healthy tissue. Research shows that vitamin C (VC), vitamin (VK3) has anti-cancer features and using this compound, ratio 100:1 (VC:VK3), shows an increase of synergistic cytotoxicity. Cell's damage are available of oxidative stress (caused by vitamins) during the reaction between free radicals compose reactive oxygen species (ROS) or their precursors, such as hydrogen peroxide. In this work, studied mentioned substrates cytotoxicity against tumor cells.

ISSN

Long-term Tillage and Cropping System Related Soil Organic Carbon Pool Stabilization on Climate Change Retention

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Abstract

Soils organic carbon (SOC) pools have been estimated in a long-term field experiment where conventional tillage and minimized tillage systems with cereal crop rotation (winter wheat, spring barley and spring rape) and cover crop treatment were adjusted. It was estimated, that even while over the 16 years the mean annual temperature increased on average by 1.14 °C and mean annual precipitation expanded on average by 113 mm, soil tillage systems as well as steady plant rotation and cover crop treatment have influenced the accumulation of SOC. However, along the conventional tillage the pools of SOC were not increasing. Though, while shallow rotovating, cover cropping and no-tillage have been processed, the accumulation of SOC have increased on average by 1.5 folds in ploughed (0-20 cm) horizon. Therefore, in uppermost (0-10 cm) mineral soil layer along the minimized tillage the C:N ratio and microbial biomass accumulation have been increasing. Consequently that, due to the increase in soil C:N ratio and lower mineralization of soil microbial biomass, minimized soil tillage system following the climate change intensifies the accumulation of SOC as well as humification.

Keywords: climate change, long-term experiment, tillage and cropping system, soil organic carbon, microbial biomass carbon

ISSN

Chemical analysis of Phenolic Compounds And evaluation of Radical Scavenging Activity in *Geranium Macrorrhizum L.*, Using Spectophotometric and Chromatogpraphic Methods

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Abstract

The aim of the analysis is to determine *Geranium macrorrhizum L.* properties' related to non-volatile compounds, to compare this plant from different years and the impact on the non-volatile compounds. To this end, the analysis is based on the determination of the total amount of phenolic compounds, flavonoids and radical scavenging activity of *Geranium macrorrhizum L* using spectrophotometric and chromatographic methods.

In the past, *Geranium macrorrhizum L*. was used in medicine. It is also called "Rock crane's Bill". It is a perennial plant which was found in Bulgaria but today, it is naturalized everywhere in temperate regions [1]. This aromatic plant has been introduced in the Botanical Garden of Vytautas Magnus University. According to literature, this plant has some antimicrobial, antioxidant properties thanks to its flavonoids, phenolic acids and vitamin contents. The analysis was carried out with two types of sample: 2014 and 2015. The comparison between the two years can show differences depending on the climate.

To get results, raw material of *Geranium macrorrhizum L* was extracted by methanol (75%) for spectrophotometry. Different spectrophotometry methods were carried out according to phenolics compounds, flavonoids and radical scavenging activity. For the determination of phenolic compounds, a Folin reagent triggered a redox reaction. For flavonoids, a solution with methanol, acetic acid (33%), aluminum chloride was prepared to obtain results by colorimetric method. Finally, radical scavenging activity was measured with the reduction of 2,2-diphenyl-1-picrylhydraxyl (DPPH). Rutin was used for the calibration as equivalent for quantitative determination of phenolic compounds.

The analysis has shown that Geranium macrorrhizum L. has bioactive compounds which can be used in medicine. This analysis introduces the chemical composition of this aromatic plant which could be used for its antioxidant properties [3]. Moreover, there are differences according to the year, climate have an impact on the plant's composition.

Keywords: Geranium macrorrhizum L., non-volatile compounds, phenolic compounds, flavonoids, radical scavenging activity, aromatic plant

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Chemicals analysis of phenolic compounds and evaluation of radical scavenging activity in *Leonurus sibiricus* L. methanolic extracts

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Abstract

The aim of these chemical analyses is to determinate total content of phenolic compounds, total content of flavonoid compounds and radical scavenging activity of *Leonurus sibiricus L*. methanolic extracts in the different parts of the plant such as the stems, the leaves, the seeds and a mixture which is representative of the plant.

The object of these analyses is *Leonurus sibiricus L*. It is a biennial and annual herbaceous plant of Lamiaceae family and it is native to Asia, more especially from China, Mongolia and the South of Siberia. Nowadays, it is mostly cultivated in the coastal region of Brazil and in the Chiapas region of Mexico. The extracts studied come from Mexico and were harvested during the year 2017. According to the literature, *Leonurus sibiricus L*. is widely used in folk medicine in Asia for puerperal and menstrual diseases [1], indeed the seeds are considered to be constructive and aphrodisiac. In Chinese medicine, dried plant is prescribed as a tonic. The leaves are used to treat postpartum bleeding or painful menstruation in women [2]. Today the plant is a stimulant of the respiratory tract and affects motor endings: its roots and its leaves are used as febrifuge [3]. In this study, the amount of non-volatiles compounds was performed using spectrophotometry methods. The amount of phenolic compounds was evaluated using Folin–Ciocalteu reagent spectrophotometry method. Colorimetric aluminium chloride method was carried out to determinate the total content of flavonoid compounds. Lastly, the radical scavenging activity was estimated by DPPH (2,2-diphenyl-1-picrylhydrazyl).

Total content of phenolic compounds, flavonoid compounds and radical scavenging activity was evaluated in different parts of the plant (stems, leaves, seeds, mixture – representative sample of the whole plant).

Keywords: *Leonurus sibiricus L*., phenolic, flavonoid, radical scavenging activity, spectrophotometry methods

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Modeling of Optimal Antibiotics Concentration in Bacteria with PHREEQ Tool

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Abstract

PHREEQC is a computer program developed by the US Geological Survey (USGS) and has become the standard for water chemistry. In present PHREEQC is implementing in biochemistry and environment science where a computational tools are needed. This study is an attempt to model practical appliance and to compute optimal antibiotics (tetracycline, penicillin G and penicillin V) concentration at with the accumulation of antibiotics in bacteria E. coli reaches 0.5 mg per 1 g of bacteria at solution pH ≈ 5.0.

This study used simplified model of extracellular water including of Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} Cl^{-}, HPO_4^{-2} and HCO_3^{-} ions, antibiotics (tetracycline, penicillin G, penicillin V) and data sets of thermodynamic parameters for use in PHREEQC. Uptake of antibiotics by bacteria was described and computed as adsorption of neutral species of antibiotics with their log K values 2.36 for tetracycline, 2.24 for penicillin G and 2.26 for penicillin V respectively.

Using PHREEQC was modeled 0.5 mg accumulation of each antibiotic per 1 g of bacteria when concentration in solution of tetracycline was 100 mg/L, penicillin G was 300 mg/L and penicillin V was 300 mg/L. While antibiotics where added pH value of solution was changed from 5.5 at initial point to 4.97 at final point.

ISSN

Human Mitochondrial DNA Variability In Investigation Kazakh Populations

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Abstract

The distribution of human genetic variability is the result of thousand years of human evolutionary and population history. The investigation of the our present project indicate that the analysis of the mtDNA variation useful in genetics field Kazakh population. The particular features of the mtDNA D-loop regions, including high average mutation rate and also determine its usefulness in genetic studies. For instance, the reconstruction of the phylogeny is straightforward because the lineages are passed through the matriline with the only changes generated by mutation.

However, most studies of human evolution that have included mtDNA sequences have been confined to the D-loop Hypervariable Region. The study aims filling in the knowledge of the genetic history of kazakhstan population. We studied 24 Kazakh populations from unrelated families with different regions of Kazakhstan territory. Extracted DNA from peripheral blood samples of people aliquots and used to construct Human mtDNA D-loop Hypervariable Region sequencing libraries that were then amplified by the long-range polymerase chain reaction (PCR) using primers specific to the library adaptors and reading DNA polymerase. The sequence of two hypervariable regions (HVR-I and HVR-II) at the following nucleotide positions: 29-285,172- 408,15997-16236 and 16159-16401. Mitochondrial DNA was obtained DNA fragments from the D-loop mitochondrial regions were captured and sequenced on the Illumina Miseq platform. Miseq data that paired reads were merged and the results human mtDNA sequences alignment was made with the revised cambridge reference sequence (rCRS). The date was analyzed using the mtDNA MiSeq Reporter (MSR) plugin, interpreted using the BaseSpace® mtDNA Variant Processor v1.0 App (Illumina, San Diego, USA) software. The mtDNAmanager software was used to determine haplogroups.

Our results indicated that haplogroup D appears at a high frequency with his subclade (D2, D4, D4o, D5, D5/G, 70-80%, less frequency H,G, N,C, X,T, I,J,B 20-30%). The study of modern populations in order to reconstruct the migration that have participated in to spread of our ancestry of the planet.

ISSN

The Influence of Phosphorus Compounds on the Variety of Higher Aquatic Vegetation and Ichthyofauna of the River Seret

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Abstract

Introduction. The **aim** of our investigation is to study content of phosphates in the components (water, biotic compone regularity nts- higher aquatic vegetation) of the river Seret and determination of reasons and regularity of their redistribution and migration depending on hydrological, hydro chemical and biotic conditions of aquatic environment, and to study the species composition of ichtyofauna.

Models and methods of research. The content of phosphates (PO_4^{3-}) in the water was determined with the help of formation phosphosus molybdenum blue using ascorbic acid as deoxidizer. The content of overall phosphorus in higher aquatic vegetation was defined with the help of sample mineralization by dry ashing with the formation of salts of Orthophosphoric acid and further photometric determination of phosphorus coloured in the yellow colour of the compound. The systematic composition of fish fauna of the river Seret is provided with the help of bony fish system by J. Nelson (2006).

Results of research and discussion. In the river Seret the concentration of phosphates varied from the minimum index in September (0,021 mg/dc³) to the maximum index in August (0,046 mg/dc³). From April till May the content of the element has slightly increased and in July it dropped by 1,77. From the period from June till August the concentration of the element increased in 2,09, and after this it as much decreased. By the end of vegetation season the concentration of phosphates increased by 1,71.

The highest content of phosphorus in *Glyceria maxima* L was observed in May. The high content of phosphorus in *Lemna minor* L is noticed as well (23 mg/kg) as a result of active plant vegetation in June and July. In September the *Lemna minor* is absent as it is a thermophilic plant and doesn't vegetate in autumn. High vegetation of *Glyceria maxima* and *Ceratophyllum demersum* L. is observed as both of them are cold-water species. The content of phosphorus is much higher in *Glyceria maxima* than in *Ceratophyllum demersum* (4,5 mg/kg). The concentration of phosphoric compound increases in September as a result of secondary pollution due to the death of a significant number of plants *Ceratophyllum demersum* (5,1 mg/kg), *Glyceria maxima* -1,9 mg/kg. The ichthyofauna of the river Seret includes 19 species, which belong to 7 families, 4 types and 3 subtypes. Over the period of research 828 samples of fish was caught. The most numerous are represented by the family of *Gasterosteidae* 472 samples (57%); the second place – the *Cyprinidae* family 257 samples (31 %); the third place *Percidae* family 65 samples (7,8%). The family *Esocidae* are represented in smaller numbers (16 samples, 1,9%), *Balitoridae* (14 samples, 1,7%), *Odontobutidae* (3 samples 0,4%), *Cobitidae* (1 sample 0,1%).

Keywords: phosphates, aquatic plant, *Glyceria maxima* L., ichthyofauna, *Gasterosteidae, Cyprinidae, Esocidae, Balitoridae, Odontobutidae.*

ISSN

The Impact of Divalent Metals Ions and Colistin on Lipopolysaccharides' Concentration in *Escherichia Coli* Suspension

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Abstract

Lipopolysaccharide (LPS) is a glycolipid, which is a part of the outer membrane of gram-negative bacteria. The structure of LPS consists of polisaccharydes and the toxic part called lipid A. The imunne system of human has a strong response to LPS molecules and can raise a septic shock in human's organism. The object of this research is to evaluate the effect of divalent metal Zn2+, Mg2+ and Fe2+ ions or antibiotic Colistin (or Polymyxin E) on the change of the concentration of lipopolysaccharides *E. coli* KMY-1 bacteria. The lowest concentration of LPS was determined when *E. coli* bacteria were affected by 0,035 mg/ml of Colistin solution. Optical density of bacteria suspension was equal to 1 in every experiment. It was investigated that divalent metal ions influenced the growth of the *E. coli* bacteria and the release of the LPS. The most effective ion was Zn2+, which had decreased the LPS concentration comparing with Mg2+ or Fe2+.

Keywords: lipopolysaccharides, *E. coli*, colistin, divalent, metal, ions.

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Detection of *Borrelia* Species in Small Mammals in Lithuania

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Abstract

Borrelia is a genus of bacteria of the spirochete phylum, which causes borreliosis – a zoonotic, vector-borne disease transmitted by ticks and lice to human and animals. Small mammals are important reservoirs hosts for different *Borrelia* pathogens. The aim of the study was to estimate the prevalence of *Borrelia* spp. in different species of small mammals collected in Lithuania. A total of 238 small mammals belonged to the nine species *Apodemus flavicollis* (n=150), *Myodes glareolus* (n=34), *Mus musculus* (n=15), *Micromys minutus* (n=14), *Apodemus agrarius* (n=10), *Microtus oeconomus* (n=9), *Microtus agrestis* (n=4), *Microtus arvalis* (n=2) and *Sorex araneus* (n=3) were captured with live-traps in different locations of Lithuania in 2010, 2015–2016. DNR extracted from animal urine bladder was examined for the presence of *Borrelia* by using conventional, multiplex and nested PCR, and sequence analysis. *ospA* gene and 16S (rrsA) – 23S (rrlA) intergenic spacer region (ITS) were used as targets. *Borrelia* spp. was detected in 28 (11.7 %) of small rodents. In total, 11.7% (20/150) of *Apodemus flavicollis*, 5.9% (2/34) of *Myodes glareolus*, 2 of the 4 *Microtus agrestis*, one of the 2 *Microtus arvalis*, and 3 of the 9 *Microtus oeconomus* were found to be infected with *Borrelia* pathogens. The genotyping of *ospA* gene and sequence analysis of partial 16S (rrsA) – 23S (rrlA) ITS region of *Borrelia* indicated the presence of two *Borrelia* species: *B. afzelii* from the *Borrelia burgdorferi* sensu lato complex, and *Borrelia miyamotoi* belonging to the relapsing fever group. Phylogenetic analysis of ITS region sequences demonstrates that *Borrelia* isolates from small rodents are 99-100% similar to those *B. afzelii* and *B. miyamotoi* isolates derived from patients, ticks and rodents. This is the first report of *B. miyamotoi* found in small rodents in Lithuania.

Keywords: *Borrelia afzelii*, *Borrelia miyamotoi*, rodents, Lithuania

ISSN

Rare And Threatened Habitats Of Transcarpathia (Ukraine)

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Abstract

The Ukrainian Carpathians is the only part of the Carpathian mountain system that has not yet entered the program of Natura 2000 network, and this fact prevents to create the integral network of the natural region. The first step towards the integration of the Ukrainian territory into the pan-European network was making inventory and sozological estimation of natural habitats (including those of Natura 2000) of Transcarpathia bordering on four country members of the EU.

Here, in Transcarpathia, 54 habitats of Natura 2000 (14 of which are priority ones) and 114 natural habitats were found which were singled out according to the system of habitat type classification worked out for the model region of the Ukrainian Carpathians. 64 types of those (more than a half) are under one or another type of danger, and one habitat is considered to be vanished. As a whole, this index, like those of rarity habitats according to the categories, resembles the indices in the other European countries. Among the habitats of mountain territories, rarity and threatened ones are rocky habitats, alpine and subalpine grasslands (especially on calcareous substrates) and notably raised bogs that almost all are under the danger of degrading and vanishing.

Along with the wetlands in mountain regions the habitats in the plain part are also in the critical state, twenty-one types of those are described in the "Habitats" Directive. They are concentrated mainly on the volcanic hills and in the flood-plains of major rivers where the whole diversity of region's habitats can be found. Taking into account the number and area of the habitats, we can state that rare habitats for Transcarpathia are all those of dry (xerothermic) grasslands, shrub and forest communities.

Some water habitats are rare and threatened, first of all, that of "Water courses of plain to mountain levels with the Ranunculion fluitantis as well as Callitricho-Batrachion vegetation" (3260) and "Oligotrophic to mesotrophic standing waters with vegetation of the Littorelletea uniflorae and/or Isoëto-Nanojuncetea" (3130) with the communities of Marsilea quadrifolia that has entered the Bern Convention.

The habitat of "Riparian mixed forests of Quercus robur, Ulmus laevis and Ulmus minor, Fraxinus excelsior or Fraxinus angustifolia, along the great rivers (Ulmenion minoris)" (91F0), deserves special attention. These forests are concentrated in the flood-plains of the Latoritsa and Borzhava Rivers. Some areas of these forests in the lower reaches of the Borzhava River, to judge by their structure and age over 200 years, can be thought of as equivalents of primeval ecosystems (quasiprimeval forests) and considered as standard areas for the similar forest formations of the world importance.

Keywords: habitat, Natura 2000, Transcarpathia, Ukraine.

ISSN

Investigation of Ticks-borne Encephalitis Virus in Norway

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Abstract

Tick-borne encephalitis (TBE) becomes one of the most important diseases of humans in many parts of Europe and has been currently identified as a major health problem in many countries.

In Norway TBE was first diagnosed in 1998 and occurred sporadically, although over the past decade the number of TBE cases has increased and parts of the southern coast have been established as endemic. TBE is a notifiable disease in Norway. According to Norwegian Surveillance System for Communicable Diseases, the annual number of reported cases of TBE in Norway has increased from 2 to 14 cases during the last ten years.

The aim of the present study was to confirm the existence of TBEV endemic foci and determine the spread of TBE in different localities of Norway. The ticks were collected at nine locations along the southern coast of Norway during June-July in 2009. A total of 1542 ticks were collected. All collected ticks were identified as *Ixodes ricinus.* The ticks were pooled according to sampling site, development stage and sex. RNA was extracted from ticks' pools using RNeasy Mini Plant Kit (Qiagen, Hilden, Germany). For the detection of TBEV specific RNA, primers and a probe of a quantitative real-time RT-PCR protocol according to Schwaiger and Cassinotti (2003) were used. A total of 251 pools were examined and TBEV RNA was detected in 5 pools. These results prove the presence of TBEV in four locations in southern Norway, broaden the current knowledge of tick-borne encephalitis dissemination and will help raise awareness of TBEV infections in Norway.

Keywords: tick-borne encephalitis, *Ixodes ricinus*, Norway, real-time RT-PCR.

ISSN

Antifungal effects of *Monarda Didyma* L. Alone and in Combination with Enilconazolum

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Fungi are present everywhere in indoor and outdoor environments. *Trichophyton* spp. are fungal species that known as tinea infections in various areas in humans and animals. Enilconazolum is used antifungal drug for the treatment of both superficial and deep infections caused by *Trichophyton*. Moreover, the therapeutic response may be slow, and thus inappropriate for treatment of patients with severe or rapidly progressive infections. Essential oils are one of the most promising groups of natural compounds for the development of safer antifungal agents. Many essential oils are only fungistatic and high concentrations are needed for fungicidal activity. To enhance the efficacy of essential oils, the combined use of different oils has been evaluated recently for synergistic effects. The combination of essential oils with synthetic antifungals will probably result in a more effective therapy. Antifungal agents were tested against fungal isolates using the agar diffusion method. We used the essential oil *Monarda Didyma* L. The results of the agar diffusion tests indicate that these essential oil is significantly potent against *Trichophyton* spp. MIC assay results exhibited strong inhibition of 9 mm in most of the experimental concentrations of 50 % and 1 % of agar diffusion test. The MIC synergism of the essential oil *Monarda Didyma* L. with enilconazolum were remarkably decreased and agar diffusion test exhibited inhibition of 20 – 30 mm sterile zone. The combination and synergism essential oils *Monarda Didyma* L. and enilconazolum may reduce the efficacious dose of enilconazolum and minimize the side - effects of enilconazolum.

Keywords: essential oils, *Monarda Didyma* L., enilconazolum.

ISSN

Detection of *Hymenoscypus Fraxineus* in *Fraxinus Excelsior* Plants Derived on Mature Embryo Culture

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Fraxinus excelsior L. (common ash) is one of the important and valuable tree species. During over 15 years, massive dieback of *Fraxinus excelsior* has been observed in Europe and also in Lithuania. The identified pathogenic fungus *Hymenoscypus fraxineus* was the cause of common ash stands dieback. A species-specific DNA primer situated in the internal transcribed spacer (ITS) region of the ribosomal DNA, which can be used to detect the fungus in diseased host material. The aim of this study was to assess the rate of *Hymenoscypus fraxineus* presence in the common ash (*Fraxinus excelsior* L.) saplings using DNA specific primers for detection of patogen. The common ash plants *in vitro* were obtained from mature embryos of selected visually healthy trees from four common ash populations of Lithuania. After seed sterilisation the embryos were isolated and cultured on Murashige and Skoog medium without additional plant growth components. The plantlets with true leaves and shoots were succesfully transffered to Jyffy tablets for aclimatization *ex vitro* conditions. Survived plants during adaptation period were planted to pots with turf substrate and transfered to greenhouse and after two month growth they were moved to nursery garden. After wintering period under nursery garden conditions some seedlings didn't survive. On first decade September of 2016 year the leaves from 153 survived common ash trees were collected and DNA extracted using modified CTAB method. The results confirmed the presence of *Hymenoscypus fraxineus* in 15 common ash trees (9,8 % of the total sample), while the remaining trees (90,2 %) were free of this pathogen. This study suggests that isolated mature embryo culture is valuable technique for screening healthy seedlings of *Fraxinus exelsior* L. to conserve this species.

Keywords: *Fraxinus excelsior* L., embryo culture, *Hymenoscypus fraxineus*, DNA primer

ISSN

Pine Fungal Diseases and Their Management in Public and Private Nurseries

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Abstract

Concerns over the health of trees in the Europe has increased dramatically in recent years with the appearance of several emerging fungal pathogens previously unknown in the European countries which can affect growth and cause mortality of many trees species. Public urban green spaces (parks, forests, green roofs and community gardens) have played an important role in reducing the level of pollutions and creating the better living conditions for people. A good management at private and public nurseries is important to ensure growth of good quality plants. Nursery managers are responsible for the day-to-day operations of a nursery. In the present study, we have established questionnaire to analyze and evaluate information from managers responsible for forests containing a substantial component of pines in their mix of tree species. The key questions were related with the susceptibility of pine to *Dothistroma septosporum* and *Lecanosticta acicola* pathogens and knowledge about how they spread. These fungal pathogens cause needle necrosis and premature needle loss, resulting in substantial growth loss. In February of 2017, the 32 public and private land managers were interviewed. The analysis of questionnaire answers shows that *Pinus sylvestris, Pinus mugo, Pinus nigra* are the main pine species grown in Lithuania. The respondents indicated *Pinus strobus* and *Pinus ponderosa* pine species as having the lowest resistance for fungal diseases. Some nurseries are imported pines seeds and seedlings in Lithuania from the southern and central parts of Europe. Pines growing in Lithuania are exported to the northern, eastern, southern and western European countries. This is the potential disease pathways. Respondents' answers demonstrated that a lot of private and public nurseries have not enough knowledge about fungal diseases (their spread, plant susceptibility, and etc.). To ensure successful disease management, the sufficient quantity of information for nurseries should be provided.

Keywords: pine, fungal, diseases, nurseries, management

ISSN

Factors that Determine Shoot Viability and Root Development during *in Vitro* Adaptation and Propagation of Birch (*Betula pendula*)

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Betula pendula Roth (silver birch) *in vitro* shoot culture method is used to improve vegetative propagation of this economically valuable species in order to obtain healthy material for rot-free short rotation plantations. The aim of this study was to evaluate and improve *in vitro* technologies for birch selection and propagation. Experiments were conducted on seven silver birch genotypes. Birch explants (shoot segments with vegetative buds) were placed into glass culture tubes (150 × 20 mm) containing nutrient medium. Two variants of medium were used for comparison: hormone-free and cytokinin-enriched, with the latter containing 25 μmol*l^{-1} of 6-benzylaminopurine (BAP). The percentage of viable shoots and the morphometric characteristics of developing plants were evaluated.

The results showed that a shorter storage time between cutting of branches and disinfection of explants (one week instead of two weeks) was more favourable for the viability of explants. Also the results showed that cytokinin did not have a positive impact on the viability of explants during the very first *in vitro* adaptation period, but, when used afterwards, it increased callus formation and subsequent regeneration of adventitious shoots.

Among the six silver birch genotypes that had been planted *in vitro*, the genotypes 01BPL115 and 52BPL171 were distinguished for their viability. These two genotypes produced stable tissue cultures, but also had considerable differences from each other. After twelve months of culture, 01BPL115 genotype was characterized by intensive growth and well-developed root system, while genotype 52BPL171 regenerated from callus relatively weak shoots that did not form roots.

In turn, cytokinin (BAP), although necessary for effective shoot regeneration, was found to have a negative effect on root formation in birch culture. Thus, in order to improve birch propagation and to obtain rooted clones, it is important not only to be careful with disinfection and *in vitro* adaptation approach, but also to target exogenous cytokinin only at certain stages of *in vitro* adaptation.

Keywords: *Betula pendula* Roth, vegetative propagation, *in vitro* culture, cytokinin

ISSN

Identification of Gender in Full – Sibling Progeny of Hybrid Aspen Using RAPD Markers Linked to Putative Sex Determination Locus in *Salicacea* Family Plants

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The inability to determine gender at early age can create problems in advanced-generation breeding schemes of trees. The aim of the research was to define gender (female or male) by specific RAPD markers in early growth stage of new individuals of hybrid aspen that were produced making hybridization under controlled conditions. In this study, we analysed F2 progenies of combinations ♀(P.tremuloides x P.tremula) x ♂ (P.tremuloides x P.tremula), ♀(P.tremuloides x P.tremula) x ♂(P.alba x P.tremula). Progenies of *in vitro* culture growth tested using RAPD markers for female gender determination OPK-20_{400}, OPK-20_{500}, OPK-20_{800}[1], UBC-354_{560}[2], and male - $S60_{1800}$[3]. Individuals were differentiated into two families: PT34 family included 34 hybrids and PTA43 family – 43 hybrids. Total 77 samples of progeny individuals (including parents) were analysed using gender specific RADP markers.

Results of the study defined the fragments of DNA markers OPK-20_{400}, OPK-20_{500}, OPK-20_{800}, UBC-354_{560}, and $S60_{1800}$ at different frequency of distribution in the progenies according to gender. In the PT34 family, primer $S60_{1800}$ was determined in frequency of 70.6%, and in the PTA43 family its frequency was 60.5%. Primer S60 corresponded to parents (males). The frequencies of primer UBC-354 were 2.9% in the PT34 family, and 7% in the PTA43 family. OPK-20 400bp, 500bp and 800bp fragment's frequencies in the PT34 family were 0%, 14.7%, and 79.4%, respectively, and in the PTA43 family it were 2.3%, 16.3%, and 37.2% respectively. Fragments 500bp and 800bp of primer OPK-20 corresponded to parent (female).

It can be concluded that identified markers linked to putative sex determination locus in *Salicacea* family plants are informative in hybrid aspen individuals too.

Keywords: gender, RAPD markers, progeny, aspen, hybrid.

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ISSN

Molecular identification of *Lipoptena cervi* and *Lipoptena fortisetosa*

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Deer keds (genus *Lipoptena*), are hematophagous parasites which typically infest wild ruminants like roe deer, red deer and moose. However, the information on the distribution and abundance of deer keds on their mammalian hosts is still scarce, especially in Lithuania. The aim of present study was to identify species of deer keds parasitizing cervids in Lithuania. The furs of cervids (roe deer, red deer and moose) harvested through the hunting were examined for deer keds. Among examined animals, moose were found the most heavily infested with deer keds (more than 2500 deer keds were removed from one adult male). Taxonomic identification of deer keds was based on description of morphological characteristics of pupae and adults and using molecular methods. Genomic DNA was isolated from pupae and wingless deer keds. Two set of primers as described by Lee et al. (2016) were used in nested PCR to amplify the mitochondrial cytochrome oxidase c subunit I (*cox 1*) gene of deer keds. Our investigation demonstrates that in Lithuania cervids are infested with two species of deer keds. The partial COI gene sequences of deer ked obtained in this study showed 99% similarity with *Lipoptena cervi* and 97% with *L. fortisetosa* sequences deposited in GenBank. The present study is the first molecular identification of deer keds *Lipoptena cervi* and *Lipoptena fortisetosa* in Lithuania.

Keywords: deer keds, *Lipoptena cervi*, *Lipoptena fortisetosa*, COI gene

ISSN

The Prevalence Of *Rickettsia* spp. Pathogens In Ticks From Migratory Birds

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Birds are increasingly considered important in the global dispersal of tick-borne pathogens. The aim of this study was to investigate the presence of the spotted fever group (SFG) rickettsiae in ticks feeding on passerine migrating birds and living in their nests. A total of 284 I. ricinus ticks (109 larvae and 175 nymphs) were collected from birds at Ventės Ragas ornithological station in 2013 and 2014 years, and 2767 I. lividus ticks (1 male, 4 females, 7 nymphs and 2755 larvae) were found in sand martin (*Riparia riparia*) nests from three breeding colonies in 2013 and 2015 years. PCR analysis was used to detect *Rickettsia* spp. by targeting gltA and 17-kDa genes. *Rickettsia* pathogens were detected in 10.2 % of the *I. ricinus* ticks and in 89 % of I. lividus ticks. Sequence analysis of partial 17-kDa gene of *Rickettsia* isolates derived from *I. ricinus* ticks reveals presence of human pathogenic *R. helvetica* and *R. monacensis*. Rickettsia isolates from *I. lividus* ticks were closely related to *Rickettsia vini*. The presence of *Rickettsia vini* is reported for the first time in Lithuania.

Keywords: *Ixodes lividus, Ixodes ricinus,* migratory birds, *Rickettsia* spp.

ISSN

Geomagnetic storm, strong solar wind and stream interaction region affect for cardiovascular system

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Abstract

Recent research shows that not only geomagnetic storms (GS), but also other heliophysical indicators affect human health. The main goal of this research is to evaluate geomagnetic storms, solar wind velocity, and flow of stream interaction regions (SIR) in the formation of influence patients for acute coronary syndromes the health status. In research we used daily heliophysical data from 2001 till 2003 (Solar activity period). The data of 1391 patients, who were hospitalized at the Hospital of Lithuanian University of Health Sciences, were used. Data analysis was performed using multivariate logistic regression, binary indicators of the health of the patient using the response factors - categorizes heliophysical indicators. It was found that the hospitalization day at Solar wind speed ≥ 600km/s, more than 50% increased risk of acute coronary syndrome (ACS) in patients with hypertension, diabetes and kidney disease. SIR events increase risk of arrhythmias more than two times. In patients, hospitalized during GS or 1-2 days after their increased the risk of hyperglycemia over 1,5-fold. GS lasted more than one day at Solar wind ≥ 600km/s over 2,5 times increased of myocardial infarction with ST elevation. In patients with the metabolic syndrome the risk of ACS increased over 1,5 times during GS and on 1-2 days before and after. The results obtained suggest that the 1-2 days prior to GS, GS, 1-2 days after GS, the high Solar wind velocity, and SIR can be identified as independent risk factors in humans.

Keywords: Geomagnetic activity, human health, space weather, acute coronary syndrome.

ISSN

Investigation of *Toxoplasma Gondii* and *Bartonella* Spp. Pathogens in Domestic Cats in Lithuania

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Abstract

Toxoplasmosis and Cat Scratch Disease (CSD) are the most common zoonotic diseases of cats that can be transmitted to humans. Toxoplasmosis is caused by widespread zoonotic protozoan parasite *Toxoplasma gondii* that has both a simple and a complex life cycle, Domestic and wild felids are usually asymptomatic and they are the definitive hosts, and all warm-blooded vertebrate species – including humans – can serve as intermediate hosts. CSD is a bacterial infection caused by *Bartonella henselae* and *B. clarridgeiae* which is generally spread to people through cat bites or scratches. The aim of the present study was to determine the seroprevalence of *T. gondii* and to identify *Bartonella* species using molecular detection in cats from Lithuania. Blood samples were collected from 46 cats in pet clinics and animal shelters in Kaunas. Antigen rapid test was used to detect the presence of antibodies against *T. gondii* in the serum or blood samples. *T. gondii* infection was detected in 31 % (15/46) of cats. Molecular analysis using 16S–23S internal transcribed spacer (ITS) target allowed detection of *Bartonella* DNA in 14 % (7/46) of cats. Sequence analysis of partial ITS region of *Bartonella* isolates revealed the presence of two *Bartonella* species in cats – *B.henselae* and *B.clarridgeiae.* This study is the first report on prevalence of *T.gondii* and molecular detection and prevalence of *B.henselae* and *B.clarridgeiae* in cats in Lithuania, which allows for better evaluation of the zoonotic risk potential to the Lithuanian people.

Keywords: *Toxoplasma gondii, Bartonella henselae, Bartonella clarridgeiae,* cats, Lithuania

ISSN

The Use Of Economic Research In The Biodiversity Of Sessile Invertebrates (Examples Of Liverpool Docks)

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Background: Space is a limitative factor for sessile invertebrates, therefore it is essential to understand some factors such as competition and predation which drive obtain of space.
Materials and methods: This study proposed 2 hypotheses 1) selective removal of the strongest competitors may shift the relative dominance of competing species and increase diversity; and 2) predator exclusion may favour large solitary ascidians over other organisms. Hypotheses were studied using five treatments: control panel, cage, control (open) cage, disturbance (removal of dominant organisms) and disturbed cage treatments. Twenty PVC panels (10 × 10 cm) were used as a base to grow organisms and 10 steel cages (12 × 12 × 12 cm in dimension) were fitted around PVC panels these treatments.
Results: Removal of dominant organisms (*Botryllus schlosseri* and *Bugula stolonifera*) did not affect species diversity, while absence of dominant species favoured solitary ascidians (*Ciona intestinalis*). Moreover predator exclusion did not increase the number of solitary ascidians, because small predators could fit through mesh.
Conclusions: Findings indicate that these factors determined by many different processes including the size distribution of predators and seasonal patterns of sessile invertebrates.

Keywords: sessile invertebrates, predators, dominant specie, space.

ISSN

Molecular Detection of Filarial Parasites In Mustelidae From Lithuania

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Abstract

Filariasis is a parasitic disease caused by an infection with roundworms of the Filarioidea subfamily. The main vector of this parasite is mosquitoes. This disease belongs to the group of diseases called helminthiases. The aim of this study was to investigate the presence of filarial parasites in Mustelidae from Lithuania. A total of 127 animals belonging to the six species of Mustelidae family *Neovison vison* (n=52), *Martes foina* (n=23), *Martes martes* (n=21), *Mustela putorius* (n=21), *Meles meles* (n=7), *Lutra lutra* (n=3) were collected from roads or harvested by hunters. DNA was extracted from the spleen samples, and then used for PCR analysis. Two primer sets amplifying fragments of ITS-2 region and COI gene were used for molecular identification of helminthes. Amplification occurred in two samples obtained from *Neovison vison* and *Martes martes*. Sequence analysis of partial ITS-2 region of sample derived from *Neovison vison* show 99 % similarity with *Toxocara cati* sequences from GenBank. Sequences analysis of partial ITS-2 region and COI gene indicated the presence of filarial parasites in *Martes martes*. The sequences of ITS-2 region and COI gene obtained in the present study have 89 % and 91 % similarity to *Brugia spp*. sequences deposited in the GenBank, respectively. This is the first molecular detection of *Toxocara cati* in *Neovison vison* and filarial parasites in *Martes martes* in Lithuania.

Keywords: *Toxocara, Filarioidea, Mustelidae.*

ISSN

Rickettsia Pathogens in Red Squirrel (*Sciurus vulgaris*) and Their Ectoparasites

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Abstract

Rickettsia is vector-borne, gram-negative intracellular bacteria. This zoonotic pathogen cause infectious diseases worldwide. The aim of this study was to investigate the prevalence of *Rickettsia* spp. infection in red squirrels and their ectoparasites using molecular tools. A total of 33 red squirrels victims of road traffic were found in Lithuania during 2010-2016. Squirrels were found to be infested with *Ixodes ricinus* ticks (154) and *Ceratophyllus sciurorum* fleas (40). *Rickettsia* DNA was detected in 4 (12.1%) squirrels and 35 (22.7%) *Ixodes ricinus* ticks. *Rickettsia* spp. was not found in any fleas. Sequence analysis of 17kDa gene fragment showed that sequences were identical to *Rickettsia helvetica*.

Keywords: *Rickettsia*, squirrel, ectoparasites, Lithuania.

ISSN

Detection of *Babesia* spp. in Collected Ticks from deer in Lithuania

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Abstract

Human babesiosis is a zoonotic disease caused by protozoan parasites of the *Babesia* genus, primarily in the Northeastern and Midwest United States due to *B. microti*, and Western Europe due to *B. divergens*. The current understanding of human babesiosis epidemiology is that many infections remain asymptomatic, especially in younger or immune competent individuals, and the burden of severe pathology resides within older or immunocompromised individuals. However, transfusion-transmitted babesiosis is an emerging threat to public health as asymptomatic carriers donate blood and there are as yet no licensed or regulated tests to screen blood products for this pathogen. Further, new *Babesia* spp. have been identified globally as agents of severe human babesiosis, suggesting that the epidemiology of this disease is rapidly changing, and it is clear that human babesiosis is a serious public health concern that requires close monitoring and effective intervention measure.

In Lithuania not many research experiments have been performed on finding *Babesia* genus. We performed series of experiments on *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from deers in various regions in Lithuania. *Babesia* spp. were detected in 4 (1.82 %) out of 220 samples. Sequences analysis showed that ticks were infected with *Babesia venatorum* and *Babesia microti* parasites.

Keywords: *Babesia microti*, *Babesia venatorum*, ticks, *Ixodes ricinus*

ISSN

Design of *Theileria* spp. Specific Primers and DNA Amplification Protocols for Molecular Characterization

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Abstract

Climate change is happening in much faster and stronger way than expected. One of global climates change most discussed consequences is its effect on infectious diseases. Even though it is not clear how infectious diseases will spread, or how their occurrence will change, current assumption is that, climate conditions are what limit the distribution of infectious diseases. This assumption is particularly true for tick – borne diseases, where relationship between vectors that spread disease and climate they are living in is well defined. Therefore, tick – borne diseases spread is going to change with the change of the climate.

Theileria genus consists of intracellular protozoan that cause infectious disease in its host and are transmitted by ticks. The pathogen not only has complex life cycle comprising of both, the host and vector, but also has few individual species that has no cure of now and cause great loss in industry yearly. A lot is known of *Theileria* species that are distributed in tropical regions, but there is lack of information on species spread in Europe. There is almost no information on vectors that are responsible of distributing *Theileria* species in Europe. Because of that and unavoidable consequences of climate change *Theileria* species that are spread in Europe have to be very well observed and investigated. There is a need of a highly specific and sensitive method specific for *Theileria* detection.

Using PCR and primers specific for *Theileria* genus, there is a possibility to define not only animals that are carrying the pathogen but also its vectors. One of possible genes for creating molecular primers to catch all species within genus of *Theileria* is 18S rRNR. In this study we look at V4 variable region of 18S rRNA gene for possible molecular primers, design them and optimize PCR for *Theileria* spp. detection.

Keywords: *Theileria*, PCR, pathogen, tick – borne, diagnostics, primer design.

ISSN

Detection of *Anaplasma phagocytophilum* Infection in Collected Ticks from deer in Lithuania

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Abstract

Anaplasma phagocytophilum is an emerging tick-borne pathogen of global public health and veterinary significance. Wild ungulates are suggested to serve as reservoirs for *A.phagocytophilum* in Europe. In this study we investigated the occurrence of *A. phagocytophilum* in ticks (*Ixodes ricinus* and *Dermacentor reticulatus*) collected from deer in Lithuania. *A. phagocytophilum* were detected in 49 (22.3 %) out of 220 samples. Five *Anaplasma*-positive PCR products were purified and sequenced. Sequencing the 380 bp of the *msp4* gene revealed two different sequence types. Two types of sequences differed from each other at 33 nucleotide positions. Types of sequences from this study matched those derived from roe deer, red deer, sika deer and ticks (*Ixodes ricinus* and *Dermacentor reticulatus*) in other Europe countries.

Keywords: *Anaplasma phagocytophilum,* ticks, *Ixodes ricinus, Dermacentor reticulatus.*

ISSN

In vitro Analysis of DNA Damage of Bleomycin Electrotransfered Cells Using Comet Assay

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Currently, there is a need for individual, local tumor treatment with chemotherapeutical drugs. One of the methodologies for local anticancer drug delivery is electroporation. This phenomenon is induced when electric field is applied to the cells, by increasing transmembrane potential of the cell membrane. Temporal hydrophilic electropores are created in the membrane when electroporation threshold is reached. Created electropores are like bridge to hydrophilic anticancer drugs to enter affected cells. This way local (only electroporated cells) drug delivery method is obtained. At the moment local anticancer drug delivery via electroporation is available in clinics and is termed as electrochemotherapy. Mainly during electrochemotherapy treatment anticancer drug bleomycin (BLM) is used. This drug induces DNA breaks, causing cell death. However, to our knowledge there is no studies published, that indicate the quantification of DNA damage induced by BLM electrotransfer. Here we performed the *in vitro* analysis of DNA damage of BLM electrotransfered cells using the technique of Comet assay.

Chinese hamster ovary (CHO) cell line was used as an object for BLM electrotransfer. Electroporation was performed with 1 HV (1400 V/cm) pulse. Used BLM concentration was from 2 mg/ml to 20 μg/ml. Electroporation was performed in electroporation medium (pH 7.1, conductivity 0.1 S/m, osmolarity 270 mOsm). Colonogenic assay was performed to evaluate cell viability.

Electroporated cells were resuspended in low melting agarose (0.5 %), put on objective glass and covered with cover slip, 70 min after electroporation. Afterwards cells were kept in lysis buffer for 24 hours. Thereafter electrophoresis for 30 min in alkaline buffer (pH 13) was performed with voltage at 0.74 V/cm and 300 mA current. Fluorophore ethidium bromide (1 μg/ml) that binds to DNA was used to obtain visualization of the DNA damage under fluorescent microscope. Obtained cell DNA\Ethidium bromide complex fluorescence images were processed with open access software ImageJ plugin OpenComet v1.3.1.

Results from performed experiments indicate a sufficient cell DNA damage after BLM electrotransfer to the cells. 80 % of the DNA damage was obtained after BLM electrotransfer at anticancer drug concentration of 2 mg/ml, 70 % at 200 μg/ml and 30 % at 20 μg/ml **.** Even though cell DNA damage decreases when BLM concentration is decreased, the killing effect of DNA electrotransfer stays the same. In all used concentrations cell viability after BLM electrotransfer was around 1 %. Similar experiments were performed with BLM incubation with analog concentrations. No DNA damage or viability decrease was observed.

Here we performed the *in vitro* analysis of DNA damage and viability of BLM electrotransfered cells. We showed what bleomycin electrotransfer at concentration of 20 μg/ml is sufficient for cell viability decrease to 1 %, however DNA damage decreased from 80 % at 2 mg/ml BLM concentration to 30 % at BLM 20 μg/ml.

Keywords: Electroporation, Bleomycin, Comet Assay, DNA damage, Cancer.