

Impact of aqueous extract of *Viscum album* on different organisms

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The phytochemical and pharmacological potential of *Viscum album* extracts are widely used for the treatment of various diseases in many countries. The effect of aqueous extract from different mistletoe host trees (*Abies alba*, *Acer saccharinum*, *Malus domestica*, *Pinus sylvestris*) on model objects (*Allium cepa* and *Drosophila melanogaster*), bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*), and fungi (*Aspergillus niger*, *Heterobasidion annosum*, *Inonotus obliquus*) was evaluated. The influence of mistletoe extracts on *A. cepa*, *D. melanogaster* and basidiomycetes species *H. annosum* and *I. obliquus* was demonstrated for the first time. The study findings revealed that the impact of mistletoe aqueous extract exhibits considerable variation, influenced by multiple factors. These factors include the specific tree from which mistletoe is obtained, the concentration used, and the organism targeted. Mistletoe extracts caused the appearance of cells with pathologies in the meristematic tissues of *A. cepa* at different stages of the plant cell cycle. A clear cytostatic effect on *A. cepa* and *D. melanogaster* was shown, with the highest effect of mistletoe extract obtained from *A. saccharinum*. The main action of the solution on cells is probably manifested in the inhibition of cell division according to the results of the cytotoxicity assessment of aqueous extracts of mistletoe. Antibacterial activity of concentrated mistletoe extract obtained from *A. saccharinum* was found only against *S. aureus* with zone of growth inhibition 11.0 ± 0.1 mm. In the assay on agar plates the tested extracts stimulated the mycelial growth of *A. niger* 1.85 times higher compared to the control, but inhibited the growth of *H. annosum*, *I. obliquus* at 65.5% and 62.2%, respectively. However, the biomass production of *H. annosum* was strongly dependent on the host mistletoe tree of the aqueous extract, and both the inhibition of mycelial synthesis of the wood-destroying fungus and its stimulating effect on mycelial accumulation were found. A negative effect (1.46 times decrease in biomass) of *H. annosum* was found in the case of application of mistletoe extract obtained from *A. saccharinum*. The highest stimulation effect on fungus (1.53 times increase in biomass) was established by using mistletoe extract prepared from *A. alba*. In this study the main effects on living organisms used were indirectly caused by the host trees of mistletoe extracts. The overall negative impact was associated with the host tree *Acer saccharinum*.

Keywords: mistletoe; aqueous extract; host trees; onion; fruit flies; antimicrobial activity; fungi species.

Introduction

Viscum album L. is a well-known evergreen plant thriving as a hemiparasite, and relying on over 450 species of host trees across Europe, Asia, and North Africa (Becker 2000; Walas et al., 2022). Mistletoe is considered a threat to tree growth and forest health. *Viscum album* reduces nitrogen content and photosynthesis of host leaves, causes decreased leaf size and tree vitality, shoot die-off distal to the mistletoe, a worsening of the quality and volume of wood produced, higher mortality, as well as reduced flowering and fruiting, and increased susceptibility to damage from insects and fungi (Pilichowski et al., 2018; Camarero et al., 2019; Thomas et al., 2022).

All parts of *V. album* contain a large number of different chemical compounds. There are fats, amino acids, enzymes, glycoproteins (lectin, such as viscumin and agglutinin, as well as mistletoe lectin (Ladokun, 2015)), polypeptide (viscotoxin) and other proteins of small molecular weight (Segneau et al., 2022; Nicoletti, 2023), oligo- and polysaccharides, flavonoids, triterpenic acids and others (Kienle & Keine, 2010; Marvibaigi et al., 2014; Kleszken et al., 2022). *Viscum album* has been

widely used in folk medicine for a long time. Now mistletoe is used for the prevention and treatment of various diseases, such as atherosclerosis, hypertension, bronchial asthma, inflammatory kidney diseases, diabetes mellitus, etc. (Singh et al., 2016; Suveren et al., 2017; Szurpnicka et al., 2020; Park et al., 2021). Mistletoe extracts are given to cancer patients either as alternative therapy or commonly as complementary treatment in addition to conventional neoplasm therapy (Huebner et al., 2014; Matthes et al., 2020; Thronicke et al., 2022). Nowadays, humanity is faced with the emergence of new diseases, their transfer to other organisms and their spread to larger and larger areas. Also a big problem is the resistance of many pathogens to chemical components that have been massively used in recent years. In view of this, the study of the effect of plant components on different types of cells and organisms is relevant. Several authors argue the necessity to discover of the activity of *V. album* extracts, including the toxic effects (Zhang et al., 2016; Nicoletti, 2023).

A preliminary review showed that the majority of modern works are dedicated to the investigation of the effects of white mistletoe extracts on animal cells and human tissues. Mostly, the therapeutic effect in a number of diseases is studied (Szurpnicka et al., 2020; Valle et al., 2023). Different

studies are devoted to the antibacterial activity of *V. album* extracts (De-liorman et al., 2001; Abualhasan et al., 2014; JideFaleye & Olatunya 2016; Shah et al., 2017; Oubaid et al., 2022). The antimicrobial activity has been connected with antitumour properties (Ertürk, 2003, 2010; Oguntoye et al., 2008; Hussain et al., 2011). Information on antifungal effects of extracts is still scarce. Only a few reports are concerned with antifungal activity of *V. album* extracts (Ertürk, 2010; Hussain et al., 2011; Akalazu et al., 2016; Keskin & Ceyhan-Guvensen, 2018). The effect of mistletoe extract on pathogenic wood-destroying fungi as well as on plants has not yet been studied. Therefore, the investigation of this issue can have both scientific interest and practical application.

The purpose of this study was to reveal the effect of aqueous mistletoe extract on model objects such as *Allium sepa*, *Drosophila melanogaster*, some fungi and bacteria species.

Materials and methods

Plant material. To study the effect of substances from the green parts of *V. album*, leaves and young shoots of mistletoe grown on maple (*Acer saccharinum* L.), apple (*Malus domestica* Borkh.), pine (*Pinus sylvestris* L.) in the forestry "Pushcha-Vodytsia", Kyiv and fir (*Abies alba* Mill.) in the forestry "Solotvinske LG", Ivano-Frankivsk region were collected. The plant material was washed from dust, dirt and insects and used to prepare an extract solutions.

Preparation of aqueous extracts of white mistletoe. The prepared plant material was crushed as much as possible using liquid nitrogen and poured with hot distilled water. Several options of preparation contained 30 g of the sample and 60 mL of water (1:2), 10 g and 100 mL (1:10), 10 g and 200 mL (1:20), 10 g and 500 mL (1:50) and 10 g and 1000 mL (1:100) were used in the study. The mixture was heated and stirred for 20 min. Then the solutions were covered and left for extraction for a day at room temperature. After a day, it was centrifuged (under conditions of 5000 rpm for 5 min and 14500 rpm for 3 min) and the plant component was filtered. The extract was stored in a fridge. Before adding the extract to the nutrient medium, the solutions were disinfected using filters "Millipore" TYPE PH 0.03 µm.

Effect of mistletoe extracts on plant objects. Onion roots (*Allium cepa* L.) was used to evaluate the effect of the extract on the mitotic activity of plant cells. Plants were germinated under daylight and room temperature for several days on prepared solutions and on settled water (control). Grown roots with a length of 0.5–1.0 cm were fixed by Carnois fixative (alcohol-acetic solution 3:1) and stored in the refrigerator. A cell division synchronisation method was used to capture the moment of maximum number of meristematic divisions. The samples were transferred to a fridge and left at 5 °C for a few days before being returned to normal growing conditions and fixed. Before viewing, the meristematic tissues of the root were stained with a 1% solution of acetoorcein for 30 minutes at a temperature of 70 °C. Chemical maceration of the tissues was not carried out.

The number of cells at different stages of the cell cycle, the number of cells without disturbances, the number of cells with mitotic pathologies and chromosomal aberrations were counted. Cells that, in addition to the main nucleus, had micronuclei, an irregularly shaped nucleus, or a fragmented nucleus were noted separately. At least 13,000 cells were used for each variant of the experiment. The experiment was repeated three times.

Preparations were analyzed by light microscopy (AxioStar microscope, Zeiss); photographs were taken by an AxioCam MRc5 digital camera (Zeiss) and AxioVision 4.7 (Zeiss) and ImageJ software.

Effect of mistletoe extracts on animal objects. *Drosophila melanogaster* Meigen larvae were used as a model object. Test tubes with flies were stored in a clean place, without the influence of direct sunlight at a temperature of 25 °C with constant maintenance of the required level of moisture in the nutrient medium. Composition of growing medium was 6 g of agar, 80 g of wet yeast, 50 g of sugar, 50 g of semolina and 4 mL of propionic acid per 1 liter of water. To evaluate the effect of water extracts of white mistletoe on *D. melanogaster*, they were added to test tubes with a nutrient medium. 4 mL of nutrient medium with white mistletoe solution was added to 18 test tubes. 3 tubes were without the addition of the extract (control). 3 females and 2 males of the Oregon R line (<http://flybase.org/reports/FBsn0000276.html>) were planted in each tube. After the flies

were planted, the tubes were kept until the larvae hatched. Then the parents were planted. When the offspring passed to the imago stage, they were seared and counted. The survival of each group of flies was estimated as the ratio of the number of flies in the experimental group to the number of flies obtained in the control group.

Effect of white mistletoe extracts on bacteria. Pathogenic (gram+ and gram-) bacteria *Staphylococcus aureus* Rosenbach UCM B-4001 (ATCC 65388), *Escherichia coli* (Migula) Castelanis and Chalmers UCM B-901 (ATCC 6633), *Klebsiella pneumoniae* (Schoeter) Trevisan UCM B-7623 were kindly supplied from the Ukrainian Collection of Microorganisms (UKM, UCM) from the D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The bacterial cultures were grown in Mueller Hinton Agar (Merck). The antimicrobial activity of the crude white mistletoe extract (1:2) was studied by agar well diffusion technique (Balouiri et al., 2016). Bacterial strains were grown in Mueller Hinton Broth medium (MHB, Merck) for 24 h at 37 °C. Bacterial suspensions contained 10⁶ CFU/mL of each bacterium were prepared and plated on Mueller Hinton Agar. Paper discs (7 mm in diameter) were placed on the agar to load 10 µL of mistletoe extract sample. Inhibitory zones after incubation for 24 hours at 37 °C were determined.

Effect of white mistletoe extracts on fungi. Basidiomycetes *Inonotus obliquus* (Fr.) Pilát, 1877 and *Heterobasidion annosum* (Fr.) Bref 361 were kindly supplied from the IBK Mushroom Culture Collection of the M. G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (Bisko et al., 2020). Micromycete *Aspergillus niger* Tiegh. IFBG 134 was obtained from the Collection of Microorganisms and Plant Lines for Food and Agricultural Biotechnology of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine. Fungi were stored in a refrigerator at a temperature of 4 ± 1 °C in test tubes with potato-dextrose agar (PDA, Sigma). Fungi of each species were transplanted into Petri dishes with PDA and incubated at a temperature of 26 ± 1 °C until the dish was completely overgrown. In the phase of active growth of cultures, mycelial disks with a diameter of 8 mm were cut and used as inoculum for experiments.

Two series of experiments were conducted. In the first series, crude extracts at different concentrations were tested for their effect on fungal growth on solid media. Cooled to 40 °C Sabouraud dextrose agar (SDA, Sigma) medium was mixed with previously prepared sterile mistletoe extract (2 mL). After the medium solidified, one fungal disk was placed in the centre of the Petri dish. The effect of extracts was determined by measuring the diameter of the colonies with a digital vernier (Fisher Scientific, Waltham, MA, USA) for *A. niger* and *H. annosum* (14-th *I. obliquus*) on 7th day and expressed as the percentage inhibition of mycelial growth compared to the negative control, according to the formula (Alfredsen et al., 2008): antifungal activity (AFA) was calculated:

$$\text{AFA} = 100 \times (\text{GC} - \text{GT})/\text{GC},$$

where GC = hyphal growth on the control medium and GT = hyphal growth on medium with extracts.

In the second series of experiments, the concentrated white mistletoe extract (1:2) was tested for producing of *H. annosum* biomass. The flasks (500 mL) with Sabouraud liquid media and the addition of the extract 1:2 (2 mL) or in the control version (aliquot of distilled water) were inoculated with the mycelium (three disks), and then incubated at a temperature of 26 ± 2 °C for 14 days. Fungi growth was assessed by the absolutely dry mass of mycelium, which was filtered, washed with distilled water and dried at 105 °C to a constant weight, followed by weighing on analytical weights.

Statistical analysis. All experiments were performed in triplicate, and were expressed as means ± SE (standard error of the mean). Statistical processing of experimental results was detected by one-way ANOVA analysis with the Tukey test ($P < 0.05$ was considered to indicate a statistically significant difference), according to the consideration of Bonferroni's correction using the Statistica Software Package (version 11.5, USA).

Results

Effects of aqueous extracts of white mistletoe from different host plants on *A. cepa*. On evaluating the cytotoxic impact of white mistletoe

aqueous extracts on plant cells significant variations during the germination stage of *A. cepa* roots were observed. Thus, on solutions with a concentration of 1:2, plants almost did not form roots, while the bulb itself retained viability and grew green parts. The significant concentration of the active substances of the solution had a cytostatic effect on *A. cepa* root cells, and did not allow them to develop. This effect was confirmed for extracts of mistletoe collected from different host trees (maple, apple, pine and fir). The root growth also depended on extract concentration. The best growth was found on the extract with a concentration of 1:100, and the worst ones on 1:10. It was also noticed that at concentrations of 1:10 and 1:20 the roots became brittle very quickly and fell off on the second or third day. At 1:2 solution concentration roots rotted approximately on the second day of growth. On increasing the extract concentration the destruc-

tive process of the bulb accelerated, for example, on the fourth day after immersion for 1:10 and after seven days for 1:100 solution.

The effect of mistletoe extract on the proliferative activity of meristematic cells also decreased with reduced concentration. In the roots from all the experimental samples, a shift in the pool of mitoses in time was observed, which also indicates a certain cytostatic effect of the solutions. At each stage of the cell cycle, constant monitoring of the cell number allowed us to identify certain features in the roots that grew on extracts prepared from mistletoe collected from different host trees. A statistically significant decrease in proliferative activity compared to the control was noted for all extract samples (Fig. 1). The smallest decrease in mitotic activity was observed under the action of mistletoe extract from pine, and the highest decline for mistletoe from maple.

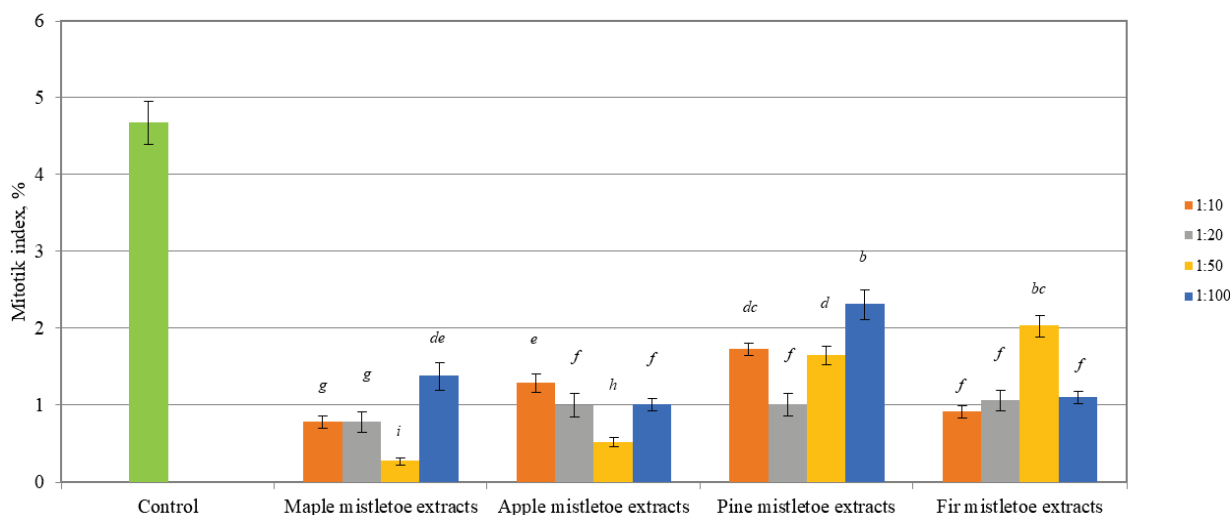


Fig. 1. The intensity of cell division in the root meristem of *Allium cepa* at grown on aqueous extracts at concentration 1:10, 1:20, 1:50, 1:100 of white mistletoe collected from different host trees: the data were analyzed using one-way ANOVA with Tukey test ($P < 0.05$, $x \pm SE$, $n = 3$) with Bonferroni's correction, values with the same letter are not significantly different

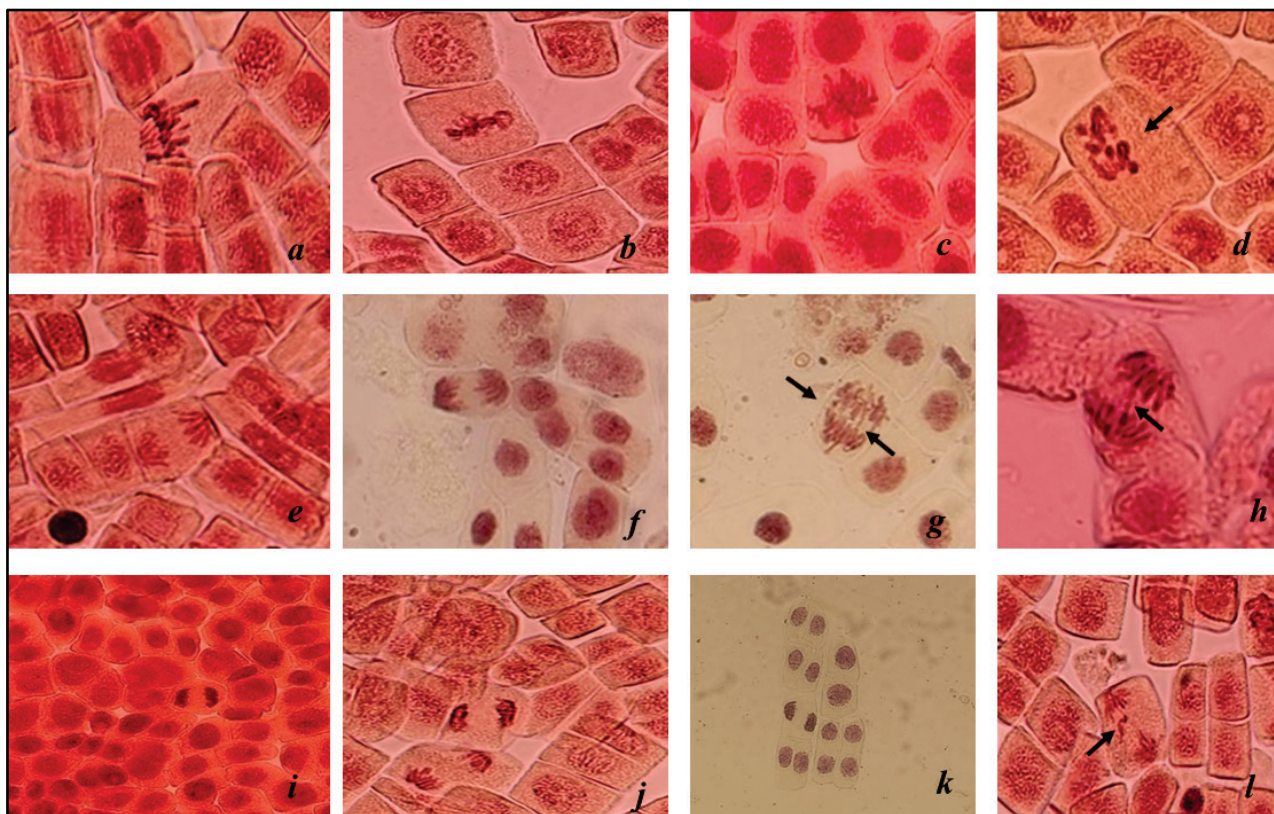


Fig. 2. Different stages of the cell cycle in the meristematic tissues of *Allium cepa* roots: *a-c* – metaphase, *e-f* – anaphase and *i-k* – telophase without pathologies: arrows show agglutination of chromosomes (*d*), lagging chromosomes (*l*), chromatid (*g*) and chromosomal bridges (*h*)

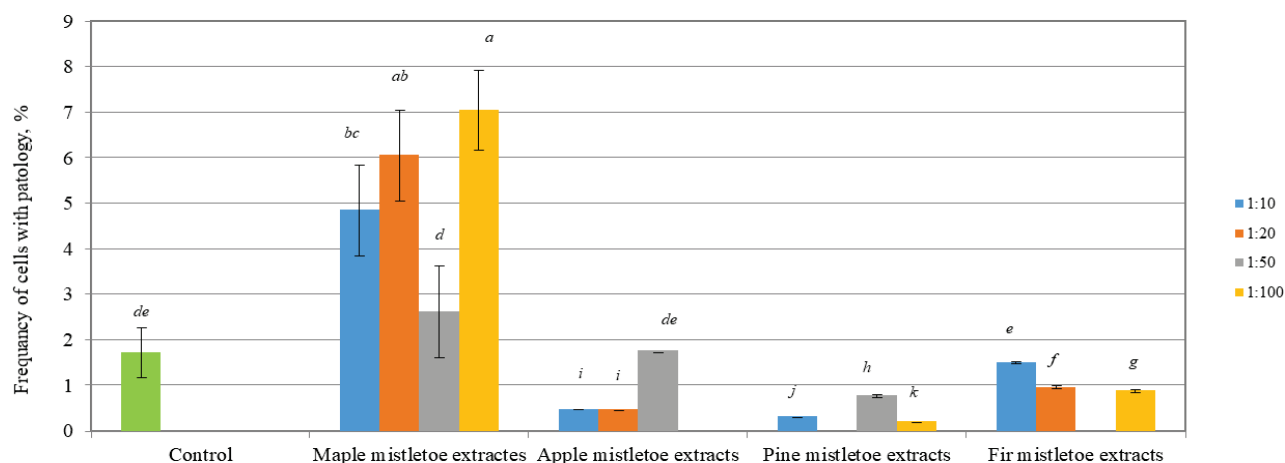


Fig. 3. The frequency of mitotic pathologies in the cells of the root meristem of *Allium cepa* growing on aqueous extracts at concentration 1:10, 1:20, 1:50, 1:100 of white mistletoe collected from different host plants: the data were analyzed using one-way ANOVA with Tukey test ($P < 0.05$, $x \pm SE$, $n = 3$) with Bonferroni's correction, values with the same letter are not significantly different

Cells with mitotic pathologies and chromosome aberrations were monitored since they can be seen as indicators for the detection of possible genetic damage at influence of the extracts (Fig. 2). In the vast majority of cells mitosis was without pathologies, which allows us to estimate the overall level of genotoxicity of aqueous extracts of mistletoe as rather insignificant. In the control sample ($1.73 \pm 0.55\%$) the level of pathologies showed the spontaneous mutational process and the effects of intracellular metabolites. In meristematic tissues of *A. cepa* growing on extracts of mistletoe from apple, pine and fir this indicator remained at the control level or was slightly lower than this value. However, for all concentrations (except the 1:50 concentration) of maple extract, a statistically significant increase in this indicator was observed (Fig. 3).

In each experimental variant, a different spectrum of mitosis pathologies and chromosome aberrations was observed. Only chromosomal and chromatid bridges were noted for the samples growing on apple extract. In addition to bridges, lagging and agglutination of chromosomes were established under the action of mistletoe extracts from pine and fir. There were five types of aberrations in the control. Of them, 47.1% were over-ran, 35.3% were bridges, among others, lagging and agglutination of chromosomes, multipolar mitoses. For samples from maple, the spectrum consisted of bridges, lagging and agglutination of chromosomes, multipo-

lar mitoses, nuclear fragmentation were also noted. The integral rate of single-cell damage was quite low. Among the interphase cells there were cells that had a fragmented nucleus. The highest number of such cells (0.11%) was observed at exposition by 1:20 concentration solution.

Effect of the aqueous extract of mistletoe on the larval stages of *Drosophila melanogaster* and bacteria. There was no significant differences between results obtained at solution concentrations of 1:10, 1:20, 1:50, and 1:100 in nutrient medium for growing of *D. melanogaster*. The number of viable individuals was 99.1–99.7%. Whereas at adding a solution with a concentration of 1:2 the destruction of flies (100%) was established on two days after the start of cultivation. The greatest effect was found, so in further studies only this extracts concentration was used.

Concentrated extract (1:2) of maple mistletoe was evaluated for antibacterial activity. The studies showed that the extracts were found not to display antibacterial activity against *E. coli*, *S. aureus*, and *K. pneumoniae*. Weak antibacterial activity of aqueous extract of white mistletoe collected from maple at a concentration of 1:2 was revealed only against *S. aureus*. The zone of growth inhibition was 11.0 ± 0.1 mm.

Effect of aqueous mistletoe extracts on fungi. In this investigation the effect of mistletoe extracts on mushroom growth depended on the mushroom species and tested extract concentration in agar plate assay (Fig. 4, 5).

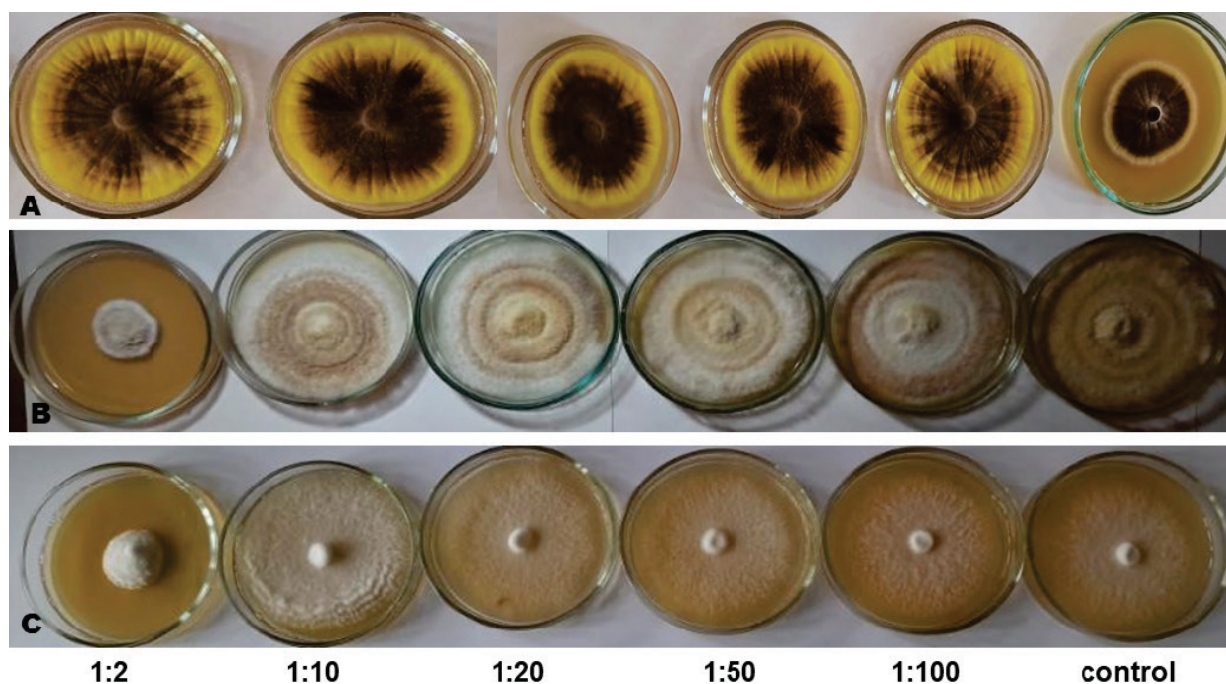


Fig. 4. Effect of aqueous mistletoe extract on fungi *Aspergillus niger* (a) and *Heterobasidion annosum* on 7th day (c) of growth on Sabouraud-dextrose agar medium: the concentration of aqueous extracts were 1:10, 1:20, 1:50, 1:100 of white mistletoe

It was found that all extracts concentration stimulated *A. niger* growth (Fig. 4a). Moreover, growth of this fungus was 1.85 times higher on the medium with concentrated extract (1:2) compared to control. However, antifungal activity was also observed also in the case of concentrated extract (1:2) application. Growth inhibition of *H. annosum* was established on 65.5% (Fig. 4b), and *I. obliquus* on 62.2% (Fig. 4c).

Effects of host tree of mistletoe aqueous extract on biomass accumulation of *H. annosum* were observed (Fig. 5). Biomass production ranged from 6.0 ± 0.4 to 13.5 ± 0.6 g/L. The highest stimulation effect (increase in biomass production by 1.53 times) on fungus was found by using mistletoe extract prepared from fir. The negative impact on the biomass synthesis of *H. annosum* by 1.46 times less than in the control was noted for application of maple mistletoe extract. Moreover, this extract caused the formation of a significant amount of calcium oxalates crystals, which can be explained as a physiological reaction of the fungus to a toxic metabolites of maple and hypothesized to regulate intracellular pH (pH 3.5).

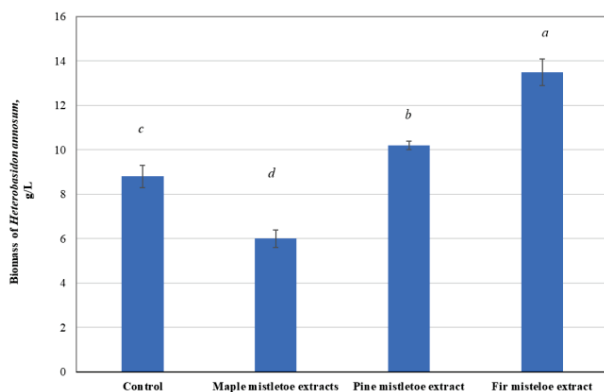


Fig. 5. Effect of host tree of mistletoe aqueous extract on biomass production of *Heterobasidion annosum*: the data were analyzed using Tukey test ($P < 0.05$, $x \pm SE$, $n = 3$) with Bonferroni's correction, letters *a-d* indicate statistically significant differences

Discussion

To evaluate the effects or damage causing tested agents, it is necessary for the sample to undergo continuous mitotic division, which will allow the detection of toxic effects and changes that occur during the cell cycle. Cytotoxicity tests using *in vivo* plant test systems, such as *A. cepa*, have been confirmed by several researchers (Teixeira et al., 2003; Bosio & Laughinghouse, 2012). As a result of this study, certain cytological features were found in the root meristem of *A. cepa* under the influence of aqueous extracts of mistletoe collected from different host trees. The obtained findings showed the cytostatic effect of aqueous solutions of white mistletoe which weakens with decreasing concentration. Chromosome and chromatid bridges (59.5%) are the most common pathologies in the studied cells of *A. cepa*. Therefore, the action of the active components of mistletoe extract precisely on the structure of chromosomes makes normal cell division impossible, for example, chromosomes cannot disperse to the poles due to the joined areas. This effect is dependent on the host plant where the white mistletoe grows. The most pronounced impact was observed when utilizing extracts derived from mistletoe grown on maple trees, which can be attributed to the specific chemical compounds transferred from the host tree to the parasite. This finding aligns with previous research highlighting the influence of the host tree on the metabolic composition of mistletoe (Pietrzak et al., 2017; Stefanucci et al., 2020; Jäger et al., 2021; Majeed et al., 2021; Pietrzak & Nowak, 2021). In addition, the qualitative and quantitative composition of phytocomponents in different plant organs can vary greatly (Yousefvand et al., 2022).

This study represents the first documentation of the cytostatic effect of mistletoe extracts on *A. cepa*. Early cytostatic effect of mistletoe extracts has been shown on rabbit sperm (Halénar et al., 2017).

The effect of aqueous extracts on insects has been tested only fragmentarily. It was found that aqueous extracts from *V. album* inhibited monoamine oxidase activity and increased the levels of serotonin and seroto-

nin receptor 5-HT_{1A} in *Galleria mellonella* L. (Lepidoptera) larvae (Szurpnicka et al., 2022). The *Drosophila* model is one of the most versatile and easy models used to examine the pharmacological effects of plants/plant-derived components. However, this first report of influence of mistletoe extract on *D. melanogaster* is presented. The toxicity of substances in mistletoe extract is confirmed by the mortality of *D. melanogaster*. A significant mortality rate of flies was established when they were in contact with different concentrations of the extract. Obviously, the larval stages of midges could not develop precisely because of the inhibition of the processes of cell division.

Drosophila melanogaster is recognized as a pest that can cause damage and spread diseases among fruit and berry crops. And therefore, the search for means of control without the involvement of synthetic chemical compounds, which can get into food products, is underway. Compounds derived from plant matter are safer, cheaper and more effective. Thus, a significant toxic effect on *D. melanogaster* of extracts of such plants as *Euphorbia prostrata* Aiton, *Parthenium hysterophorus* L., *Fumaria indica* (Hausskn.) Pugsley, *Chenopodium murale* L. and *Azadirachta indica* A. Juss was found (Riaz et al., 2018). The peculiarity of these plants is that they belong to the so-called unwanted plants or weeds. Accordingly, using them (or more precisely, the compounds obtained from them) as bioinsecticides, several problems can be solved at once. A similar situation is observed with mistletoe because there is a problem of dealing with it as a parasitic plant and a pest. As a result of the research, the cytostatic effect of the aqueous extract of white mistletoe on *D. melanogaster* was confirmed. Presumably, the larval stages of midges could not develop precisely because of the inhibition of the processes of cell division, which is the basis of the body's growth. The obtained results can become the basis for further study of the effect of mistletoe extracts on both fly larvae and other insects.

The assessment of the level of cytotoxicity of mistletoe aqueous extracts on two model objects (*A. cepa* and *D. melanogaster*) revealed that the main effect of the solution on the cells is probably manifested in the inhibition of their division.

There is growing interest in the application of plant extracts as a promising new antimicrobial agent since commercial synthetic drugs have shown several side effects on humans. We observed a very low reproducibility of antibacterial activity in extracts from *V. album*. The antibacterial activity of mistletoe aqueous extract was found only against *S. aureus*. However, the results against other bacteria were not promising. Since the extract tested in the current investigation is crude one, use of purified extract could give clear and better results. Different extracts of *V. album* have a higher antibacterial effect against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Proteus vulgaris* (Ertuck, 2003, 2010; Hussain et al., 2011; JideFaleye & Olatunya, 2016; Shah et al., 2017; Sklyar et al., 2011; Oubaid et al., 2022). At the same time, Oguntoye et al. (2008) showed that aqueous and ethanolic extracts of *V. album* did not inhibit growth of *S. aureus* in contrast to weak antibacterial action against *P. aerogenosa* and *E. coli*. The difference in the results of this investigation for antibacterial activity and those of other researchers may be due to the use of different parts of the plant, solvents for the preparation of extracts, host plants from which the mistletoe was collected and different assayed microorganisms or strains.

The current study showed that mistletoe extracts vary in their effect on growth of the tested fungi. The extracts stimulated mycelial growth of *A. niger*, but inhibited the growth of *H. annosum* and *I. obliquus* in agar plate assay. These differences in reaction to the extract are an indicator that plant extracts could have constituents with variable effects on fungi. In contrast to these results, some studies have demonstrated inhibition growth of *A. niger* by methanolic extract from *V. album* leaves (Sadananda et al., 2014; Akalaru, 2016). In addition, researchers have found two compounds, caprylamide and linoleic acid methyl ester, as the main antifungal compounds present in *V. album* leaves.

This study represents the inaugural investigation into the impact of mistletoe extract on basidiomycetes growth. Maple mistletoe extracts inhibited the growth of *H. annosum* and *I. obliquus*.

It should be noted that *Acer platanoides* L. extract was found to be the most effective of the bark extracts studied in reducing the growth of tested basidiomycetes such as *Fomitopsis pinicola* (Swartz: Fr.) Karst., *Hetero-*

basidion parviporum (Niemelä & Korhonen) and *H. annosum* in agar plate test (Alfredsen et al., 2008).

The biomass production of *H. annosum*, however, was significantly dependent on the host tree of the mistletoe aqueous extract, as both inhibition of mycelial synthesis of this wood-destroying fungus and its stimulating effect on mycelium accumulation were observed. The negative effect of mistletoe extract on the mycelium of *H. annosum* was confirmed under conditions of growth in liquid medium. The stimulating effect of other mistletoe extracts on this wood-destroying fungus may be related to its biological nutrient requirements, as it is predominantly associated with spruce, fir, pine in its habitat, and is able to infect other conifers.

Moreover, mistletoe extract derived from samples collected from maple can be a potentially valuable biological control agent against the wood-destroyed fungus *H. annosum*. Since the root and butt rot caused by *H. annosum* is one of the most destructive diseases of conifers in the northern temperate regions of the world, particularly in Europe (Asiegbu et al., 2008), fungicides based on natural substances can be used as an eco-friendly alternative in forest treatment for minimizing the disease spread.

The observed results showed that the main effects on tested living organisms were indirectly caused by the host trees of mistletoe extracts, and the overall negative impact was associated with maple. This tendency was clearly demonstrated in experiments on *A. cepa* and *H. annosum*. It should be noted that the toxicity of maple (*Acer*) has been described previously in medical anti-cancer experiments (Gonzales-Sarrias et al., 2011, 2012).

Conclusions

This is the first report on the influence of mistletoe extract on *A. cepa*, *D. melanogaster* and basidiomycetes such as *H. annosum* and *I. obliquus*. The findings revealed a diverse range of effects exerted by mistletoe aqueous extract, contingent upon factors such as the source tree, concentration, and targeted organism.

As such, the results of this study show a clear cytostatic effect of the aqueous extract of white mistletoe on two model objects: *A. cepa* and *D. melanogaster*. The assessment of the level of cytotoxicity of mistletoe aqueous extracts on these different model objects revealed that the main effect of the solution on the cells is probably manifested in the inhibition of their division. Weak antibacterial activity of mistletoe aqueous extract was found only against *S. aureus*. Tested extracts stimulated mycelial growth of *A. niger*, but inhibited the growth of *H. annosum* and *I. obliquus* in agar plate assay. The biomass production of *H. annosum* was strongly dependent on the host tree of mistletoe aqueous extract, and both inhibition of mycelial synthesis of the wood-destroying fungus and its stimulating effect on mycelial accumulation were found.

The conducted research is the basis for further investigation of biologically active metabolites of mistletoe, their biological stability and search for areas of their promising application.

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The authors declare that they have no conflict of interest.

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