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ОСОБЛИВОСТІ ПЕРЕДАЧІ ІНФОРМАЦІЇ У ФУНКЦІОНАЛЬНИХ МЕРЕЖАХ КОРИ ПІД ЧАС СПРИЙНЯТТЯ НЕЙТРАЛЬНИХ ОБЛИЧ В УМОВАХ ЕМОЦІЙНОГО ВПЛИВУ

Дослідження спрямоване на вивчення та моделювання емоційної активності функціональних мереж у корі головного мозку людини з використанням методів щільності спектра потужності (PSD) і детрендової ентропії фазового перенесення (PTE). Увагу зосереджено на виявленні змін у когнітивних механізмах, спричинених поданням нейтральних людських обличчя як рідкісних стимулів серед зображень обличчя із негативним чи позитивним забарвленням. Дані ЕЕГ реєстрували під час сприйняття й оброблення зображень нейтральної міміки людини, представленої серед позитивних і негативних обличчя у двох серіях зображень, що було проаналізовано за допомогою методів щільності спектра потужності та детрендової ентропії фазового перенесення. Також зареєстровано і проаналізовано активність кори головного мозку у стані спокою з відкритими та закритими очима. Для аналізу обрано окремі ЕЕГ-діапазони (θ та β) на основі їх значень у когнітивних механізмах. Топографія спектральної щільності потужності відповідала загальноприйнятим ідеям, що описують механізми сприйняття й оброблення зорових подразників. Метод ентропії фазового перенесення не був ефективним для аналізу стану спокою. Результати аналізу, проведеного за допомогою методу ентропії фазового перенесення, виявили проблеми диференціації нейтральних обличчя, коли вони представлені в позитивному емоційному контексті. Під час презентації нейтральних обличчя в негативному емоційному контексті спостерігалися посилені процеси мотиваційного кодування та саморефлексії. Висновки: ентропія фазового перенесення та спектральна щільність потужності продемонстрували свою ефективність у аналізі механізмів оброблення емоційних зорових подразників, опосередкованих у різних зонах кори.

Ключові слова: ЕЕГ; емоції; вираз обличчя; функціональна мережа.

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ОСОБЕННОСТИ ПЕРЕДАЧИ ИНФОРМАЦИИ В ФУНКЦИОНАЛЬНЫХ СЕТЯХ КОРЫ ВО ВРЕМЯ ВОСПРИЯТИЯ НЕЙТРАЛЬНЫХ ЛИЦ В УСЛОВИЯХ ЭМОЦИОНАЛЬНОГО ВЛИЯНИЯ

Исследование направлено на изучение и моделирование эмоциональной активности функциональных сетей в коре головного мозга человека с использованием методов плотности спектра мощности и детрендовой энтропии фазового переноса. Внимание было сосредоточено на выявлении изменений в когнитивных механизмах, вызванных представлением нейтральных человеческих лиц как редких стимулов среди изображений лиц с отрицательной или положительной окраской. Методы: данные регистрировали при восприятии и обработке изображений нейтральной мимики человека, представленной среди эмоциональных лиц в двух сериях изображений, и проанализировали с помощью методов плотности спектра мощности и детрендовой энтропии фазового переноса. Также зарегистрированы и проанализированы активность коры мозга в состоянии покоя с открытыми и закрытыми глазами. Результаты: для анализа были выбраны отдельные ЭЭГ-диапазоны (θ и β) на основании их роли для когнитивных механизмов. Топография спектральной плотности мощности отвечала общепринятым идеям, описывающим механизмы восприятия и обработки зрительных раздражителей. Метод энтропии фазового переноса не был эффективным для анализа состояния покоя. Результаты анализа, проведенного методом энтропии фазового переноса, обнаружили проблемы дифференциации нейтральных лиц, когда они представлены в положительном эмоциональном контексте. Во время презентации нейтральных лиц в негативном эмоциональном контексте наблюдались усиленные процессы мотивационного кодирования и саморефлексии. Выводы: энтропия фазового переноса и спектральная плотность мощности продемонстрировали свою эффективность в анализе механизмов обработки эмоциональных зрительных раздражителей, опосредованных в различных зонах коры.

Ключевые слова: ЭЭГ; эмоции; выражение лица; функциональная сеть.

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INFLUENCE OF *PHOLIOTA* SPP. (STROPHARIACEAE, BASIDIOMYCOTA) MYCELIAL BIOMASS ON SEED GERMINATION AND SEEDLINGS GROWTH OF *LEPIDIUM SATIVUM* L. AND *CUCUMIS SATIVUS* L.

Basidiomycetes represent a very diverse group of eukaryotic organisms in terms physiological parameters. Some organisms such as plants or fungi release certain secondary metabolites, which can affect the organisms around them. Some of the substances released by mushrooms could have effects on the growth and further development of nearby plants. Studies of fungi and their biologically active components have grown significantly, with the aim of potential introduction to various biotechnological processes. The allelopathic effect of *Pholiota* species has been investigated in this study. Mycelial biomass of seven screened *Pholiota* species were tested to study cucumber (*Cucumis sativus* L.) and lettuce (*Lepidium sativum* L.) seed germination and the sprouting growth. The results of our experiment showed that the biomass of the species of the genus *Pholiota* did not affect the germination of seeds of both plant species. 100% seed germination was recorded in both control and experimental samples. The addition of mycelial biomass of the *Pholiota* species led to a suppressive allelopathic effect, which affects seed germination, the length of the studied plant (both shoots and roots), as well as changes the morphology of the roots (pubescence, changes in lateral roots). The inhibitory effect on sprouting length was 8,6%-87,1% in the case of *C. sativus* and 42,2%-91,8% if specify for *L. sativum* in dependence on *Pholiota* species. Allelopathic properties of *Pholiota subochracea*, where sprouting growth ratio did not exceed 12.9%, compared to the control group, should be noted. This result suggests that *Pholiota* mushrooms have a significant regulatory effect on lettuce and cucumber sprouting growth. The given results suggest that the studied species may play a significant role in relationships within ecosystems.

Keywords: mushroom allelopathy, *Pholiota*, sandwich method bioassay.

Introduction. Allelopathy includes all types of chemical interactions among plants and microorganisms. Allelochemicals influence patterns in features that affecting the vegetation communities, seed germination and the sprouting growth, plant defence nutrient chelation, prevent nutrient uptake of target plants and regulation of soil biota in ways that affect decomposition and soil fertility [5, 8, 18, 22].

Weeds account for not more than 1% of the total plant species on the earth, nevertheless they cause great problems to humankind by interfering in food production, health, economic stability, and welfare [20]. Moreover, as far as it is known, 255 weeds from 92 crops have become stable to 163 herbicides all over the world [9]. That is why the weed control issues demand new ways of solving.

The use of natural products that can serve as natural herbicides is a promising direction because of their greater safety for humans and environment [14]. The phenomenon of allelopathy was studied in terms of plant interaction [6, 8, 12], less often in relation to other organisms [15, 17, 21]. Data on allelopathic interactions of fungi have recently emerged, nonetheless, there are still a lot of wild mushrooms, whose activity have not been described well, including *Pholiota* species [17, 21].

But there are no data about allelopathic influence of cultured mushroom mycelia. Besides, mushrooms are known as an excellent source of nutrients inclusive of macronutrients and bioactive compounds [11, 23, 25], however, there is still insufficient information on their allelopathic properties in relation to other organisms.

The purpose of this study was the necessity for a broader study of the allelopathic properties of *Pholiota* genus, namely seven species from the IBK Mushroom Culture Collection. According to the system proposed in the ninth

edition of Ainsworth & Bisby's Dictionary of the Fungi, species of the genus *Pholiota* belong to the family Strophariaceae, Basidiomycota of the kingdom Fungi [13]. World diversity is unknown, but the approximate number of species of the genus *Pholiota* (Fr.) P. Kumm. – about 200–300 species, 25 of them are reported in Ukraine [4, 27]. Most species of this genus are saprotrophs, which are active wood-destroying organisms. Most species are inedible due to bitter pulp, some species are edible, poisonous are absent. *Pholiota nameko*, *Pholiota adiposa* – cultivated on an industrial scale in Southeast Asia as valuable edible mushrooms [7, 19].

Materials and methods. Seven strains of seven *Pholiota* species from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of the Ukraine were investigated [2] (Table 1) as well as standardized seeds of the model plants *Lepidium sativum* L. and *Cucumis sativus* L.

Table 1. List of the studied *Pholiota* species and strains

Species	IBK strain
<i>Pholiota adiposa</i> (Batsch) P. Kumm.	2169
<i>Pholiota alnicola</i> (Fr.) Singer (syn. <i>Flammula alnicola</i> (Fr.) P. Kumm.)	2406
<i>Pholiota aurivella</i> (Batsch) P. Kumm.	2605
<i>Pholiota limonella</i> (Peck) Sacc.	2335
<i>Pholiota nameko</i> (T. Ito) S. Ito & S. Imai	2154
<i>Pholiota squarrosa</i> (Oeder) P. Kumm.	2010
<i>Pholiota subochracea</i> (A.H. Sm.) A.H. Sm. & Hesler	2535

Mushroom cultures were grown on glucose peptone yeast (GPY) liquid media; g/l: glucose – 25.0; peptone – 3.0; yeast extract – 3.0; MgSO₄ × 7 H₂O – 0.25. The medium was sterilized using an autoclave (1 Atm.) Strains grown on GPY medium with agar-agar (20 g/l) was used as inoculum. Mycelial discs with a diameter of 5 mm were cut with a sterile steel tube at a distance of 8–10 mm from the edge of the active growth of the colony and placed in flasks (volume of 500 ml) with GPY media (200 ml). The mycelia were incubated at a temperature of 26±0,1 °C for 21 days. The biomass was washed with distilled water and dried at 60 °C, which was subsequently used in its native form.

To determine the effect of mycelial biomass used a modified technique – sandwich method bioassay [17]. Dry biomass (0.1 g) was ground to a powdery state and left under UV radiation for 2 hours to obtain sterility. Agar medium without nutrients and trace elements was used as a substrate, SA: agar-agar – 10 g/l, water – 1 l. Dry mycelial biomass was applied in an even layer in a Petri dish (d=85 mm), which was covered with 8 ml of SA and left to solidify completely, then another 8 ml of medium was added on top and left to solidify this media again. Then 10 seeds of *L. sativum* or *C. sativus* were decomposed on the surface of the agar medium at equal distances. The seeds were not subjected to additional processing before the experiment and no stratification was performed Petri dishes with SA without addition of mycelial biomass were used as control. Studies of allelopathic activity were carry out in a thermostat at a temperature of 26 ± 0.1 °C. Each experiment was conduct in triplicate.

On the 3rd day, the number of germinated seeds was recorded and the radicle and hypocotyl of the plant were measured. During the analysis of the data paid attention to the following parameters: the length of the root, its pubescence, the development of lateral roots, the length of the shoot, the total length of the plant. Morphometric measurements were performed using the program AxioVision 4.8.2.

The percentage of the radicle and hypocotyl growth ratio of the lettuce and cucumber sproutings was calculated for each sample compared with the control by the following formula: Growth ratio (%) = 100 × (average of sample length/average of control length) [17]. Inhibitory effect was calculated as a mathematical difference between 100% and the value of growth ratio. Statistical analysis was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Results. Allelopathic interactions involved growth inhibition in both *L. sativum* and *C. sativus* species (Fig. 1–8). A comparison was performed to study the level of increase or decrease in growth ratio, root and shoot length for both species.

Cucumis sativus

The total percentage of *C. sativus* seed germination with the addition of *Pholiota* biomass was 100% compared to the control group. As a result of the experiment, there was a detection of growth inhibition of both roots and shoots of cucumber sproutings due to *Pholiota* biomass exposure (Fig. 1–4).

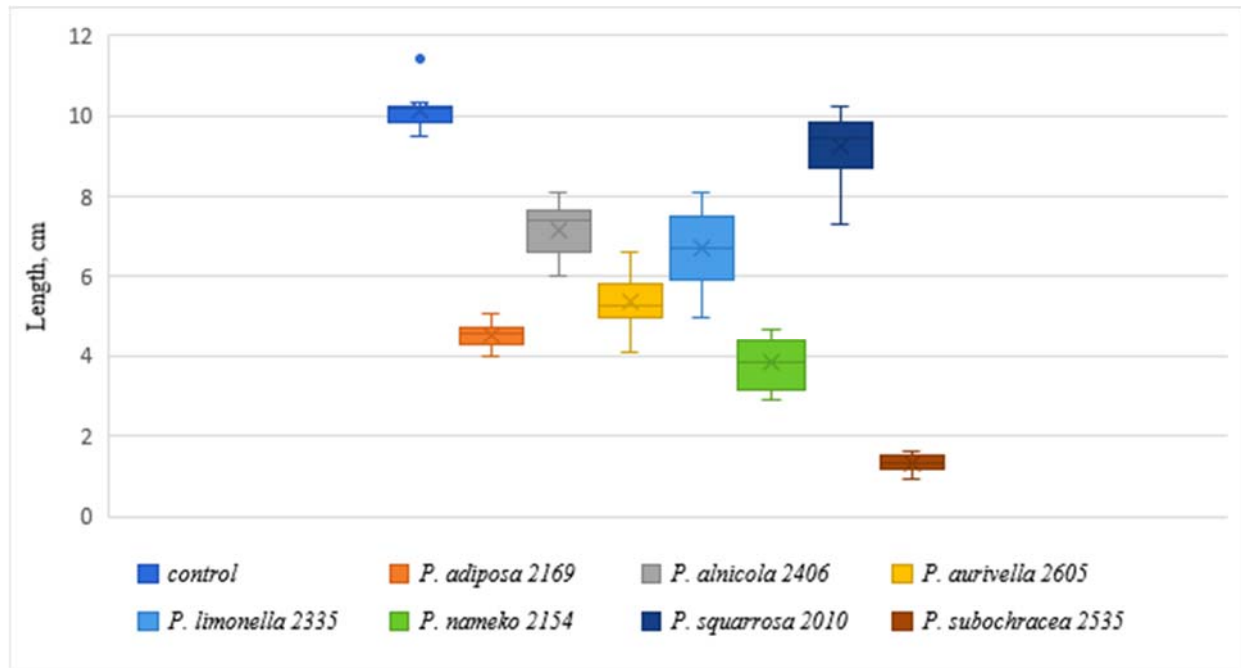


Fig. 1. Influence of *Pholiota* species biomass on *C. sativus* sprouts

As can be seen from Fig. 1 the allelopathic inhibition of *Pholiota* species biomass was recorded in all cases compared with control samples. The addition of *Pholiota* mycelia led to suppression of *C. sativus* sprouting growth.

P. subochracea, *P. nameko* and *P. adiposa* biomass have the most negative effect on seed growth, unlike *P. squarrosa*, which has almost no effect on growth (Fig. 2).



Fig. 2. *Cucumis sativus* sproutings (control) (A); with the addition of *Pholiota squarrosa* 2010 biomass (B) and *Pholiota subochracea* 2535 biomass (C); 3 days of germination, stroke length 1 cm

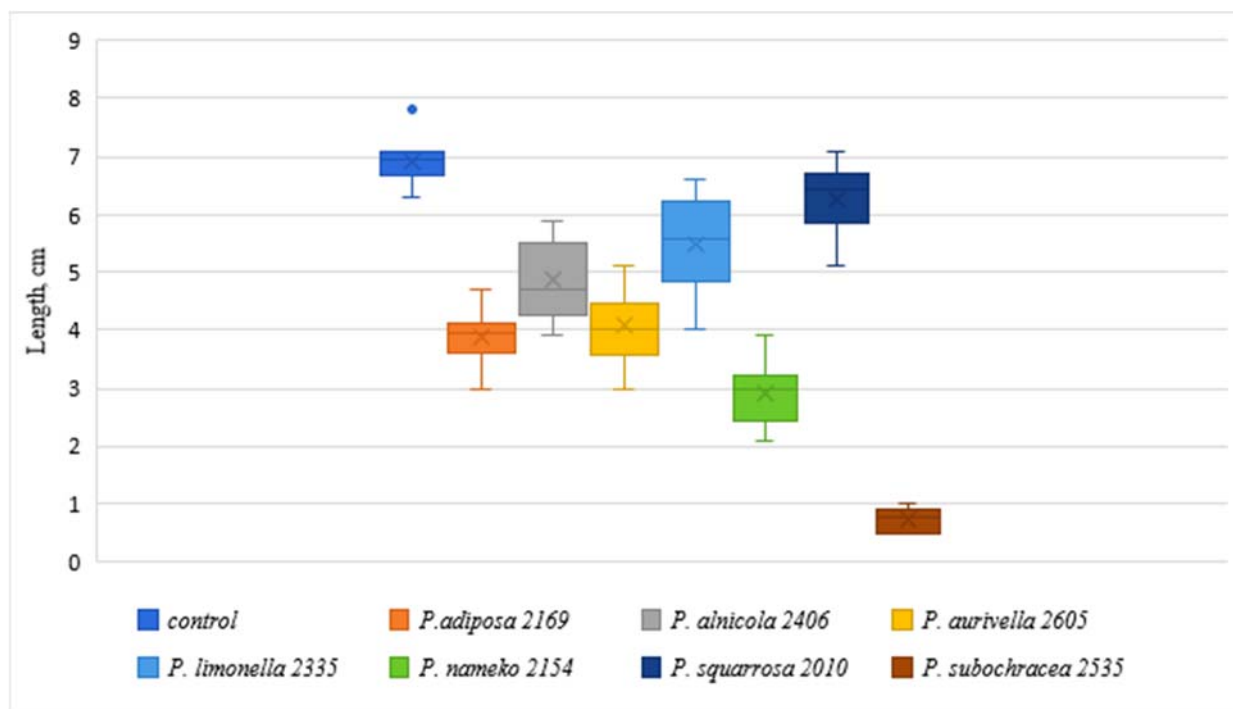


Fig. 3. Influence of *Pholiota* species biomass on the length of *C. sativus* roots

To examine the effect on the seed germination of *C. sativus* by *Pholiota* species a comparison was performed on root growth ratio (Fig. 3). Addition of *P. squarrosa* mycelia led to the least inhibition of *C. sativus* root growth, the average value of root length in this case decreased by 9,6% compared to the control group. Moreover, we did not record any morphological changes in the nature of pubescence or the number of lateral roots (Fig. 2A, 2B). In contrast to the previous case, exposure of *P. subochracea*

biomass on cucumber seeds, the seed germination ratio is only 10,4%. In addition, significant changes were recorded in the appearance of the sprouting – almost no pubescence and significant differences in the number and length of additional roots (Fig. 2C). For other *Pholiota* species, the roots growth ratio ranges from 42,1% (*P. nameko*) to 79,3% (*P. limonella*) (Fig. 3). Changes in pubescence were also recorded compared to control samples for such species as *P. adiposa*, *P. aurivella* and *P. limonella*.

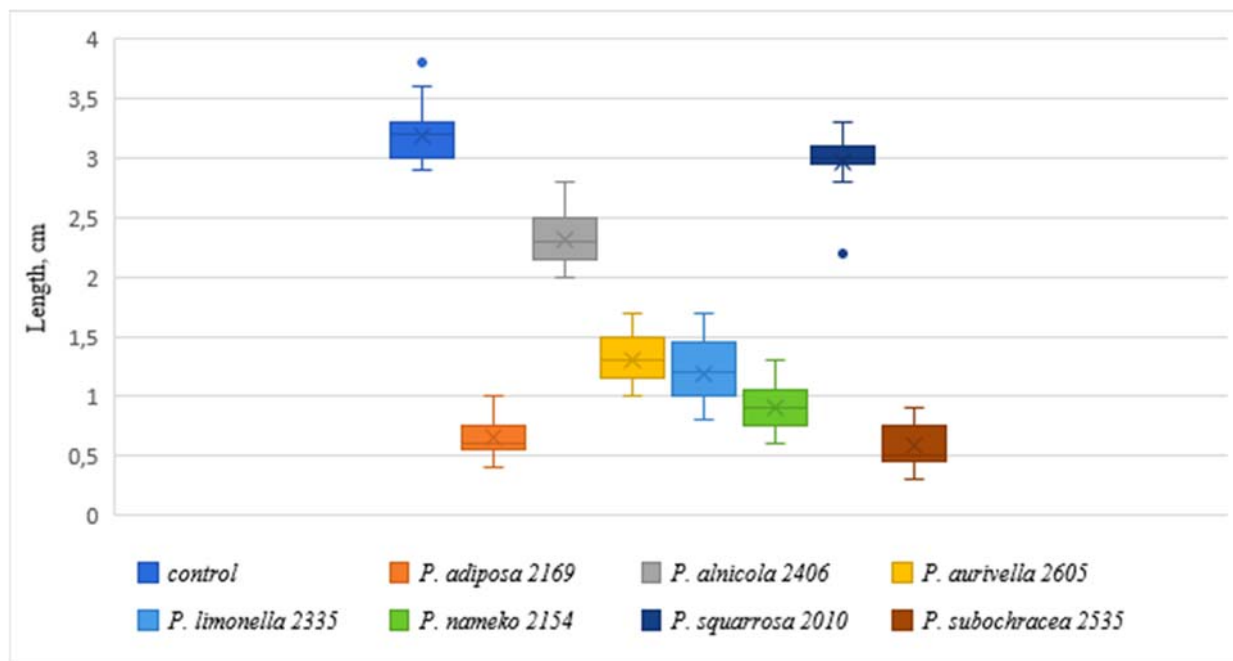


Fig. 4. Influence of *Pholiota* species biomass on the length of *C. sativus* shoots

As in the case of roots, the length of the shoot in the control experiment was greater than in experiments using *Pholiota* biomass, which indicates the inhibitory effect of mushroom species (Fig. 4). Maximum and minimum

shoot growth ratio as in the previous case were observed for *P. squarrosa* and *P. subochracea* and were 93,1% and 18,2%, respectively. There are no changes in case of the

shoot morphology in comparison with control samples were observed.

Lepidium sativum

The total percentage of *L. sativum* seed germination with the addition of *Pholiota* biomass was 100% compared to the

control group. Change in the length of the lettuce sproutings roots and shoots due to the effects of *Pholiota* species biomass shown in Fig. 5–8.

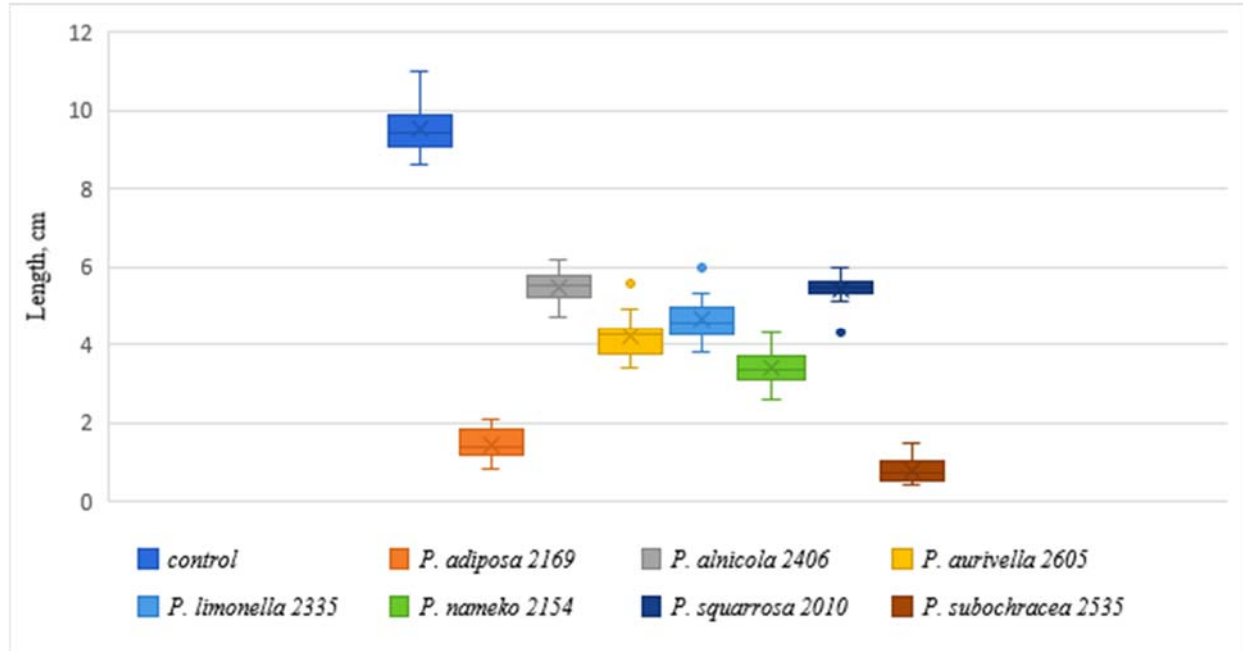


Fig. 5. Influence of *Pholiota* species biomass on *L. sativum* sprouts

The biomass of all species showed a significant allelopathic effect on the growth of *L. sativum* both roots and shoots, and consequently the plant as a whole (Fig. 5). The strongest inhibitory effect (91,8% and 84,85%) on sprout

growth was exerted by the biomass of a number of such species as *P. subochracea* and *P. adiposa* respectively (Fig. 6), while other showed decrease lettuce growth ratio by 42,8%-64,4% compared to the control group.



Fig. 6. *Lepidium sativum* sproutings (control) (A); with the addition *Pholiota adiposa* 2169 biomass (B) and *Pholiota subochracea* 2535 biomass (C); 3 days of germination, stroke length 1 cm

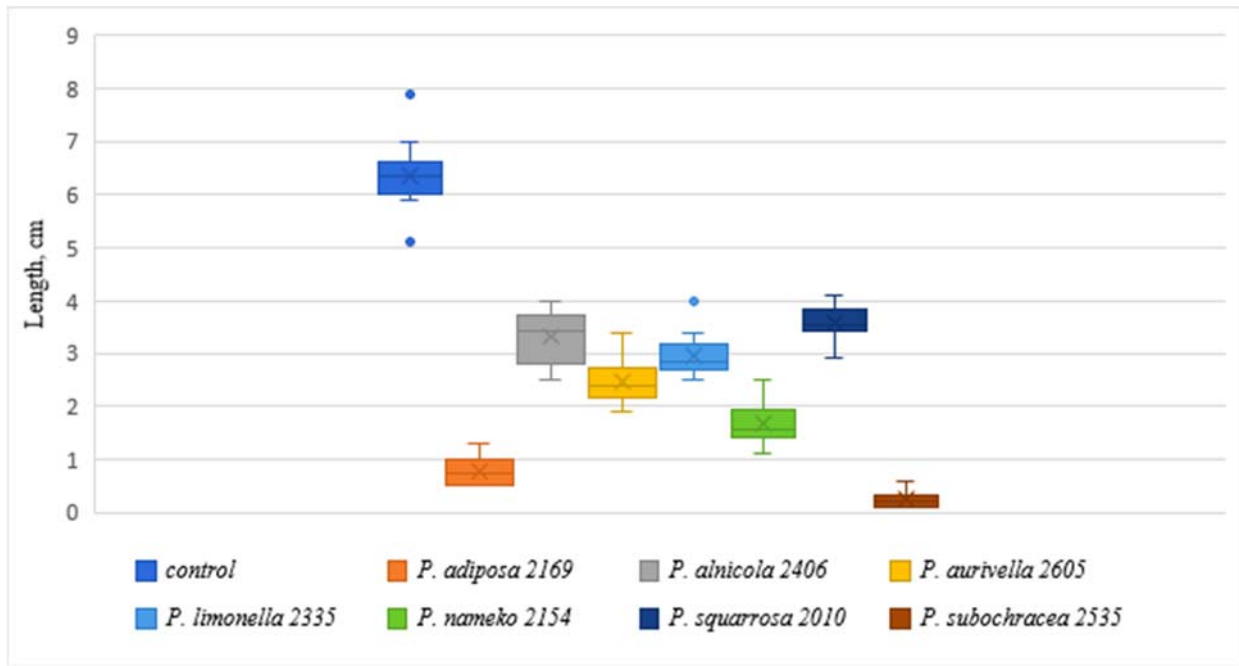


Fig. 7. Influence of biomass of *Pholiota* species on the length of *L. sativum* roots

Data in the diagram shown in Fig. 7 shows that in the case of adding *Pholiota* species biomass to lettuce seeds, roots growth is inhibited in all cases. The most noticeable allelopathic effect was exerted by two species – *P. adiposa* and *P. subochracea*, where the growth ratio were only 12,1% and 3,78%, in accordance. It is also worth noting the

reduction of root pubescence in all cases of *Pholiota* species biomass addition in comparison with control sproutings (Fig. 6). The highest roots growth ratio, and therefore the least depressant effect, was found for *P. alnicola* (52,1%) and *P. squarrosa* (56,4%) (Fig. 7).

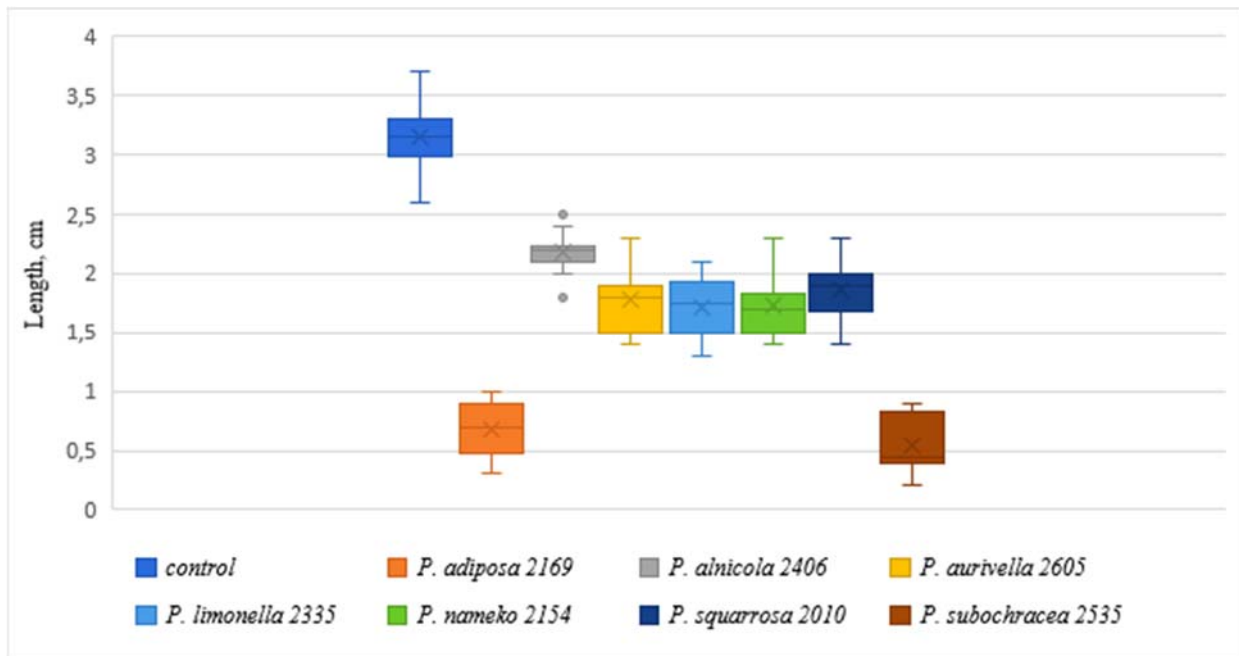


Fig. 8. Influence of *Pholiota* species biomass on the length of *L. sativum* shoots

Analyzing the data from Fig. 8, we can say that the greatest suppression of shoot growth was due to the addition of biomass of *Pholiota* species – *P. adiposa* and *P. subochracea*, where the growth ratio was 21,3% and 17,1%, in accordance. The least inhibitory effect was recorded for *P. alnicola*, where the shoots growth ratio was 69,2%. There are no changes in case of the morphology of

the shoot in comparison with control samples were observed (Fig. 6).

Discussions. Allelopathy is the chemical interaction between plants, including stimulatory as well as inhibitory influences [16]. In this study, we found that the adding *Pholiota* mycelial biomass has significant allelopathic effect

on the seed germination and growth of *Lepidium sativum* and *Cucumis sativus*.

Previous reports showed that mushroom fruiting bodies, including some of *Pholiota* species are potential for being allelopathic [17]. The strongest growth-inhibiting species belonged to *Mycenaceae*, *Cortinariaceae*, *Clavariaceae*, *Lyophyllaceae*, *Entolomataceae*, *Strophariaceae*, and *Tricholomataceae* of the phylum *Basidiomycota*. The fruiting bodies of two *Pholiota* species was taken to analyze the allelopathic effect – *P. spumosa* (Fr.) Singer and *P. terrestris* Overh. Moreover, *Pholiota spumosa* with some other species exhibited the highest levels of *Lactuca sativa* L. hypocotyl elongation inhibition – 25.1% [17]. In our work, the inhibitory activity of *Pholiota* species mycelia ranged from 87.1% (*P. subochracea*) to 8.6% (*P. squarrosa*) in the case of cucumber and 91.8% (*P. subochracea*) – 42.2% (*P. alnicola*) in case of lettuce seeds. In addition to the fact that we used other *Pholiota* species, the higher effect of allelopathic inhibition can be explained by the higher mushroom biomass samples than in research by Osivand et al. [17].

Ridwan with colleagues determined the structure of isolated compounds from *P. lubrica*, and then all compounds were subjected to the plant regulatory assay against the lettuce [21]. Comparing the existing data it is possible to draw a common conclusion about the inhibitory properties of *Pholiota* genus.

Brassicaceae have been used as model plant family to study the effects on induction of plant responses including defence traits, as they are characterized by the presence of a rather specific inducible defense system [24, 26]. Therefore, analyzing obtained data, we can conclude about the essential allelopathic effect of fungi of *Pholiota* genus on both – the traditionally model family *Brassicaceae* (*Lepidium sativum*) and the representative of other family like *Cucurbitaceae* (*Cucumis sativus*).

Besides the apparent inhibitory effect on sproutings, changes in root morphology depending on the *Pholiota* species were recorded. For sproutings of *L. sativum* and *C. sativus* there were changes in pubescence, compared with control samples, for cucumber were also noted changes in the size and number of lateral roots.

Conclusions. Influence of *Pholiota* species extracts on seed germination and the sprouting growth performance of *Lepidium sativum* and *Cucumis sativus* were investigated. Inhibitory allelopathic effect of mycelial biomass was observed for all parts of the sprouting – both root and shoot.

Significant allelopathic properties were found for *P. adiposa*, *P. nameko* and *P. subochracea* on *C. sativus* sprouts and for *P. adiposa*, *P. subochracea* on *L. sativum* sprouts, where inhibitory effect on both seedling sprouting species was more than 50%. It should be noted almost no inhibitory effect on *Cucumis sativus* with addition *P. squarrosa* mycelium (growth ratio was 91,39%). Should be specified, that there were cases when the inhibitory effect did not exceed 10% – with the addition of *Pholiota* biomass during germination of cucumber seeds, while for lettuce, this this figure ranges from 40 to 45%.

The results of this work show that the studied *Pholiota* species mycelia contains allelochemical compounds, that might be the potential candidates for future investigations for the development of herbicides based on its secondary metabolites and their allelopathic inhibitory effects.

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ВПЛИВ МІЦЕЛІЮ *PHOLIOTA* SPP. (*STROPHARIACEAE*, *BASIDIOMYCOTA*) НА СХОЖІСТЬ НАСІННЯ Й РІСТ *LEPIDIUM SATIVUM* L. ТА *CUCUMIS SATIVUS* L.

Basidiomycetes є вельми розмаїтою групою еукаріотичних організмів за фізіологічними параметрами. Деякі організми, такі як рослини або гриби, здатні виділяти певні вторинні метаболіти, які можуть впливати на оточуючі організми. Нині кількість досліджень грибів та їхніх біологічно активних компонентів значно збільшилася у зв'язку з потенційним упровадженням у різні біотехнологічні процеси. Деякі речовини, що виділяються грибами, можуть впливати на ріст і подальший розвиток рослин, що є поруч. У цьому дослідженні вивчено алопатичну дію представників роду *Pholiota*. Біомаса грибів семи досліджуваних видів роду *Pholiota* обрана для вивчення впливу на проростання насіння озіркіє (*Cucumis sativus* L.) та салату (*Lepidium sativum* L.). Результати нашого експерименту показали, що біомаса видів роду *Pholiota* не впливала на схожість насіння обох видів рослин. Стовідсоткову схожість насіння фіксували як у контрольних, так і в експериментальних зразках. Додавання вегетативного міцелію видів *Pholiota* spp. призвело до пригнічуючого алопатичного ефекту, який впливає і на проростання насіння, і на довжину досліджуваної рослини (пагонів і коріння), а також змінює морфологію коренів (опушення, зміни бічних коренів). Інгибіуючий ефект на довжину проростків становив 8,6%–87,1% у випадку *C. sativus* та 42,2%–91,8% для *L. sativum* залежно від видів роду *Pholiota*, що використовувалися. Слід відмітити алопатичні властивості *Pholiota subochracea*, де коефіцієнт росту проростків не перевищує 12,9% порівняно з контрольною групою. Цей результат свідчить про те, що представники роду *Pholiota* мають значний регулятивний вплив на ріст салату й озіркіє. Наведені результати дають можливість припустити, що досліджені види можуть відігравати значну роль у взаємовідносинах усередині екосистем.

Ключові слова: алопатія грибів, *Pholiota*, сандвич-метод біодосліджень.

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ВЛИЯНИЕ МИЦЕЛИЯ *PHOLIOTA* SPP. (*STROPHARIACEAE*, *BASIDIOMYCOTA*) НА ВСХОЖЕСТЬ СЕМЯН И РОСТ *LEPIDIUM SATIVUM* L. И *CUCUMIS SATIVUS* L.

Basidiomycetes представляют весьма разнообразную группу эукариотических организмов по физиологическим параметрам. Некоторые организмы, такие как растения или грибы, способны выделять определенные вторичные метаболиты, которые могут влиять на окружающие организмы. Количество исследований грибов и их биологически активных компонентов значительно выросло в связи с возможностью потенциального внедрения в различные биотехнологические процессы. Некоторые вещества, выделяемые грибами, могут влиять на рост и дальнейшее развитие растений, находящихся рядом. В этом исследовании изучено аллопатическое действие представителей рода *Pholiota*. Биомасса грибов семи исследуемых видов рода *Pholiota* была выбрана для изучения влияния на прорастание семян огурцов (*Cucumis sativus* L.) и салата (*Lepidium sativum* L.). Результаты нашего эксперимента показали, что биомасса видов рода *Pholiota* не влияла на всхожесть семян обоих видов растений. Стопроцентную всхожесть семян фиксировали как в контрольных, так и в экспериментальных образцах. Добавление вегетативного мицелия видов *Pholiota* spp. привело к подавляющему аллопатическому эффекту, который влиял и на прорастание семян, и на длину исследуемого растения (побегов и корней), а также менял морфологию корней (опушение, изменения боковых корней). Ингибирующий эффект на длину проростков составлял 8,6%–87,1% в случае *C. sativus* и 42,2%–91,8% для *L. sativum* в зависимости от используемых видов рода *Pholiota*. Следует отметить аллопатические свойства *Pholiota subochracea*, где коэффициент роста проростков не превышал 12,9% по сравнению с контрольной группой. Этот результат свидетельствует о том, что представители рода *Pholiota* имеют значительное регулятивное влияние на рост салата и огурцов. Приведенные результаты позволяют предположить, что исследованным видам может принадлежать значительная роль во взаимоотношениях внутри экосистем.

Ключевые слова: аллопатия грибов, *Pholiota*, сандвич-метод биодоследований.