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Allelopathic influence of *Pholiota* (*Strophariaceae*, *Basidiomycota*) spp mycelial biomass on seed germination and seedlings growth of *Lepidium sativum* L. and *Cucumis sativus* L.

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ABSTRACT

We studied the allelopathic effects of mushroom *Pholiota* (Fr.) P. Kumm. species on seed germination and seedlings growth of *Cucumis sativus* L. and *Lepidium sativum* L. The mycelium of *Pholiota* species suppressed the seed germination, shoot and root length of test plants and also changed the morphology of roots (pubescence, changes in lateral roots). The inhibition in sprout length was 8.6 %-87. 1 % in *C. sativus* and 42.2 %-91.8 % in *L. sativum* depending on *Pholiota* species. Allelopathic effects of *Pholiota subochracea* (A.H.Sm.) Hesler, drastically decreased the germination to 12.9 % than control.

Keywords: Allelopathy, *Cucumis sativus* L., *Lepidium sativum* L., Mushroom, Mycelial biomass**,** *Pholiota*, sandwich method bioassay

INTRODUCTION

Allelopathy includes all types of chemical interactions among plants and microorganisms. Allelochemicals affects the vegetation, seed germination and the sprout growth, plant defence, nutrients chelation, nutrients uptake of target plants and regulate soil biota to affect the decomposition and soil fertility (4,6,15,18).

Weeds are about 1 % of total plant species on earth, nevertheless they cause great interferance in food production (16). Till now, 255 weeds have become resistant to 163 herbicides (7). That is why the weed control issues require new ways for weed management. The use of natural products as natural herbicides is a promising direction because of their greater safety for humans and environment (11). The phenomenon of allelopathy has been much studied in plant interactions (5,6,10), than on other organisms (12,14,17).

Data on allelopathic interactions of fungi have recently emerged, nonetheless, there are still many wild mushrooms, whose allelopathic activity has not been studied, including *Pholiota* species (14,17). But there are no data about allelopathic influence of cultured mushroom mycelia on plants. Besides, mushrooms are an excellent source of nutrients including macronutrients and bioactive compounds (9,21,19), however, there is still insufficient information on their allelopathic effects on other organisms.

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The *Pholiota* (Fr.) P. Kumm, genus (*P. nameko* (T.Ito) and *P. adiposa* (Batsch) specie fruits are used to obtain biologically active substances with medicinal properties. We selected the lettuce (*Lepidium sativum* L.) and cucumber (*Cucumis sativus* L.) as these are commonly used as test plants in Allelopathy Research. This study aimed to determine the allelopathic Potential of *Pholiota* genus on seed germination and seedlings growth of Cucumber and lettuce.

MATERIALS AND METHODS

In spring 2023, effects of 7-strains of *Pholiota* species (Table-1) from the *IBK* Mushroom Culture Collection, M.G Kholodny Institute of Botany, National Academy of Sciences, Ukraine (41°24'12.2"N 2°10'26.5"E) were investigated (2) on seeds germination and seedlings growth of model plants *Lepidium sativum* L. and *Cucumis sativus* L.

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

Species	*IBK* strain
Pholiota adiposa (Batsch) P.Kumm.	2169
Pholiota alnicola (Fr.) Singer (syn. *Flammula alnicola* (Fr.) P. Kumm.)	2406
Pholiota aurivella (Batsch) P.Kumm.	2605
Pholiota limonella (Peck) Sacc.	2335
Pholiota nameko (T.Ito) S.Ito and S.Imai	2154
Pholiota squarrosa (Oeder) P.Kumm.	2010
Pholiota subochracea (A.H.Sm.) A.H.Sm. and 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; $MgSO_4 \times 7\ H_2O$ – 0.25). Strains grown on GPY medium with agar-agar (20 g/L) were used as inoculum. Mycelial discs with 5 mm dia 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 with 200 ml GPY media. The mycelia were incubated at 26±0. 1 ºC for 21 days. The biomass was washed with distilled water and dried at 60 °C, which was subsequently used.

To determine the effects of mycelial biomass, we used modified sandwich bioassay method, described by Osivand *et al*. (14). Dry biomass (0.1 g) was ground to powder and was sterilized under UV radiation for 2 h. Agar medium without nutrients and trace elements (SA) was used as a substrate. Mycelial biomass layer was applied in Petri dishes (d=85 mm), it was covered with 8 ml of SA and left to solidify completely, then another 8 ml medium was added on top and again left to solidify. Then 10 seeds of *L. sativum* or *C. sativus* were sown on the surface of the agar medium at equal distances. Petri dishes with SA but without mycelium biomass were used as control. Each experiment was done in triplicate. Studies were done at 26 ±0.1 ºC.

On 3rd day, the number of germinated seeds was recorded and the radicle and hypocotyl of the plants were measured. Data was collected for following parameters: Root length, its pubescence, development of lateral roots, shoot length, total length of plant.

The radicle and hypocotyl growth ratio (%) of lettuce and cucumber seedlings was calculated for each sample compared with control by following formula:

Growth Ratio (%) = 100 × (Mean sample length/Mean control length).

The treatments were replicated thrice in complete randomised design.

Statistical Analysis: Microsoft Exel (Microsoft Corp., Redmond, WA, USA) software, were used for statistical processing of data.

RESULTS

Allelopathic interactions caused growth inhibition in both test species *L. sativum* and *C. sativus* (Fig. 1-8). A comparison was done to study the increase or decrease in growth ratio, root and shoot length for both species.

CUCUMIS SATIVUS

The *C. sativus* seed germination (%) with addition of *Pholiota* biomass was 100 % compared to control group. The application of *Pholiota* biomass inhibited the growth of both roots and shoots of cucumber seedlings (Fig. 1-4).

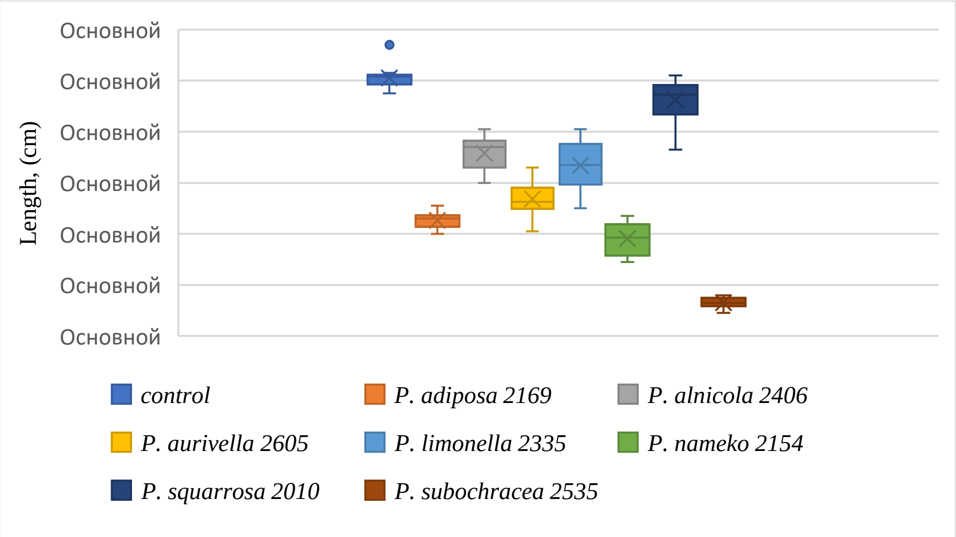

Figure 1. Influence of *Pholiota* species biomass on *C. sativus* sprouts

The application of *Pholiota* species biomass caused allelopathic inhibition in all cases compared with control samples (Fig. 1). The addition of *Pholiota* mycelia suppressed *C. sativus* sprout growth. *P. subochracea* biomass had the most negative effects on seedling growth, unlike *P. squarrosa*, which has no effect on growth (Fig. 2).

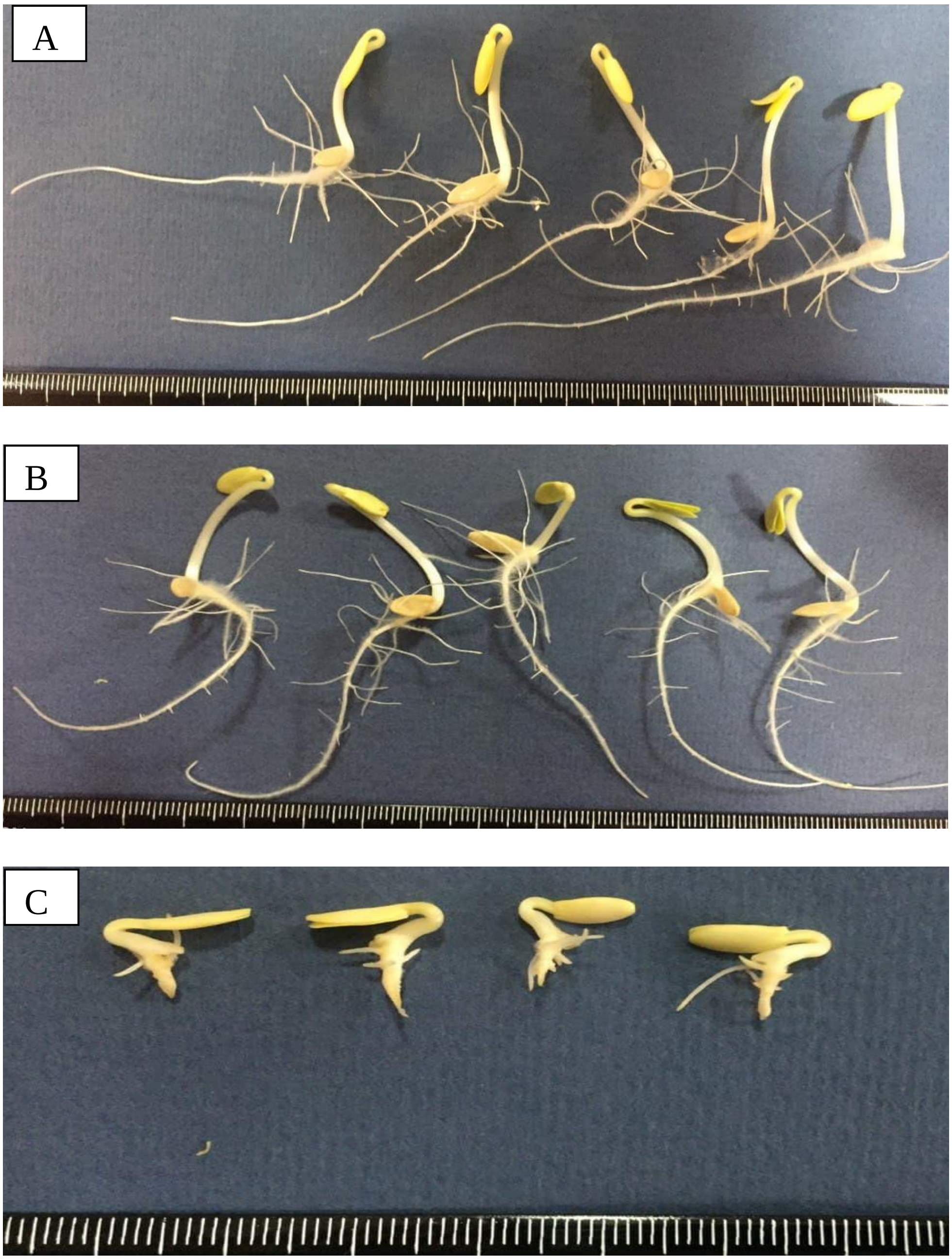

Figure 2. *Cucumis sativus* sprouts (A). Control, (B). with biomass of *Pholiota squarrosa* 2010, (C). with biomass of *Pholiota subochracea* 2535. Germination after 3 days.

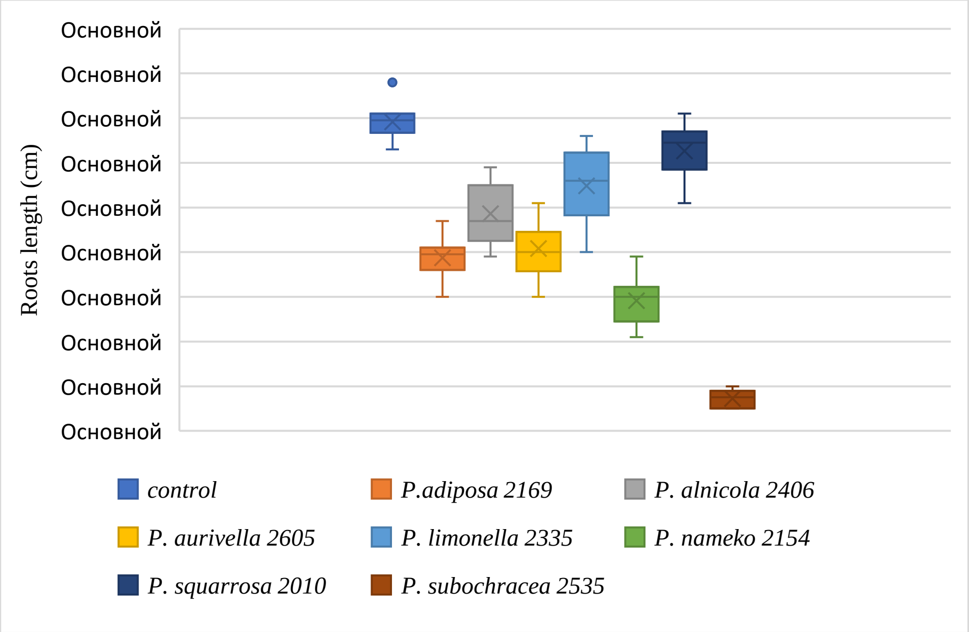

Figure 3. Influence of *Pholiota* species biomass on the roots length of *C. sativus*

To examine the effects on the seed germination of *C. sativus* by *Pholiota* species a comparison was done on root growth ratio (Fig. 3). Addition of *P.squarrosa* mycelia caused least inhibition in *C. sativus* root growth, the root length was decreased by 9.6 % than control. Moreover, there were no 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 was only 10.4 %. In addition, significant changes were recorded in the appearance of the sprouts-almost no pubescence and there were 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 over control samples for species such as *P. adiposa*, *P. aurivella* and *P. limonella*.

Figure 4. Influence of *Pholiota* species biomass on the shoot length of *C. sativus*

Similar to roots, the shoot length in control was greater than with addition of *Pholiota* biomass, which showed the inhibitory effects of mushroom species (Fig. 4). Maximum and minimum shoot growth ratio with *P. squarrosa* and *P. subochracea* were 93.1 % and 18.2 %, respectively. There were no changes in shoot morphology than control samples.

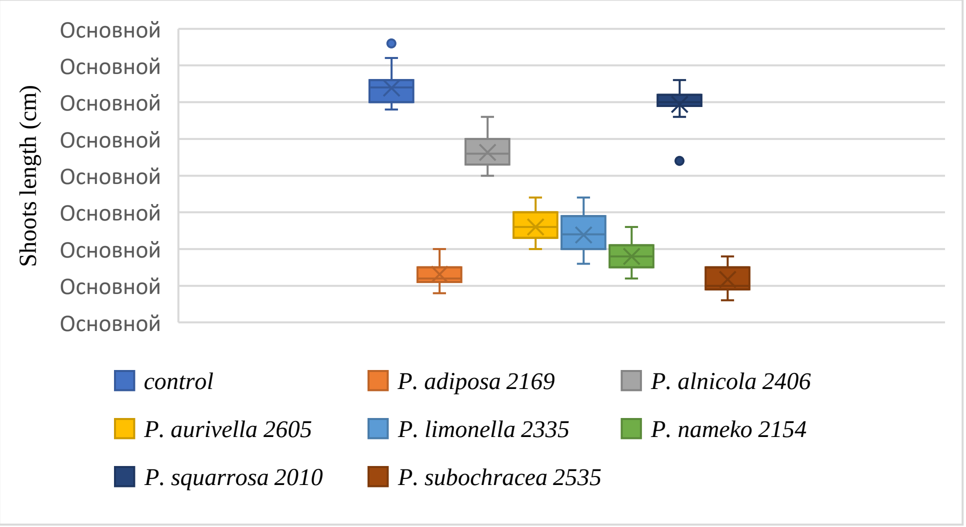

Figure 4. Influence of *Pholiota* species biomass on the shoots length of *C. sativus*

LEPIDIUM SATIVUM

The total seed germination (%) of *L. sativum* with addition of *Pholiota* biomass was 100 % compared to control. *Pholiota* species biomass changed the length of lettuce sprout roots and shoots (Fig. 5-8).

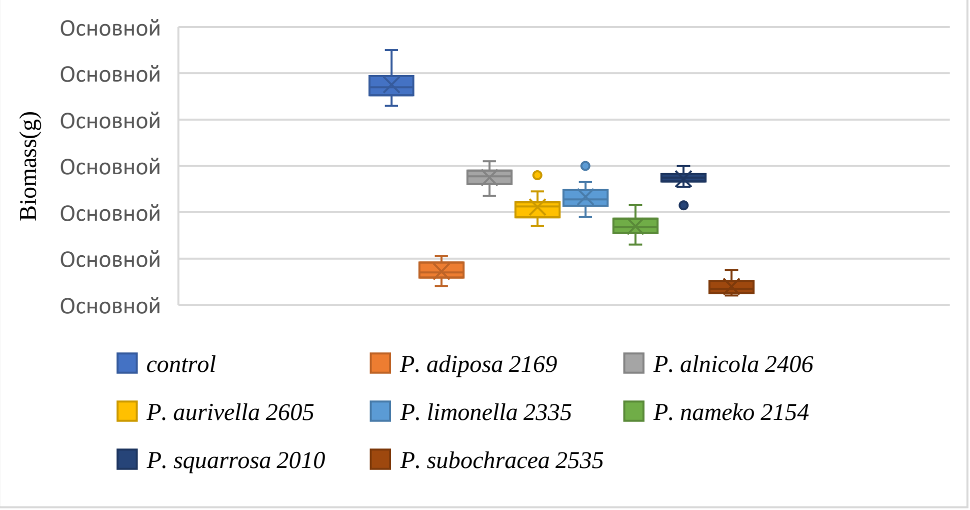

Figure 5. Influence of *Pholiota* species on biomass of *L. sativum* sprouts

The biomass of all species showed significant allelopathic effects on the growth of *L. sativum* both roots and shoots, and consequently the plant as a whole (Fig. 5). The strongest inhibitory effects (91.8 % and 84.85 %) on sprout growth were exerted by the biomass of *P. subochracea* and *P. adiposa* respectively (Fig. 6), while, other spp decreased lettuce growth ratio by 42.8 %-64.4 % compared to control.

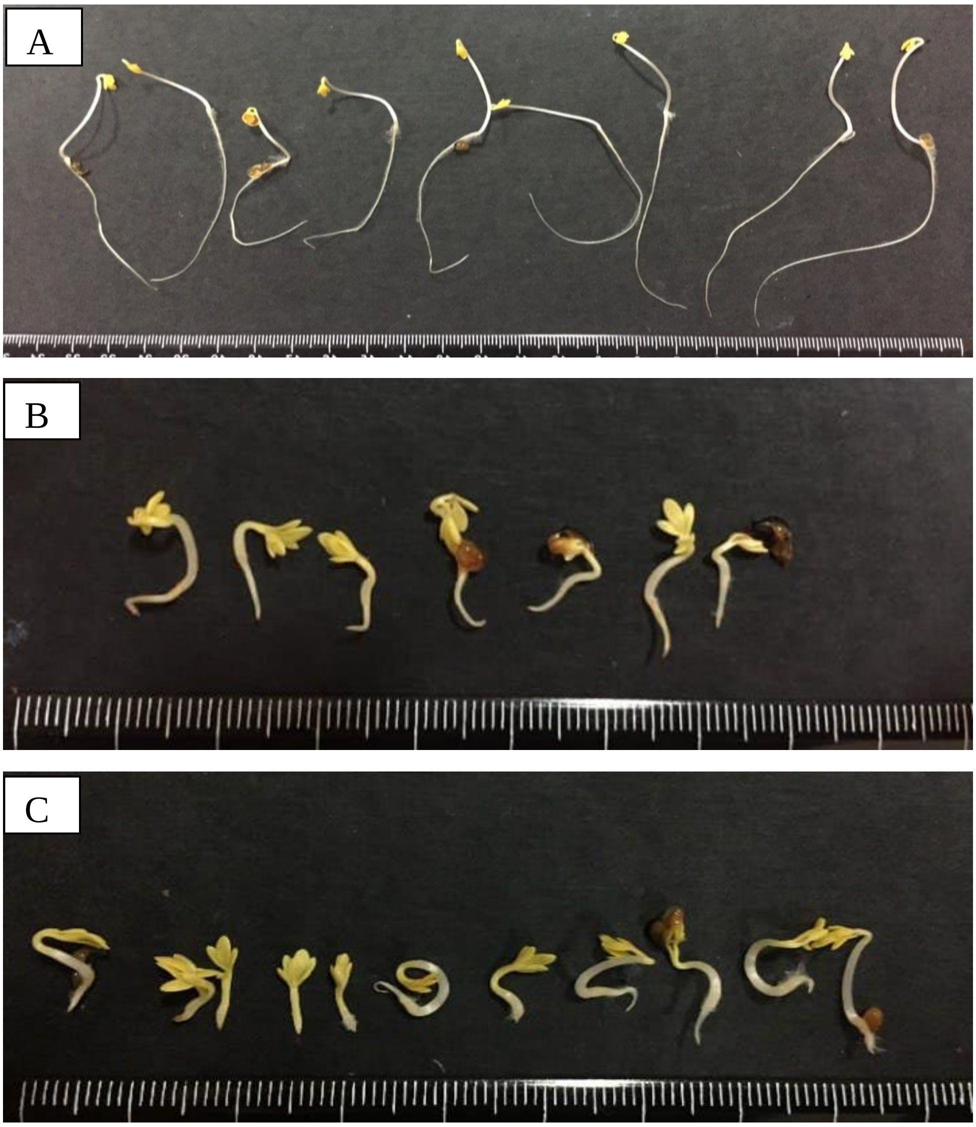

Figure 6. *Lepidium sativum* sproutings (control) (A); with biomass of *Pholiota adiposa* 2169 (B) biomass of *Pholiota subochracea* 2535 (C). Germination after 3 days.

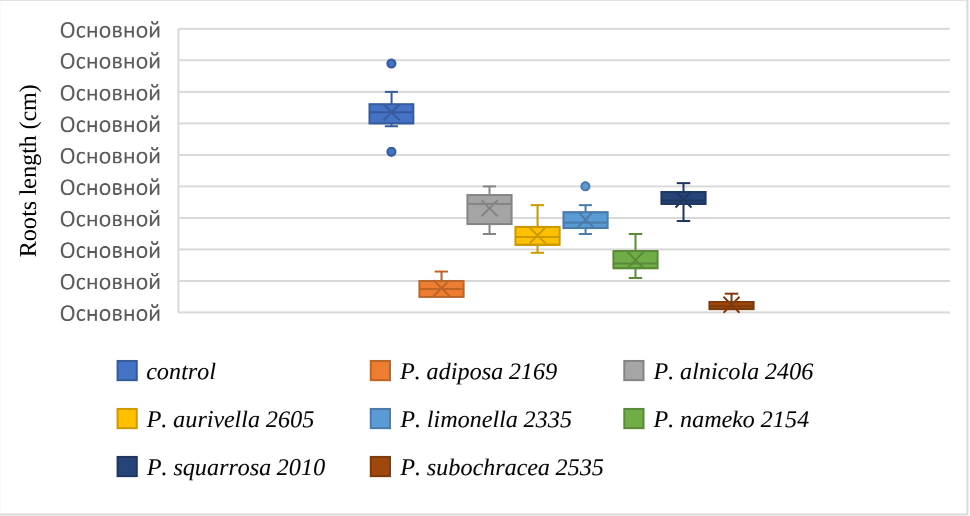

Figure 7. Influence of biomass of *Pholiota* species on the roots length of *L. sativum*

Addition of *Pholiota* species biomass to lettuce seeds, inhibited roots growth in all cases (Fig. 7). 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 % respectively. It is also worth noting that the reduction of root pubescence in all cases of *Pholiota* species biomass addition in comparison with control sprouts (Fig. 6). The highest roots growth ratio (i.e. least inhibitory effect) was found for biomass of *P. alnicola* (52.1 %) and *P. squarrosa* (56.4 %) (Fig. 7).

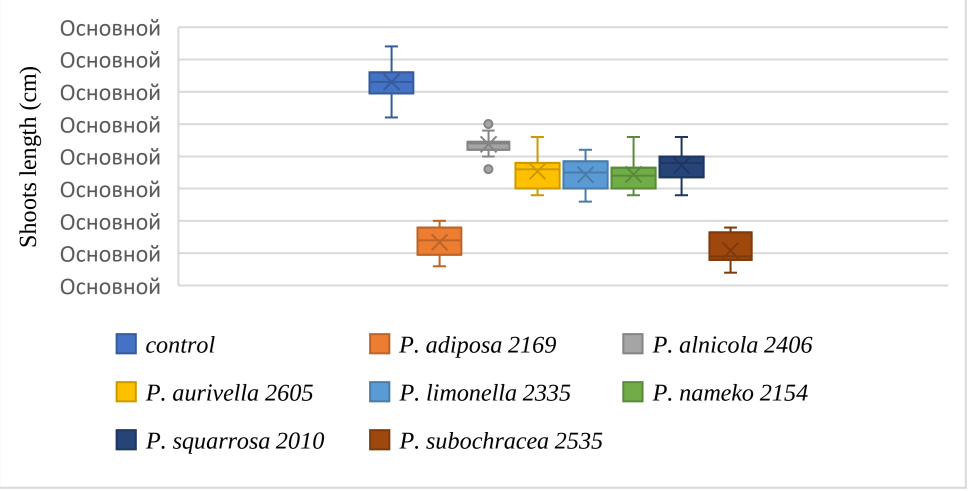

Figure 8. Influence of *Pholiota* species biomass on the shoots length of *L. sativum*

Analyzing the data from Fig. 8, 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 % respectively. The least inhibitory effect was recorded for *P. alnicola,* where the shoots growth ratio was 69.2 %. There were no changes in morphology of the shoot in comparison with control (Fig. 6).

DISCUSSION

Allelopathy is the chemical interaction between plants, including stimulatory as well as inhibitory influences [13]. In this study, we found that the addition *Pholiota* biomass had significant allelopathic effects on the seed germination and seedlings growth of *Lepidium sativum* and *Cucumis sativus*.

Previous reports showed that mushroom fruiting bodies, including some of *Pholiota* species have allelopathic potential. The strongest growth-inhibiting species belonged to *Mycenaceae, Cortinariaceae, Clavariaceae, Lyophyllaceae, Entolomataceae, Strophariaceae* and *Tricholomataceae* families of the phylum *Basidiomycota*. The fruiting bodies of two *Pholiota* species (*P. spumosa* (Fr.) Singer and *P. terrestris)* were used to analyze their allelopathic effects. Moreover, *Pholiota spumosa* with some other species exhibited the highest inhibition 25.1 % of hypocotyl elongation (14). In our work, the inhibitory activity of *Pholiota* species mycelia ranged from 87.1 % (*P. subochracea*) to 8.6 % (*P. squarrosa*) in cucumber and 91.8 % (*P. subochracea*) and 42.2 % (*P. alnicola*) in lettuce seeds. In addition to the fact that we used other *Pholiota* species, the higher effects of allelopathic inhibition can be explained by the higher mushroom biomass samples than in previous research (14).

Ridwan *et al.* (17) determined the structure of isolated compounds from *P. lubrica*, and then all compounds were bioassayed against the lettuce. Comparing our data, we 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 characterised by the presence of a rather specific inducible defence system (20,22). Therefore, analyzing obtained data, we concluded about the essential allelopathic effect of fungi of *Pholiota* genus on both the traditional model family *Brassicaceae* (*Lepidium sativum*) and the representative of other family *Cucurbitaceae* (*Cucumis sativus*).

Besides there were apparent inhibitory effects on sprouts, changes in root morphology depending on the *Pholiota* species. In sprouts of *L. sativum* and *C. sativus,* there were changes in pubescence, compared with control samples in cucumber, there were also 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 effects of mycelia biomass were observed on both root and shoot seedlings of both test plants. Significant allelopathic properties were found for 3-*Pholiota* species (*P. adiposa*, *P. nameko* and *P. subochracea*), which suppressed the seedlings species more than 50 %. It should be noted, 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 showed that the studied *Pholiota* species mycelia contained 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.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

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