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The influence of biomass and species extracts of *Ganoderma* P. Karst on the seeds germination and the growth of *Lepidium sativum* L.

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Abstract

To determine the allelopathic effects of mycelial biomass of investigated strains was used a modified sandwich method: between two layers of agar medium was added 0.1 g of dry crushed biomass, followed by the introduction of seeds on the top layer. To determine the effect of methanol and ethyl acetate extracts, extract - soaked filter paper discs in sterile petri dishes were used, to which 2 ml of sterile water was added after evaporation of the solvents, then seeds were added. Aqueous extracts were adjusted to 2 ml with sterile water and impregnated the filter paper discs, after which the seeds were added. Biomass of all investigated strains shows allelopathic influence: the strongest - mycelium *G. tsugae* Murrill 2024 - inhibits plants growth by 80.9%, the weakest - *G. carnosum* Pat. 2502 - inhibits plants growth by 30.7% compared to control. Addition of 20 µl of *G. sinense* J.D. Zhao, L.W. Hsu & X.Q. Zhang 2516 aqueous extract stimulates shoot growth compared to the control by 19.3%. Ethyl acetate extracts of *G. tsugae* 2024 showed the highest allelopathic influence, which increased with increasing.

Key words: Ganoderma, Ganoderma extracts, mushroom allelopathy, plant growth regulation, sandwich method.,

INTRODUCTION

The species of the genus *Ganoderma* P. Karst are wood-destroying fungi which are responsible for causing white-rot of numerous tree species [8]. These fungi have long been known for their positive influence on human organism and have been used in traditional eastern medicine for centuries. The most distinguished representetive of this genus is *Ganoderma lucidum* (Curtis) P. Karst., [5]. The variety of grate number of recent scientific studies has reported that the fruit bodies, spores and mycelia of fungi of this genus contain a different biologically active compounds, including: polysaccharides, triterpenoids, proteins, aminoacids, cytokinins, etc [1, 3, 7, 14, 16]. As known, cytokinins stimulate plant cell division and may affect their growth, however there is lack of studies to confirm or refute the effect of biomass or extracts derived from *Ganoderma* fungi on the growth of higher plants [13]. In nature, species of this genus secrete enzymes, including laccase, manganese peroxidase, lignin peroxidase etc [11] and thus have a direct influence on trees. Such properties make *Ganoderma* fungi the interesting objects for determining their influence on the growth of higher plants.

It has been reported that aqueous extracts of various basidiomycetes can both inhibit and stimulate the growth and the rate of seeds germination of *Pinus banksiana* Lamb., and other plants and lichens [6]. It was also established that some mycorrhizal fungi are capable of secreting substances that show allelopathic effect on neighboring non-symbiotic plants [15]. Osivand *et al.* conducted a large-scale experimental work to determine the allelopathic effects of fruit bodies obtained from 289 different wild species of mushrooms; but the properties of *Ganoderma sp*. were not investigated in this study [9]. *Lepidium sativum* L. is important model object in botanical researches, also it`s a popular edible plant, at all over the world. So, we used it for our experiment to show the influence of *Ganoderma* P. Karst species mycelium biomass and its extracts on seeds germination and plant growth of *L. sativum.* It was first investigation of allelopathic activity of different Ganoderma species, and it`s make a new perspectives to use these fungi against invasive species plants or a plants that harm agriculture.

MATERIALS AND METHOD

Mycelia of 9 strains, 6 species of *Ganoderma* P. Karst. fungi from The IBK Mushroom Culture Collection of M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine [4] (Table 1) and standardized seeds of the model plant *L. sativum* L. were used in this research.

Species	Strain	Strain origin
Ganoderma applanatum (Pers.) Pat	1899	Isolated from the fruit body, Ukraine, Crimea, 2006.
Ganoderma carnosum Pat.	2502	Obtained from "Mycoforest type culture collection", Slovakia, 2016.
G. lucidum P. Karst.	1904	Isolated from the fruit body, Ukraine, Crimea, 2006.
Ganoderma resinaceum Boud.	2477	Isolated from the fruit body, Ukraine, 2016.
	2503	Isolated from "Mycoforest type culture collection", Slovakia, 2016.
Ganoderma sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang	2516	Obtained from "Mycoforest type culture collection", Slovakia, 2016.
Ganoderma tsugae Murrill	1848	Obtained from "HAI", Haifa, Israel, 2005
	2024	Obtained from Tavria State Agrotechnological University collection, 2014 .
	2566	Obtained from "Mycoforest type culture collection", Slovakia, 2016 .

Table 1. List of investigated strains and species of the genus *Ganoderma*

Mycelia were grown using submerged liquid substrate method of fungal cultivation. Peptone Yeast Extract Glucose Broth (PYG) used for cultivation consisted of glucose – 25 g/L; peptone – 3 g/L; yeast extract – 3 g/L; MgSO4 – 0,25 g/L; KH2PO4 – 1 g/L; K2HPO4 – 1 g/L; pH – 6,0. The culture conditions were maintained at temperature - 26 ±1°C; agitation speed - 120 rpm; using 500 ml Erlenmeyer flasks with 100 ml of media for 14 days. Obtained biomass was dried at 60 °C and used in further experiments in native state on

seeds grows, or for extract preparation

Extract preparation:

1) Methanol extract was obtained by extracting 2 g of biomass in 6 ml of absolute methanol (HANEYWELL, CHROMASOLVTM Gradient for HPLC, gradient grade, ≤99.9%) for 7 days at 4°C, procedure was repeated twice.

2) To obtain the ethyl acetate extract 2 g of biomass was extracted in 6 ml of ethyl acetate (HANEYWELL, CHROMASOLVTM Gradient for GS, gradient grade, ≤99.92%) for 7 days at 4°C, procedure was repeated twice.

3) The aqueous extract was obtained as a fraction during the isolation of ganoderic acids by the classical method [12]: 0.1 g portion of mycelium was extracted with 70% methanol at 4°C temperature twice a week; following that the resulting solution was evaporated on a rotavap to obtain a dry substance, which later was washed off by 5 ml of hot water. Afterwards the resulting substance was mixed with 5 ml of chloroform for further extraction of ganoderic acids, and the aqueous fraction was used in this experiment.

Determination of the effects of mycelial biomass on seeds:

To determine the effect of mycelial biomass we used the modified technique described by Osivand et al [9]. A portion of dry biomass (0.1 g) was ground to a powder form. To obtain sterility, the mycelium underwent UV radiation for 2 hours. For a substrate agar medium lacking nutrients and microelements (non-nutrient agar) was used. Mycelial biomass was applied in a uniform layer in Petri dish Ø 85 mm, which was covered with 8 ml of non-nutrient agar afterwards and left until complete solidification; later another 8 ml of medium was added on top and left to solidify as well. In the following procedure 10 seeds of *L. sativum* were spread on the surface of the non-nutrient agar at equal distances.

Petri dishes with non-nutrient agar and no mycelial biomass added were used as controls.

The number of germinated seeds was recorded on the 3rd day and the stem and root length of the plant was measured.

Determination of the effects of *Ganoderma* mycelium extracts on seeds:

1) Methanol and ethyl acetate extracts of *Ganoderma tsugae* Murrill 2024 in volume of 20, 50 and 100 µl were adjusted to 1 ml with pure methanol / ethyl acetate and applied evenly to a disc of filter paper that completely covered the Petri dish. In 24 hours, after the solvent had completely evaporated, the paper was moistened with 2 ml of sterile water and 10 *L. sativum* seeds were added to a Petri dish. In the control sample, the corresponding solvent (1 ml), instead of extracts, was used to moisten the filter paper.

2) The aqueous fraction, obtained during the isolation of ganoderic acids from the mycelium of *G. tsugae* 2024 and *G. sinense* J.D. Zhao, L.W. Hsu & X.Q. Zhang 2516, in the volume of 0.02 to 0.5 ml was adjusted to 2 ml with distilled sterile water and evenly applied on the filter paper covering the Petri dish. Afterwards, 10 seeds of *L. sativum* were added. Petri dishes with water of the same volume without extracts were used as controls. The number of germinated seeds was recorded on the 3rd day and the stem and root of the plant was measured. When analyzing the data, the following indicators were taken into consideration: root length, stem length, total plant length. Every experiment was repeated three times. Scale diagrams, constructed using Microsoft Exel software, were used for statistical processing,

RESULTS AND DISCUSSIONS

The influence of biomass:

The influence of mycelial biomass on *L. sativum* roots growth shows that *Ganoderma* mycelia exhibit significant herbicidal activity against L. sativum. According to the data provided in Fig.1, the roots of the plants grown in the control media were much longer compared to the roots of the plants cultivated under the effects of mycelial biomass of the investigated strains and species of genus Ganoderma: *G. sinense* 2516; *G. resinaceum* 2503, 2477; *G. tsugae* 2566, 2024, 1848; *G. applanatum* 1899; *G. lucidum* 1904; *G. carnosum* 2502. The most considerable negative influence on *L. sativum* root growth was shown by all strains of *G. tsugae* and *G. lucidum* 1904. The influence of *G. tsugae* 2024 biomass need to be highlighted considering the decrease of average root length by 82.4% compared to the control experiment, and inability of 7 of 30 seeds to develop the root. The growth of L. sativum roots was slightly less affected by the biomass of all strains of *G. resinaceum*, as well as *G. applanatum* 1899 and *G. sinense* 2516. Compared to the above species, the biomass of G. carnosum 2502 inhibited the growth of *L. sativum* roots the least (28.7%) as to the control group (Fig. 1).

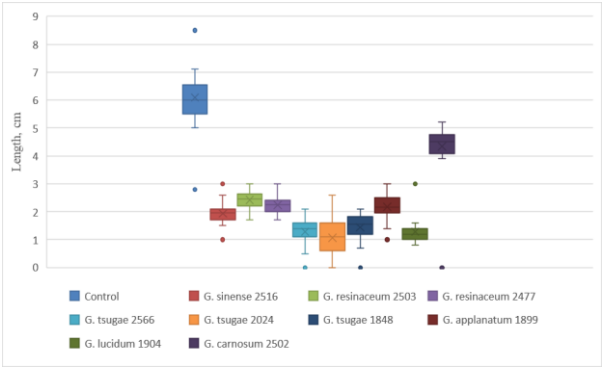

Figure 1. The influence of mycelial biomass of different *Ganoderma* strains on *L. sativum* root length

The data distribution in Fig. 2 indicates a significant inhibitory effect of mycelial biomass of the studied species on the growth of *L. sativum* stems. Repeating the results obtained from the experiments on roots, the stem length in the control group was longer than in the experiments where the biomass of *Ganoderma* fungi was used. The addition of *G. tsugae* 2024 mycelium resulted in inhibition of stem growth of *L. sativum*, the average value of stem length in this experiment decreased by 78.3% compared to the control group, at the same time 16.7% of seeds in the sample did not germinate. The other 7 strains (besides *G. carnosum* 2502) also showed a strong allelopathic effect, the average values of stem length under the influence of mycelia biomass of *G. sinense* 2516; *G. resinaceum* 2503, 2477; *G. tsugae* 2566, 1848; *G. applanatum* 1899; *G. lucidum* 1904 are similar to that in *G. tsugae* 2024 and significantly lower than the value obtained in the control experiment. The exception is the strain of *G. carnosum* 2502, its biomass inhibited stem growth by 34%, compared with the average value of the control sample (Fig. 2).

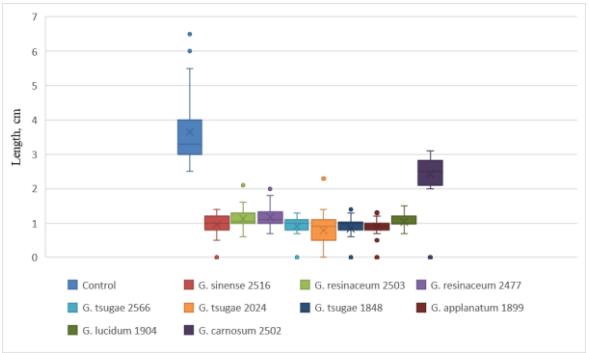

Figure 2. The influence of mycelial biomass of different *Ganoderma* strains on *L. sativum* stem length

Summing up the results of the influence of mycelial biomass of *Ganoderma* fungi, illustrated in Fig. 3, it can be

concluded that the vast majority of species and strains investigated in the work had a significant negative influence on the growth of roots and stems of *L. sativum*. The most significant inhibitory effect was shown by *G. tsugae* 2024 biomass: the linear size of the plant decreased by 80.9%, on the average, compared to the control sample. The biomass of other strains of *G. tsugae*, as well as *G. lucidum* 1904 affected the plant size in the similar to *G. tsugae* 2024 way, slightly weaker effect was demonstrated by the biomass of *G. resinaceum* strains, *G. sinense* 2516 and *G. applanatum* 1899. The mycelial biomass of *G. carnosum* 2502 differed from other species, in terms of its allelopathic properties, showing a lower inhibitory effect on the total plant length, the linear size of which was reduced by 30.7% compared to the control group (Fig. 3).

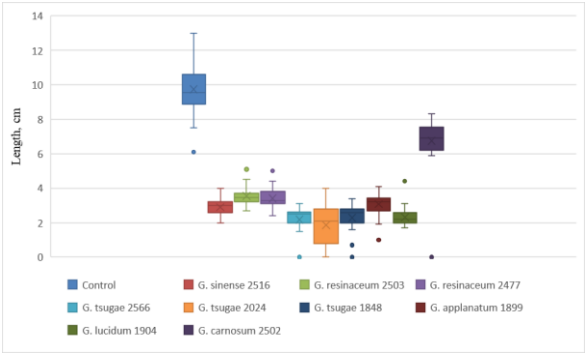

Figure 3. The influence of mycelial biomass of different *Ganoderma* strains on *L. sativum* (root and stem length)

Osivand et al. (2018) established the influence of fruit bodies of 289 fungi species collected in natural conditions on the growth of *Lactuca sativa* var Great Lakes 366 seeds[9]. Fifty four species that were examined showed significant allelopathic effects. Thus, the fruit bodies of *Calocybe gambosa* (Fr.) Singer suppressed root growth by 2.8%, *Heimiella japonica* Hongo - up to 10.2%. The strongest inhibitory effect on stem growth was demonstrated by the biomass of following species: *Xeromphalina tenuipes* (Schwein.) A.H. Sm. - 11.4%; *Entoloma clypeatum* (L.) P. Kumm. - 28.4%. Several species had a considerable allelopathic effect on the overall growth of the plant (root and stem length): *X. tenuipes* - 5.1% on root growth, 11.4% on stem growth; *Leucopaxillus septentrionalis* Singer & A.H. Sm. - 14.8% and 21.4%, respectively.

However, the authors in their study used 10 µg of biomass per 10 ml of agar medium; such concentration is lower than the one we used in our work, this explains the weaker allelopathic effect obtained from Osivand experiments [8].

Araya (2004) investigated the influence of 59 wild mushrooms on the *Lactuca sativa* var Great Lakes 366 growth [2]. Among the studied species was *G. lucidum*. Percent of inhibition with adding 50 mg *G. lucidum* was 55,44% for root and only 8,37% for stem. In our study adding of *G. lucidum* 1904 biomass inhibited the root length on 80,6% and the stem length on 29%.

Regeda et al. (2021) established the influence *of Pholiota spp.* mycelial biomass on seed germination and seedlings growth of *Lepidium sativum* L. and *Cucumis sativus* L at the same conditions with our experiment [10]. But in it`s study all seeds are germinated with adding mycelium biomass of any *Pholiota* species. *P. subochracea* and *P. adiposa* shown a higher inhibitory effect on length of *L. sativum*, to comparison with our best result (*G. tsugae* 2024 biomass). These species inhibited plant growth by 91.8% and 84.85% vs 80,9% in our study.

We first have established the influence of mycelial biomass of 6 species, 9 strains of the genus *Ganoderma* on the *L. sativum* growth. Biomass of all species and strains showed a significant allelopathic effect on the growth of both roots and stems of *L. sativum*, and accordingly the growth of total plant. The growth of roots, stems and plants in general was inhibited mostly by *G. tsugae* 2024 biomass - by 82.4%, 78.3%, and 80.9%, respectively, compared to the control (Figs. 1, 2, 3). The lowest inhibitory activity on the growth of roots, stems and plants in general was shown by biomass of *G. carnosum* 2502 - by 28.8%, 34%, and 30.7%, respectively, compared with the control group (Figs. 1, 2, 3).

The effects of aqueous extracts on *L. sativum* growth

The data, shown in Fig. 4, represent that *L. sativum* roots, grown in pure water lacking mycelium extracts, did not have a significant advantage in development. Most of the aqueous extracts affected the root of *L. sativum* very slightly, except for the extract from *G.sinense* 2516 biomass at the concentration of 500 µl. The addition of *G.sinense* 2516 aqueous extracts decreased the average root length value by 36% compared to the average value from the control sample, data is statistically significant (p = 0.001).

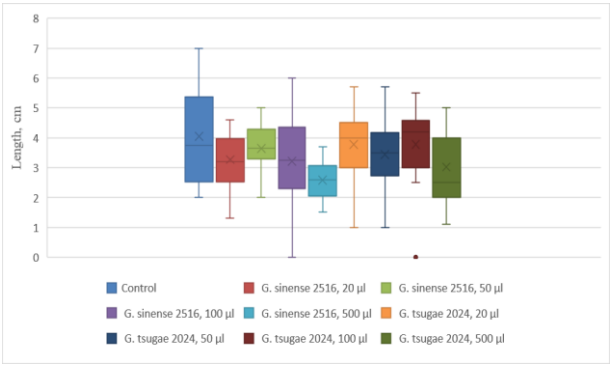

Figure 4. The influence of aqueous extracts of different *Ganoderma* strains on *L. sativum* root length

According to the data distribution shown in Fig. 5, addition of aqueous extracts from the mycelia of selected species can positively affect the growth of *L. sativum* stems. Thus, the introduction of the lowest concentration of *G. sinense* 2516 mycelium extract (20 µl) stimulated the growth of the stem compared to the the control group (which grew on Petri dishes with water), and dishes with aqueous extracts of other studied fungi. The diagram depicted in Fig. 5 shows that the introduction of 20 µl of extract stimulated the growth of the stem, increasing the average value of its length by 19.3% compared with the control group, the data is statistically significant (p = 0.001). Such stimulating effect may be related to the possible presence of cytokines in the mycelium of *G. sinense* 2516, which enhanced the stem growth [13]. Any other concentrations of this extract had no effect on stem length.

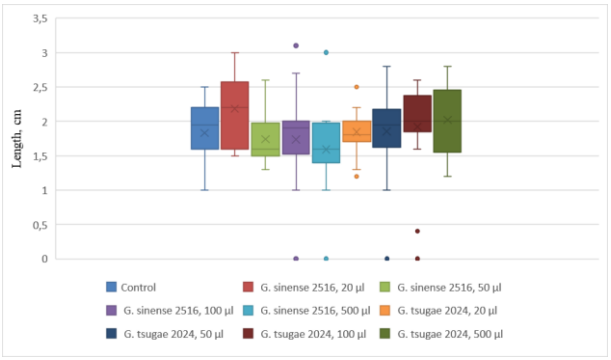

Figure 5. The influence of aqueous extracts of different *Ganoderma* strains on *L. sativum* stem length

The distribution of data groups in the diagram shown in Figure 6 illustrates that most of the aqueous extracts of *G. sinense* 2516 and *G. tsugae* 2024 mycelial biomass did not have a significant effect on the growth of *L. sativum*. Addition of 500 µl of *G. sinense* 2516 mycelium extract reduced plant length by 23.9% compared with the control, the data is

statistically significant (p = 0.001). The results show that the aqueous extracts of mycelial biomass of the selected species did not have the significant impact on the total growth of plants or their individual organs. It is possible that further studies with a greater variety of extracts and their concentrations will demonstrate other effects.

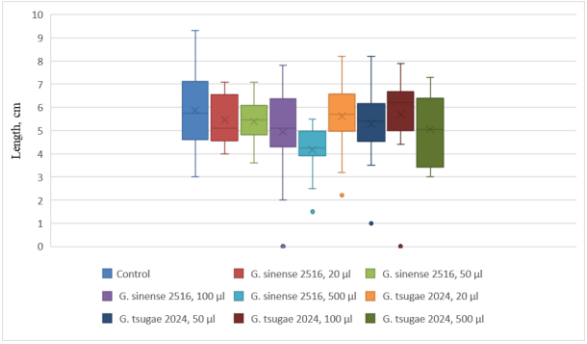

Figure 6. The influence of aqueous extracts of different *Ganoderma* strains on *L. sativum* (root and stem length)

The effects of methanol and ethyl acetate extracts on *L. sativum* growth:

Since the biomass of *G. tsugae* 2024 demonstrated the strongest allelopathic effect on the germination of *L. sativum* seeds, it was decided to obtain methanol and ethyl acetate extracts from mycelium and to investigate their effects on the plant growth.

The diagram shown in Fig. 7 demonstrates the strong allelopathic effect of methyl acetate extracts on the L. sativum roots growth, the higher concentrations directly increased the suppression effect. Methanol extracts had slighter inhibition effect on the root growth of the plant. The addition of 20 µl of methyl acetate extract decreased the average sample value by 61% compared with the control sample. The introduction of 50 µl of methyl acetate extract reduced the root length by 88.9% comparing with the control and most of the seeds (14 of 20) did not germinate at all. The addition of 100 µl of ethyl acetate extract from the *G. tsugae* 2024 mycelium inhibited root growth completely by 100%. Thus, ethyl acetate extract of *G. tsugae* 2024 mycelium provided a considerable allelopathic effect on the roots growth of *L. sativum*. Methanol extract of *G. tsugae* 2024 had a weaker inhibitory effect on the growth of lettuce roots, which did not depend on the concentration. The decrease of root length with the addition of samples with 3 concentrations (20, 50, and 100 µl) was in the range of 28.8-32.6% (Fig.7).

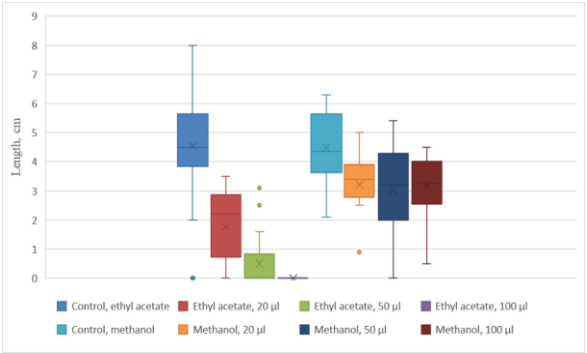

Figure 7. Comparison between effects of methanol and ethyl acetate extracts of *G. tsugae* 2024 mycelium on *L. sativum* root length

A similar situation is observed while investigating the extracts effects on *L. sativum* stem growth, with some differences in the action of methanol extracts. The diagram in Fig. 8 shows that the addition of 20 µl of ethyl acetate extract of *G. tsugae* 2024 mycelium resulted in inability of some seeds to germinate (7 of 20), the average sample value is 67.4% lower than in the control group. The addition of 50 µl of extract suppressed stem growth even more significantly - the number of non-germinated seeds doubled (15 out of 20), and the average length of germinated seeds was 87.2% shorter than in the control group. The introduction of 100 µl of ethyl acetate extract of *G. tsugae* 2024 inhibited the growth of the *L. sativum* stems completely. Despite the allelopathic effect on root growth, the addition of 100 µl of methanol extract did not inhibit the stem growth, compared to the control group. The average values of these groups are almost similar. The addition of 20 µl and 50 µl of methanol extract of *G. tsugae* 2024 suppressed stem growth, the average sample values are 17.7% and 27%, respectively. Ethyl acetate extracts demonstrated stronger allelopathic effect on the germination of stems compared to methanol extracts following the results obtained in experiment with roots.

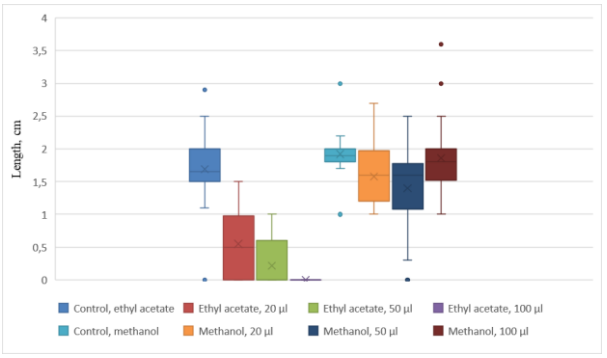

Figure 8. Comparison between effects of methanol and ethyl acetate extracts of *G. tsugae* 2024 mycelium on *L. sativum* stem length

The results indicated that ethyl acetate and methanol extracts of *G. tsugae* 2024 both had allelopathic effect on the growth of *L. sativum* plants. The studies confirmed that ethyl acetate extract significantly increased plant growth inhibition, in direct ratio with increasing concentration. The addition of 100 µl of this extract completely prevents seed germination (Fig. 9). Different concentrations of methanol extract also showed an allelopathic effect, although it was less significant compared with the ethyl acetate (Fig. 9).

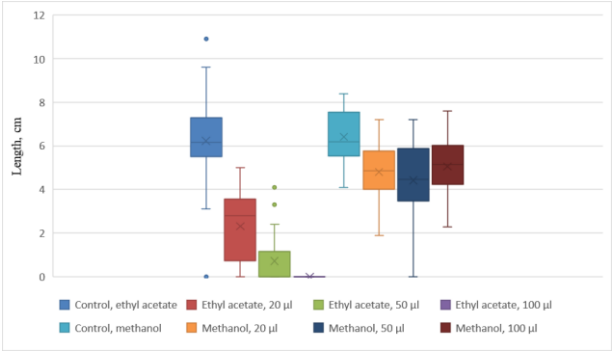

Figure 9. Comparison between effects of methanol and ethyl acetate extracts of G. tsugae 2024 mycelium on L. sativum (root and stem length)

CONCLUSIONS

The biomass and different extracts from investigated *Ganoderma* strains and species can have allelopathic or stimulating effect on the germination of seeds and total growth of plants and individually on its various parts.

The biomass of different strains of one of the same species of *Ganoderma* has different allelopathic effect on the seed germination and growth of L. sativum.

The biomass of 8 *Ganoderma* strains: *G. applanatum*, *G. lucidum*, *G. resinaceum* (2 strains), *G. sinense*, *G. tsugae* (3 strains) had a strong exceptionally allelopathic effect on the roots and stems (as well as a whole plant) growth of L. sativum. Total plant length with adding of biomass was shorter on 69,3-80.9% compared to the control group.

The addition of *G. carnosum* 2502 biomass demonstrated the weakest allelopathic effect compared to the biomass of

other species and strains used – total plant lenght was on 30.7% shorter compared to the control group.

A low concentration (20 µl) of *G. sinense* 2516 mycelium aqueous extract acted as a stem growth stimulant – stem was on 19.3% longer, in comparsion with control group.

Aqueous extracts did not have a significant influence on plant growth, but the addition of 500 µl of *G. sinense* 2516 mycelial biomass extract had a low allelopathic effect on root growth and total plant growth.

Ethyl acetate extract of *G. tsugae* 2024 had strong allelopathic effect on the total growth of *L. sativum* and on its individual parts. The effect was increasing with the increase in concentration. At 100 µl ethyl acetate mycelium extracts inhibited seed germination completely.

Different concentrations of *G. tsugae* 2024 methanol extract also demonstrated allelopathic effect, but it did not depend on the concentration level. The addition of 100 µl of extract did not inhibit the stem growth at all.

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