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The influence of biomass of *Ganoderma* species on seed germination and seedlings growth of *Cucumis sativus* L.

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Abstract: The allelopathic effect of biomass of *Ganoderma* species (9 strains of 6 species) on *Cucumis sativus* seeds germination and growth of root and shoot was determined by using a modified sandwich method. It was demonstrated that the biomass of some investigated strains shows allelopathic activity. Mycelia of *Ganoderma resinaceum* 2477 and *Ganoderma lucidum* 1904 inhibits the growth of *Cucumis sativus* by 61%, mycelia of *Ganoderma sinense* 2516, *Ganoderma tsugae* 2024 and *Ganoderma carnosum* 2502 increased the average length of the shoots by 35.7- 42.9% compared to the control group. These results suggest that *Ganoderma* species have a regulatory effect on cucumber sprouting growth.

Key Words: *Ganoderma, allelopathy, plant growth, sandwich method*

INTRODUCTION

Allelopathic relations between plants, microorganisms and mushrooms play an important role in all types of ecosystems. Studies of mushroom allelopathy began in 1937 [Araya, 2005]. Previous reports showed that fruit bodies of different mushroom species and their extracts possess allelopathic activity on the growth of *Latuca sativa* L. [Araya, 2005], *Vigna radiate* L. [Putra, 2020], *Cucumis sativus* L. and *Lolium perenna* [Salim et al., 2020]. The allelopathic activity was determined for more than 200 species mushrooms from different systematical and ecological groups [Araya, 2005; Putra, 2020; Regeda et al., 2021; Salim et al., 2020]. These properties can be used for creating some herbicides against invasive species, pest species, etc. In most studies was used fruit bodies of wild mushrooms, but a large number of fungi fruit bodies can`t be cultivating in artificial growth conditions. And growing a fruit bodies of popular cultivated fungi species takes a lot of time and resources. According to these facts, there is a problem of using fungi to obtain herbicides on industrial scope. So, for solving this problem could be used mycelium biomass, cultivated by liquid static or submerged culture [Bisko et al., 2012; Hu et al., 2018; Kim et al., 2005].

The aim of this study was the investigation of allelopathic properties of mycelial biomass of *Ganoderma* species.

MATERIAL AND METHODS

Mycelia of nine strains of six *Ganoderma* species from the IBK Mushroom Culture Collection of M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine [Bisko et al., 2020] (Tab. 1) and standardized seeds of the model plant *Cucumis sativus* L. were used in this research.

Mycelia were grown on glucose peptone yeast (GPY) liquid media, g/l: glucose – 25.0; peptone – 3.0; yeast extract – 3.0; $MgSO_4$ x $7H_2O$– 0.25; KH_2PO_4 – 1.0; K_2HPO_4 – 1.0; pH – 6.0. The media was sterilized using an autoclave (1 atm.). Mycelia were grown on GPY medium with agar-agar (20 g/l) crushed in a homogenizer and used as inoculums (10% v/v). The mycelia were incubated in Erlenmeyer flasks (volume 500 ml) with GPY media (100 ml) at a temperature of 26±0.1 ºC during 14 days in submerged conditions (agitation speed – 120 rpm). 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 [Osivand et al., 2018]. 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 (SA) was used as a substrate: 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 15 seeds of *C. sativus* were decomposed on the surface of

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

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	Obtained 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 International Centre for Cryptogamic Plants and Fungi University of Haifa, Haifa, Israel, 2005
	2024	Obtained from Tavria State Agrotechnological University collection, Melitopol, Ukraine, 2014.
	2566	Obtained from "Mycoforest type culture collection", Slovakia, 2016.

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 the addition of mycelial biomass were used as control. Studies of allelopathic activity were carried out in a thermostat at a temperature of 26 ± 0.1 ºC. Each experiment was conducted in triplicate.

On the 3rd day, the number of germinated seeds, the length of the root and shoot, the total length of the plant were recorded. Morphometric measurements were performed using the program AxioVision 4.8.2.

Statistical analysis was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

The obtained results indicate that the total percentage of *C. sativus* seed germination with the addition of biomass of different investigated strains was 100% compared to the control group with the exception of the biomass of *G. resinaceum* 2477, *G. resinaceum* 2502, and *G. applanatum* 1899, which suppressed seed germination on 6.6%, 13.3% and 20% accordingly.

As a result of the experiment, there was a detection of the influence of *Ganoderma* spp. biomass on the growth of roots, shoots, and sproutings of cucumber. Compared to the control group, only the addition of mycelium of *G. sinense* 2516 does not significantly affect the development of *C. sativus* roots: the average values of its length lie within the statistical error (Fig. 1). It was demonstrated that the strongest allelopathic effect to the roots of *C. sativus* exhibits biomass of *G. resinaceum* 2477 - the average length of the roots is the least of all samples and lower than the average in the control group by 55.8% (Fig. 1). Adding biomass of other studied strains (*G. sinense* 2516; *G. resinaceum* 2503; *G. tsuga*e 2566, *G. tsugae* 2024, *G. tsugae* 1848; *G. applanatum* 1899; *G. carnosum* 2502) also had a depressing effect on the *C. sativus* growth roots. In this case, the allelopathic action was manifested in reducing the length of roots on 20% - 52.3% (Fig. 1). Thus, the experimental data indicate that the biomass of studied strains of *Ganoderma* species has a negative or neutral effect on the growth of the roots of *C. sativus*.

It was demonstrated that biomass of *G. sinense* 2516, *G. tsugae* 2024 and *G. carnosum* 2502, reliably positively affects the growth of *C. sativus* shoots (Fig. 2). The addition of mycelium of the above strains increased the average length of the shoots by 35.7%, 42.9% and 42.9%, accordingly, in comparison with the average length in the control group (Fig. 2). The average length of the shoots in experiments with the addition of mycelia of *G. resinaceum* 2503, *G. tsugae* 2566, and *G. tsugae* 1848 lie within the statistical error compared with the control group. Thus, the biomass of these strains does not affect the development of *C. sativus* shoots. The addition of biomass of the another studied strains led to the suppression of *C. sativus* shoots (Fig.2). The strongest allelopathic inhibition

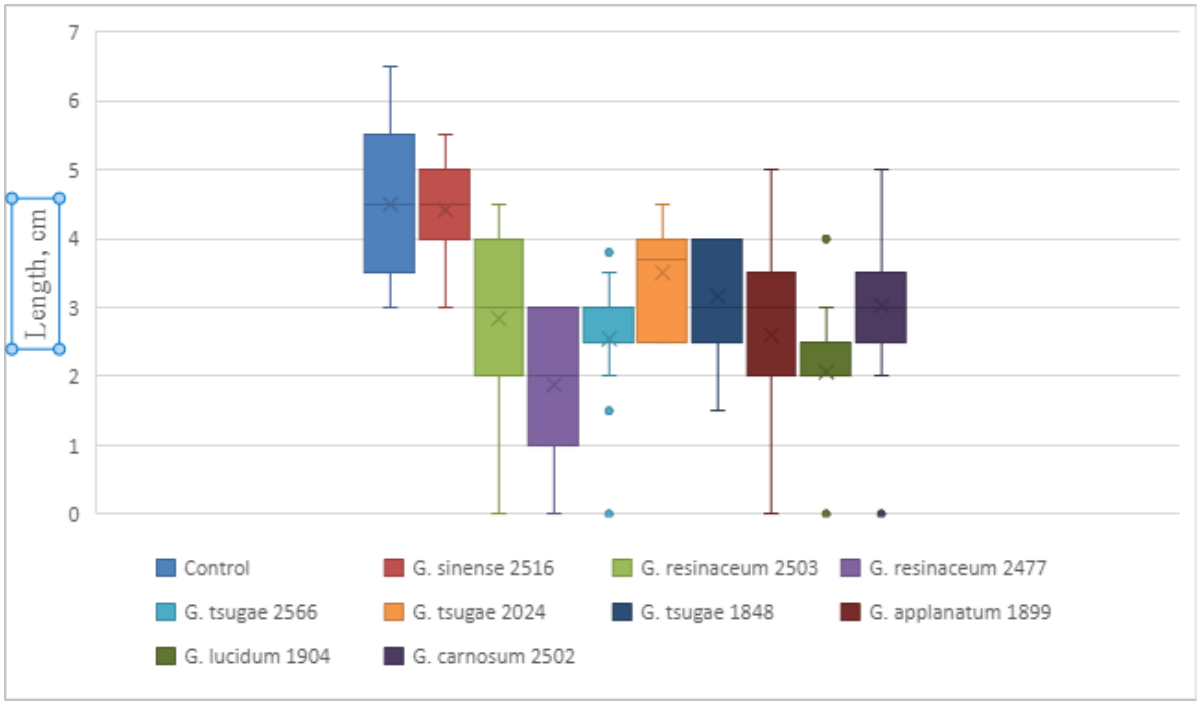

Figure 1. Influence of *Ganoderma* species biomass on the length of *Cucumis sativus* roots on the 3rd day germination at 26.0 ± 0.1 °C.

of the growth of the shoots affected biomass of *G. applanatum* 1899; - the average length of shoots in case of its addition was 39.3% smaller than in the control group (Fig. 2). Thus, the experimental data indicate that the biomass of studied strains of *Ganoderma* species has a positive, negative, or neutral effect on the growth of the shoots of *C. sativus.*

Obtained results about the influence of *Ganoderma* species biomass on *C. sativus* growth sprouting (roots and shoots) was represented on figure 3. It can be argued that the addition of biomass *G. sinense* 2516 acts as a weak stimulator of *C. sativus* growth - the average length plant in experimental group was 5% larger than in the control group. The average value of *C. sativus* plant

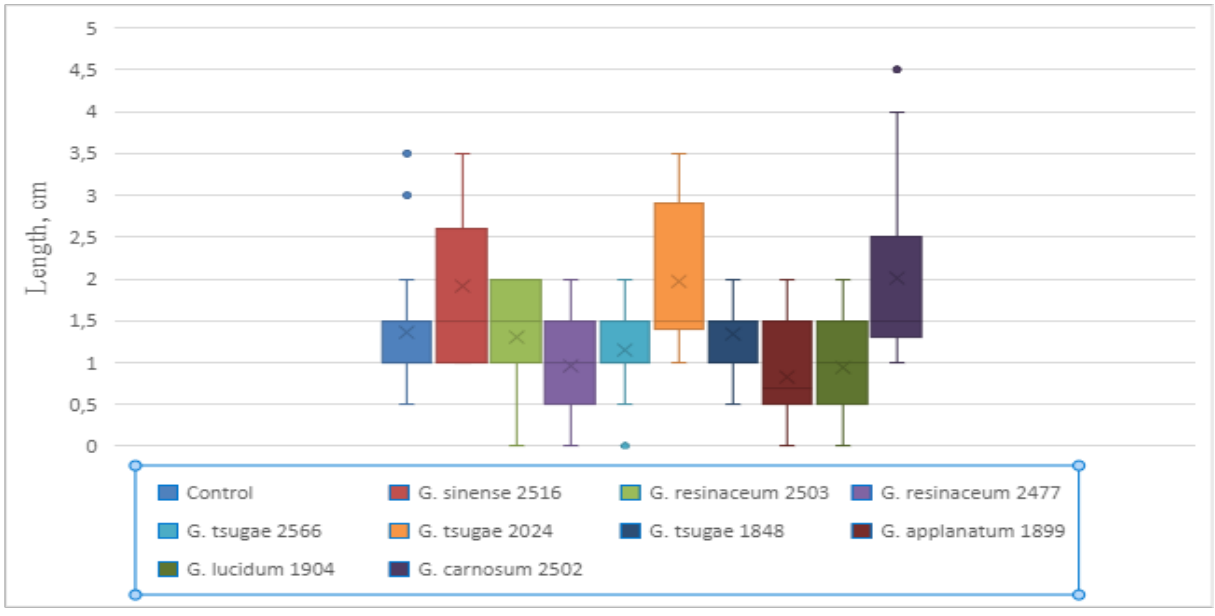

Figure 2. Influence of *Ganoderma* species biomass on the length of the *Cucumis sativus* shoots on the 3rd day germination at 26.0 ± 0.1 °C.

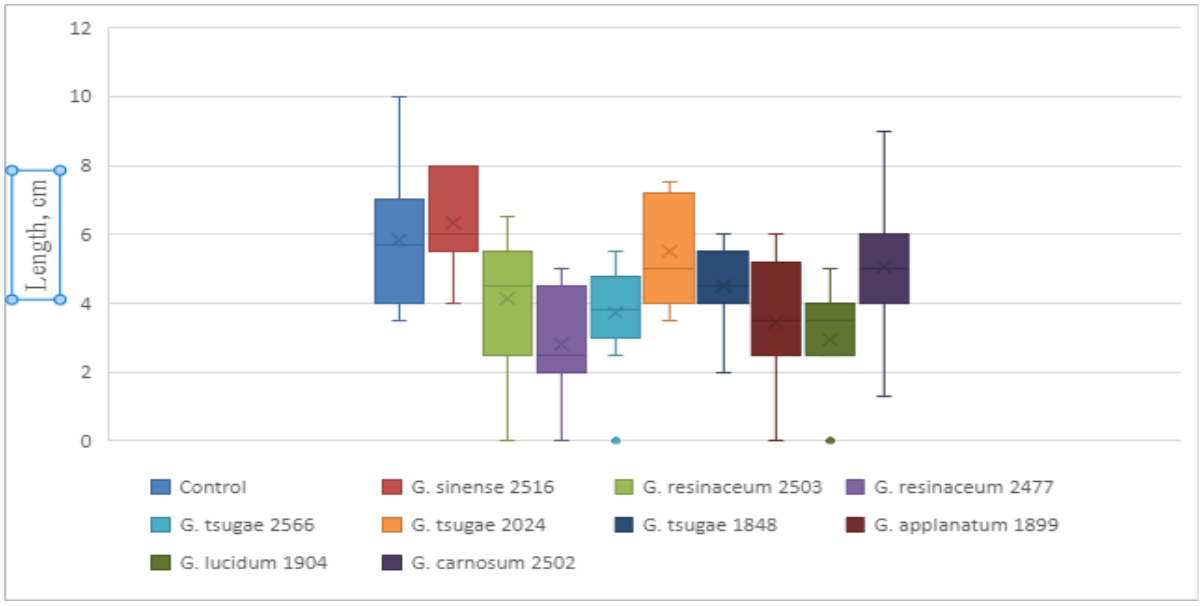

Figure 3. Influence of *Ganoderma* species biomass on the length of the *Cucumis sativus* (roots and shoots) on the 3rd day germination at 26.0 ± 0.1 °C.

in a group with the addition of mycelium *G. resinaceum* 2477 and *G. lucidum* 1904 was on 61% smaller than in control group. The biomass of another studied strains with the exception *G. tsugae* 2024 inhibited the growth of *C. sativus* plant (Fig. 3). It can be concluded that in general, biomass of various strains of *Ganoderma* species effects on the growth of *C. sativus* (roots and shoots) as positively as negatively.

Results of our morphometric experiments indicate on the significant decrease of the roots pubescence in all cases of adding biomass of *Ganoderma* species (Fig. 4).

species of *Pholiota* has different allelopathic effect on the seed germination and growth of *Lepidium sativum* L. and *Cucumis sativus* L. This study has shown that the mycelium biomass made an allelopathic effect on the growth of higher plants. It provides opportunities for future researches of fungi allelopathic activity, especially easy-cultivated mycelium biomass, with the subsequent perspectives for the development of herbicides.

Figure 4. Comparison of control group length of *Cucumis sativus* (1), and the groups with adding *Ganoderma lucidum* 1904 (2) & *Ganoderma resinaceum* 2477 (3) biomass.

It should be noted the allelopathic strain specificity of *Ganoderma* species was studied as regards seed germination, length of roots, shoots and the plant total length of *C. sativus*. Our results agree with the data of L. Regeda [Regeda et al., 2021] which determined that biomass of a different strains of one of the same

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Ganoderma növlərinin biokütləsinin toxum cücərməsinə təsiri və *Cucumis sativus* L. fidan böyüməsi

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Ganoderma növlərinin (9 ştammdan ibarət 6 növ) biokütləsinin *Cucumis sativus* (xiyar) toxumlarının cücərməsinə, kök və cücərtilərin böyüməsinə allelopatik təsiri sendviç metodundan istifadə edərək müəyyən olunmuşdur. Tədqiq edilmiş ştammlardan bəzilərinin biokütləsi allelopatik aktivliyə malikdir. Kontrolla müqayisədə, *Ganoderma resinaceum* 2477 və *Ganoderma lucidum* 1904 miselisi *Cucumis sativus* bitkisinin inkişafını 61% yubadır, *Ganoderma sinense* 2516, *Ganoderma tsugae* 2024 və *Ganoderma carnosum* 2502 miselisi isə çücərtilərin orta uzunluğunu 35.7-42.9% artmışdır. Bu nəticələr göstərir ki, *Ganoderma* növlərinin xiyar bitkisinin böyüməsinə tənzimləyici təsir göstərir.

Açar sözlər: *allelopatiya effekti, bitki böyüməsi, sendviç-metod*

Влияние биомассы видов *Ganoderma* напрорастание семян и рост проростков *Cucumis sativus* L.

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Аллелопатический эффект биомассы видов *Ganoderma* (9 штаммов из 6 видов) на прорастание семян и рост проростков и всход *Cucumis sativus* (огурец) определяли с помощью модифицированного сендвич-метода. Было показано, что биомасса некоторых исследованных штаммов проявляют аллелопатическую активность. Мицели *Ganoderma resinaceum* 2477 и *Ganoderma lucidum* 1904 подавляли рост *Cucumis sativus* на 61%, а мицели *Ganoderma sinense* 2516, *Ganoderma tsugae* 2024 и *Ganoderma carnosum* 2502 увеличивали среднюю длину побегов на 35.7-42.9% по сравнению с контрольной группой. Эти результаты указывают на то, что виды рода *Ganoderma* оказывают как стимулирующее, так и ингибирующее действие на прорастание семян и рост проростков огурца.

Ключевые слова: *аллелопатический эффект, рост растений, сэндвич-метод*