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THE EFFECT OF LARVAE HYDROLYSATES ON THE GROWTH AND PHOTOSYNTHETIC APPARATUS OF BEANS

B a c k g r o u n d . ***Biostimulants are increasingly seen as an effective tool for sustainable agriculture, as their action is based not on increasing the amount of nutrients applied, but on improving the efficiency of their nutrient assimilation by plants and enhancing plant resistance to stress. Hydrolysates obtained from insect larvae (Hermetia illucens*** **L.**, ***Tenebrio molitor*** **L.**, ***Zophobas morio Fabricius) are characterized by high antioxidant and cytoprotective activity and a rich amino acid composition. The aim of this study was to investigate the effect of larval hydrolysates on the growth and photosynthetic apparatus of beans.***

M e t h o d s . ***After planting the seeds, they were watered once with hydrolysates of Hermetia illucens, Tenebrio molitor, and Zophobas morio (Zm, Tm, and Hi) at two concentrations each (0.1 % or 0.2 %). The control group was watered with water. The percentage of seed germination, seedling growth, and seedling energy index were determined on the 10th day of germination. On the 15th day, the seedlings were re-treated with hydrolysates. A specific hydrolysate was applied to the roots at concentrations of 0.1 % or 0.05 %, or applied to the leaves (sprayed) at concentrations of 0.1 % or 0.2 %. The growth of seedlings after treatment, the above-ground and below-ground vegetative mass of plants, and the content of chlorophylls a and b and carotenoids in the leaves were determined.***

R e s u l t s . ***Treatment with insect hydrolysates did not significantly affect the overall germination of seeds and the growth of bean seedlings. The seedling energy index showed the stimulating effect of 0.1 % Zophobas morio hydrolysate and the inhibitory effect of 0.1 % T. molitor hydrolysate. Repeated treatment with 0.1 % hydrolysates of Z. morio and H. illucens stimulated bean growth in both types of irrigation. Root and foliar irrigation with insect hydrolysates tended to decrease the content of chlorophyll a and carotenoids and increase the content of chlorophyll b. Only spraying with Z. morio showed a sharp increase in chlorophyll b and a moderate increase in carotenoids, indicating an enhancement of defense mechanisms under such treatments.***

C o n c l u s i o n s . ***Among the reagents studied, 0.1 % Zophobas morio hydrolysate performed best, stimulating defense mechanisms at the photosynthetic system level when applied to leaves and growth processes when applied to roots with this reagent. H. illucens hydrolysate stimulated bean growth, while T. molitor had a predominantly neutral or inhibitory effect.***

K e y w o r d s : ***Hermetia illucens, Tenebrio molitor, Zophobas morio, chlorophylls, carotenoids, seed germination.***

Background

Agriculture has traditionally played a key role in Ukraine's socio-economic development. The active use of inorganic fertilizers between 2000 and 2020 led to nutrient asymmetry (the accumulation of excess nitrogen). Recent studies show that since the beginning of the invasion in 2022, there has been a persistent deficit of nitrogen, phosphorus, and potassium nutrients, excessive nutrient removal by crops, and a prolonged decline in soil organic matter content (Reimer et al., 2020; Litvinova et al., 2021; Medinets et al., 2025). In these conditions, strategies that allow for increased productivity without additional environmental impact become important, in particular through the optimization of plant nutrition and the use of biological growth stimulants. Biological fertilizers and biostimulants are increasingly seen as an effective tool for sustainable agriculture, as their action is based not on increasing the amount of nutrients applied, but on improving the efficiency of their nutrient assimilation, modifying plant physiological processes, regulating root nutrition, optimizing metabolism, enhancing antioxidant protection, and increasing stress resistance (Calvo et al., 2014; du Jardin, 2015; Yakhin et al., 2017; Ammar et al., 2023; Prisa et al., 2023; Arinaitwe et al., 2025). A promising group of biostimulants for research are protein hydrolysates of plant or animal origin, as they can modulate nitrogen and carbon metabolism, enhance antioxidant protection, and influence the functioning of the photosynthetic apparatus (Colla et al., 2017; Carillo et al., 2022; Pasković et al., 2024). In recent years, insects have attracted increasing attention due to the hyperaccumulation of proteins and fats through consumption and, accordingly, waste utilization, with their subsequent use as a food source for animals and humans (Seong Hyeon et al., 2012; Jong Bin et al., 2014; Tomberlin, & van Huis, 2020; Rumbos, & Athanassiou, 2021; Ogello et al., 2025). Of particular interest are hydrolysates obtained from insect larvae (*Hermetia illucens* L., *Tenebrio molitor* L., *Zophobas morio* Fabricius), which have been described as having high antioxidant and cytoprotective activity, as well as a rich amino acid composition (Zielińska et al., 2017; Firmansyah, & Abduh, 2019; Batish et al., 2020; Araujo et al., 2025; Cho et al., 2025). Despite this, there are practically no experimental studies on the effect of insect hydrolysates on plants: only one study on the use of *T. molitor* hydrolysate is known, in which growth parameters were evaluated without analyzing photosynthetic activity (Szopa et al., 2023). Among the practical applications of insect processing products in crop production, there is only information about frass. Frass *H. illucens* increased bean yield and improved biological nitrogen fixation compared to mineral NPK and organic fertilizer (Chepkorir et al., 2024). Similar results for *T. molitor* and *Z. morio* frass were obtained on table beets (Przemieniecki et al., 2024), where frass and vermicompost increased plant biomass and altered the structure of the rhizosphere. At the same time, frass is an organic substrate consisting of chitin, feed residues, microbes, and insect waste products, and differs significantly from protein hydrolysates. Therefore, the results for frass cannot indicate the effectiveness of insect protein hydrolysates, but they do point to the promise of insect products in general.

In the context of food security and protein independence, the EU pays particular attention to legumes. Beans (*Phaseolus vulgaris* L.) combine high nutritional value with the ability to fix nitrogen symbiotically, making them an important element of sustainable agrosystems (Çelmeli et al., 2018).

The main objective: to investigate the effect of hydrolysates of *Hermetia illucens, Tenebrio molitor,* and *Zophobas morio* larvae on the growth and photosynthetic apparatus of beans.

Methods

To obtain hydrolysates, distilled water was added to the larval biomass at a ratio of 1:1 by weight and homogenized using a blender. Sodium pyrosulfite (16 g per kg of homogenate) was added to the resulting homogenates, left for 24 hours at +40 °C. The next, an equal amount of water was added and left for another 12 hours at the specified temperature. After filtration, the resulting hydrolysates (hereinafter Zm – hydrolysate of *Zophobas morio* (black soldier fly), Tm – hydrolysate of *Tenebrio molitor* (black soldier fly) larvae; Hi – hydrolysate of *Hermetia illucens* (mealworm) larvae) were used for further research. For the study, we used seeds of common beans (*Phaseolus vulgaris* L.) of the Igolskaya variety: early maturing, high-yielding, resistant to diseases and lodging, with high taste qualities. The plant is bushy, compact – 40–50 cm high, does not require support. The seeds are cylindrical in shape and white. In order to improve selection and germination, the bean seeds were soaked in water for 12 hours before planting. Soaking helps soften the seed coat, facilitating the penetration of water and oxygen, which are necessary for germination. This helps the seeds germinate faster and more uniformly, and also allows non-viable seeds to be identified and removed. Visually high-quality, well-filled seeds with intact seed coats and approximately the same size were selected. The seeds were not treated with fungicides to avoid possible interactions between different active ingredients. Two seeds were planted in each hole, with 10 holes in each experimental group. The seeds were planted in Peatfield universal soil with a pH of 5.5–6.5 without biological additives. After planting, the seeds were watered once with 20 ml/hole of Zm, Tm, and Hi hydrolysates in two concentrations each (0.1 % or 0.2 %). The control group was watered with water. The plants were grown under greenhouse temperature conditions: with a daytime temperature of +25 °C and a nighttime temperature of +20 °C, which is close to the optimal conditions for planting seeds in the soil. The effectiveness of hydrolysates on the germination and growth of beans was determined. The percentage of seed germination was determined on the 10th day of germination, when the last individual seeds germinated; later, the seeds did not germinate. Seedling growth was measured on the 10th and 15th days to determine the effect of initial watering with different doses of hydrolysates on the germination and growth of beans.

Percentage of germination = (number of germinated seeds / total number of seeds taken for germination) x 100

The seedling energy index was determined on the 10th day according to Srivastava (2015).

Seedling energy index = (germination % x seedling length)

On day 15, the seedlings in each group were divided into two groups of 10 each and re-treated with hydrolysates. For example, Zm hydrolysate was applied to the roots at concentrations of 0.1 % or 0.05 %, or sprayed onto the leaves at concentrations of 0.1 % or 0.2 %. The length of the seedlings was measured 5 and 10 days after treatment and compared with the length of these plants before treatment (increase).

The above-ground and below-ground vegetative mass of the plants was weighed, after which leaf samples were taken to determine their chlorophyll and carotenoid content.

The content of chlorophylls a and b and the total carotenoid content were determined according to Sumanta et al. (2014). The pigments were extracted with 96 % ethyl alcohol, and the absorption of the leaf extract solution was measured at 664 nm, 649 nm, and 470 nm. The content of photosynthetic pigments was presented in mg/g of raw material.

Statistical processing of the results was performed using Prism Graphpad 8 (GraphPad; La Jolla, CA, USA). The reliability of the results was determined using multifactorial ANOVA with Tukey's correction.

Results

The results of the study showed that the obtained hydrolysates do not significantly affect the germination of bean seeds, although there is a certain tendency to stimulate germination, especially with *Zophobas morio* hydrolysate at a concentration of 0.1 % (Fig. 1a). The best results were also obtained after treatment with this hydrolysate in terms of sprout length, while the hydrolysate from *Tenebrio molitor*, compared to the Zm hydrolysate, had a significantly worse effect on sprout growth.

The sprout energy index, which combines both important indicators, seed germination rate and sprout growth, shows that treatment with Zm hydrolysate at a concentration of 0.1 % increased the sprout energy index by 1.5 times (Fig. 2). Tm hydrolysate had a slightly lower seedling energy index, while in other experimental groups the results were similar to the control.

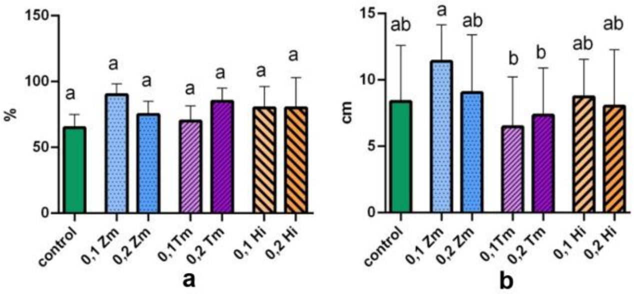

Fig. 1. Effect of larval hydrolysates on seed germination rate (a) and bean sprout length on day 10 (b)
Notes: different letters indicate significant differences within the parameter (P < 0.05) according to the results of Tukey's multiple comparison test.

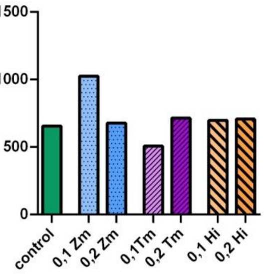

Fig. 2. Energy index of bean seedlings after treatment with various larval hydrolysates on the 10th day of germination

Treatment of 15-day-old sprouts with larval hydrolysates during the first 5 days had a positive effect on the growth of all experimental groups, both under watering and spraying conditions.Ten days after treatment, the trend continued, with only the group sprayed with 0.5 % Zm showing a decrease in growth rate (Fig. 3 a, b). Plant growth was best stimulated by watering with 0.1 % Zm and 0.1 % Hi, as well as spraying with 0.1 % Zm, 0.1 % and 0.2 % Hi.

Repeated treatment of plants with larval hydrolysates showed that, in general, the chlorophyll a content in bean leaves after both irrigation and spraying with hydrolysates did not differ significantly from that in control plants. However, both for irrigation and spraying, there is a tendency for a more positive effect of hydrolysates from *Zophobas morio* in both concentrations, and a tendency to decrease the chlorophyll *a* content with different treatments with other hydrolysates (Fig. 4 a). Chlorophyll *b* content did not differ significantly from the control when plants were watered with hydrolysates, but it increased significantly after spraying with *Zophobas morio* hydrolysate, particularly at a concentration of 0.2 % (Fig. 4 b). Treatment with other hydrolysates did not significantly affect the chlorophyll content compared to the control group; however, in most cases, this parameter was significantly lower than in the 0.2 % Zm group. Spraying also produced better results for both chlorophyll *a* and chlorophyll *b*.

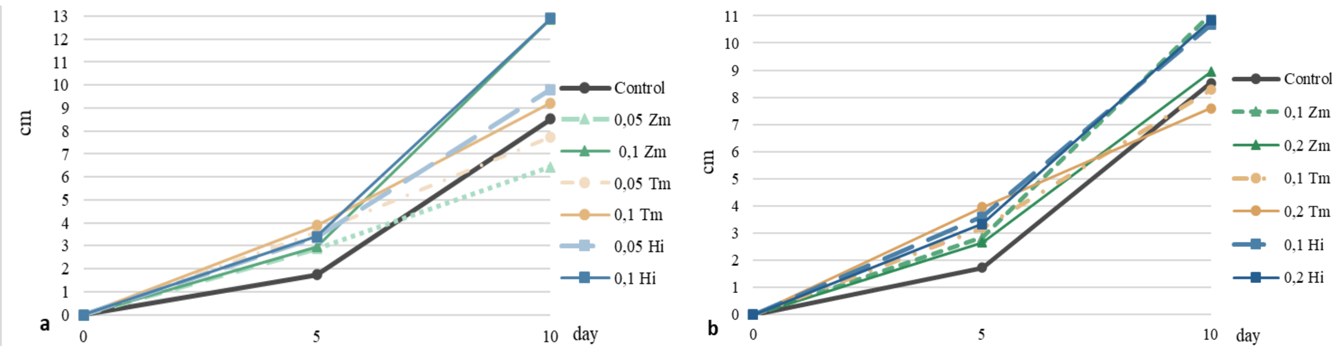

Fig. 3. Dynamics of bean sprout length growth during 5–10 days after repeated treatment with larval hydrolysates under the following conditions: a) root irrigation; b) spraying (n = 20)

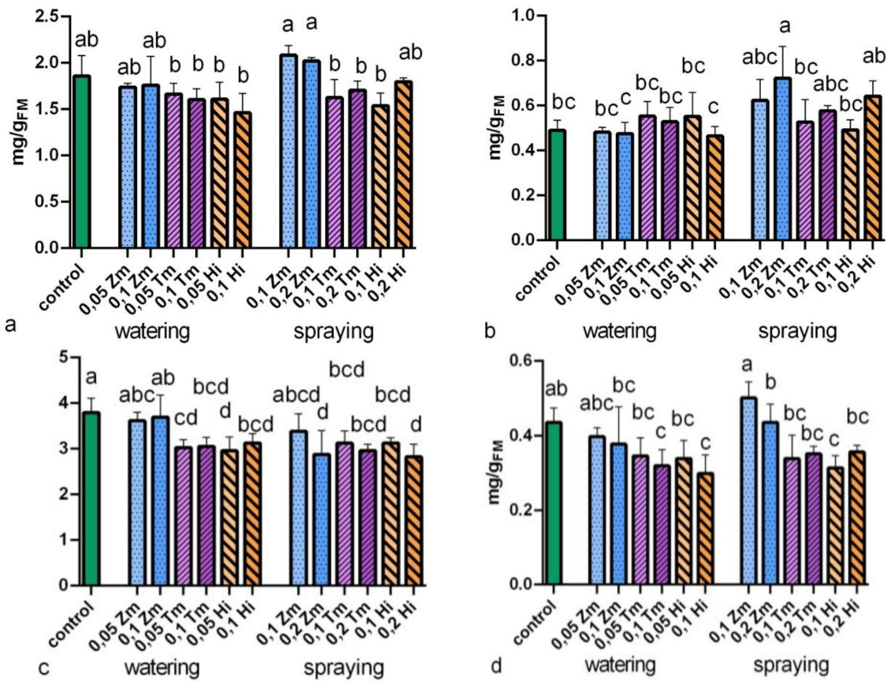

Fig. 4. Chlorophyll and carotenoid content in bean leaves after treatment with larval hydrolysates: a) chlorophyll *a*; b) chlorophyll *b*; c) ratio of chlorophyll *a* to chlorophyll *b*; d) carotenoids
Notes: different letters indicate significant differences within the parameter ($P < 0.05$) according to the results of Tukey's multiple comparison test.

When watered with hydrolysates from *Tenebrio molitor* and *Hermetia illucens*, there was a tendency to decrease the content of chlorophyll *a* and increase the content of chlorophyll *b*, which significantly reduced the ratio of chlorophyll *a/b* in these experimental groups compared to the control group (Fig. 4 c). At the same time, watering with hydrolysates from *Zophobas morio* did not cause a shift in the chlorophyll *a/b* ratio compared to the control. The deviation from the control of the chlorophyll *a/b* ratio in plants treated by spraying with Zm and Hi hydrolysates at a concentration of 0.2 % was caused by increased chlorophyll *b* synthesis, which may indicate the stimulation of adaptive responses, while the decrease in the chlorophyll *a/b* ratio after spraying with 0.1 %, 0.2 % Tm and 0.1 % Hi hydrolysates is associated with a decrease in chlorophyll *a* content, indicating the destructive effect of such treatment. A significant decrease in carotenoid content can also be noted when watering with 0.1 % Tm and 0.1 % Hi hydrolysates and spraying with 0.1 % Hi hydrolysate, which also indicates the negative effect of these hydrolysates (Fig. 4 d). Spraying with Zm hydrolysate at concentrations of 0.1 and 0.2 % showed a tendency to stimulate protective mechanisms at the carotenoid level.

Studies of the above-ground and below-ground mass of bean plants did not reveal any significant differences between the experimental and control groups after treatment with larval hydrolysates (Fig. 5 a, b). This is primarily due to the asynchronous germination of plants and, accordingly, the large dispersion of plant sizes within groups. However, there is a tendency toward a decrease in the above-ground and below-ground mass of plants after any treatment with Tm hydrolysate and no negative effect on vegetative mass accumulation when watered with 0.1 % Zm, 0.05 % Hi, and 0.1 % Hi hydrolysates and after spraying with 0.1 % and 0.2 % Zm.

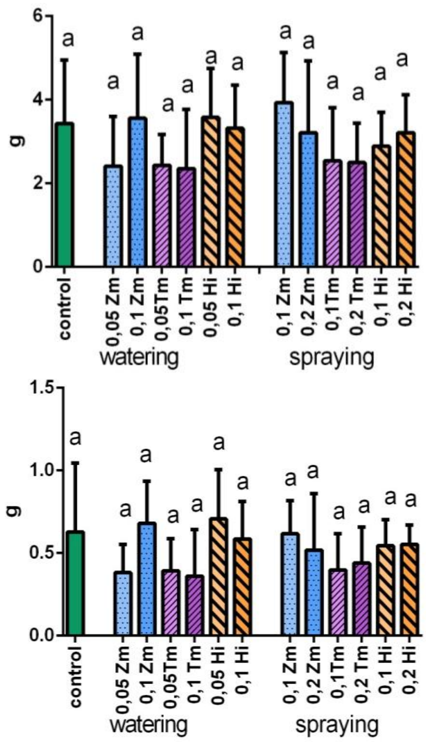

Fig. 5. Above-ground (a) and below-ground (b) bean mass after treatment with larval hydrolysates (n = 15). Different letters indicate significant differences within the parameter (P< 0.05) according to the results of Tukey's multiple comparison test

Discussion and conclusions

It is known that protein hydrolysates of plant and animal origin can alter the regulation of nitrogen and carbon metabolism, affect photosynthetic parameters, and enhance antioxidant protection (Colla et al., 2017; Pasković et al., 2024; Sun et al., 2024). On the other hand, photosynthetic activity is one of the most sensitive links to any agronomic interventions (du Jardin, 2015; Yakhin et al., 2017).

According to a study by Riolo, individual peptide fractions of *H. illucens* demonstrated cytoprotective effects, in particular a reduction in the formation of reactive oxygen species (Riolo et al., 2023). The production of *T. molitor* protein hydrolysates with high antioxidant activity has also been described (Leal et al., 2025). However, their effect on the photosynthetic apparatus of plants has not been studied. In our work, we found that *H. illucens* and *T. molitor* hydrolysates have an inhibitory effect on carotenoids, which are important links in the antioxidant protection of the photosynthetic system. A comparison of the composition of *T. molitor* and *H. illucens* protein hydrolysates showed similarities in amino acid profile and antioxidant activity (Leal et al., 2025). Our results on the effect of both hydrolysates on beans showed a similar effect overall.

Z. morio hydrolysates also contain numerous bioactive peptides with antioxidant and antiperoxide properties (Araujo et al., 2025). In our study, treatment with this hydrolysate had a neutral effect on chlorophyll a and carotenoids, but stimulated the synthesis of chlorophyll b, which is one of the important links in the protective mechanisms of the photosynthetic system.

According to the literature, *T. molitor* hydrolysates were used to treat plants, which contributed to the improvement of growth parameters and root system development (Szopa et al., 2023), while according to our results, there was a tendency to decrease above-ground and below-ground mass. No similar experiments with *H. illucens* and *Z. morio* hydrolysates have been found in the open literature. In our studies, these hydrolysates showed a certain stimulating effect on bean growth. At the same time, it should be noted that the methods used to obtain the hydrolysates were not identical: autohydrolysis was used in our study, whereas Szopa and colleagues employed 60 % sulfuric acid, which can significantly affect the content of these hydrolysates. It should be noted that under the same conditions of obtaining hydrolysates, the sample from *Z. morio* was relatively transparent, while the samples from *H. illucens* and *T. molitor* were significantly cloudier with a large number of impurities. *Z. morio* larvae are characterized by their ability to effectively digest plastic, polystyrene, or polyethylene, thanks to the bacteria that live in their intestines (Li et al., 2020; Rumbos, & Athanassiou, 2021). Due to the presence of more active biological compounds, hydrolysis of homogenates from *Z. morio* larvae can probably occur to low molecular weight compounds without the need to add exogenous enzymes or acids. Whereas, to obtain more effective hydrolysates from *T. molitor* and *H. illucens*, it may be necessary to use more aggressive hydrolysis methods.

Treatment with insect hydrolysates at concentrations of 0.1 % and 0.2 % did not significantly affect the overall germination of seeds and the growth of bean seedlings. The seedling energy index showed a stimulating effect of 0.1 % *Z. morio* hydrolysate and an inhibitory effect of 0.1 % *T. molitor* hydrolysate. Repeated treatment with 0.1 % hydrolysates of *Z. morio* and H. illucens stimulated bean growth in both root and foliar irrigation.

Root and foliar irrigation with insect hydrolysates tended to decrease chlorophyll *a* content, increase chlorophyll *b* content, and decrease carotenoid content. The most stable indicators of both pigments were observed in the variants with *Z. morio*. Only spraying with 0.2 % *Z. morio* showed a sharp increase in chlorophyll b, and with 0.1 % *Z. morio*, there was a tendency to increase carotenoids, which indicates a strengthening of protective mechanisms during such treatments.

A comparison of the application methods revealed fundamental differences in their effects. Watering proved to be more effective in stimulating growth, providing the highest growth rates, which indicates a more efficient uptake of biologically active substances through the root system. Spraying, on the other hand, had a stronger effect on the pigment profile, particularly in the *Z. morio* variants, where a significant increase in chlorophyll *b* and carotenoids was observed. In general, spraying caused weaker systemic changes than watering, but its effect was more noticeable at the level of photoprotective pigments.

Thus, the following conclusions can be drawn.

Among the reagents studied, 0.1 % *Z. morio* hydrolysate showed the best performance, stimulating defence mechanisms at the level of the photosynthetic system when applied to leaves and promoting growth when applied to roots. *H. illucens* hydrolysate stimulated bean growth, while *T. molitor* had a predominantly neutral or inhibitory effect.

Authors' contributions: Iryna Shevchenko – data validation, article writing; Andriy Nikolayuk – data validation; Nataliia Nuzhyna – conceptualization, formal analysis, article writing and editing.

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ВПЛИВ ГІДРОЛІЗАТІВ ЛИЧИНОК НА РІСТ І ФОТОСИНТЕТИЧНИЙ АПАРАТ КВАСОЛІ

Вступ. ***Біостимулятори дедалі частіше розглядаються як ефективний інструмент сталого землеробства, адже їхня дія ґрунтується не на збільшенні кількості внесених поживних речовин, а на підвищенні ефективності їх засвоєння рослинами та посиленні стійкості рослин до стресів. Для гідролізатів, отриманих із личинок комах (Hermetia illucens L., Tenebrio molitor L., Zophobas morio Fabricius), характерні висока антиоксидантна й цитопротекторна активність і багатий амінокислотний склад. Метою цієї роботи було дослідити вплив гідролізатів личинок на ріст і фотосинтетичний апарат квасолі.***

Методи. ***Після висаджування насіння одноразово полили гідролізатами Hermetia illucens, Tenebrio molitor та Zophobas morio (Zm, Tm і Hi) у двох концентраціях кожним (0,1 % або 0,2 %). Контрольну групу поливали водою. Визначали відсоток схожості насіння, ріст проростків та індекс енергії проростків на 10-й день пророщування. На 15-ту добу проростки повторно пройшли оброблення гідролізатами. Певним гідролізатом поливали під корінь у концентраціях 0,1 або 0,05 %, або поливали по листку (обприскували) у концентраціях 0,1 або 0,2 %. Визначали приріст проростків після оброблення, надземну та підземну вегетативну масу рослин, уміст хлорофілів a та b і каротиноїдів у листках.***

Результати. ***Оброблення інсектними гідролізатами достовірно не впливало на загальну схожість насіння і ріст проростків квасолі. Показник індексу енергії проростків виявив тенденції стимуляційного ефекту 0,1 % гідролізату Zophobas morio та пригнічувальну дію 0,1 % гідролізату T. molitor. Повторне оброблення 0,1 % гідролізатами Z. morio та H. illucens стимулювало ріст квасолі за обох типів поливу. Прикореневий і позакореневий полив інсектними гідролізатами переважно мав тенденцію до зменшення вмісту хлорофілу a та каротиноїдів і збільшення вмісту хлорофілу b. Лише обприскування із Z. morio показало різке підвищення хлорофілу b та помірне зростання каротиноїдів, що свідчить про посилення захисних механізмів за такого оброблення.***

Висновки. ***Серед досліджених реактивів найефективнішим виявився 0,1 % гідролізат Zophobas morio, за використання якого спостерігали стимуляцію захисних механізмів на рівні фотосинтетичної системи при обробці по листку та ростових процесів за прикореневої обробки. Гідролізат H. illucens стимулював ріст квасолі, а T. molitor переважно мав нейтральну або інгібувальну дію.***

Ключові слова: ***Hermetia illucens, Tenebrio molitor, Zophobas morio, хлорофіли, каротиноїди, схожість насіння.***

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