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Assessment of the Toxicological Safety of Soil after the Application of Zoohumus *Hermetia illucens*

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ABSTRACT

The problem of finding environmentally friendly organic fertilizers is obvious. The aim of the work was to determine safe doses of *Hermetia illucens* L. zoohumus application to low fertility soil. A field experiment was conducted on plots of 1 m^2. Zoohumus was applied to the soil in doses of 0.5, 1, 1.5, 2, 3 kg/m^2. The field germination of *Triticum aestivum* L. wheat, the growth of its aboveground parts on the 14th day and 117th day of the experiment were determined, and also conducted bioassays on the chemotaxis of *Paramecium caudatum* Ehrenberg and bioluminescence of *Escherichia coli* Migula. On the 14th day of the experiment the germination of T. aestivum was reduced (by 56-77% compared to the control, r = -0.72). However, on the 117th day of the experiment, an increase in the number of shoots and their length compared to the control was shown, the maximum being for the variants of 0.5-1.5 kg/m^2 of zoohumus ($p<0.05$). Bioassays made it possible to clarify the optimal doses of zoohumus application to the soil. Safe options for soil fertilization were additives of zoohumus from 0.5 to 1.0 kg/m^2. Thus, the effectiveness of H. *illucens* zoohumus as an organic fertilizer was confirmed and it was proven for the first time that the soil does not change toxicological indicators when optimal dosages are observed.

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INTRODUCTION

The growing population of the Earth requires the production of more agricultural products, as the UN predicts that the world's population will reach 9.7 billion people by 2050 (UN, 2016). This will result in a 56% increase in food demand (Alexandratos, 2012; McKenzie & Williams, 2015). It is obvious that the extensive path of agricultural development has already exhausted itself. Many soils need to be improved, marginal soils make up about 15% of agricultural land worldwide and 21% of the world's total land resources (UN, 2019; Jumaniyazov et al., 2023), about 10% of the total agricultural land is saline or subject to salinization processes (Shahid et al., 2018). As a result, 15% to 36% of agricultural land is commercially unprofitable (Wood et al., 2000; Jumaniyazov et al., 2023). Therefore, the task of finding technologies and innovative ways to maintain soil quality and restore soil fertility is very urgent.

The use of various types of fertilizers is a classic and effective method. However, it entails many environmental risks.

Russia is one of the largest producers of mineral fertilizers in the world. According to the

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Russian Fertilizer Producers Association, 54.8 million tons were produced in 2020, which is 13% of the entire world market, 34.2 million tons were exported, it accounts for 40% of all foreign supplies. The negative environmental effects of mineral fertilizers are known (Bisht & Chauhan, 2020). For example, excessive application of nitrogen fertilizers disrupts the balance of organic matter and can also cause soil acidification. Phosphorus fertilizers can contribute to the eutrophication of surface waters, and the heavy metal impurities they contain pollute the soil (Bijay-Singh & Sapkota, 2023). Also, with excessive use of mineral fertilizers, the enzymatic activity of the soil decreases (Strukova & Malyukova, 2009), it becomes compacted, its natural structure is lost, macro- and microelements are washed out, which affects the intensity of photosynthesis, reduces the resistance of plants to diseases, and this leads to increased use of pesticides (Prishchepa, 2000).

A number of countries make a practice of developing organic farming and are actively introducing the use of organic fertilizers. For example, France adopted a program for the development of the organic sector (CAP 2023-2027), according to which farmers who practise organic farming will receive financial bonuses from the state. It is planned that by 2027, 18% of French farmland should be reoriented to the production of organic products. However, not all substrates are suitable for use in organic fertilizers. Organic waste (sludge from treatment facilities, domestic water, biological waste from farms, wastewater from the food industry), which could potentially be used to fertilize soils, can accumulate heavy metals (Carpanez et al., 2022). Some organic fertilizers are a source of microplastics in the soil, especially small ones (<100 μm) (Zhao et al., 2023).

The product of processing organic matter by the larvae of the fly *Hermetia illucens* L. (Stratiomyidae (Diptera)), which is also known in scientific literature as the black soldier fly, is of interest. Their use in environmentally friendly technologies, firstly, will help to achieve the processing of problematic organic waste: plant and food residues, feces, bird droppings, pig farm wastewater (Diener et al., 2011). Secondly, during larval processing, a loose organic substrate with a particle size of 1-3 mm, dark or light brown in color (depending on the raw material being processed) is formed – larval frass, which we consider it appropriate to call *zoohumus*. It contains nutrients accumulated in the larvae (Pendyurin et al., 2020).

It is known that *H. illucens* larvae can reduce the amount of pathogens (Lalander et al., 2015; Klammsteiner et al., 2020) and chemical pollutants (Lalander et al., 2016) contained in organic waste to levels acceptable for use in agriculture (Bernal et al., 2009), which is important for reducing the risks of infectious diseases of cereals in the fields. It was shown that black soldier fly zoohumus can become a sorbent capable of extracting various ions from soils, in particular Zn^{2+} (Sverguzova et al., 2021).

Biological contamination is also prevented. Food passing through the digestive tract of the larvae is enzymatically broken down, enriched with the products of the larval activity, which exhibit antimicrobial properties against many microorganisms (Hattori, 1995; Nitbani et al., 2022). As a result, the survival rate of pathogenic bacteria in the zoohumus of *H. illucens* is reduced by 90-93%; on the 9th day Salmonella spp. bacteria are not detected in chicken manure after processing by larvae (Lyashchev et al., 2022a). Various fungal pathogenic communities, mainly mold communities, completely disappear from organic substrates (Kuznetsova et al., 2021). The population of housefly (*Musca domestica* L.) in poultry farms and pig faeces is reduced by 94-100% (Awasthi et al., 2020).

Zoogumus has a complex positive effect on the soil. Due to its high moisture capacity and moisture resistance, this fertilizer slows down soil drying by 18 days and improves its structure (Pendyurin et al., 2023). The soil is enriched with organic matter and macronutrients. The main nutrients are found in it in the form of various compounds with humic acids. Unlike coprolite (vermicompost), zoohumus *H. illucens* contains an increased content of organic matter (1.3 times), total nitrogen in dry matter (2 times), total phosphorus (P_2O_5) and potassium (K_2O)

(Lyashchev et al., 2022*a*). In addition, zoohumus contains growth stimulants, biologically active substances that increase plant resistance to diseases and pests, chitin, as well as growth biostimulants (Ravi et al., 2020).

Thus, a large array of data is accumulated on the technology of obtaining zoohumus using *H. illucens* larvae, the composition and properties of the resulting product, and its effect on the physicochemical properties of the soil. However, there is still no data on the effect of zoohumus on the toxicological properties of soils under natural conditions. Obtaining such data using model plants and other test organisms is necessary to justify the doses of fertilizer application and its environmental safety.

The purpose of this work was to conduct field tests of *H. illucens* zoohumus in a series of different dosages, determine its phytotoxicity for soft spring wheat *Triticum aestivum* L. and soil ecotoxicity in bioassays for *Escherichia coli* and *Paramecium caudatum*.

MATERIAL & METHODS

The colony of insects for zoohumus production was cultivated in the Industrial Entomology Laboratory of the Northern Trans-Urals State Agrarian University (Tyumen, Russia). The colony was maintained at a temperature of 26±0.5°C, relative humidity of 65–75%, and a photoperiod of 16:8 L:D h (Lyashchev et al, 2022 *b*). The larval diet was chicken feed for chickens, with the addition of an equal volume of water (Sheppard et al., 2002). The zoohumus used in the experiment was accumulated for six months. Before adding to the soil, the fresher and older zoohumus were mixed.

The microplot field experiment was conducted on low-fertility arable soil. The experimental area was a wasteland overgrown with weeds. The site preparation included plowing and clearing weeds (manually). The humus content was measured by Tyurin's method in modification by the Central Institute for Agrochemical Surveys (TsINAO) (GOST (State Standard) 26213-91). This method is based on the oxidation of organic matter with a solution of potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid and the subsequent determination of trivalent chromium equivalent to the content of organic matter using a spectrophotometer at a wavelength of 590 nm. The content of organic matter (in percent) is calculated using a calibration graph constructed using mixtures of oxidizer and reducer taken in concentrations specified by the method. The content

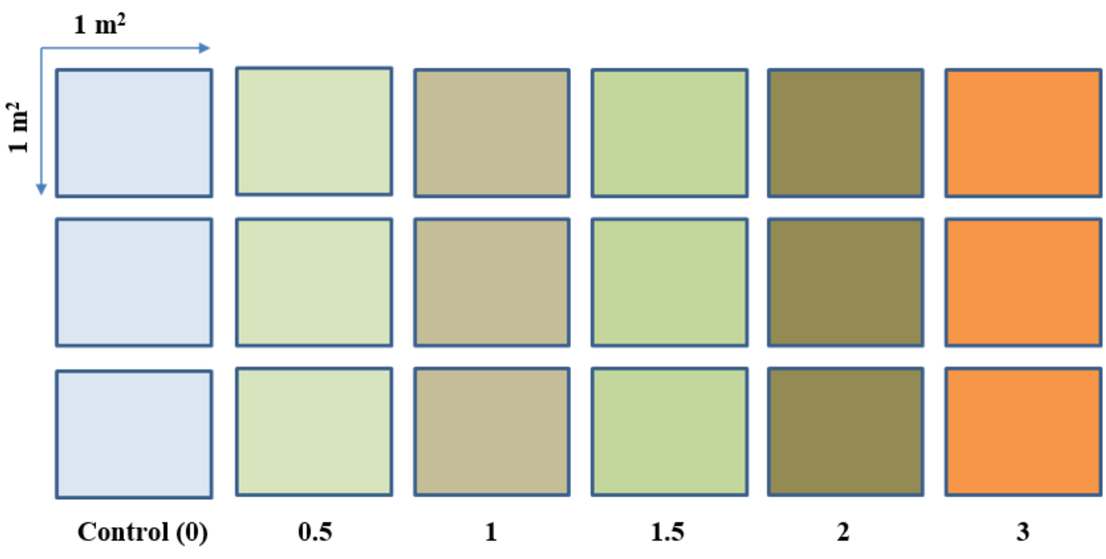

Fig. 1. Overall design of Experiment, zoohumus concentration, kg/m²

Table 1. Climate monitoring during field work in 2023 (according to the website "Weather and Climate[1]")

	Average monthly temperature norm, °C	Actual temperature, °C	Norm of precipitation amount, mm	Actual precipitation amount, mm
May	12.0	14.9	44.0	1.0
June	17.0	16.5	61.0	88.0
July	18.7	22.0	86.0	55.0
August	16.1	16.7	60.0	21.0
September	10.0	12.4	45.0	9.0

[1] Weather Monitoring Site: www.pogodaiklimat.ru

of organic matter in the soil is 2.3%. The granulometric composition of the soils (0-15 cm) was represented by a fraction of physical sand (91%), lithomorphic (7%) and biomorphic (2%) inclusions. Zoohumus was added to the soil in doses of 0.5, 1, 1.5, 2, 3 kg/m^2 (corresponding to 5, 10, 15, 20 and 30 t/ha). We did not apply zoohumus or other fertilizers to the control areas. The area of each microplot was 1 m^2, the experiment was repeated three times (Fig. 1). The distance between the test plots was at least 50 cm. 30 g of soft spring wheat *T. aestivum* seeds were added to each plot, which corresponds to approximately 750 grains. The initial seed germination was 96±4% (experiment in 5 repetitions, 25 seeds each).

Climate conditions have a significant impact on the growth and development of wheat sprouts in the vegetative and generative stages of ontogenesis. Lack of moisture and heat can significantly reduce seed germination. The table shows climate monitoring data from May to September in Tyumen (table).

The deviation from the norm of average monthly temperature was from -0.5°C in June to +3.3°C in July. The summer period was generally characterized by reduced precipitation, in particular in May, July and August. In order to avoid the death of seedlings in sandy soil during periods of insufficient moisture, the soil was watered as the soil dried out.

The effect of *H. illucens* zoohumus on the field germination of wheat seeds and the growth of seedlings after sowing (day 14) and before harvesting (day 117) was studied in comparison with the control. To do this, on the 14th day of the experiment, all visually detectable shoots were counted, and at the end of the experiment, the number of productive shoots was counted by counting ears of wheat.

Soil bioassays was carried out at the end of the experiment. Soil samples were collected using the envelope method (Kapanadze et al., 2019). Eluate bioassays for acute toxicity by reactions of microorganisms *Paramecium caudatum* Ehrenberg and *Escherichia coli* Migula were carried out. To do this an aqueous extract was prepared using natural water and air-dry soil 1:4. The chemotaxis of *Paramecium caudatum* Ehrenberg was assessed using the Biotester device (Russia) (FR 1.39.2015.19241, 2015) and the bioluminescence of a bacterial preparation based on *Escherichia coli* Migula was assessed using the Biotox-10M device.

The test function of *P. caudatum* is chemotaxis, the device was a Biotester (Russia) (FR 1.39.2015.19241, 2015). The test function of *E. coli* was bioluminescence, the device was Biotox-10M (Russia) (PNDF T 14.1:2:3:4.11-04, 2010). The test reaction time was 30 minutes. Water extracts from the soil (distilled water) were tested, with a phase ratio of 1:4, the contact time of water and soil was 2.5 hours, including 2 hours on a rotator (100 revolutions per minute) and 30 minutes settling. The filler liquid was filtered through a decontaminated paper filter. The reactions of microorganisms to extracts from the soil and the cultivation medium were compared. The formula was used for calculations:

$$T = \frac{T_c - T_{ex}}{T_c}$$

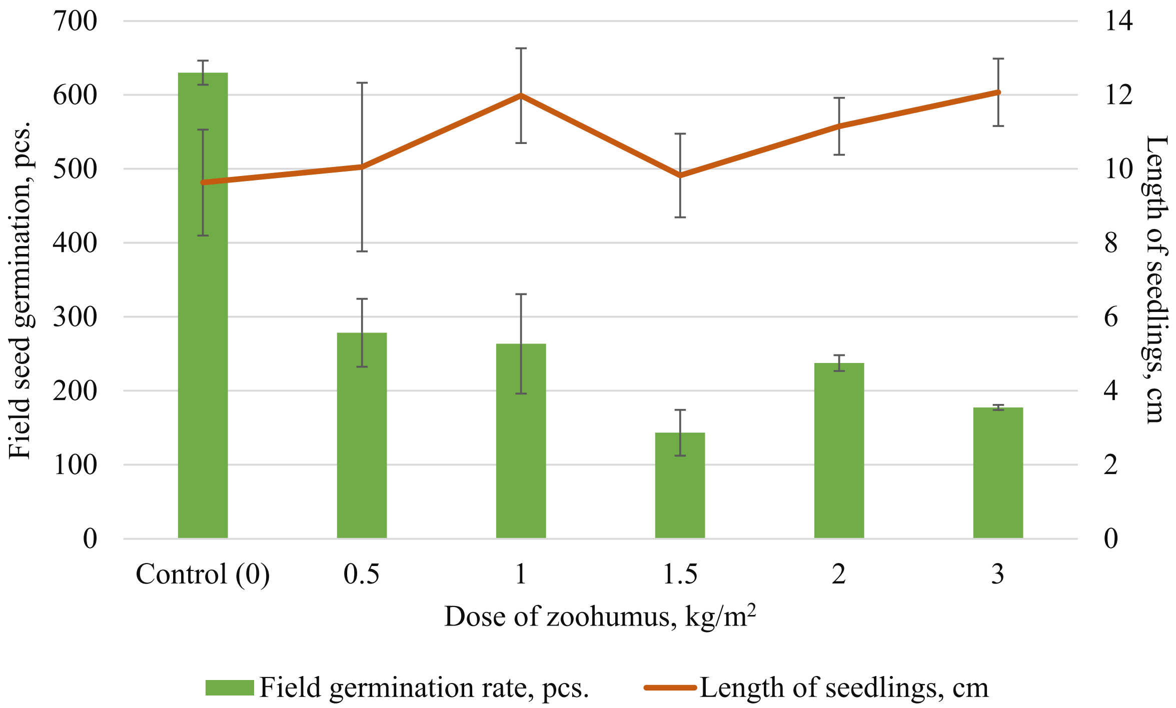

Fig. 2. The influence of *H. illucens* zoohumus on the field germination of wheat seeds and the length of seedlings on the 14th day of the experiment

where T_c is the toxicity index for a relatively clean environment –control, T_{ex} is the toxicity index for the tested environment.

Statistical processing of the results was carried out in R version 4.4.2. The results of measurements and other experimental data had a normal distribution. Next, the relationship of the indicators was determined by the Pearson linear correlation coefficient (r), p = 0.05. The results were presented as x±SD, where x is the average value and SD is the mean square deviation of the parameter.

RESULTS AND DISCUSSION

The effects of different zoohumus additions to the soil on the growth and development of wheat seedlings were studied. Field germination and seedling growth were assessed 14 days after sowing (Fig. 2).

The first assessment of plant response to zoohumus addition to the soil (14 days after sowing) showed that field germination of wheat was reduced in all experimental variants (fig. 2). The decrease in the indicator was from 56 to 77% compared to the control. The most pronounced inhibitory effect was noted in the variant with a dosage of 1.5 kg of zoohumus per experimental plot, as well as 3 kg, where germination was 23 and 28% of the control, respectively. The relationship between the dosage and the value of the field germination indicator is confirmed by a strong inverse correlation of these indicators (r = -0.72).

Despite a decrease in field germination of seeds, further study of developing shoots showed that their linear dimensions do not decrease (fig. 2). On the contrary, in all experimental variants with the use of zoohumus, an increase in plant growth was noted: the average values varied from 9.82 to 11.98 cm, while the average length of shoots in the control was 9.63±1.43 cm. A direct correlation of the average degree "length of shoots – weight of the additive" was revealed (r = 0.67). The maximum effective addition of zoohumus was 3 kg per 1 m^2. Such application of fertilizer increased the size of shoots by 25% relative to the control ($p < 0.05$). In the other variants, no reliable differences in this indicator were found, but the tendency to increase the length of shoots when adding zoohumus to the soil remained.

The primary increase in soil phytotoxicity as a result of the application of organic fertilizer is known. The work (Santolin et al., 2024) showed that 9 out of 17 studied organic fertilizers are products with restrictions, and their use is possible in combination with mineral fertilizers. A decrease in germination under the influence of an aqueous extract of black soldier fly zoohumus was previously recorded in the work of Pushkareva et al. (2023). At a dosage of zoohumus over 5% in an aqueous substrate on which wheat was germinated, inhibition of germination and inhibition of root growth were noted in direct proportion to the dosages. Such phytotoxicity, as well as toxicity due to field germination in our case, can be associated with both the chemical structure of zoohumus (Rani et al., 2022; Fuhrmann et al., 2022), its physical properties, in particular the ability to retain moisture, and with the completeness of the processing of organic matter and its "maturity" (Chiam et al., 2021; Meyers et al., 2024).

Thus, the initial decrease in field germination could be due to the increased content of ammonium and salts in zoohumus; it is known that at a dosage of 15-20 t/ha, black soldier fly zoocompost can have toxic effects (Alattar et al., 2016; Chiam et al., 2021). During the processing of organic matter by black soldier fly larvae, a fairly large amount of ammonia is formed (Parodi et al. 2020; Boakye-Yiadom et al., 2022), which can reduce germination (Haden et al., 2011; Beesigamukama et al., 2021). Therefore, fresh zoohumus, rich in ammonium, could have increased phytotoxicity until the ammonia concentration decreased.

Simultaneous activation of some plant parameters and inhibition of others at the early stages of their development may be due to the ability of the zoohumus microbiome to produce volatile organic compounds (VOC) (Rani et al., 2022). VOC are compounds with a low molecular weight (<300 Da) that can spread through soil and air due to their high vapor pressure and ability to diffuse through air and water-filled pores over fairly long distances. The effect of volatile organic compounds depends on their concentration and can have a positive or negative effect on plant growth (Meldau et al., 2013; Park et al., 2015). For example, benzonitrile exhibits herbicide properties. Bailly and Weisskopf (2012) described in their studies that the growth of *Medicago sativa*, *A. thaliana* and *N. tabacum* was inhibited by dimethyl sulfide secreted by soil bacteria of the genera *Stenotrophomonas, Chromobacterium* and *Burkholderia*. Considering that zoohumus is enriched with the microflora of the digestive tract of black soldier fly larvae, its introduction into the soil, where its own natural microbiome is formed, could cause active production of VOC as a protective reaction by soil and zoohumus microbes until the latter is completely assimilated.

The chemical composition of zoohumus also depends on the completeness of the processing of organic residues by larvae. Immature compost often contains phytotoxins, including phenolic compounds, organic acids and excessive amounts of salts, which can negatively affect plant growth (Luo et al., 2018).

The initial decrease in seedling density reduced competition for resources, which was reflected in the activation of seedling growth compared to the control. This was probably also facilitated by the gradual mineralization of zoohumus components in the soil.

The morphophysiological parameters of soft spring wheat were re-evaluated at the end of the experiment – at the harvest stage (117 days). It was found that the use of high doses of zoohumus (3 kg per experimental plot) did not lead to a change in the number of plants in this variant compared to the control (Fig. 3). Moderate doses (0.5–2 kg/m^2) stimulated the development of wheat plants. On average, the number of shoots in these variants exceeded the control data by 27%. The plants grown using Black Soldier fly zoohumus were characterized by the development of two productive stems from one seed, which explains the quantitative excess of shoots over planting material. We explain this phenomenon by the increased nitrogen

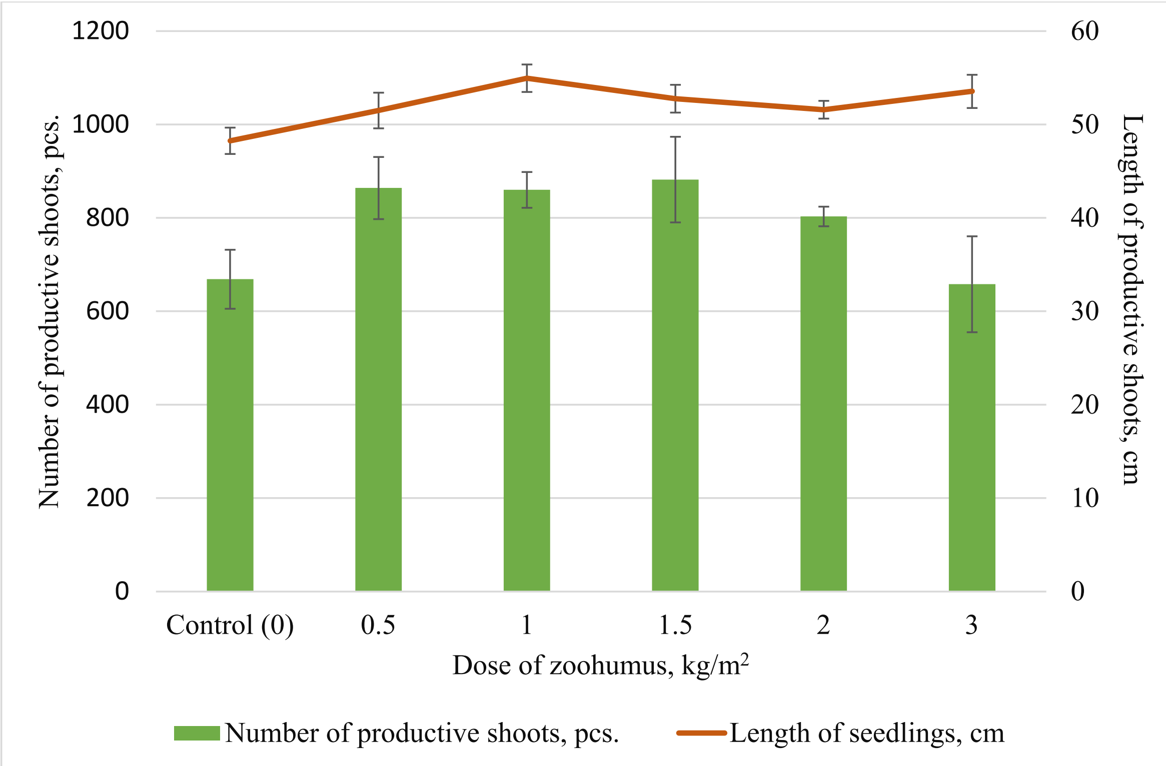

Fig. 3. The influence of *H. illucens* zoohumus on the number of productive shoots of wheat and their length at the end of the experiment

content in the soil, which is necessary for plants to form proteins and amino acids, as well as for cell division.

The maximum positive effect on the number of shoots was observed with a zoohumus dosage of 0.5 to 1.5 kg/m^2 ($p<0.05$). Interestingly, it was the addition of 1.5 kg/m^2 at the early stage of seed development that inhibited their germination to the greatest extent. This effect can be characterized as time-dependent. The inversion of the plant response to zoohumus during ontogenesis is associated with its gradual biodegradation. On the one hand, there is a dispersion of herbicide-like and synthesis of growth-stimulating VOC, ammonia hydrolysis, and on the other hand, the supply of available nutrients to plants is activated, moisture is released, and the soil microbial community is rebuilt. The stimulating effect of zoohumus on plant development was previously noted (Pushkareva et al., 2023). In this study, zoohumus extracts accelerated the formation of the third leaf in wheat, the entry into the earing stage occurred 10 times faster than in the variant without zoohumus, the amount of dry biomass was 60% higher than in the control.

By the harvest stage, it is obvious that zoohumus stimulated the linear dimensions of soft spring wheat (fig. 3). The most pronounced effect on wheat growth was demonstrated by the addition of zoohumus at 1 and 3 kg/m^2 – 13.8 and 11% higher than the control, respectively ($p<0.05$). The stimulation is associated with the gradual mineralization of zoohumus, the consumption by plants of released macro- and microelements, as well as available organic substances. The least effect was observed with the addition of zoohumus (0.5 kg/m^2), where growth activation was not significant. However, no clear dose-effect relationship was found, the correlation coefficient (r) was 0.55, indicating medium correlation strength. The literature contains works on the effect of black soldier fly zoohumus on the growth of plant organs, and our study is consistent with the general trends (Rosmiati et al., 2017; Beesigamukama et al., 2021; Abdessan et al., 2024).

According to the analysis of the morphophysiological parameters of the experimental wheat, the optimal dose of zoohumus application is 1 kg/m^2. Such an additive leads to the germination of the largest number of seeds, maximum growth of seedlings at the first stages of development and at the harvest stage.

Tests on unicellular organisms of different systematic affiliation were chosen for bioassay, since it is unicellular organisms that form the basis of edaphon. The results obtained are shown in Figures 4 and 5.

In the bioassay on ciliates *P. caudatum*, the control sample had a slight inhibition of the chemotaxis of the organisms (fig. 4). According to the method used, the values of toxicity indices below 0.40 c. u. belong to toxicity group I – non-toxic. Minimal zoohumus additives equal to 0.5 and 1 kg/m^2 did not significantly affect *P. caudatum* ($p>0.05$). A further increase in the additive led to a natural decrease in toxicity indices, i.e., stimulation of the vital activity of protozoa compared to the control levels and small additives. If we extrapolate the effect to soil communities, it can be called an adaptation to an increase in the proportion of introduced organic matter in the soil. Ciliates in the soil community are predators, and they respond positively to an increase in organic matter, since an increase in its concentration is followed by a natural increase in the population of bacteria and microfungi – their food base. Such cascade effects have been demonstrated in the case of soil application of polycyclic aromatic hydrocarbons: protozoa indirectly modulated PAH degradation by regulating bacterial and fungal communities (Du et al., 2024).

Cascade effects are extremely difficult to predict and are best avoided in any ecosystem. In terms of organic fertilizer, the best-case scenario is to maintain toxicological parameters at the control level. Therefore, the *P. caudatum* bioassay showed that the optimal dose of zoohumus application is 0.5–1 kg/m^2.

In the bacterial bioassay, significant stimulation of bioluminescence of *E. coli* was observed. It is known that bacteria are the first to respond to an increase in the proportion of organic matter in the soil, followed by fungi and protozoa (Yang et al., 2021; Porras-Alfaro, 2024). In general, this confirms the hypothesis given above. This is a typical situation for soil bioassay,

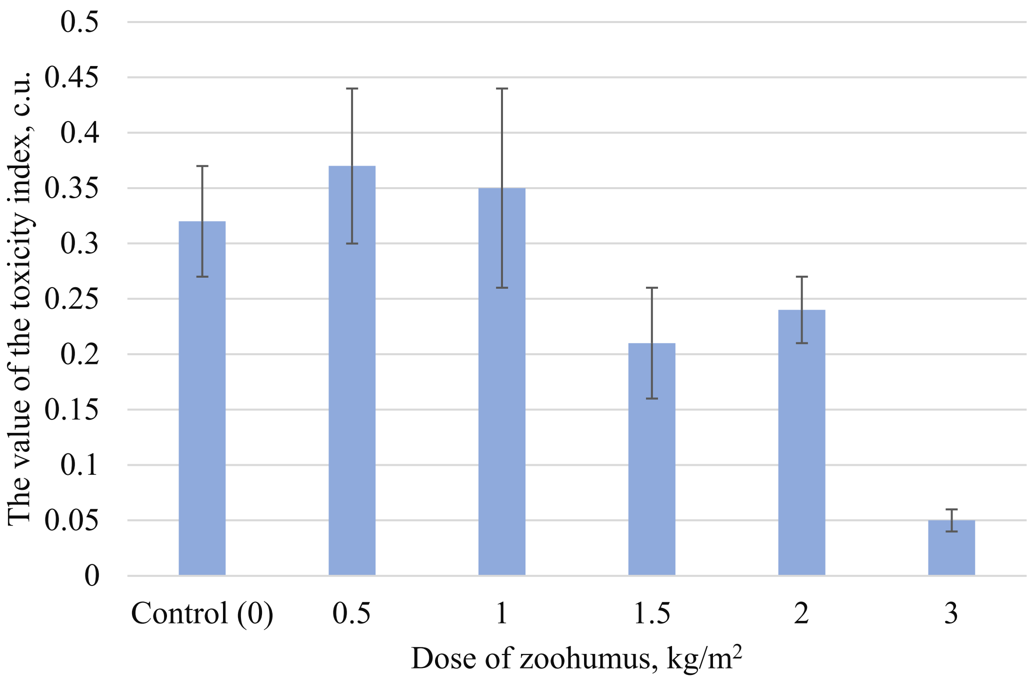

Fig. 4. Responses of *P. caudatum* to aqueous extracts of soils treated with *H. illucens* zoohumus

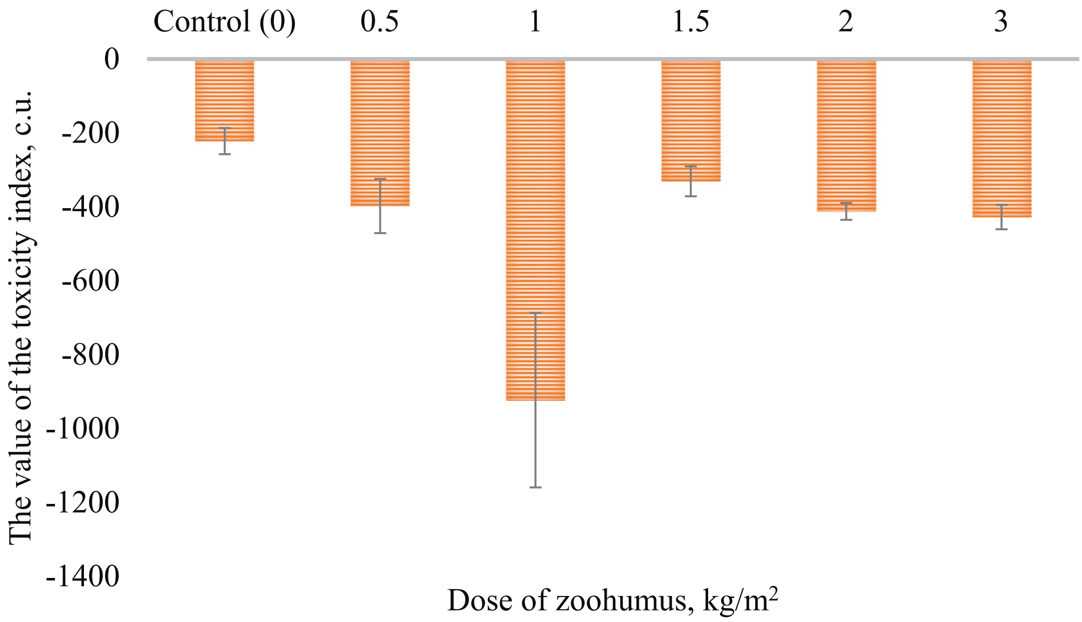

Fig. 5. Responses of *E. coli* to aqueous extracts of soils treated with *H. illucens* zoohumus

since natural mineral and organic substances migrating into the aqueous extract from the soil affect bacteria by the hormesis type. An increase in stimulation was observed in response to the introduction of 0.5 and 1 kg of the preparation per 1 m^2 of soil: 1.8 and 4.2 times compared to the control ($p < 0.05$). Then the toxicity indices decreased, approaching the control values. At the same time, bacterial inhibition relative to the control was not observed, which indicates the absence of toxicity. There are studies where hormetic reactions of organisms are presented as a mechanism for increasing resistance to pollution (Fan et al., 2021; Erofeeva 2023). Therefore, the use of zoohumus in doses above 1.5 kg/m^2 is not recommended, as it neutralizes this function.

The two conducted bioassys confirmed that when applying 1.5 kg/m^2 of zoohumus or more, there is a transition from the positive effect of zoohumus on the soil to a gradual decrease in the effect. Therefore, to improve the properties of poor soils, an application rate of 0.5–1 kg/m^2 of zoohumus is suitable.

An effective dose of fertilizer must be environmentally safe. According to the standard adopted in Russia for organic fertilizers based on livestock products (IS 33830—2016, 2018), organic fertilizer should not contain benzo(a)pyrene, polychlorinated biphenyls, pathogenic and disease-causing microorganisms, including enterobacteria (pathogenic serovars of E. coli, salmonella, proteus), enterococci, staphylococci, clostridia, bacilli, enteroviruses, protozoa, helminth eggs. They may contain small impurities (mg/kg) of heavy metals (2–130), pesticides (0.1), coliforms and enterobacteria. According to these indicators, as well as the minimum content of nitrogen, phosphorus and potassium in total, zoohumus of black soldier fly larvae complies with this standard. However, a standard for zoohumus *H. illucens* has yet to be developed.

CONCLUSION

The presented work confirms that zoohumus *H. illucens* is a promising fertilizer for poor soils. In addition to growth stimulation, we noted the development of double spikelets, which contributes to obtaining the greatest yield. The economic benefit from using this fertilizer is also obvious: the rate of compost or manure application is 30 to 100 t/ha, and zoohumus *H.*

illucens will require 3-10 times less.

An effective safe dosage of zoohumus was established - 1 kg / m^2 (10 t/ha) based on the responses of spring soft wheat and test organisms *P. caudatum* and *E. coli*. Higher dosages either inhibited growth or reduced germination, and also suppressed the activity of test microorganisms. A lower dosage was also insufficient for reliable growth of seedlings.

Besides, it was shown for the first time that black soldier fly zoohumus in field conditions is characterized by an inhibitory effect on the field germination of spring soft wheat. During the vegetation process, an inversion of plant responses occurs. As a result, the morphophysiological indicators of plants grown on soils with the introduction of zoohumus exceed the control data.

GRANT SUPPORT DETAILS

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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