Development of effective substrates for growing *Hermetia illucens* larvae with a high protein content and assessment of the effect of the obtained zoocompost on phytonematode

N A Ushakova¹, Zh V Udalova¹, S V Zinovieva¹ and N Yu Garmash²

¹A N Severtsov Institute of Ecology and Evolution of RAS, 33, Leninskij prosp., Moscow, 119071, Russia
²Federal research center “Nemchinovka”, 6, Agrokhimikov St., Novoivanovskoye, Odintsovsky District, Moscow Province, 143026, Russia

E-mail: udalova.zh@rambler.ru

**Abstract.** The larvae of the black soldier fly *Hermetia illucens* are actively used for bioconversion of a wide range of organic residues, and the insects themselves are an excellent source of feed protein, and waste products can be used as fertilizers. It is noted that the excrement of larvae and the residual mass of undigested substrates significantly affect soil biota. In this article, the effect of the composition of feed mixtures for the life of *H. illucens* larvae on the protein-fat composition of larvae, the biophilic composition of the obtained composts and the possibility of using zoocompost to control one of the most pathogenic types of root-knot nematodes on tomatoes have been studied. It has been shown that feed mixtures based on potatoes, apples, corn, and including sunflower meal, wheat bran or pine sawdust make it possible to obtain competent larvae with a high protein content. All obtained zoocompost significantly suppresses the development of the nematodes *Meloidogyne incognita*. However, mixing coniferous sawdust in the feed mixture reduces the conversion of the substrate, and the resulting zoocompost does not have a growth-promoting effect on tomato plants.

1. **Introduction**

The larvae of *Hermetia illucens* are phytosaprophages that use organic substances as feed substrates, including various wastes from food and agricultural industries [1]. Bioconversion of organic substrates using *H. illucens* allows solving not only the problem of waste management, but also obtaining a protein feed product for farm animals and aquaculture, as well as bio-fertilizer (zoocompost). Obtaining fodder protein of insects, including the larvae of the black soldier fly *H. illucens* on an industrial scale is a promising innovative direction in the world [2, 3]. For Russia, the value of insect protein production has increased significantly due to the need to saturate the country's domestic market with domestic agricultural products. An important condition for the successful implementation of the task is the development of effective and cost-effective feed mixtures for growing *H. illucens* larvae, which is associated with the study of the growth and development of the insect on various substrates, assessment of substrate conversion and biological significance of metabolic products - excretory secretions that determine the properties of the obtained zoocompost. Zoocompost formed by the larvae of the black soldier fly *H. illucens* consists of the remains of an undigested feed substrate, excrement,
and the remains of the insect’s chitinous outer cover during metamorphosis. It was noted that zoocompost is promising for use as a stimulator of plant growth and development [4, 5]. Enriching the soil with various biologically active substances using zoocompost can change soil biota, as indicated by the aggressiveness of *H. illucens* larvae and their ability to eliminate pathogenic bacteria and fungi [6]. The biologically active substances of excretory secretions of larvae may contain compounds that directly inhibit the development of soil organisms, as well as compounds that induce plant resistance to pathogens. Phytoparasitic nematodes belong to the most pathogenic soil organisms. They cause losses of about 12.3% of global agricultural production [7], while the economic damage exceeds $125 billion per year [8]. The decrease in the yield of field, vegetable, industrial, fodder, and fruit crops caused by phytohelminths can be up to 90%. In addition to a direct decrease in yield, they carry viruses, fungal and bacterial diseases, reduce the effectiveness of mineral and organic fertilizers, lead to mass death of plants in drought and overwinter, reduce the quality of the crop, causing rotting of food products with bacteria and mushrooms. Effective and environmentally friendly ways to limit the development of these parasites are absent. The use of organic substrates as a safe tool for controlling parasitic plant nematodes is proposed [9].

In this article, the possibility of growing larvae of the black soldier fly *H. illucens* on feed mixtures containing organic waste, with an assessment of the composition of the obtained larvae and the characteristics of zoocompost, including their effect on the phytonematode, has been investigated.

2. **Research materials and methods**

Rearing of *H. illucens* larvae was carried out according to the standard method [10] on theoretically developed feed mixtures using substandard fruit and vegetables components and grain components, dm, %: option No. 1: potatoes - 30, apples - 30, crushed corn - 14, sunflower meal - 10, coniferous flour sawdust - 5, skim milk - 11; option No. 2: potatoes - 23, apples - 24, wheat bran - 6, crushed corn - 10, sunflower meal - 9, feeding yeast - 5, water - 23; option No. 3: potatoes -24, apples - 25, crushed corn - 17, sunflower meal - 8, feeding yeast - 5, water - 21.

Substrate conversion is the difference between the dried initial feed substrate and the dried residue obtained after separation of the grown larvae, expressed in % of the dry initial mass [11]. All experiments were performed in 3 replicates. In samples of zoocompost, indicators characterizing the intensity of the processes of microbial transformation of a number of biophilic elements were studied [12]. To assess the ability of 3 types of zoocompost to influence the development of tomato plants and suppress the development of the root-knot nematode *Meloidogyne incognita*, 1.5-month-old seedlings of F1 Gamayun tomatoes susceptible to the root-knot nematode (resistance index to the root-knot nematode ~ 30%) were planted in separate 500 ml vases in soil for vegetable and flower crops (Peter Peat universal). At the same time, on options, zoocompost was introduced under the root system of tomatoes at a dose of 13 g per plant. The dose is selected based on the previously obtained results of experiments with zoocompost based on the vital products of a black soldier fly *H. illucens*. At the same time, plants were infected with the root-knot nematode *M. incognita* in the amount of 1000 larvae per plant. Infected plants without treatment by zoological compost were considered as a control group. It was repeated tenfold. Plants were grown in laboratory conditions at a temperature of 25°C, maintaining 70% humidity and a photoperiod of 16/8 hours. After 40 days, the biometric parameters of plants (mass and length of aboveground organs) were taken into account and the degree of infection of the root system with the root-knot nematode on a 4-point scale was assessed [13]. Using a Carl Zeiss stereoscopic microscope, the infection of the root system of *M. incognita* tomatoes (number of galls per gram of root) and the morphological and physiological state of nematodes (size of females and their fertility) were evaluated. Statistical processing was carried out in the software package Statistica 12.

3. **Results and discussion**

An analysis of the influence of the feed substrate on the development of larvae showed that all tested feed mixtures yielded approximately the same biomass of larvae within 160-170 g of dry larvae per 1
kg of dry weight of the initial feed mixture (table 1). The difference is not significant, \((p > 0.05)\). The conversion of the substrate was at the level of 55-60\%, the difference is not significant \((p > 0.05)\). It should be noted that the substrate in option No. 1 was subjected to biological conversion to a lesser extent, which is possibly due to the introduction of indigestible coniferous sawdust containing lignocellulose into the feed mixture.

**Table 1.** The effect of the substrate on the biomass of *H. lucens* larvae and its conversion processes.

| Option No. | Biomass yield, green weight, g one-kg dry substrate | Biomass yield of dm, of one-kg dry substrate | Conversion of substrate,\% |
|------------|--------------------------------------------------|---------------------------------------------|---------------------------|
| 1          | 536.1±11.0                                       | 160.8±3.3                                   | 55.4±2.2                  |
| 2          | 522.9±14.3                                       | 156.9±4.3                                   | 58.6±1.2                  |
| 3          | 579.3±22.6                                       | 173.8±6.7                                   | 59.8±3.6                  |

The biochemical composition of the larvae also basically did not differ significantly (table 2). There was 40-45\% of, about 30\% of fat, 14-16\% of chitin and sugar. However, the phosphorus content was significantly higher in option No. 1 with coniferous sawdust.

**Table 2.** Biochemical composition of larvae on different feed substrates.

| Indicators, % | Option, No. | Method of determination |
|---------------|-------------|-------------------------|
|               | 1           | 2                       | 3                       |
| Protein       | 39.32±4.8   | 44.81±3.5               | 40.89±3.8               | GOST 32044.1-2012         |
| Fat           | 30.81±6.4   | 26.77±5.2               | 29.19±6.7               | GOST 32905-2014           |
| Chitin        | 16.12±3.4   | 14.08±2.9               | 15.97±3.2               | GOST 31675-2012           |
| Starch, sugar | 13.75±2.8   | 14.34±5.1               | 13.95±4.9               | Calculation method        |
| Phosphorus    | 0.84*±0.02  | 0.40±0.01               | 0.35±0.01               | Atomic absorption method  |

* \(p \leq 0.05\)

All zoocompost obtained after separation of the grown larvae from the remains of the substrate contained a complex of biophilic elements and were enriched in phosphorus, nitrogen, and potassium (table 3).

**Table 3.** The total content of biophilic elements in zoocompost.

| Option | Gross forms | Active forms | |
|--------|-------------|--------------|-----------------|
|        | P           | K            | N               | C               | C/N          | P             | K             | N-NH3          | |
|        | % dry weight| mg/100 g     |                 |                 |               |               |               |                |                |
| 1      | 5.3         | 2.24         | 2.3             | 41.12           | 1.79          | 863.00        | 4235.00       | 1545.00        |                |
| 2      | 3.5         | 2.10         | 1.06            | 41.68           | 3.93          | 950.00        | 5269.00       | 2293.00        |                |
The treatment of tomato seedlings with zoocompost in the studied doses not only did not inhibit the development of plants (table 4), but in some cases had a growth-stimulating effect, which was expressed in an increase in the length of the indeterminate Gamayun hybrid and an increase in the green mass of the plant (options 2 and 3). Significant differences in biometric indicators of plants were only for 3 options (at p = 0.05). Apparently, despite the high content of essential elements in option 1, the presence of coniferous sawdust containing phytoncide terpenes did not stimulate plant growth, and they were at the control level in this option.

Regarding the infection of the root system with the root-knot nematode, all options showed an inhibitory effect on the penetration and development of the nematode. The infection score in the treatment options decreased by 1.8-3 times. In the control option, there is a significant formation of isolated galls along the entire length of the root, as well as large fused galls - singalls, which indicates a massive infection of the roots. In the treatment options, isolated galls prevailed in the first third of the root length, and not along the entire length. And only on some roots there were small singalls. All treatment options for infection rates significantly differed from the control (at p = 0.05). It should be noted that the ratio of the weight of the root to the weight of the aerial organs in the control option was 2 times higher, since the roots were much heavier due to the formation of numerous galls, compared with the treated plants.

As can be seen from table 4, the number of galls formed on the roots, depending on the treatment option by zoocompost, was 1.5–3.5 times less. The size of galls also significantly differed in the case of treatments of infected plants, so the average gall size decreased by 3.2–4.3 times (at p = 0.05). Indicators of inhibition of the development of the nematode are the size of the female and its fertility. From table 4 it is seen that these indicators were significantly reduced for all treatment options (at p = 0.05).

Table 4. The effect of zoocompost obtained using H. illucens larvae on the biometric parameters of tomatoes, their infection with M. incognita and the development of the nematode.

| Treatments option | Height of plant, cm | Weight of overgrown organs, g | Weight of roots, g | m<sub>root</sub>/m<sub>overgrown organs</sub> | Infection score | Number of galls /g | Size of galls, mm:mm | Size of female, mm:mm | Number of eggs in the egg sack |
|-------------------|---------------------|--------------------------------|-------------------|--------------------------------|-----------------|--------------------|---------------------|-------------------------|-----------------------------|
| control           | 28±6.6              | 8.5±3.72                       | 1.6±0.56          | 0.18                           | 3.2±0.60        | 252±99.5          | 11.7±4.77          | 0.33±0.047              | 167±14.4                    |
| 1                 | 31±10.2             | 8.2±5.21                       | 1.0±0.37          | 0.11                           | 1.1±0.50        | 71±29.5           | 2.7±0.69           | 0.28±0.008              | 79±17.1                     |
| 2                 | 36±13.1             | 9.0±4.71                       | 0.9±0.39          | 0.10                           | 1.6±0.72        | 122±27.1          | 2.8±0.95           | 0.25±0.025              | 65±14.3                     |
| 3                 | 41±9.6              | 11.2±3.45                      | 1.2±0.40          | 0.10                           | 1.7±0.48        | 163±23.5          | 3.6±1.68           | 0.25±0.016              | 80±10.6                     |

Thus, the introduction of zoocompost under tomato plants susceptible to the root-knot nematode significantly reduces the pathogenic effect of the nematode, which is reflected in a significant decrease in the formation of galls on the roots and inhibition of the development of the nematode itself.

4. Conclusion
In the laboratory, three economically attractive variants of feed mixtures were evaluated to obtain biomass of the larvae of the black soldier fly H. illucens based on substandard products, such as potatoes, apples, corn, and including sunflower meal, wheat bran or pine sawdust. All components are available on the Russian market in sufficient quantities. When developing on these feed mixtures, the larvae contained protein of 40% or more, and fat in the range of 25-30%, which indicates the potentially high protein-energy feeding qualities of the larvae. Zoocompost obtained after separation
of the larvae contained all the main nutrients with relatively high phosphorus content and can be used as an organic fertilizer to increase soil fertility. However, the properties of zoocompost are affected by the composition of the feed mixture on which the larvae were grown, which must be taken into account when developing a method for applying zoocompost and evaluating its effect on different types of plants.

Regardless of the composition of the substrate on which the larvae were grown, all zoological compost obtained was able to stimulate plant immunity to phytopathogenic nematodes and inhibit the development of these helminths. Zoocompost obtained by growing larvae on a phytosubstrate without bran was shown to be the most effective in terms of growth-promoting activity in combination with a root-knot nematode-inhibiting effect (option No. 3). However, mixing coniferous sawdust in the feed mixture reduced the conversion of the substrate, and zoocompost did not have a growth-stimulating effect on tomato plants.

The resulting zoological compost can be a promising tool for improving soil, protecting plants, increasing soil fertility and plant productivity; however, they need further research on different types of plants and in different dosages.

Using the technology of growing larvae of the black soldier fly H. illucens makes it possible to bio-utilize substandard fruit and vegetable and grain substrates to obtain feed protein of larvae and zoocompost with unique effectiveness against phytopathogenic nematodes.

Acknowledgements
This work was financially supported by the Ministry of Education and Science, project No. 075-11-2019-070.

References
[1] Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S., De Meulenaer B, Michiels J, Eeckhout M, De Clercqband P and De Smet S 2017 Sci Food Agric. 97 2594–600
[2] Diener S, Solano NMS, Gutiérrez FR, Zurbrügg C and Tockner K 2011 Waste Biomass Valor. 2 357–63
[3] Müller A, Wolf D and Gutzeit HO 2017 Z Naturforsch. 72 351–63
[4] Kalova M and Borkovcova M 2013 Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis. 61 77-83
[5] Cai M, Zhang K, Zhong W, Liu N, Wu X, Li W, Zheng L, Yu Z and Zhang J 2019 Waste and Biomass Valor. 10 265–73
[6] Lee J, Kim Y M, Park Y K, Yang Y C, Jung, B G and Lee B J 2010 J. Vet. Med. Sci. 80 736–40
[7] Sasser J N and Freckman DW 1987 Vistas on Nematology (Society of Nematologists) eds JA Veech and DW Dickson (Hyattsville MD) pp7–20
[8] Chitwood D J 2003 Pest.Manag. Sci. 59 748-53
[9] Kranti K V, Patil J A, Yadavi S and Patil J A 2019 Journal of Pharmacognosy and Phytochemistry 8 713-20
[10] Bastrakov A I and Ushakova O N 2014 Eurasian Union of Scientists 8 105–7
[11] Ushakova N A, Bastrakov A I, Karagodin V P and Pavlov D S 2018 Success in modern biology. 138 172–82
[12] Zvyagintsev D G 1991 Methods of soil microbiology and biochemistry (Moscow: Moscow State University) pp 47–58
[13] Zinovieva S V, Ozeretskoyuskaya O L, Iliinskaya L I and Vasyukova N I 1995 Rus. J. Nematol. 3 65–7