Recycling biological waste using the fly *Hermetia illucens*, environmental risks and biosafety for Russia

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Abstract. The processing of biowaste by fly larvae *Hermetia illucens* is a unique technology for recycling products of human life. Fly larvae grown from biological waste can be a suitable raw material in animal feed production. During the analysis of references it was found that *H. illucens* is not a pest, so cultivation of this plant species does not require increased precautions. This species is not classified as potentially invasive for Russia and is not listed as a quarantine species. This insect currently has an almost cosmopolitan distribution (except in cold regions) and no significant adverse environmental impacts have been identified for it. The production of *H. illucens* larvae has been found to have much less environmental impact (e.g., greenhouse gas emissions) than production of other protein feeds of animal origin. The production of *H. illucens* larvae may serve the purposes of sustainable development of the Russian agro-industrial complex. The industrial cultivation of *H. illucens* requires compliance with generally accepted safety measures to prevent people from swallowing the eggs of this insect.

1. Introduction
The Black lion cub (*Hermetia illucens* (Linnaeus, 1758)) (Diptera) has now been brought by humans to tropical, subtropical and temperate regions around the world. Biotechnology using the black lionfly fly allows for the recycling of solid organic waste. This converts waste into biohumus and feeds for animals with high nutrient properties. Therefore, the commercial importance of this species in the world and in Russia has increased significantly in recent years. Ecological and biological safety of growing this insect species is poorly studied. The introduction of even knowingly useful species can pose a certain threat to natural communities and biodiversity. It is necessary to analyze scientific literature to identify possible ecological risks of *H. illucens* cultivation on industrial scale in Russia.

2. Use of *H. illucens* flies in animal husbandry

2.1 Feeds with *H. illucens* fly larvae as an alternative to expensive feeds
Animal feed with *H. illucens* is now considered an alternative to fishmeal, which is used to feed carnivorous fish and added to the diets of other animals because of its high content of protein and lipids [1]. The relevance of fishmeal in aquaculture is well known, but high demand for various fish-
processing products has led to reduced production and higher prices for fishmeal, leading to the search for a low-cost alternative to this feed for fish farming [2]. *H. illucens* larvae enriched with omega-3 fatty acids are formed when the diet of these larvae is supplemented by fish by-products [3]. Such "enriched" larvae are a suitable food for fish that do not produce significant differences in growth in comparison with conventional fishmeal for trout feeding *Oncorhynchus mykiss* [4].

It is known that *H. illucens* larvae feed on a variety of organic substrates and are already used for waste processing [5]: manure, straw, food waste, faecal sludge, animal by-products, kitchen waste, etc. It should be noted that *H. illucens* larvae are considered suitable for feeding and have been studied in detail in this direction [2]. It is known that *H. illucens* is superior to crickets and worms in feed conversion ratio. The main property of these fly larvae is their ability to accumulate lipids from their diet. Therefore, the fly larvae can be processed into biodiesel and be an energy source. In combination with nitrogen-containing additives, this insect can be used as a fertilizer. The development time of the *H. illucens* larvae is more than three weeks, one larvae consumes a large amount of substrate and reaches a large size [6]. All these advantages make *H. illucens* a popular organism, which is actively introduced into modern production.

The *H. illucens* fly larvae turn organic waste into nutrient-rich biomass, which is actively used for feeding animals. It has been established that the diet affects all studied parameters of the larvae and products of their vital functions. However, it has previously been shown that regardless of the diet, larval fat consisted mainly of saturated fatty acids with a predominance of lauric acid [5].

2.2 Use of *H. illucens* larvae in bird feeds

This species of fly at the larva stage is a valuable source of nutrients, which allows it to be used as a feed additive for birds. Differences in growth characteristics and nutrient values of *H. illucens* larvae and pupae grown on plant waste have been analyzed and compared [7]. An increase in the expression level of genes of acetyl-SoA-carboxylase, fatty acid synthase, lipase, and acyl-SoA-dehydrogenase, which is associated with the active inclusion of lipid metabolism pathway, was found. It was found [7] that the larvae of the fifth age showed the greatest ability to bioconvert the substrate into its own biomass.

2.3 Application of *H. illucens* larvae as fish food

In today's scientific world, the impact of replacing fishmeal food with food containing *H. illucens* larvae is under study. It was shown that the inclusion of 10% of *H. illucens* larvae in the fish diet significantly improved growth rates compared with control variant, and significantly increased histometric measurements [4].

The experiment [6,8] in which Atlantic salmon (*Salmo salar* L.) was fed in a diet with partial or complete substitution of fishmeal for food with *H. illucens* larvae mixed with organic waste is known. The aim of the study was to evaluate the quality of fish fillets by studying their physical and chemical properties [9]. The results showed that the complete replacement of fishmeal with food with *H. illucens* larvae did not affect the physico-chemical properties of fish fillets [9].

2.4 Influence of *H. illucens* fly larvae-based feed on cattle health and productivity (cattle feed)

Major livestock companies today face problems associated with the preservation of young cattle, reduced productivity and high mortality caused by gastrointestinal diseases. The dairy period of feeding young cattle is the most important one for cattle breeding, as it is in this period that the animal body's need for external nutrients increases, as the cat body has not yet formed sustainable enzymatic systems. Melanin is a powerful antioxidant that can promote long-term storage of feed. Melanin is partially digested in the animal body and partially acts as an enterosorbent in the intestines of cattle. Researchers in their work used melanin protein-energy supplement derived from the larvae of the fly *H. illucens*. After feeding there was a positive dynamics of growth and development of young cattle: the state of intestinal microflora has improved, there was a decrease in cases of calf diarrhea [10].
3. Biosafety of *H. illucens* flies production

3.1 Environmental benefits associated with the use of organic waste as a raw material for growing *H. Illucens*

In animal feed, the main source of proteins and lipids are soybean products, fishmeal and fish oil. At the same time, fish stocks are declining and the potential for further expansion of agricultural land is already largely exhausted. The reduced availability and high cost of these resources have challenged the livestock sector to develop more sustainable and affordable alternatives. Insects are a potential source of valuable animal feed protein, which has become increasingly important in the last decade. Commercially growing insects require much less farmland than feed plants. Some insect species, including the black lion fly (*H. illucens* L.), can be grown on organic waste, which reduces environmental pollution [11]. Organic waste is a reservoir of bacteria, viruses, and parasites that can infect animals and humans [11]. The development and implementation of cost-effective and safe disposal methods is necessary to reduce the risk of disease spread. Common methods of waste disposal, including composting, incineration, anaerobic digestion with biogas production, are not always the most economically justified and environmentally safe [12]. Consequently, insects such as *H. illucens*, which can feed on biological waste, are of particular importance. In this case the waste is turned into high-protein feed. In addition, waste processed by insects can be used as organic fertilizer. Consequently, from an environmental perspective, insect production can be part of sustainable production systems with relatively low carbon dioxide emissions [13].

Feeds prepared from *H. illucens* have antimicrobial action and inhibit the multiplication of a number of pathogenic bacteria [11,14-16]. An additional advantage of the use of *H. illucens* larvae is that the processing of waste with their help not only reduces the smell of rotting organisms as a result of microbial processing and production of bacteriostatic, bactericidal, and fungicidal compounds, but also modifies the microflora of manure by reducing the content of harmful microorganisms and preventing egg-laying of other species of flies [17-19].

3.2 Ecologically safe products obtained with the use of *H. illucens*

A priority research topic for environmental safety is proving that there is no risk of contamination with pathogens in animals that consume feed prepared from *H. illucens* larvae that have been fed on potentially dangerous substrates such as biomass from slaughterhouses. Larvae that develop on manure, faeces, animal corpses and humans may present potential health risks to animals and humans. Manure is not considered to be a reservoir of pathogens such as cattle spongiform encephalitis, etc., but pathogens may persist in slaughterhouse waste treated by *H. illucens* and may persist in fly larvae. Until scientifically proven safety documents are available for products derived from *H. illucens*, European manufacturers are advised that insects used as animal or human feed should not be fed with manure and meat substrates [20].

Organic waste may contain persistent pollutants such as heavy metals. Cadmium may be accumulated in *H. illucens* larvae when feeding some waste. The rate of accumulation of this heavy metal in the prepupae varies from 2.32 to 2.94. The concentration of lead in the prepupae is much lower than its concentration in the feed. The pre-harvest zinc bioaccumulation ratio decreased as its concentration in the feed increased from 0.97 to 0.39. Accumulation of some heavy metals limits the use of *H. illucens* in bioconversion of cadmium contaminated waste into animal feed [15].

The development of larvae in chicken litter and other substrates subjected to bioconversion reduces the content of microorganisms, e.g., *Escherichia coli* and *Salmonella enterica*, and chemical contamination. In addition, microbial contamination can be eliminated during processing of insects, as well as during thermal treatment of the substrate, which is used to feed them at 120 °C for 45 minutes. To eliminate pathogens, *H. illucens* biomass can be processed by drying at 105-120°C [12,18,20,21]. Finished products should be checked for the presence of human and animal pathogenic microorganisms.
An important role in the decontamination of organic waste using black lion cubs is played by unique, in comparison with the microflora of other insects, bacteria in the intestines of larvae of this fly species. Bacterial communities contribute to the bioconversion of rotting organics to produce useful antibacterial, antifungal and probiotic compounds that can improve soil condition [19].

3.3 Prospects for the introduction of H. illucens into the natural surroundings of Russia

In recent decades, invasive foreign economic species of plants and animals, including insects, have been recognized as a significant international problem. For many alien species, no adverse impacts have been reported, but some insects can spread rapidly and have significant negative impacts on ecosystems, human health and are dangerous crop pests. Alien infestation is a critical factor in the transformation of natural ecosystems, reducing biodiversity and harming economies [22,23].

There is also increasing interest in Russia in the study of invasive species, the development of scientifically sound measures to conserve biological diversity and the reduction of negative consequences of biological invasions. In regions outside the natural range to which alien species are introduced, there is a need for additional research on reproduction, bioecological connections, ontogenesis, distribution methods and other features of the species' biology, as well as assessment of possible economic damage and development of measures to prevent it. These studies should be carried out not only for quarantine species, but also for potentially invasive species. Special attention should also be given to insects that are intentionally introduced into new areas.

The list of invasive plants in Russia includes 354 species. The unified list of quarantine objects of the EAES as of January 1, 2019 includes 233 quarantine objects, including 132 objects - insects and ticks. Of these, the list of quarantine objects distributed in the Russian Federation includes 42 objects, including 23 insect species [24]. With the increase in trade turnover between countries, an increasing number of alien species are being detected in different regions.

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The black lion cub (H. illucens) is not listed as a quarantine species, regulated non-quarantine pests, pests (plant pests, stock pests, etc.), pathogens or pathogen vectors. However, the construction of a facility for bioconversion of organic waste using this species of fly requires a comprehensive environmental risk assessment and the development of technologies that take into account environmental safety. This problem has been identified by the authors of several scientific works. For example, 14 alien species of two-winged flies (Diptera) have been registered in Slovenia, including H. illucens. Presumably, the main route of introduction of the black lion cub is the deliberate import as a biocontrol agent for domestic flies (Musca domestica L.) and animal feed. The potential impact of this species on natural biodiversity has not been studied [25,26].

In Panama, H. illucens is damaging the banana crop. Female species lay eggs on ripe yellow fruits. The larvae do not penetrate the fruit but leave stains on it, which makes it unfit for sale [27]. For Russia, this feature of H. illucens is not economically significant.

H. illucens is widespread in subtropical and temperate regions of Europe; acclimatization of this species in southern regions of Russia is not excluded. In 2019, this species was first recorded in Russia in the Krasnodar region [28]. It is possible that H. illucens may exist in natural conditions in the Crimea, Republic of Adygea, Krasnodar and Stavropol Territories, Karachay-Cherkessia, Kabardino-Balkaria, Chechen Republics, Republic of Ingushetia and Dagestan. In more northern regions, including the Belgorod region, acclimatization of Hermetia illucens is unlikely.

It has been noted that H. illucens successfully competes with harmful synanthropic species of flies and other species for substrates such as bird litter and corpses [29,30], so the impact of the species on coprophage biodiversity in natural biocoenoses is not excluded.

Specific parasitoids have been registered in Hermetia illucens [31]. The impact on biodiversity of foreign organisms that may be introduced together with Hermetia illucens also needs to be studied and should be taken into account when assessing the ecological risks of production.
3.4 Myasis
As a rule, adult species do not consume anything but water or nectar, do not approach people, do not bite, tolerate or spread any infectious diseases [20]. If imperfect technology is used and if safety precautions are not followed, *H. illucens* may present some danger to human health [20].

It has so far been established that while adult species, *H. illucens*, larvae and pupae are safe for humans, in rare cases their eggs may pose a certain danger. There are several cases of *H. illucens* larvae (intestinal myasis) and skin myasis infestation of the digestive tract. Infestation is likely to occur when eggs or fly larvae are swallowed on unwashed fruit and eggs get into skin wounds. The larvae are resistant to digestive enzymes and may develop in the gastrointestinal tract. Black lion larvae are also likely to cause miasis in animals [20,32-34]. Intestinal myases caused by *H. illucens* have been observed in a 7-year-old girl in Malaysia [34], a 26-year-old woman in Cuba [33], and a 71-year-old woman in Costa Rica [35]. Several cases of *H. illucens*-induced furuncular cutaneous myiasis have been reported in people infected in tropical countries, particularly in East Africa [32,35]. Thus, diseases associated with the black lion cub are extremely rare.

4. Conclusion
The processing of biowaste by *H. illucens* fly larvae is a unique technology for processing products of human economic activity. *H. illucens* is not a harmful organism, so the cultivation of this species does not require increased precautions. For Voronezh, Belgorod and more northern regions of Russia, this species is not classified as potentially invasive and is not listed as a quarantine species. The insect is currently almost cosmopolitan (except in cold regions) and no significant adverse environmental impacts have been identified. The production of *H. illucens* larvae has significantly less environmental impact (e.g., greenhouse gas emissions) than the production of other protein feeds of animal origin. Fly production may serve the purpose of sustainable development of the Russian agro-industrial complex. The industrial cultivation of *H. illucens* requires compliance with generally accepted safety measures to prevent people from swallowing the eggs of this insect.

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