Abstract

The main aim of this doctoral dissertation was to determine the possibility of using post-breeding waste (so-called frass) obtained during the rearing of the *H. illucens* insect as biofertilizers. However, since *H. illucens* larvae can be used for entomoremediation, including the utilization of waste and/or contaminated biomass, this research also addressed this aspect. Post-production residues of legume seeds, beans and peas, as well as mycotoxin-contaminated (molded) bean waste were used as the feed substrate for bioconversion by the larvae. This approach allows for the recycling of nutrients contained in the waste biomass through bioconversion, both to insect biomass and to post-breeding residues in the form of frass. The first publication (**P1**) is a review article aimed at demonstrating the potential applications of the *H. illucens* insect in both industry and agriculture. This work presents the growing interest in this insect genus in the scientific community, reflected in the steadily growing numer of publications, particularly since 2018. Rearing *H. illucens* generates biomass rich in proteins, fats, and chitin. The wide range of applications of this insect includes the production of protein and fat for animal feed. Additionally, the possibility of producing chitin and chitosan is being investigated, as well as the production of biodiesel from fats and biogas from post-breeding residues (frass – a mixture of uneaten food substrate, larval excrements, dead individuals, and larval cuticle (decayed after each molt stage) enriched with larval-associated microorganisms). The review also discusses the antimicrobial properties of insect extracts, as well as the larvae's ability to utilize and bioconvert waste biomass. Increased insect production on an industrial scale also results in an increase in the amount of frass, which can be used as an biofertilizer. A review of articles on frass as a biofertilizer identified gaps in knowledge and aided in developing the experimental portion of the research included in this doctoral dissertation.

The aim of the second publication (**P2**) was to characterize the basic fertilizing properties of frass, produced after bioconversion by *H. illucens* larvae of waste bean and pea seeds, which, due to their poor quality, were unsuitable for other purposes. This type of waste was selected because of its naturally high protein content (and thus high nitrogen content), which was expected to result in nitrogen-rich rearing residues. Additionally, the frass was subjected to an extended maturation process (lasting 10 months) in order to stabilize its properties. Fresh frass from the bean variant was rich in plant-available NH_4^+ ions and high 8

total N content, as well as macro- and micronutrients. However, it also possessed characteristics that could result in phytotoxic effects, namely, excessively high electrical conductivity (EC) and a low carbon/nitrogen ratio (C/N). Maturation of the bean frass led to positive changes in these parameters. In the case of fresh pea frass, it had a lower nitrogen content than the bean variant, but better EC and C/N values, which are within the optimal range according to the literature. Maturation of pea frass caused the C/N ratio to increase significantly, which may result in nitrogen immobilization in the soil environment.

The third publication **(P3)** aimed to demonstrate the entomoremediative features of *H. illucens* larvae on a substrate naturally contaminated with mycotoxins produced by the fungus *Fusarium* (present in a bean variant), by examining changes in the concentration and diversity of these compounds in larvae, fresh frass and frass after maturation. Despite the presence of mycotoxins in the substrate, studies have shown no accumulation in the larval bodies. The method of processing the contaminated biomass with larvae allows for the disposal of this type of waste, obtaining contamination-free larval biomass and reducing the concentrations of some mycotoxins. Bioconversion by the larvae led to a decrease in concentrations below the detection level of deoxynivalenol, monoacetoxyscirpenol, diacetoxyscirpenol, and T-2 toxin in the frass. The concentration of nivalenol also decreased, becoming undetectable after frass maturation. In the case of HT-2 toxin and zearalenone (and its metabolites α-zearalenone and β-zearalenone, which were not present in the substrate), their concentrations increased in fresh frass. Further measurements in matured frass revealed higher concentrations of HT-2 toxin and zearalenone, combined with a reduction in α- and β-zearalenone. This likely indicates additional fungal growth under the experimental conditions, which in turn can be prevented by changing the rearing conditions or pretreating the substrate. The reduction of some mycotoxins in frass, in the context of its use as a biofertilizer, is a positive feature, although there are no legal standards regarding maximum permissible mycotoxin concentrations in soil additives, and the dynamic of various mycotoxins in soil is still not well understood.

The aim of the fourth publication (**P4**) was to determine whether, and if so, which phytohormones were present in frass after *H. illucens* rearing. These results represent a comprehensive study of the presence of many phytohormones from the three most common classes of these compounds. It was noted that frass from bean waste, as well as the leachate (from frass) obtained during larval rearing, were characterized by high concentrations

of phytohormones such as indole-3-acetic acid, plant stress hormones, and cytokinins. These concentrations were higher than those from pea waste frass, with the exception of one cytokinin: cis-zeatin riboside. However, the pea frass leachate was rich in abscisic acid, jasmonic acid, trans-zeatin, trans-zeatin riboside, and cis-zeatin. Compared to control variants (without larvae), the phytohormone content was higher in variants with larvae present. It can be concluded that the bioconversion of bean and pea waste by *H. illucens* larvae not only allows for minimizing their quantity and obtaining fertilizer in the form of frass, but also increases its value as a plant biostimulant.

Keywords: black soldier fly, waste revalorization, biofertilizer, biostimulants, phytohormones, mycotoxins