INSTYTUT AGROFIZYKI
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WYKORZYSTYWANIE ODPADÓW PO HODOWLI *HERMETIA ILLUCENS* JAKO BIONAWOZÓW

USE OF WASTE FROM *HERMETIA ILLUCENS* REARING AS BIOFERETILIZERS

Rozprawa doktorska

Doctoral thesis

Rozprawa doktorska przygotowana pod kierunkiem

Promotora: prof. dra hab. Andrzeja Bieganowskiego

oraz

Promotora pomocniczego: dra inż. Piotra Bulaka

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OŚWIADCZENIE PROMOTORA ROZPRAWY

Oświadczam, że niniejsza rozprawa została przygotowana pod moim kierunkiem i stwierdzam, że spełnia ona warunki do przedstawienia jej w postępowaniu o nadanie stopnia naukowego.

Data..... Podpis promotora rozprawy.....

OŚWIADCZENIE PROMOTORA POMOCNICZEGO ROZPRAWY

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Data..... Podpis promotora pomocniczego rozprawy.....

OŚWIADCZENIE AUTORA ROZPRAWY

Świadomy odpowiedzialności prawnej oświadczam, że niniejsza rozprawa doktorska została napisana przeze mnie samodzielnie i nie zawiera treści uzyskanych w sposób niezgodny z obowiązującymi przepisami.

Oświadczam również, że przedstawiona rozprawa nie była wcześniej przedmiotem procedur związanych z uzyskaniem stopnia naukowego w wyższej uczelni.

Oświadczam ponadto, że niniejsza rozprawa jest identyczna z załączoną wersją elektroniczną.

Data..... Podpis autora rozprawy.....

Pragnę wyrazić najszczersze podziękowania
***prof. dr hab. Andrzejowi Bieganowskiemu**, promotorowi głównemu,*
*oraz **dr inż. Piotrowi Bulakowi**, promotorowi pomocniczemu,*
za profesjonalne prowadzenie, merytoryczne wsparcie
oraz liczne wskazówki, które pozwoliły na ukierunkowanie badań.
Dziękuję za cenny czas poświęcony na konsultacje,
wyrozumiałość oraz życzliwość okazywaną podczas wspólnej pracy.

Streszczenie

Zasadniczym celem niniejszej rozprawy doktorskiej było określenie możliwości zastosowania odpadów pohodowlanych (tzw. frassu), powstających podczas hodowli owada *Hermetia illucens,* jako bionawozów. Jednakże, z racji, że larwy *H. illucens* można wykorzystywać do procesu entomoremediacji, a więc m.in. zastosowania tych owadów do utylizacji biomasy odpadowej i/lub zanieczyszczonej, niniejsze badania dotyczyły również tego aspektu. Jako substrat pokarmowy do biokonwersji przez larwy użyto pozostałości poprodukcyjnych nasion roślin strączkowych – fasoli oraz grochu oraz skażonych mykotoksynami (zapleśniałych) odpadów fasoli. Podejście to pozwala na recykling składników odżywczych zawartych w odpadowej biomasie poprzez biokonwersję, zarówno do biomasy owadziej, jak i do pozostałości pohodowlanych w formie frassu.

Pierwsza publikacja (**P1**) jest artykułem przeglądowym, który miał na celu wykazanie możliwości zastosowania owada *H. illucens* w przemyśle i rolnictwie. Praca ta przedstawia wzrost zainteresowania tym gatunkiem w świecie naukowym, na co przekłada się stale rosnąca liczba publikacji, w szczególności od 2018 roku. Hodowla *H. illucens* generuje biomasę, która bogata jest np. w białka, tłuszcze czy chitynę. W szerokim zakresie zastosowań tego owada mieści się produkcja białka i tłuszczu na cele paszowe. Dodatkowo badana jest możliwość pozyskiwania chityny i chitozanu, jak również produkcji biodiesla z tłuszczów oraz biogazu z pozostałości pohodowlanych (frassu – mieszaniny nieprzejedzonego substratu pokarmowego, odchodów larw, martwych osobników oraz kutikuli larw (zrzucanej po każdym etapie linienia) wzbogaconych w mikroorganizmy związane z larwami). W przeglądzie omówiono również właściwości przeciwdrobnoustrojowe ekstraktów z owada, jak i zdolności larw do utylizacji oraz biokonwersji biomasy odpadowej. Zwiększona produkcja owada na skalę przemysłową powoduje jednocześnie wzrost ilości frassu, który może zostać wykorzystany jako bionawóz. Przegląd artykułów dotyczących frassu jako bionawozu pozwolił na wyznaczenie luk w wiedzy i pomógł w opracowaniu części doświadczalnej badań ujętych w niniejszej rozprawie doktorskiej.

Celem drugiej publikacji (**P2**) było scharakteryzowanie podstawowych cech nawozowych frassu, powstałego po biokonwersji przez larwy *H. illucens* odpadowej frakcji nasion fasoli i grochu, które z powodu niskiej jakości nie nadawały się do innych celów. Ten rodzaj odpadów został wybrany z powodu naturalnie wysokiego poziomu białka (a więc również azotu), co powinno przekładać się na uzyskanie bogatych w azot pozostałości pohodowlanych.

Dodatkowo frass poddano procesowi przedłużonego dojrzewania (trwającego 10 miesięcy), co miało zapewnić stabilizację jego właściwości. Świeży frass z wariantu fasoli był bogaty w dostępne dla roślin jony NH_4^+ oraz charakteryzował się wysoką całkowitą zawartość N, a także makro- i mikroelementy. Jednakże posiadał również cechy, które mogłyby skutkować efektem fitotoksycznym, mianowicie, zbyt wysokie przewodnictwo elektryczne roztworu (EC - electrical conductivity) i niski stosunek węgla do azotu (C/N). Dojrzewanie frassu z fasoli doprowadziło do korzystnej zmiany tych parametrów. W przypadku świeżego frassu z grochu stwierdzono, że miał on niższą zawartość azotu niż wariant z fasoli, ale z kolei lepsze wartości EC i C/N, będące według literatury w optymalnym zakresie. Dojrzewanie frassów z grochu spowodowało, że stosunek C/N zwiększył się znacznie, co może skutkować immobilizacją azotu w środowisku glebowym.

Trzecia publikacja (**P3**) miała na celu wykazanie entomoremediacyjnych cech larw *H. illucens* na substracie naturalnie zanieczyszczonym mykotoksynami wyprodukowanymi przez grzyba *Fusarium* (obecnego w wariancie zapleśniałej fasoli), poprzez zbadanie zmian w stężeniu i różnorodności tych związków w larwach, frassie świeżym oraz frassie po dojrzewaniu. Pomimo obecności mykotoksyn w substracie badania wykazały brak ich akumulacji w ciałach larw. Metoda przetwarzania zanieczyszczonej biomasy larwami pozwala więc na utylizację tego typu odpadu, umożliwiając uzyskanie wolnej od skażenia biomasy larw oraz redukcję stężeń niektórych mykotoksyn. Biokonwersja przez larwy doprowadziła do spadku stężeń poniżej poziomu detekcji deoksyniwalenolu, monoacetoksyscrirpenolu, diacetoksyscirpenolu oraz toksyny T-2 we frassie. Zmniejszyło się również stężenie niwalenolu, które po procesie dojrzewania frassu było już niewykrywalne. W przypadku toksyny HT-2 i zearalenonu (oraz jego metabolitów: α-zearalenonu i β-zearalenonu, które nie były obecne w substracie) doszło do wzrostu ich stężeń w świeżym frassie. Dalsze pomiary w dojrzewanym frassie wykazały wyższe stężenie toksyny HT-2 i zearalenonu, w połączeniu z redukcją α- i β-zearalenonu. Wskazuje to prawdopodobnie na dodatkowy rozwój grzybów w warunkach doświadczenia, czemu z kolei można zapobiec poprzez zmianę warunków hodowli lub wstępnej obróbki substratu. Redukcja niektórych mykotoksyn we frassie, w kontekście jego wykorzystania jako bionawozu, jest pozytywną cechą, mimo iż nie istnieją normy prawne dotyczące dozwolonych maksymalnych stężeniach mykotoksyn w dodatkach do gleby, jak również, dynamika różnych mykotoksyn w glebie nie jest nadal dobrze zbadany.

Celem czwartej publikacji (**P4**) było sprawdzenie, czy (a jeśli tak to jakie) fitohormony są obecne we frassie po hodowli *H. illucens*. Wyniki przedstawiają szeroko zakrojone opracowanie dotyczące występowania wielu fitohormonów należących do trzech najpowszechniejszych klas tych związków. Odnotowano, że frass, jak również odcieki uzyskane podczas hodowli larw (z frassu) z odpadu fasoli, charakteryzowały się wysokimi stężeniami takich fitohormonów jak: kwas indolo-3-octowy, hormony stresu roślinnego oraz cytokininy. Stężenia te były wyższe niż we frassie z odpadów grochu, z wyjątkiem jednej cytokininy: rybozydu cis-zeatyny. Natomiast odciek z frassu z grochu był bogaty w kwas abscysynowy, kwas jasmonowy, trans-zeatynę, rybozyd trans-zeatyny oraz cis-zeatynę. W porównaniu z wariantami kontrolnymi (bez larw) zawartość fitohormonów była wyższa w wariantach, w których larwy były obecne. Można stwierdzić, że biokonwersja odpadów fasoli i grochu przez larwy *H. illucens* nie tylko pozwala minimalizować ich ilość i uzyskiwać bionawóz w postaci frassu, ale także podnosi jego wartość jako biostymulatora roślin.

Słowa kluczowe: czarna mucha, rewaloryzacja odpadów, bionawóz, biostymulatory, fitohormony, mykotoksyny

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 number 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

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

Spis treści

1. Wykaz publikacji wchodzących w skład rozprawy doktorskiej

Niniejsza rozprawa doktorska oparta jest na zbiorze czterech powiązanych tematycznie artykułów naukowych, z czego trzy zostały już opublikowane (oznaczone w dalszej części jako **P1** ÷ **P4**). Wszystkie prace zamieszczono na końcu niniejszego opracowania i stanowią jego integralną część.

Niniejsze opracowanie nie zawiera wszystkich informacji przedstawionych w opublikowanych pracach. Kryterium doboru tych informacji była potrzeba wykazania, że cykl publikacji stanowi logiczną i merytorycznie spójną całość, odpowiadającą tytułowi doktoratu:

„Wykorzystywanie odpadów po hodowli *Hermetia illucens* jako bionawozów"

P1. Kaczor M., Bulak P., Proc-Pietrycha K., Kirichenko-Babko M., Bieganowski A., 2023. The variety of applications of *Hermetia illucens* in industrial and agricultural areas – review. Biology 12, 25. DOI: 10.3390/biology12010025.

Impact Factor: 3,80. Punktacja wg listy Ministerstwa Nauki i Szkolnictwa Wyższego: 100.

P2. Kaczor M., Bieganowski A., Wiącek D., Bulak P., 2025. Black soldier fly frass from seed waste of nitrogen-rich legumes – How long-term maturation affects the fertilizer properties? Journal of Environmental Management 373, 123752. DOI: 10.1016/j.jenvman.2024.123752.

Impact Factor: 8,4. Punktacja wg listy Ministerstwa Nauki i Szkolnictwa Wyższego: 200.

P3. Kaczor M., Bulak P., Kosicki R., Twarużek M., Bieganowski A., 2025. Advancing mycotoxin degradation in agricultural waste – insights from *Hermetia illucens* larvae and frass safety analysis. Journal of Insects as Food and Feed. https://doi.org/10.1163/23524588-bja10296

Impact Factor: 4,7. Punktacja wg listy Ministerstwa Nauki i Szkolnictwa Wyższego: 70.

P4. Kaczor M., Bulak P., Waligórski P., Bieganowski A., 2026. Linking waste recycling and sustainable agriculture: Phytohormone-rich biofertilizer from black soldier fly frass. Journal of Cleaner Production 548, 147855. https://doi.org/10.1016/j.jclepro.2026.147855

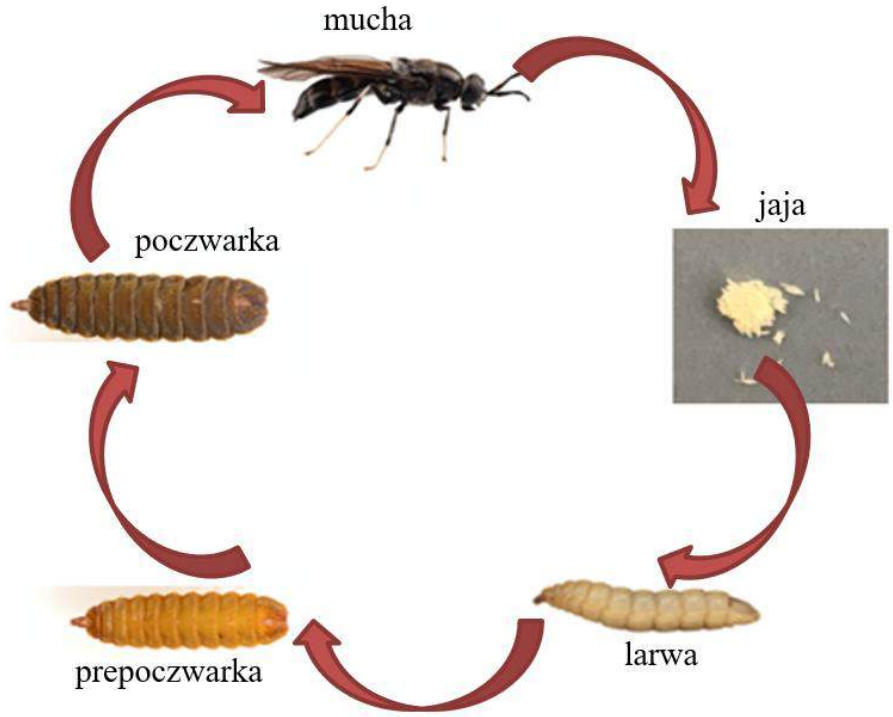
Impact Factor: 10,0. Punktacja wg listy Ministerstwa Nauki i Szkolnictwa Wyższego: 140.

2. Wprowadzenie

2.1. *Hermetia illucens* (Black Soldier Fly/Czarna Mucha)

Jednym z najliczniejszych rzędów wśród owadów jest rząd muchówek (Diptera). Przedstawicielem tego rzędu jest m.in. *Hermetia illucens* (potocznie nazywana czarną muchą żołnierską lub po prostu czarną muchą, ang. black soldier fly), która należy do w rodziny lwinkowatych (Startiomyidae). Mucha ta pochodzi z terenów obu Ameryk, a obszar jej występowania obejmuje strefy tropikalne i subtropikalne. Jednakże, ze względu na hodowlę na skalę przemysłową, jak również postępujące ocieplanie się klimatu, dochodzi do jej rozpowszechnienia i coraz częściej jest spotykana także w krajach strefy umiarkowanej (Kaya i in. 2021). *H. illucens* jest owadem holometabolicznym, co oznacza, że jej cykl rozwojowy obejmuje stadium poczwarki i przebiega z przeobrażeniem całkowitym (Rys. 1). Larwy *H. illucens* mają barwę jasną (mlecznożółtą). Bezpośrednio po wykluciu z jaj zaczynają intensywnie pobierać pokarm, gromadząc zapasy energetyczne wykorzystywane w kolejnych etapach rozwoju. Okres larwalny *H. illucens* trwa kilkanaście dni (ok. 18), w zależności od temperatury oraz rodzaju substratu żywieniowego (De Smet i in. 2018) i dzieli się na siedem etapów, podczas których larwy przechodzą linienie (zrzucanie egzoszkieletu) (Fabian i in. 2025). Optymalne warunki środowiskowe podczas hodowli larw obejmują wilgotność substratu, w którym żyją, na poziomie 60-70% oraz temperaturę w zakresie 27-30 °C. Podczas ostatniego etapu larwalnego, larwy nazywane prepoczwarkami zmieniają barwę na szarobrązową i poszukują siedlisk o niższej wilgotności, aby przejść do kolejnego etapu rozwoju. Następnie owad przybiera postać ciemnej, szarobrązowej poczwarki, która traci zdolność poruszania się w wyniku utwardzenia (sklerotyzacji) zewnętrznej warstwy oskórka. W tej fazie, w warunkach optymalnych, pozostaje ok. 10 dni (De Smet i in. 2018). Po tym okresie z poczwarki wydostaje się dorosłe imago – mucha, pozostawiając tzw. wylinkę. Dorosłe osobniki żyją jedynie do 8 dni, a ich jedynym zadaniem jest rozmnażanie (De Smet i in. 2018). Muchy czerpią energię z zapasów zgromadzonych w stadium larwalnym, jednak mogą przyjmować wodę (Bertinetti i in. 2019).

Rys. 1. Schemat cyklu rozwojowego muchy *Hermetia illucens*.



Hodowla *H. illucens* jest zasadniczo łatwa do prowadzenia, gdyż jej larwy charakteryzują się dużą odpornością na niekorzystne warunki, pobierają duże ilości pokarmu w krótkim czasie (budując przy tym bogatą w białko biomasę larwalną), a ich cykl rozwojowy jest względnie krótki (Nabaterega i in. 2025). Larwy są saprofagami i mają niewielkie wymagania względem jakości pokarmu, co pozwala na wykorzystanie i rewaloryzację szerokiego zakresu odpadów biologicznych.

Cechy te sprawiają, że *H. illucens* stała się dobrym kandydatem do produkcji przemysłowej na cele paszowe, m.in., dla ryb, drobiu, trzody chlewnej oraz innych zwierząt hodowlanych. W 2017 roku przyjęto Rozporządzenie Komisji (UE) 2017/893, które zmieniło m.in. przepisy dotyczące stosowania przetworzonego białka zwierzęcego, dopuszczając wykorzystanie przetworzonego białka pozyskanego od określonych gatunków owadów jako składnika pasz dla wybranych zwierząt gospodarskich. Akt ten, poprzez wprowadzenie kategorii „owadów hodowanych" w ramach regulacji paszowych i określenie warunków ich stosowania, stworzył podstawy prawne do rozwoju przemysłu produkującego wykorzystując surowiec owadzi i produkty owadzie (białko, tłuszcze) na cele paszowe w Unii Europejskiej. Choć sektor paszowy stanowi główny kierunek wykorzystania *H. illucens*, nie jest jedynym możliwym zastosowaniem tego owada. Jednakże, z racji,

że w skład niniejszej rozprawy doktorskiej wchodzi przegląd literatury w temacie wykorzystywania *H. illucens* w rolnictwie i przemyśle (**P.1**), rozwinięcie tematu przedstawiono w dalszej części rozprawy.

2.2. Rośliny strączkowe jako roślinne źródło białka

Rośliny strączkowe swoją nazwę zawdzięczają owocom, które wytwarzają, zwanymi strąkami. Do grupy tej należą m.in. fasola, groch, soczewica, ciecierzyca czy bób. Są one przedstawicielami roślin jednorocznych, uprawianych najczęściej w celu produkcji nasion. Nasiona cechują się wysoką, na tle innych roślin, zawartością białka (20-40%) (Haque i in. 2016). Dodatkowo stanowią źródło takich związków jak błonnik, witaminy (w szczególności z grupy B), minerały (m.in. wapń, potas, fosfor, magnez, miedź, cynk) czy antyoksydanty (Mophosa i Jideani 2017). Spożywanie nasion roślin strączkowych sprzyja niższemu spożyciu tłuszczów nasyconych, które mogą przyczyniać się do wzrostu częstości występowania tzw. chorób cywilizacyjnych, m.in. poprzez podnoszenie poziomu cholesterolu we krwi (Mitchell i in. 2009).

Jednocześnie sama uprawa roślin strączkowych wywiera korzystny wpływ na środowisko glebowe poprzez ich symbiotyczny związek z bakteriami brodawkowymi, które posiadają zdolności biologicznego wiązania azotu atmosferycznego (Kebede 2021). Mikroorganizmy te, a przede wszystkim przedstawiciele rodzaju *Rhizobium*, są zdolne do przekształcenia azotu cząsteczkowego (N_2) w formy dostępne dla roślin, równocześnie wzbogacając glebę w ten pierwiastek. W efekcie uprawa roślin strączkowych stanowi element zrównoważonego rolnictwa, a jej stosowanie w płodozmianie jest rekomendowane w celu poprawy żyzności i struktury gleby (Graham i Vance 2003). W ostatnich latach można odnotować wzrost zainteresowania uprawą roślin strączkowych, na co wpływają zmiany w preferencjach żywieniowych ludzi. Coraz większy odsetek populacji przechodzi na diety oparte na produktach roślinnych, co jest przejawem rosnącej świadomości ekologicznej i dostrzegania skutków klimatycznych, z którymi wiąże się produkcja białka zwierzęcego (emisja gazów cieplarniach, duże zapotrzebowanie na powierzchnię, wysokie zużycie wody) (White 2016). Skutkiem tych zmian jest zwiększenie podaży roślin strączkowych, stanowiących wartościowy zamiennik źródła białka. Według Organizacji Narodów Zjednoczonych do spraw Wyżywienia i Rolnictwa (Food and Agriculture Organization of the United Nations - FAO) światowa produkcja nasion tych roślin w roku 2022 wynosiła ok. 96 mln ton, a szacuje się, że w roku 2032 wzrośnie do ok. 125 milionów ton. Natomiast w trakcie przetwarzania, przede wszystkim

na etapie czyszczenia, sortowania i mielenia, powstają odpady, które mogą stanowić od 5 do nawet 25% całkowitej masy surowca (Karaca i Nickerson 2022).

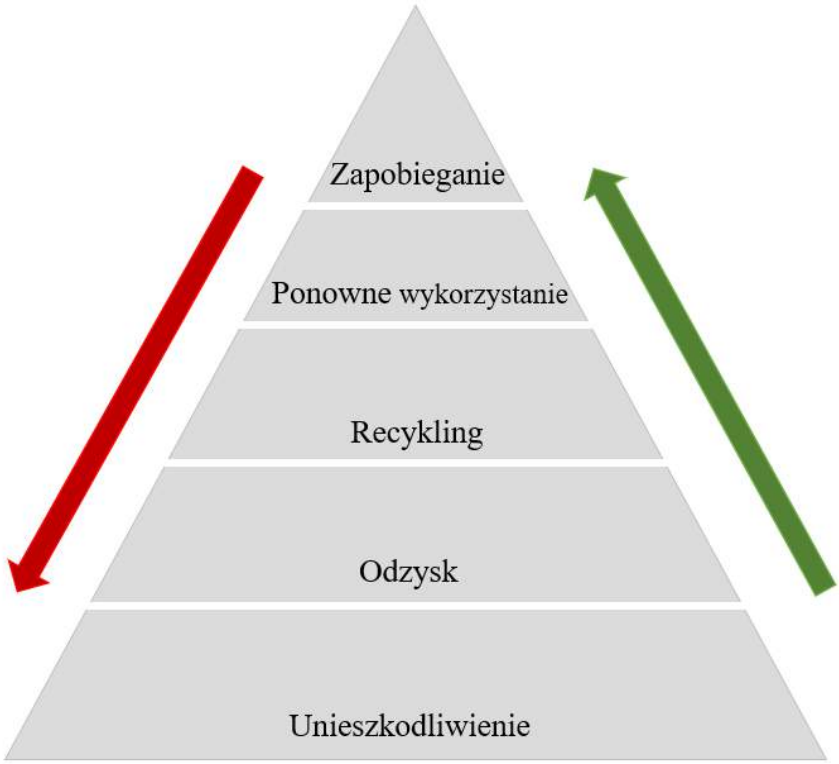
W trakcie przechowywania lub procesów przetwórczych nasion roślin strączkowych istnieje ryzyko wystąpienia zakażenia grzybicznego (Pavicich i in. 2024). Szczególną rolę odgrywają tu takie rodzaje, jak *Fusarium*, *Aspergillus* czy *Penicillium*, zdolne do syntezy metabolitów wtórnych zwanych mykotoksynami. Związki te wykazują silne działania toksyczne (hepatotoksyczne, immunospresyjne, karcerogenne) i spożyte przez ludzi lub zwierzęta mogą stanowić zagrożenie dla zdrowia i życia (Bryde 2007). Czynniki sprzyjające rozwojowi grzybów oraz produkcji mykotoksyn obejmują m.in. nieodpowiednie warunki przechowywania, zbyt wysoką wilgotność czy uszkodzenia mechaniczne nasion. Zanieczyszczenie mykotoksynami istotnie obniża jakość produktów, a w przypadku przekroczenia dopuszczalnych stężeń, produkty te muszą być wycofane z cyklu produkcji i zutylizowane (np. przez spalenie w kontrolowanych warunkach), generując straty ekonomiczne i dodatkowe koszty (Chen i in. 2020). Odpady poprodukcyjne bogate w składniki odżywcze powinny znaleźć zagospodarowanie w sposób ograniczający marnowanie biomasy i umożliwiający jej rewaloryzację. Jedną z metod może być biokonwersja za pomocą larw *H. illucens*.

2.3. Gospodarka cyrkularna

Wskutek nieustannie rozwijającej się i rosnącej populacji człowieka, środowisko naturalne, w tym klimat, osiągają niebezpieczne poziomy deterioracji. Przejawia się to m.in. wzrostem średniej temperatury Ziemi, zanieczyszczeniem powietrza, wód i gleb, spadkiem żyzności gleb, ograniczaniem terenów zielonych, utratą bioróżnorodności roślin i zwierząt, etc. Aby zapobiec dalszemu pogarszaniu się warunków klimatycznych i jakości środowiska, kraje Unii Europejskiej wdrożyły strategię transformacji gospodarczej, tzw. „Europejski Zielony Ład", której celem jest osiągnięcie neutralności klimatycznej. Zmiany obejmują m.in. takie sektory jak przemysł (ograniczenie emisji gazów cieplarnianych), energetyka (odchodzenie od konwencjonalnych paliw kopalnych) oraz rolnictwo (ograniczenie stosowania chemicznych środków ochrony roślin i nawozów). Jednym z kluczowych założeń tej strategii jest gospodarka o obiegu zamkniętym, czyli maksymalne wykorzystanie materiałów będących już w obiegu, przy minimalizacji wytwarzania odpadów oraz zapotrzebowania na surowce pierwotne. Najlepsza opcja opiera się również na wykorzystaniu odpadów, w myśl polityki zero waste, jako źródła nowych zasobów.

Działania te powinny przynosić korzyści zarówno ekologiczne, jak i ekonomiczne (Morseletto 2020). Podstawą tej idei jest tzw. hierarchia postępowania z odpadami w Unii Europejskiej, wprowadzona poprzez Dyrektywę Parlamentu Europejskiego i Rady (2008/98/WE), która określa działania mające na celu ograniczenie niekorzystnego wpływu odpadów na środowisko naturalne. Koncepcję tę przedstawia się w formie piramidy (Rys. 2). Na jej szczycie, jako najbardziej optymalna opcja, znajduje się prewencja obejmująca zapobieganie powstawaniu odpadów oraz ograniczania ich ilości. Kolejny poziom stanowi „ponowne użycie" (reuse), wskazując, że w sytuacji, gdy odpady są nieuniknione, najlepszym rozwiązaniem jest ich ponowne wykorzystanie. Jeśli nie jest to możliwe, odpady przechodzą na kolejny poziom piramidy - recyklingu. Recykling pozwala na odzysk surowców, bądź ich składników, zmniejszając konieczność eksploatacji nowych, pierwotnych materiałów. Przedostatnim procesem jest odzyskiwanie energii, a podstawa piramidy obejmuje unieszkodliwienie odpadów.

Rys. 2. Schemat przedstawiający hierarchię postępowania z odpadami w Unii Europejskiej



2.3.1. Bioremediacja jako element gospodarki cyrkularnej

Szeroko rozumiana bioremediacja jest procesem polegającym na wykorzystywaniu organizmów żywych do usuwania lub neutralizowania zanieczyszczeń stanowiących zagrożenie dla środowiska. Najczęściej i w ujęciu klasycznym termin ten odnosi się do zastosowania bakterii. Gdy dotyczy innych grup organizmów, zwykło się stosować bardziej precyzyjne nazewnictwo, np. w przypadku roślin – fitoremediację, a w przypadku grzybów – mykoremediację (Ayilara i Babalola 2023). W przeciwieństwie do konwencjonalnych metod remediacji, opartych zazwyczaj na reagentach chemicznych, bioremediacja lepiej wpisuje się w zasady zrównoważonego rozwoju, ponieważ nie generuje dodatkowych odpadów chemicznych powstających w trakcie procesu (Wu i in. 2023). Jej ograniczenia wynikają głównie z powolności procesu oraz są związane z doborem odpowiednich organizmów, zdolnych do adaptacji w skażonym środowisku i dysponujących aparatem enzymatycznym umożliwiającym redukcję lub unieszkodliwienie zanieczyszczeń. Z tego powodu metody bioremediacji znajdują zastosowanie zwykle w przypadku skażeń małych lub średnich. W koncepcji gospodarki cyrkularnej bioremediacja umożliwia domykanie obiegów materiałowych, ponieważ pozwala na oczyszczanie zanieczyszczonych surowców lub podłoży, przywracając im wartość użytkową. Oczyszczone materiały mogą być ponownie użyte w procesach produkcyjnych, co ogranicza potrzebę sięgania po zasoby pierwotne i redukuje presję na środowisko naturalne (Bala i in. 2022). W ten sposób bioremediacja wspiera efektywność działań gospodarki cyrkularnej w kontekście środowiskowym, jak i ekonomicznym (Rizzo i 2016).

2.3.2. Entomoremediacja jako element bioremediacji

Entomoremediacja, termin powstały od greckiego słowa „entomon” (owad) oraz łacińskiego „remediation” (oczyszczać), początkowo odnosiła się do propozycji wykorzystywania kilku gatunków owadów do oczyszczania zanieczyszczonej gleby (Ewuim 2013). Z czasem definicję tę poszerzono i doprecyzowano do ”wykorzystania wyspecjalizowanych owadów oraz związanych z nimi mikroorganizmów do utylizacji, ekstrakcji, sekwestracji i/lub detoksykacji zanieczyszczeń z gleby, osadów i biomasy organicznej” (Bulak i in. 2018).

Wykorzystanie entomoremediacji wpisuje się w założenia gospodarki cyrkularnej, ponieważ polega na użyciu zalegającej biomasy odpadowej (często skażonej zanieczyszczeniami) jako substratu pokarmowego dla larw. W tym celu selekcjonuje się owady

saprofagiczne, które charakteryzują się dużą zdolnością do pobierania pokarmu oraz odpornością na zanieczyszczenia, przejawiającą się brakiem zwiększonej śmiertelności i możliwością prawidłowego rozwoju podczas ekspozycji na zanieczyszczenia. Pożądane są również mechanizmy pobierania i przetwarzania zanieczyszczeń, takie jak bioakumulacja, biodegradacja czy ich transformacja (Gwenzi i in. 2024). Zakres badań nad entomoremediacją obejmuje m.in. metale ciężkie (Iqbal i in. 2025), różnego rodzaju pestycydy (Siddiqui i in. 2022), zanieczyszczenia organiczne (Gwenzi i in. 2024), antybiotyki (Liu i in. 2020), mykotoksyny (Kaczor i in, 2025) oraz mikroplastiki (Ritchie i in. 2023). Do gatunków owadów wykorzystywanych do przetwarzania problematycznej biomasy należą m.in.: barciak mniejszy (*Achroia grisella*, Fabricius, 1794), omacnica ryżanka (*Corcyra cephalonica*, Stainton, 1866), barciak większy (*Galleria mellonella*, Linnaeus, 1758), czarna mucha (*H. illucens*, Linnaeus, 1758), mącznik młynarek (*T. molitor*, Linnaeus, 1758), trojszyk ulec (*Tribolium confusum*, Jacquelin du Val, 1863) oraz drewnojad (*Zophobas atratus*, Fabricius, 1775) (Gwenzi i in. 2024). Badania w tym kierunku wciąż się rozwijają, dlatego identyfikowane są kolejne gatunki potencjalnie przydatne w procesie entomoremediacji. Niemniej jednak obecnie to larwy *H. illucens* należą do najczęściej badanych owadów w tym kontekście.

2.3.3. Bionawozy jako sposób wykorzystania odpadów rolniczych

Nadmierne stosowanie nawozów mineralnych jest dość powszechnym zjawiskiem w intensywnym rolnictwie i wynika z dążenia do szybkiego zwiększenia plonów. Praktyka ta prowadzi jednak do licznych negatywnych konsekwencji. Nadmiar składników odżywczych ulega wymywaniu w głąb profilu glebowego, co sprawia, że stają się one trudniej dostępne dla roślin. Nawozy przedostają się do wód gruntowych i powierzchniowych, czego skutkiem jest eutrofizacja oraz zakwaszenie wodnych ekosystemów. Jednocześnie zwiększa się także emisja gazów cieplarnianych (Savci 2012). Długotrwałe nadużywanie nawozów mineralnych przyczynia się ponadto do degradacji jakości gleby poprzez obniżenie zawartości próchnicy, redukcję węgla organicznego, pogorszenie struktury gleby oraz spadek różnorodności mikrobiologicznej (Pahalvi i in. 2021).

Jedną z alternatyw dla nawozów chemicznych są bionawozy. W literaturze nadal nie ma jednej, powszechnie przyjętej definicji bionawozu (Santos i in. 2024). Według często przywoływanej definicji Vessey'a (2003) bionawóz to materiał zawierający żywe mikroorganizmy, które po aplikacji zasiedlają ryzosferę bądź tkanki roślinne i wspierają wzrost roślin poprzez zwiększenie dostępności podstawowych składników pokarmowych. Jednak

według innych autorów taki materiał mikrobiologiczny, który sam w siebie nie wzbogaca gleby w nowe składniki odżywcze, powinien być określany jedynie jako inokulant (Okon i Labandera-Gonzalez 1994). Znaczenie terminu „bionawóz" ewoluowało w czasie. Obecnie obejmuje ono również różne substancje organiczne dodawane do gleby, przy czym wyróżnikiem pozwalającym zaliczyć je do bionawozów jest ich aktywność biologiczna (główne mikrobiologiczna) (Shahzad et al., 2025). Przy tak rozszerzonym ujęciu frass owadzi również może być traktowany jako bionawóz (Ashworth i in. 2025). W przeciwieństwie do nawozów syntetycznych, które zazwyczaj są źródłem jednego dominującego pierwiastka (azotu lub fosforu), bionawozy, dzięki swojej biologicznej naturze, zawierają szeroki zakres makro- i mikroelementów niezbędnych do prawidłowego rozwoju roślin. Wspierają także odbudowę właściwości gleby i poprawę jej żyzność dzięki obecności substancji organicznych, które sprzyjają rozwojowi mikrobioty glebowej (Daniel i in. 2022).

Do produkcji bionawozów można wykorzystywać różnorodne odpady organiczne, w tym pozostałości rolnicze, które po odpowiednim przetworzeniu nabierają właściwości nawozowych (Orlandella i Fiore 2025). Podobnie pozostałości te mogą zostać wykorzystane jako substrat pokarmowy do hodowli larw, co umożliwia uzyskanie frassu, który można traktować jako swoisty odpowiednik obornika. W ten sposób możliwe jest jednoczesne zagospodarowanie odpadów rolniczych oraz otrzymanie bionawozu bogatego w składniki odżywcze.

2.4. Fitohormony jako biostymulatory

Fitohormony, inaczej hormony roślinne, to związki regulujące kluczowe procesy fizjologiczne roślin, takie jak kiełkowanie, wzrost i rozwój, różnicowanie tkanek, kwitnienie, owocowanie czy reagowanie na czynniki stresowe. Ich działanie pojawia się już przy bardzo niskich stężeniach. Do głównych grup fitohormonów należą auksyny, cytokininy, gibereliny oraz tzw. hormony stresu roślinnego (Mukherjee i in. 2022).

Fitohormony powstają endogennie w roślinach, ale mogą być również dostarczane np. wraz z nawozami pochodzenia biologicznego. Badania wykazują, że rożnego rodzaju komposty i materiały organiczne mogą zawierać fitohormony. Sienkiewicz i in. (2024) odnotowali obecność szeregu fitohormonów w kompostach, co może sugerować, że bionawozy mogą działać nie tylko jako źródło dla składników odżywczych, ale także jako źródło biostymulatorów roślinnych. W przypadku frassu owadziego większość wzmianek w literaturze naukowej ma charakter teoretyczny i jest wskazywana jako potencjalny kierunek

badań. Wydaje się, że jedyną publikacją naukową, która faktycznie określa zawartość fitohormonów we frassie owadzim, jest praca Green'a (2023). W badaniu tym frass pochodził z hodowli larw *H. illucens* na odpadach kuchennych. Oznaczono m.in. auksyny, fitohormony stresu oraz gibereliny, natomiast brakuje informacji o cytokininach. Badania ukierunkowane na oznaczenie stężeń fitohormonów we frassie stanowią dobrą podstawę do lepszego rozpoznania jego właściwości biostymulujących.

3. Cele rozprawy doktorskiej i hipotezy badawcze

Zasadniczym celem niniejszej rozprawy doktorskiej było określenie możliwości zastosowania odpadów pohodowlanych (tzw. frassu) uzyskanych podczas hodowli owada *H. illucens* jako bionawozów. Jednakże ze względu na to, że larwy *H. illucens* mogą być wykorzystywane w procesie entomoremediacji, a więc m.in. do utylizacji odpadowej i/lub zanieczyszczonej biomasy, niniejsze badania obejmowały również ten aspekt. Jako substrat pokarmowy do biokonwersji przez larwy użyto pozostałości poprodukcyjne nasion roślin strączkowych (fasoli oraz grochu) oraz skażonych mykotoksynami (zapleśniałych) odpadów fasoli. Podejście to umożliwia recykling składników odżywczych zawartych w biomasie odpadowej poprzez biokonwersję, zarówno do biomasy owadziej, jak i do pozostałości pohodowlanych w formie frassu.

W ramach realizacji celu głównego sformułowano następujące cele cząstkowe:

C1. Przedstawienie szerszego kontekstu wykorzystania owada *H. illucens* poprzez omówienie spektrum zastosowań przemysłowych i rolniczych oraz wskazanie rosnącego zainteresowania hodowlą tego gatunku na skalę przemysłową.

C2. Ocena podstawowych właściwości nawozowych frassu uzyskanego po biokonwersji odpadów nasion fasoli i grochu przez larwy *H. illucens*.

C3. Ocena zmian właściwości nawozowych frassu uzyskanego po biokonwersji odpadów nasion fasoli i grochu, zachodzących w wyniku przedłużonego okresu dojrzewania tlenowego.

C4. Ocena zmian ilości oraz składu mykotoksyn we frassie surowym oraz dojrzewanym, uzyskanym po biokonwersji przez larwy *H. illucens* odpadów nasion fasoli zanieczyszczonych pleśnią.

C5. Ocena występowania hormonów roślinnych we frassie po biokonwersji odpadów nasion fasoli i grochu przez larwy *H. illucens* oraz w odcieku z frassu powstałym podczas hodowli larw.

Cel pierwszy został omówiony w publikacji **P1**, cele **C2** i **C3** w publikacji **P2**, cel **C4** w publikacji **P3**, natomiast cel **C5** w **P4**.

Sformułowany cel główny oraz cele cząstkowe zostały postawione w oparciu o niniejsze przyjęte hipotezy badawcze:

H1. Wykorzystanie odpadów roślinnych bogatych w azot (nasion fasoli i grochu) jako substratu do skarmiania larw *H. illucens* skutkuje otrzymaniem frassu bogatego w azot.

H2. Dojrzewanie frassu prowadzi do stabilizacji jego właściwości, skutkując wzrostem wartości nawozowych.

H3. Biokonwersja przez larwy *H. illucens* substratu zanieczyszczonego grzybami pleśniowymi, a także proces dojrzewania frassu, prowadzą do spadku ilości mykotoksyn oraz spowoduje zmiany w składzie.

H4. Frass oraz odciek powstały po biokonwersji odpadów nasion fasoli i grochu przez larwy *H. illucens* zawierają hormony roślinne.

4. Materiały i metody

4.1. Publikacja przeglądowa **P1**

W celu zebrania materiału i przeprowadzenia przeglądu artykułów dotyczących wykorzystywania *H. illucens* w różnych dziedzinach przemysłu oraz rolnictwa przeszukano bazy danych Web of Science, Scopus i Google Scholar. Kombinacja słów kluczowych jakie zostały użyte w wyszukiwarkach obejmowała: „*Hermetia illucens*", „*H. illucens*", „black soldier fly". Aby przedstawić możliwe najnowsze osiągnięcia, przeglądem objęto prace opublikowane w latach 2018-2022. Artykuły zostały pogrupowane tematycznie i przedstawione w jedenastu podrozdziałach.

4.2. Hodowla owada *H. illucens*

Larwy *H. illucens* użyte w pracach przedstawionych we wszystkich publikacjach badawczych pochodziły z hodowli matecznej prowadzonej w Instytucie Agrofizyki Polskiej Akademii Nauk w Lublinie od ok. 10 lat. Hodowla ta jest prowadzona na podłożu z włókna kokosowego, które zapewnia larwom środowisko do życia oraz odpowiednią wilgotność (70 ± 10%). W celu standaryzacji warunków hodowli matecznej jako pokarm stosuje się karmę dla ryb. Jej skład (białko 24-25%, tłuszcze 3.5-5%, włókno surowe 5,8-7% oraz popiół 5,7-7%) zapewnia optymalne pożywienie do prawidłowego wzrostu i rozwoju larw (Bulak i in. 2018). Odpowiednia temperatura dla larw *H. illucens* (26 ± 1 °C) oraz ciemność są zapewniane przez inkubator laboratoryjny, w którym umieszczona jest komora z hodowlą (larwarium) – Rys. 3a. Konstrukcja larwarium umożliwia larwom (tuż przed przepoczwarzeniem) przechodzenie do wydzielonej części komory, z której są systematycznie przenoszone do insektarium (dużej komory o wymiarach 82 x 172 x 86 cm, gdzie muchy kopulują i składają jaja – Rys. 3b). Następnie jaja przenosi się do larwarium, co zamyka cykl hodowlany.

Rys. 3. a) Larwarium i b) insektarium do hodowli owada *H. illucens* w Instytucie Agrofizyki Polskiej Akademii Nauk w Lublinie.

4.3. Substraty – poprodukcyjne odpady nasion fasoli i grochu

W artykułach **P2** i **P4** jako substrat do skarmiania larw wykorzystano odpady po produkcji nasion fasoli i grochu, pozyskane od lokalnego producenta. W publikacji **P3** larwy hodowano wyłącznie na odpadach fasoli, ponieważ tylko one były zanieczyszczone pleśnią.

Odpady fasoli zawierały nasiona, które były połamane, pokurczone i przebarwione, a także występowały oznaki zanieczyszczenia pleśnią. Odpady nasion grochu składały się głównie z łusek nasiennych, jak również z mąki grochowej. Sucha masa odpadów, określona po suszeniu w 105 °C przez 24 h, wynosiła 91,31% dla odpadów z fasoli oraz 90,46% dla odpadów z grochu. Materiał doprowadzono do wilgotności 75,5 ± 5% (odpowiedniej do rozwoju larw) poprzez dodanie odpowiedniej ilości wody destylowanej. Nasiąkanie odpadów wodą trwało 12 h w obniżonej temperaturze 4 °C, aby zapobiec rozwojowi bakterii i pleśni. Następnie substraty zostały rozdrobnione za pomocą blendera kuchennego.

4.4. Przebieg doświadczenia

Do wszystkich doświadczeń użyto po tysiąc larw *H. illucens*. W doświadczeniach, których wyniki opisano w **P2** i **P3**, dawka substratu wynosiła 1 g świeżej masy na jedną larwę, co w przeliczeniu na suchą masę dawało 256,3 mg substratu z fasoli oraz 217,9 mg substratu z grochu. Hodowlę prowadzono w szczelnie zamkniętych pojemnikach, aby zapobiec ewentualnej ucieczce larw. W celu zapewnienia wymiany powietrza w przykrywkach zamontowano rurki zabezpieczone siatką, do których podłączono pompy powietrza (OXYBOOST 300 PLUS, AQUAEL). Doświadczenie prowadzono przez 30 dni w temperaturze 26 ± 1 °C. W trakcie doświadczenia pojawiające się poczwarki *H. illucens* systematycznie wyjmowano, płukano w wodzie destylowanej i 1 mM roztworze etylenodiaminotetraoctowego (EDTA) (w celu usunięcia jonów pierwiastków związanych z powierzchnią) oraz suszono ręcznikiem papierowym. Następnie poczwarki liczono, ważono (RADWAG AS310.R2 PLUS, Polska), mierzono (linijką) i zamrożono w -20 °C. Ta sama procedura dotyczyła larw i poczwarek po zakończeniu części hodowlanej doświadczeń. W przypadku larw, konieczny był dodatkowy krok, pozostawienie przemytych w wodzie destylowanej larw w pustym pudełku na 24 h. Celem tego działania było opróżnienie przewodu pokarmowego larw z niestrawionego pokarmu. Powstały frass był ważony, a następnie dzielony na dwie części. Jedna część była przeznaczona do analiz "surowej" biomasy (świeży frass), a druga została przeznaczona na dojrzewanie (dojrzały frass). Proces dojrzewania był przeprowadzony w warunkach tlenowych w temperaturze 30 ± 1 °C przez 10 miesięcy w zamkniętych pojemnikach. W tym czasie frass był mieszany szklaną bagietką co dwa dni, aby zapobiec zaskorupieniu się powierzchni na skutek utraty wilgotności. Następnie dojrzewany frass był poddawany takim samym analizom jak frass świeży.

W doświadczeniu opisanym w **P4** dawkę substratu zmniejszono do 150 mg suchej masy na jedną larwę. Decyzja ta, została podjęta w celu zoptymalizowania dawki żywieniowej i uzyskania lepszych parametrów rozwojowych larw. Kontrolą w tych doświadczeniach stanowiły substraty z odpadów z fasoli i grochu (bez dodatku larw), przechowywane w identycznych warunkach jak badane warianty z larwami i przez taki sam okres (30 dni). Analogicznie jak w **P2** i **P3**, zarówno warianty z larwami, jak i próby kontrolne, zostały umieszczone w pudełkach zamykanymi przykrywkami, w których były zamontowane otwory wentylacyjne z gumowymi rurkami podłączonymi do pompki (OXYBOOST 300 PLUS, AQUAEL). Taki sposób wentylacji nie wpływał istotnie na wartość wilgotności substratów (sprawdzane w testach wstępnych poprzez określenie zawartości suchej masy), co było

szczególnie ważne, ponieważ w tym doświadczeniu zbierano również samoistnie powstający odciek. W celu jego zebrania pojemnik z substratem miał w dnie otwory (mniejsze od rozmiaru larw) i był umieszczony w drugim pojemniku (bez otworów) – Rys. 4. Odciek przedostawał się do drugiego pojemnika grawitacyjne. Podczas trwania doświadczenia nie dodawano wody.

Rys. 4. Pojemnik, w którym prowadzona była doświadczalna hodowla larw *H. illucens*.

Wszystkie doświadczenia przeprowadzone były w trzech, niezależnych biologicznych powtórzeniach.

4.5. Metody analityczne

4.5.1. Publikacja **P2**

Sucha masa substratów, frassów oraz larw została oznaczona po ich wysuszeniu w 105 °C przez 24 h, na podstawie następującego wzoru:

$$Sucha\ masa\ (\%) = \frac{a * 100\ (\%)}{b} \qquad \text{Równanie 1}$$

gdzie:

a – masa suchej próbki (g),

b – masa świeżej próbki (g).

Podstawowa analiza składu chemicznego substratów (odpadów z nasion fasoli i grochu) obejmowała zawartości białka, tłuszczów, włókna, popiołu oraz węglowodanów. Zawartość białka została obliczona na podstawie zawartości azotu całkowitego, zgodnie z normą ISO 5983-2:2009. Zawartość azotu całkowitego oraz węgla całkowitego w substratach i frasach określono przy użyciu Thermo Scientific Flash 2000 Organic Elemental Analyzer (Stany Zjednoczone). Do oznaczenia tłuszczu wykorzystano metodę według Santos Filipe et al. (2024), polegającej na trzykrotnej ekstrakcji tłuszczów z suchej próbki w n-heksanie w stosunku 1:10 (m/obj.). Zawartość włókna została oznaczona na podstawie wystandaryzowanej metody ISO 5498:1981 przez ekstrakcję kwasowo-zasadową (w temp. 100 °C przez 30 min). Zawartość popiołu w substratach została określona po przez ich spopielenie w 550 °C. Zawartość węglowodanów została oszacowana na podstawie wzoru:

$$Węglowodany\ (\%) = 100\ (\%) - (c + d + e + f) \qquad \text{Równanie 2}$$

gdzie: c – zawartość białka (%),

d – zawartość tłuszczu (%),

e – zawartość włókna (%),

f – zawartość popiołu (%).

Procent utylizacji substratów po doświadczeniu z larwami został określony na podstawie wzoru:

$$Utylizacja\ (\%) = \frac{(h-i) * 100}{h} \qquad \text{Równanie 3}$$

gdzie:

h – sucha masa substratu użytego do doświadczenia (g),

i – sucha masa świeżego frassu uzyskanego po hodowli larw (g).

Procent utylizacji przeprowadzony mikrobiologicznie podczas procesu dojrzewania został obliczony analogicznie do sposobu obliczenia utylizacji substratów przez larwy, przy czym *h* oznaczało suchą masę świeżego frassu po biokonwersji larw (g), a *i* – suchą masę frassu dojrzewanego (g).

Współczynnik przepoczwarzenia dla larw po 30 dniach hodowli na substratach został wyliczony na podstawie:

$$Przepoczwarzenie\ (\%) = \frac{j * 100}{k} \qquad \text{Równanie 4}$$

gdzie:

j – liczba poczwarek po doświadczeniu,

k – liczba larw wziętych do doświadczenia.

Podobnie został obliczony współczynnik przeżywalności larw na substratach:

$$Przeżywalność\ (\%) = \frac{l * 100}{k} \qquad \text{Równanie 5}$$

gdzie:

l – suma żywych larw i poczwarek po doświadczeniu,

k – liczba larw wziętych do doświadczenia.

W przypadku frassów, zarówno świeżych jak i dojrzewanych, do analizy pH, EC (przewodności elektrycznej) oraz stężenia jonów amonowych oraz azotanowych wykorzystano wielofunkcjonalny miernik HQ400 (Hach Lange, Düsseldorf, Niemcy) oraz odpowiednie elektrody (PHC20101, CDC40104, ISENO318101, ISENH318101 (Hach Lange, Düsseldorf, Niemcy). Pomiary były robione w stosunku 1:10 (m/obj.) świeżej masy próbki do wody destylowanej. Dodatkowo, przy pomiarach jonów amonowych oraz azotanowych użyto odpowiednich regulatorów siły jonowej (nr kat. 2984799 i nr kat. 4447169, Permachem Reagents, HACH, Düsseldorf, Niemcy), zgodnie z zaleceniami producenta elektrod. Analiza składu pierwiastkowego substratów, frassów i larw została przeprowadzona z wykorzystaniem

optycznego spektrometru emisyjnego z indukcyjnie sprzężoną plazmą. Analizę poprzedzono odpowiednim przygotowaniem próbek poprzez przeprowadzenie mineralizacji w mieszaninie HCl i HNO_3 (w stosunku 3:1 obj./obj.) w mineralizatorze mikrofalowym Berghof Speedwave (Eningen, Niemcy). Proces ten obejmował następujące sekwencje: 15-minutowe ogrzewanie próbki od temperatury pokojowej do 140 °C, 5-minutowe utrzymanie zadanej temperatury, 5-minutowe podwyższanie się temperatury do 180 °C, 15-minutowe utrzymanie zadanej temperatury. W trakcie całej procedury ciśnienie utrzymywane było na poziomie 15 barów. Następnie analizę składu pierwiastkowego przeprowadzono za pomocą spektrometru ICP-OES (iCAP Series 6500, Thermo Scientific, Waltham, MA, Stany Zjednoczone) wyposażonego w detektor z ładowanym wtryskiem i obsługiwanego przez oprogramowanie TEVA. Parametry przepływu urządzenia wynosiły: moc i częstotliwość generatora RF – 1150 W i 27,12 MHz, natężenie przepływu gazu nośnego i pomocniczego – odpowiednio 16 l/min i 0,4 l/min, czas integracji – max. 15 sekund, prędkość pompy – 50 obr./ min oraz czas płukania – 20 sekund.

4.5.2. Publikacja **P3**

4.5.2.1 Identyfikacja grzybów w substracie

Substrat (odpad po produkcji nasion fasoli) o masie 10 g został poddany homogenizacji przy użyciu homogenizatora typu Stomacher (BagMixer 400, Interscience, Francja). Do tak przygotowanych próbek dodawano 90 ml sterylnego płynu rozcieńczającego (zawierającego 1 g enzymatycznego hydrolizatu kazeiny, 8,5 g chlorku sodu i 1000 ml wody destylowanej o pH 7,0 ± 0,2), po czym ponownie homogenizowano je przez 90 sekund. W celu określenia całkowitej liczby grzybów w próbce została zastosowana technika posiewu płytkowego. Zhomogenizowaną zawiesinę poddano serii rozcieńczeń dziesiętnych, a następnie wykonano posiewy na agarze YGC (zawierającego ekstrakt drożdżowy, glukozę i chloramfenikol) w trzech powtórzeniach. Inkubacja zaszczepionych płytek przeprowadzono w temperaturze 25 ± 1 °C przez 5-7 dni. Następnie zliczano kolonie z tych płytek, na których ich liczba mieściła się przedziale 10-100. Wyniki wyrażono jako liczbę jednostek tworzących kolonie na gram próbki. Dominujące typy grzybów anamorficznych określono na podstawie morfologii kolonii.

4.5.2.2 Analiza obecności mykotoksyn i ich metabolitów

4.5.2.2.1 Odczynniki

Użyte w analizie wzorce mykotoksyn pochodziły od Romer Labs (Tulln, Austria). Do ekstrakcji i analiz użyto rozpuszczalników i soli, takich jak acetonitryl, metanol, octan amonu i kwas octowy (wszystkie trzy o czystości LC-MS) zakupione od firmy Merck (Darmstadt, Niemcy). W celu uzyskania wody o jakości analitycznej, została ona oczyszczona za pomocą systemów Elix 3 i Simplicity UV (Merck, Darmstadt, Niemcy).

4.5.2.2.2 Przygotowanie próbek

Metodyka przygotowania próbek pochodzi z publikacji: Sulyok i in. (2006) i Varga i in. (2021). Próbkę o masie 1 g ekstrahowano 4 ml mieszaniny acetonitryl/woda/kwas octowy w stosunku 79:20:1 (obj./obj./obj.) przez 90 min w temperaturze pokojowej, z wykorzystaniem wytrząsarki Multi Reax (Heidolph, Niemcy). Następnie ekstrakt odwirowano z prędkością 7000 obrotów/min przez 10 min przy użyciu wirówki 5430 R (Eppendorf, Niemcy). Z supernatantu pobrano 0,5 ml i rozcieńczono 0,5 ml mieszaniny metanol/woda (1:4, v/v), po czym roztwór wymieszano i poddano ponownemu odwirowaniu (14500 obrotów/min przez 10 min). Następnie pobrano 80 µl supernatantu i przeniesiono do fiolki HPLC zawierającej mikroinserę, dodano 20 µl standardu wewnętrznego, a całość wymieszano. Z tak powstałego roztworu pobrano 5 µl i wstrzyknięto do systemu LC-MS/MS.

4.5.2.2.3 Analiza mykotoksyn i metabolitów

Ilościowe oznaczenie analitów zostało przeprowadzone za pomocą ultrawysokosprawnej chromatografii cieczowej sprzężonej ze spektrometrią mas (UHPLC-MS/MS). W skład układu wchodził chromatograf UHPLC Nexera (Shimadzu, Tokio, Japonia) sprzężony ze spektrometrem mas QTRAP 5500 (Sciex, Foster City, USA) ze źródłem jonizacji elektrorozpyłowej TurbolonSpray (ESI). Rozdział chromatograficzny odbył się na kolumnie Gemini C18 (150 x 4,6 mm, 5 µm) (Phenomenex, Torrance, USA). Fazę ruchomą stanowiły dwie mieszaniny metanol/woda/kwas octowy o różnych proporcjach: eluent A - 10:89:1 (v/v/v) i eluent B - 97:2:1 (v/v/v), obie zawierały dodatkowo 5 mM octan amonu. Elucja gradientowa została przeprowadzona w następujących etapach: 1) 100% eluentu A przez 2 min, 2) liniowy wzrost zawartości eluentu B do 50% w ciągu 5 min, 3) liniowy wzrost zawartości eluentu B do 100% w ciągu 14 min, 4) 100% eluentu B przez 4 min, 5) ponowne

osiągnięcie 100% eluentu A w trakcie 3,5 min. Temperatura kolumny podczas analizy utrzymywana była na poziomie 25 °C, a natężenie przepływu wynosiło 1 ml/min.

Tandemowa spektrometria mass z jonizacją elektrorozpylania (ESI-MS/MS) była wykonywana w trybie monitorowania zaplanowanych reakcji wielokrotnych, poprzez wykrywanie ujemnych i dodatnich polarności jonizacji w jednym cyklu chromatograficznym. Optymalne parametry źródła ESI obejmowały temperaturę źródła na poziomie 550 °C, ciśnienie gazu osłonowego 2,07 barów, ciśnienie gazu suszącego 5,52 barów, gaz zderzeniowy, napięcie rozpylenia jonów -4500 V i +5500 V.

Wartości granic wykrywalności (LOD) i granic oznaczalności (LOQ) zostały oznaczone przy użyciu stosunku sygnału do szumu wynoszącego odpowiednio 3 i 10, za pomocą skryptu w oprogramowaniu Analyst 1.6.3 (Sciex, Foster City, USA). Wartości odzysku oceniono, dodając mykotoksyny do czystego substratu (wolnego od zanieczyszczeń) w dwóch różnych stężeniach. Dokładność oznaczeń określono na podstawie trzech niezależnych powtórzeń.

4.5.3. Publikacja **P4**

4.5.3.1. Parametry larw

Podczas trwania hodowli co tydzień mierzono ich przyrost biomasy. W tym celu losowo pobierano do analizy po 20 larw, dokonywano pomiaru ich długości i wagi, a następnie umieszczano je z powrotem w hodowli. Stopień przeżywalności larw, jak również zawartość ich białka zostały określone zgodnie z metodyką opisaną powyżej (w części dotyczącej **P2**).

4.5.3.2. Analiza obecności fitohormonów

Określenie zawartości fitohormonów w próbkach zostało przeprowadzone z użyciem wysokosprawnej chromatografii cieczowej, a odczynniki zostały zakupione od Sigma-Aldrich Sp. z o.o. (Poznań, Polska). Standardy analityczne, w tym zarówno związki nieznakowane, jak i znakowane izotopami stabilnymi, zostały zakupione od Olchemim (Ołomuniec, Czechy). Liofilizowane próbki zostały zhomogenizowane i do każdej z nich dodano mieszaninę wzorców wewnętrznych: znakowany deuterem kwas indolooctowy, kwas abscysynowy, kwas salicylowy i kwas jasmonowy, a także znakowaną ^{15}N trans-zeatynę. Fitohormony ekstrahowano dwukrotnie roztworem metanolu, wody i kwasu mrówkowego (15:4:1, v/v/v), zgodnie z protokołem opisanym przez Dobreva i Kamínka (2002), z modyfikacjami

wprowadzonymi przez Stefancica i in. (2007). Połączone ekstrakty odparowano do sucha i rozpuszczono w 1 M kwasie mrówkowym. Ekstrakty poddano frakcjonowaniu przy użyciu kolumn do ekstrakcji w fazie stałej (SPE) Oasis MCX 30 mg (Waters). Kwaśne fitohormony (auksyny i fitohormony stresowe) wymywano metanolem, natomiast fitohormony zasadowe (cytokininy) - 0,35 M amoniakiem w 60% metanolu. Oba eluenty odparowano do sucha i odtworzono w 100 µl metanolu. Rozdzielenie chromatograficzne przeprowadzono z użyciem kolumny HPLC Supelco Ascentis RP-Amide (75 mm × 4,6 mm, wielkość cząstek 2,7 µm). W przypadku kwaśnych fitohormonów (w tym auksyn i hormonów stresu) faza ruchoma składała się z 0,1% kwasu mrówkowego w wodzie (rozpuszczalnik A) oraz mieszaniny acetonitrylu i metanolu w stosunku 1:1 (v/v) (rozpuszczalnik B). W przypadku zasadowych fitohormonów (cytokinin) faza ruchoma składała się z 0,001% kwasu octowego w wodzie (rozpuszczalnik A) i mieszaniny acetonitrylu i metanolu w stosunku 1:1 (rozpuszczalnik B). W obu przypadkach zastosowano elucję gradientową przy prędkości przepływu 0,5 ml/min. Analizę wykonano przy użyciu systemu HPLC Agilent Technologies 1260 połączonego z potrójnym spektrometrem masowym Agilent Technologies 6410 wyposażonym w źródło jonizacji elektrorozpylania (ESI). Detekcję przeprowadzono w trybie monitorowania wielu reakcji (MRM), rejestrując dwa najobficiej występujące jony produktowe dla każdego analitu. Dla każdego związku skonstruowano krzywe kalibracyjne przy użyciu odpowiednich standardów analitycznych.

4.6. Analiza statystyczna

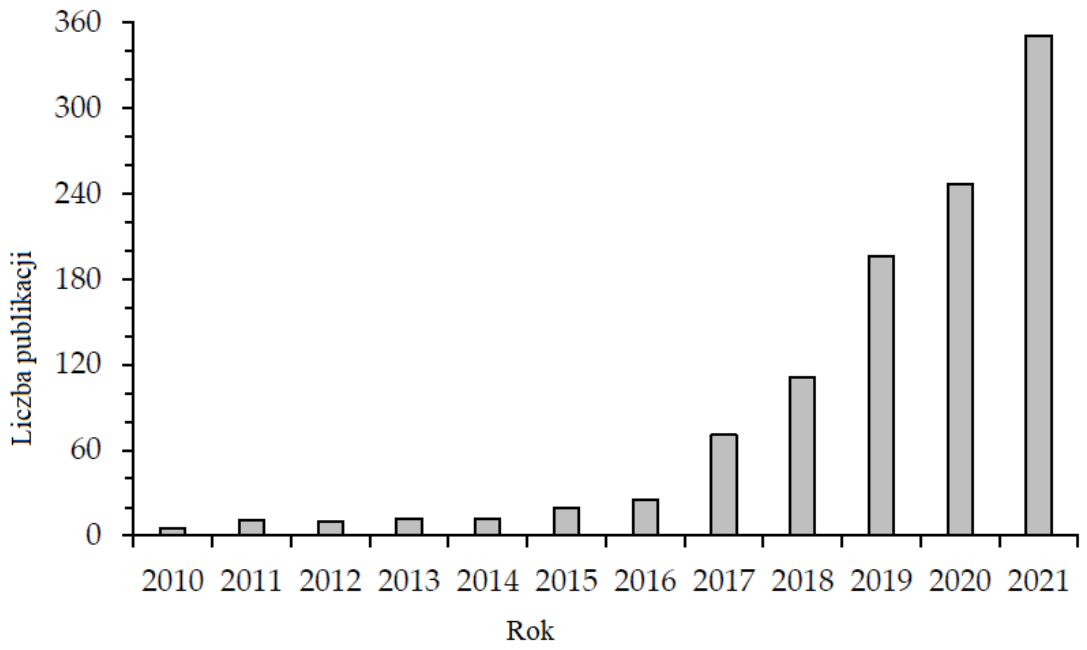
Do przeprowadzenia analiz statystycznych opisanych w **P2**, **P3** i **P4** wykorzystano program Statistica. Doświadczenia przeprowadzane były w trzech niezależnych powtórzeniach biologicznych. Liczba powtórzeń technicznych dla każdej analizy instrumentalnej wynosiła 3-5. Wyniki przedstawione zostały w formie średniej ± odchylenie standardowe. Porównywanie wartości pomiędzy dwoma wariantami (fasolowym i grochowym) polegało na określeniu istotności statystycznej różnic z użyciem testu t-Studenta ($p < 0,05$). W przypadku oceny różnic pomiędzy próbkami w obrębie danego cyklu (np. substrat, świeży frass, dojrzały frass) zastosowano test Tukey'a ($p < 0,05$). Dodatkowo w **P4,** aby uwzględnić wielokrotne testy dla różnych hormonów, wartości p ANOVA zostały skorygowane przy użyciu procedury Benjamini-Hochberg'a (FDR). Wielkość efektu została określona ilościowo przy użyciu współczynnika Cohen'a d (wykazane w suplemencie do **P4**).

5. Omówienie wyników publikacji

5.1 Różnorodność zastosowań *Hermetia illucens* w przemyśle i rolnictwie - przegląd (The variety of applications of *Hermetia illucens* in industrial and agricultural areas – review) – **P1**

Celem tej publikacji było zestawienie i uporządkowanie najnowszych badań (z lat 2018 - 2022), przedstawiających aktualny stan wiedzy o właściwościach owada *H. illucens* oraz potencjale jego zastosowań w przemyśle i rolnictwie. Od 2018 roku zaobserwowano znaczny wzrost liczby publikacji naukowych dotyczących *H. illucens* (Rys. 5). Jednocześnie poszerzał się zakres zastosowań oraz tematyka badań opisywanych w tych publikacjach. Zagadnienia te omówiono w 11 podrozdziałach, z których każdy dotyczy konkretnego obszaru zastosowań.

Rys. 5. Dynamika wzrostu publikacji na temat *H. illucens*. Informacje z bazy danych Web of Sciences, maj 2022 r. Do wyszukiwania użyto kombinacji słów kluczowych: „*Hermetia illucens*" lub „*H. illucens*" lub „Black Soldier Fly".



5.1.1 Biokonwersja biomasy odpadowej

Jedną z najbardziej charakterystycznych cech larw *H. illucens* jest ich żarłoczność oraz względna niewybredność w odniesieniu do pożywienia. Właśnie dlatego owad ten jest wykorzystywany do rewaloryzacji oraz biokonwersji odpadów organicznych, przetwarzając je w biomasę bogatą w białka i tłuszcze. Zastosowanie *H. illucens*, podobnie jak innych owadów saprofagicznych, może mieć duże znaczenie dla technologii biokonwersji w kontekście wzrostu populacji ludzkości, rosnącego zapotrzebowania na żywność, a w konsekwencji także zwiększającej się ilości odpadów organicznych. Do przebadanych dotychczas substratów należą m.in.: pozostałości rolno-spożywcze w tym przede wszystkim resztki owocowo-warzywne (Barbi i in. 2020), różnego rodzaju oborniki zwierzęce (Wang i in. 2021), odpady restauracyjne (Sprangers i in. 2017), odchody ludzkie (Dortmans i in. 2017) oraz osady ściekowe (Lalander i in. 2019). Badania te potwierdziły, że larwy *H. illucens* dobrze trawią większość związków organicznych zawartych w odpadach, z wyjątkiem ligniny i celulozy (Liu i in. 2019; Ramzy i in. 2022).

5.1.2 Pasza dla zwierząt

Wykorzystanie *H. illucens* do skarmienia zwierząt jest jednym z najlepiej zbadanych aspektów praktycznego wykorzystania tego owada. Larwy mogą być podawane w różnych postaciach: na surowo, suszone, w całości lub po zmieleniu (Yu i in. 2019), a także w postaci mączki białkowej, która może być pełnotłusta (Yu i in. 2020), częściowo odtłuszczona (Gariglio i in. 2019) lub całkowicie odtłuszczona (Lei i in. 2019). Składniki odżywcze pochodzenia owadziego mogą być również wykorzystywane jako dodatki w opracowywanych nowych formułach paszowych.

Gatunek *H. illucens* wyróżnia się korzystnym profilem aminokwasowym, co czyni go odpowiednim zarówno do zastosowań w żywieniu zwierząt, jak i potencjalnie w diecie człowieka (Oonincx i Finke 2021). Przykładowo, tłuszcz owadzi może pełnić rolę substytutu oleju sojowego, a modyfikacje diety larw, np. poprzez suplementację resztkami rybnymi, mogą prowadzić do zwiększenia udziału kwasów tłuszczowych omega-3 w ich biomasie (Barroso i in. 2017). Jednak w procesach przemysłowego wykorzystywania larw *H. illucens* często konieczny jest etap odtłuszczania, ponieważ nadmierna zawartość tłuszczu utrudnia proces mielenia. Do zwierząt, które mogą być karmione produktami na bazie *H. illucens*, należą m.in. ryby, kury, świnie, gady oraz psy. W większości przypadków takie żywienie prowadzi do korzystnych efektów w zakresie wzrostu i rozwoju zwierząt. Konieczny jest jednak

odpowiedni dobór formy produktu owadziego, dostosowany do specyficznych wymagań żywieniowych danego gatunku (Tab. 1). Przykładowo dla niektórych ryb (*Argyrosomus regius*, *Salmo salar*) bardziej odpowiednia jest jedynie suplementacja larwami *H. illucens*, nieprzekraczająca 17% świeżej masy standardowej karmy.

Tab. 1. Zestawienie zwierząt jako obiektów do badań oraz *H. illucens* jako formy pożywienia.

Zwierzę	Forma *H. illucens*	Efekt	Referencja
		Pozytywne skutki	
Pies	Dieta oparta na zbożach + 2% odtłuszczonej mączki z larw *H. illucens*	Poprawa strawności suchej masy o 1,05 razy, obniżenie poziomu TNF-α o 1,82 razy (działanie przeciwzapalne), zwiększenie poziomu peroksydazy glutationowej o 1,26 razy (działanie przeciwutleniające)	Lei i in. 2019
Tucze świń	Kukurydza, otręby pszenne i mączka sojowa + 4% suszonych i rozdrobnionych prepoczwarek *H. illucens*	Zmniejszona ekspresja cytokin prozapalnych i stężenia amin całkowitych oraz fenolu, zwiększona ekspresja cytokin przeciwzapalnych, genów bariery jelitowej oraz stężenia krótkołańcuchowych kwasów tłuszczowych (SCFA) i maślanu (efekt prebiotyczny)	Yu i in. 2019
Prosięta	Mączka rybna + 2% pełnotłustej mączki z larw *H. illucens*	Wzrost stężenia mleczanu w jelicie krętym o 1,6 razy, w jelicie ślepym o 2,2 razy oraz krótkołańcuchowych kwasów tłuszczowych (SCFA) o 1,2 razy w jelicie krętym i o 1,1 razy w jelicie ślepym (efekt probiotyczny), wzrost stężenia białka przeciwzapalnego IL-10 o 1,3 razy, spadek stężenia białka prozapalnego TNF-α o 1,3 razy	Yu i in. 2020
Kury nioski	Mączka kukurydziano-sojowa + 25% zastąpionego białka częściowo odtłuszczonym *H. illucens*	Pomimo zmniejszonej długości kosmków jelitowych, zwiększyła się ilość lotnych kwasów tłuszczowych o 1,1 razy, a ilość maślanu o 1,22 razy w jelitach	Moniello i in. 2019
Kury nioski	Mączka sojowa i olej sojowy + prepoczwarki *H. illucens*	Wzrost masy jaj o 1,1 razy, wzrost stężenia SCFA o 1,3 razy	Kawasaki i in. 2019
Kurczaki brojlery	Pasza dla kurcząt + 5% lub 10% żywych larw *H. illucens*	Zmniejszona lękliwość kur, zwiększona aktywność kur	Ipema i in. 2020
Kaczka piżmówka (*Cairina moschata domestica*)	9% częściowo odtłuszczona mączka z *H. illucens*	Spadek stężenia kwasu moczowego o 1,2 razy i kreatyniny o 1,2 razy (poprawa czynności nerek), wzrost stężenia żelaza Fe w surowicy o 1,3 razy	Gariglio i in. 2019
Okoń morski (*Dicentrarchus labrax*)	Mączka rybna + 50% suszonej mączki z larw *H. illucens*	Brak istotnych różnic w stosunku do grupy kontrolnej	Abdel-Tawwab i in. 2020
Sum afrykański (*Clarias gariepinus*)	Mączka rybna + 50% częściowo odtłuszczona mączka z larw *H. illucens*	Wzrost masy ciała o 1,5 razy	Fawole i in. 2020
Łosoś atlantycki (*Salmo salar*)	Białko kukurydziane, mączka sojowa + (200 g·kg^{-1}) mączka z *H. illucens*	Brak istotnych różnic w stosunku do grupy kontrolnej	Fisher i in. 2020
Króliki	Pokarm dla królików + 1,5% tłuszczu *H. illucens*	Hamowanie wzrostu patogenów *Pasteurella multocida* 3,17 razy, *Yersinia enterocolitica* 2,48 razy, *Listeria monocytogenes* 2,05 razy	Dabbou i in. 2020
Pstrąg tęczowy (*Oncorhynchus mykiss*)	Dieta kontrolna (gluten pszeniczny, mączka sojowa i hemoglobina) + 15% mączki z larw *H. illucens*	Wzrost liczby pożytecznych bakterii *Lactobacillus* i *Bacillus*, zmniejszenie liczby patogenów *Aeromonas* w jelitach ryb	Rimoldi i in. 2021
Samice indyków	Soja i kukurydza wzbogacone 50 g/kg tłuszczu z larw *H. illucens* (50% i 100%)	Poprawa strawności ekstraktu eterowego w jelitach Wzrost aktywności lipazy Zmniejszenie skupisk bakterii z rodzaju Bacteroides-Prevotella	Kierończyk i in. 2022
		Negatywne skutki	
Kulbin (*Argyrosomus regius*)	Częściowo odtłuszczony *H. illucens* + mączka rybna	Utrata masy ciała, spadek wydajności białkowej	Guerreiro i in. 2019
Łosoś atlantycki (*Salmo salar*)	Dieta kontrolna z pełnotłustym posiłkiem z larw *H. illucens*, zastępującym 12,5% zawartości białka oraz dieta kontrolna z pełnotłustą pastą z larw *H. illucens*, zastępującą 6,7% białka	Spadek wydajności białkowej i lipidowej oraz wskaźnika wydajności białkowej, spadek retencji fosforu	Weththasinghe i in. 2021

5.1.3 Chityna i chitozan

Chityna jest polisacharydowym biopolimerem zbudowanym z jednostek N-acetyl-D-glukozaminy oraz D-glukozaminy. Może być wykorzystywana m.in w: i) ochronie środowiska np. jako sorbent metali ciężkich, barwników czy innych zanieczyszczeń (Peter i in. 2021), ii) energetyce, np. w produkcji biosensorów czy elementów wykorzystywanych do produkcji energii (Peter i in. 2021), iii) medycynie, np. jako materiał biomedyczny, m.in. w postaci szwów chirurgicznych i opatrunków na rany (Shamshina i in. 2019).

Chityna występująca u *H. illucens* należy do formy alfa, charakteryzującej się przeciwrównoległym układem łańcuchów polisacharydowych (Faria i in. 2016). Pomimo że chitynę można wyizolować z każdego stadium rozwojowego owada, jej największe ilości występują w wylinkach, tj. pozostałościach po poczwarce po wyjściu z niej imago (Tab. 2). Wydajność ekstrakcji chityny zależy od zastosowanej metody, która może być chemiczna (z wykorzystaniem naturalnych rozpuszczalników) lub fermentacyjna.

Z chityny (również pochodzenia owadziego), na drodze procesu deacetylacji, można powszechnie wykorzystywany w gospodarce chitozan. Związek ten wykazuje m.in. właściwości przeciwutleniające (Lee i in. 2022). Guarnieri i in. (2022) potwierdzili natomiast działanie przeciwdrobnoustrojowe chitozanu (zarówno zdepigmentowanego, jak i niezdepigmentowanego) pozyskanego z larw oraz ich pozostałości (wylinek i much) wobec *Micrococcus flavus* i *E. coli*. Z kolei Alghuthaymi (2022) opracował nanokompozyt na bazie chitozanu z larw *H. illucens* oraz gumy arabskiej, z dodatkiem eugenolu i nanocząstek selenu, który wykazywał silne działanie wobec *E. coli* (MIC 15,0 µg/ml) oraz *S. aureus* (MIC 20,0 µg/ml), przewyższające aktywność ampicyliny.

Tab. 2. Całkowita zawartość chityny (%) na różnych etapach rozwoju *H. illucens* i w wylinkach.

Metoda ekstrakcji	Materiał owadzi	Zawartość chityny (%)	Stopień krystaliczności (%)	Referencja
Demineralizacja: 2M HCl (55°C, 1h, 200 rpm·min^{-1}), deproteinizacja: 2M NaOH (50°C, 18h, 200 rpm·min^{-1}), depigmentacja: 3.6% HCl (0.5h), 10-krotnie rozcieńczony NaClO (80°C, 4h, 200 rpm·min^{-1})	Larwy	3,6	33,09	Wang i in. 2020
	Prepoczwarki	3,1	35,14	
	Wylinki	14,1	68,44	
	Muchy	2,9	87,92	
Demineralizacja: 1M HCl (1:10 (m:v), temperatura pokojowa., 1h), deproteinizacja: 1M NaOH (1:25, 80°C, 1h), depigmentacja: 12-krotne powtórzenie procesu deproteinizacji	Larwy	9,5	~ 88	Soetemans i in. 2020
	Prepoczwarki	9,1	~ 95	
	Poczwarki	10,3	~ 93	
	Skórki z larw	31,1	~ 90	
	Wylinki	23,8	~ 94	
	Muchy	5,6	~ 89	
Demineralizacja:1M HCl (100°C, 0.5 h), deproteinizacja: 1 M NaOH (24h)	Wylinki	25,4	74,1	Brigode i in. 2020
	Płatki po ekstrakcji oleju	20,7	61,1	
	Muchy	78	77,8	
Acid detergent fiber - Acid detergent lignin	Wylinki	21,2	70,8	
	Płatki po ekstrakcji oleju	26,8	50,0	
	Muchy	7,9	39,0	
Demineralizacja: 1 M HCl, 22°C, 1 h, deproteinizacja: 1 M NaOH, 80°C, 24 h, depigmentacja: 9% H_2O_2, 80°C, 2.5 h	Wylinki	7,0	60	Złotko i in. 2021
Demineralizacja*:* 0.5 M kwas mrówkowy (1:10 (m:v), 1 h, temperatura pokojowa, deproteinizacja: 2 M NaOH (1:10 (m:v), 2 h, 80°C	Larwy	13,0	74	Triunfo i in. 2022
	Wylinki	31	78	
	Muchy	9,0	79	
Demineralizacja: kwas mrówkowy 0,5 M (1:10 (m:v), 1 godz., temperatura pokojowa, deproteinizacja: NaOH 2 M (1:10 (m:v)), 2 godz., 80 °C, depigmentacja: 5% H2O2 , (1:20–30), 30–60 min, 90 °C	Larwy	10.0	77	
	Wylinki	23.0	80	
	Muchy	6.0	86	

b.d. – brak danych

5.1.4. Właściwości przeciwdrobnoustrojowe

Larwy *H. illucens* są saprofagami i żyją w środowisku gnilnym, będącym siedliskiem wielu mikroorganizmów, przez co wykształciły mechanizmy obronne, w tym przeciwdrobnoustrojowe. Między innymi mają zdolność wytwarzania peptydów, które eliminują lub hamują rozwój niektórych mikroorganizmów, np. bakterii z rodzaju *Salmonella* oraz wybranych szczepów *Escherichia coli*, metycylinoopornego *Staphylococcus aureus*, czy *Helicobacter pylori*. Peptydy te zostały zidentyfikowano w transkryptomie *H. illucens* i obejmują m.in. defensyny, cekropiny, lizozymy i attaciny. W testach laboratoryjnych wykazują one silne działanie bakteriobójcze. Właściwości bakteriobójcze przypisuje się również tłuszczowi larw, szczególnie bogatemu w kwas laurynowy, który zaburza integralność zarówno ścian, jak i błon komórkowych bakterii. Dodatkowo, aktywność przeciwdrobnoustrojową mogą wykazywać metabolity grzybów symbiotycznych zasiedlających przewód pokarmowy larw, pigmenty ommochromowe z oczu much *H. illucens* oraz produkty fermentacji biomasy larw. Stwierdzono również silne właściwości przeciwdrobnoustrojowe ekstraktów z larw uzyskanych metodą ekstrakcji metanolem. Ekstrakty te hamują wzrost wielu bakterii Gram-ujemnych i Gram-dodatnich, w tym szczepów lekoopornych, a także niektórych fitopatogenów (Choi i in. 2012, Mariusch i in. 2020). Różnorodność mechanizmów i związków bioaktywnych czyni *H. illucens* atrakcyjnym źródłem naturalnych substancji o działaniu przeciwbakteryjnym, przeciwgrzybiczym, które mogą znaleźć zastosowanie w medycynie, rolnictwie oraz biotechnologii.

5.1.5. Tłuszcz i produkcja biodiesla

Zawartość tłuszczu surowego w biomasie *H. illucens* zależy od rodzaju stosowanej diety i może sięgać nawet 40% suchej masy (Zheng i in. 2012; Feng i in. 2019; Wong i in. 2020), co czyni ją potencjalnym surowcem do produkcji biodiesla. Najwyższy poziom tłuszczu występuje u larw przed przepoczwarzeniem (prepoczwarek), natomiast w stadium poczwarki obserwuje się istotny spadek jego zawartości (około 2,8-krotny) (Zhu i in. 2019). Metylowe estry kwasów tłuszczowych (FAME), będące głównym składnikiem biodiesla, mogą być wytwarzane także z triacylogliceroli pozyskiwanych z biomasy *H. illucens* w procesie transestryfikacji. Tłuszcze *H. illucens* charakteryzują się wysokim udziałem nasyconych kwasów tłuszczowych oraz niskim udziałem kwasów nienasyconych, co może pozytywnie wpływać na lepkość oraz stabilność oksydacyjną biodiesla. Skład kwasów tłuszczowych w biodieslu z larw *H. illucens* jest silnie zależny od rodzaju podłoża hodowlanego, jednak

we wszystkich przypadkach głównym składnikiem pozostaje kwas laurynowy (C12:0) (Tab. 3).

Transestryfikacja jest podstawowym etapem produkcji biodiesla z tłuszczu larw *H. illucens* i może być prowadzona z użyciem katalizatorów chemicznych (H_2SO_4, NaOH) lub enzymatycznie, z wykorzystaniem lipaz (Ishak i in. 2019). Alternatywnie można zastosować procesy niekatalityczne prowadzone w wysokiej temperaturze, osiągając wydajność porównywalną z klasyczną transestryfikacją (Jung i in. 2022). W praktycznych testach oceniono działanie zarówno oleju tłoczonego z larw (bez transestryfikacji), jak i biodiesla z tego oleju (Kamarulzaman i in. 2019). Biodiesel z larw wykazuje właściwości bardziej zbliżone do tradycyjnego oleju napędowego niż surowy olej z larw i może poprawiać parametry spalania, choć jednocześnie wiąże się ze wzrostem emisji NOx oraz zwiększonym zużyciem paliwa.

5.1.6. Produkcja biogazu

Pozostałości po hodowli *H. illucens* (frass), całe owady (larwy i muchy), jak również pozostałości po przetwórstwie owadów (np. wyekstrahowany tłuszcz larw lub pozostałości larw po ekstrakcji z nich tłuszczu) mogą być wykorzystywane do produkcji energii w postaci biogazu w procesie fermentacji beztlenowej. Najwyższy potencjał biometanu wykazywały całe larwy hodowane na odpadach spożywczych i wynosił on ok. 661-675 ml $CH_4 \cdot g^{-1}$ suchej masy organicznej (s.m.o.) (Win i in. 2018) (Tab. 4). Średni potencjał biometanu z *H. illucens* był wyższy niż w przypadku tradycyjnych substratów, takich jak rośliny uprawiane w celu pozyskiwania biomasy na cele energetyczne.

Tab. 3. Zawartość głównego składnika metylowych estrów kwasów tłuszczowych (FAME) w zależności od paszy, na której hodowano BSFL. Litera „a" przedstawia wartość obliczoną na podstawie danych przedstawionych w publikacji.

Zawartość tłuszczu surowego (%)	Główny składnik FAME	Zawartość głównego składnika FAME (%)	Dieta	Stadium rozwojowe *H. illucens*	Referencja
23,28	Kwas laurynowy	35,6	obornik bydlęcy	Larwy	Li i in. 2011
39,2	Kwas oleinowy	27,1	stała frakcja odpadów restauracyjnych	Larwy	Zheng i in. 2012a
35,7- 39,6	Kwas laurynowy	27,8	odpady stałe z restauracji i fermentowana słoma ryżowa z mikroorganizmami egzogenicznymi	Larwy	Zheng i in. 2012b
-	Kwas laurynowy	58,31	osad ściekowy	Larwy	
-	Kwas laurynowy	76,13	odpady owocowe	Larwy	Leong i in. 2015
-	Kwas laurynowy	48,06	pozostałości po wytłoczeniu oleju palmowego	Larwy	
-	Kwas laurynowy	38,43	ziarno pszenicy	Larwy	Ushakova i in. 2016
-	Kwas laurynowy	44,9	odpady spożywcze z kafeterii	Prepoczwarki	Sundera i in. 2016
33,6[a]	Kwas laurynowy	57,35	pasza dla drobiu	Prepoczwarki	
21,8[a]	Kwas laurynowy	43,65	odpad pofermentacyjny z biogazu	Prepoczwarki	Spranghers i in. 2016
37,1[a]	Kwas laurynowy	60,89	odpady roślinne	Prepoczwarki	
38,6[a]	Kwas laurynowy	57,56	odpady restauracyjne	Prepoczwarki	
31,17	Kwas laurynowy	87,46	obornik kurzy zmieszany ze słomą rzepakową	Larwy	Elsayed i in. 2020
35-40	Kwas laurynowy	65,7	Odpady z miąższu kokosa poddane fermentacji egzomikrobiologicznej	Larwy	Wong i in. 2020
57,8	Kwas laurynowy	51,8	chleb	Larwy	
46,7	Kwas laurynowy	28,6	ryby	Larwy	
40,7	Kwas laurynowy	39,9	odpady spożywcze	Larwy	
33,1	Kwas laurynowy	52,1	świeże małże	Larwy	
11,2	Kwas laurynowy	13,4	kiszone małże	Larwy	
29,7	Kwas laurynowy	32,3	zgniłe małże	Larwy	Ewald i in. 2020
20,4	Kwas laurynowy	47,4	chleb i małże10%	Larwy	
19,6	Kwas laurynowy	47,6	chleb i małże 20%	Larwy	
17,9	Kwas laurynowy	43,6	chleb i małże 30%	Larwy	
17,9	Kwas laurynowy	42	chleb i małże 40%	Larwy	
16,1	Kwas laurynowy	35,3	chleb i małże 50%	Larwy	

Tab. 4. Wydajność biometanu uzyskanego w wyniku fermentacji różnych pozostałości uzyskanych z hodowli *H. illucens.*

	Surowiec	Łączny potencjał biometanu (ml CH_4 g^{-1} s.m.o.)	Stężenie CH_4 (% obj.)	Literatura
całe owady lub ich części	Larwy po odpadach spożywczych	675 ± 118		
	Larwy po paszy dla drobiu	661 ± 29		
	Martwe muchy	570 ± 51		
	Larwy po odpadach spożywczych po ekstrakcji tłuszczy	363 ± 32	b.d.	Win i in. 2018
	Kutikula larw	343 ± 7		
	Larwy po paszy dla drobiu po ekstrakcji tłuszczy	306 ± 23		
	Całe larwy	108 ± 65		
	Całe larwy	455,87 ± n.d.	64,27	Czekała i in. 2020
frass	Odchody ludzkie poddane działaniu larw	178,9 ± 7,1	55,2 ± 0,7	Lalander i in. 2018
	Odpady spożywcze poddane działaniu larw	322,6 ± 6,4	61,4 ± 0,4	
	Pozostałości	502 ± 9	b.d.	Win i in. 2018
	Odpady po hodowli	207,9 ± 21,5	53,2 ± 3,2	Bulak i in. 2020

b.d. – brak danych

5.1.7 Entomoremediacja

Larwy *H. illucens* podczas biokonwersji biomasy odpadowej równocześnie ją utylizują zmniejszając jej ilość. Nie jest to jednak jedyny pozytywny aspekt entomoremediacji. Żerując na biomasie skażonej (np. metalami ciężkimi), larwy mogą wbudowywać te metale w swoją biomasę (Bulak i in. 2018). Badania wykazały, że metale te gromadzą się głównie w wylinkach (Gao et al. 2017). Dzięki temu dochodzi do zatężenie metali w biomasie owadów, co może umożliwić ich częściowy odzysk, np. niektórych metali ziem rzadkich (Gao i in. 2017).

Coraz większe zainteresowanie wzbudza zdolność larw *H. illucens* do degradacji zanieczyszczeń organicznych, określana mianem entomodegradacji. Badania dotyczące zanieczyszczeń gleby wykazały, że larwy BSF mogą radzić sobie z niektórymi mykotoksynami

(Leni i in. 2019; Meijer i in. 2019), węglowodorami (Fan i in. 2020), insektycydami (Meijer i in. 2021) oraz antybiotykami (Hasnol i in. 2020).

5.1.8. Frass

Po zakończeniu hodowli owadów pozostaje frass, czyli pozostałości nieprzejedzonego substratu pokarmowego wymieszanego z odchodami, wylinkami larwalnymi oraz martwymi osobnikami, wzbogaconego w mikrobiom związany z larwami. Podobnie jak w przypadku obornika zwierząt gospodarskich, frass może zostać wykorzystany jako bionawóz. Średnia zawartość głównych składników odżywczych dla roślin w różnych frassach po hodowli *H. illucens* wynosiła ok. 3,39% suchej masy dla N, ok. 2,85% suchej masy dla P_2O_2 oraz ok. 3,47% suchej masy dla K_2O (Schmitt i de Vries 2020). Przetwarzanie obornika zwierzęcego przez larwy *H. illucens* poprawia jego jakość i stabilizuje produkt poprzez przyspieszenie procesów humifikacji (Wang et al. 2021).

Jednak oprócz wartości odżywczych frass może również oddziaływać na fitopatogeny glebowe. Gebremikael i in. (2020) wykazali, że dodanie świeżego frassu zmniejszało liczebność patogennego grzyba *Rhizoctonia solani* w glebie, a w doświadczeniu z fasolą (*Phaseolus vulgaris*) stopień porażenia spadł o ok. 50%. Badania wykazały również właściwości przeciwgrzybicze ekstraktów z frassu *H. illucens*, jednak ich skuteczność zależała od podłoża, na którym hodowano owady (Arabzadeh i in. 2022). Niefiltrowany ekstrakt z larw karmionych standardową dietą Gainesville (67% woda, 17% otręby pszenne, 6,6% mąka kukurydziana, 9,9% lucerna) hamował wzrost wszystkich testowanych grzybni – *Sclerotinia sclerotiorum*, *Alternaria solani*, *Botrytis cinerea*, *Pythium capsici*, *Fusarium oxysporum* i *Rhizoctonia solani*. Z kolei ekstrakt pochodzący z larw karmionych odpadami owocowo-warzywnymi oraz piekarniczo-browarnianymi hamował jedynie *S. sclerotiorum*, *B. cinerea* oraz w mniejszym stopniu *R. solani*. Istnieją jednak również badania, takie jak te przeprowadzone przez Kawasaki i in. (2020), w których we frassie z organicznych odpadów domowych wykryto bakterie z rodziny Xanthomonadaceae wykazujące aktywność fitopatogenną. Wyniki te wskazują na potrzebę dalszych badań doświadczalnych oceniających bezpieczeństwo wykorzystania frassu jako bionawozu. Niezbędna może okazać się również konieczność opracowania i wdrożenia procedur kontroli jakości przed praktycznym zastosowaniem frassu.

Zaobserwowane efekty nawozowe obejmowały m.in. wzrost suchej masy części nadziemnej ryżu oraz zwiększenie zawartości chlorofilu w liściach przy 4% (m/m) dodatku

frassu pochodzącego z kurzego obornika (Wu i in. 2020). Mieszanki podłoża torfowego (80%) z odchodami larw *H. illucens* (20%) poprawiały wzrost bazylii, pomidora i sałaty, zwiększając suchą masę i powierzchnię liści, bez objawów stresu abiotycznego (Setti i in., 2019). Z kolei zastosowanie ekstraktu z frassu w uprawach akwaponicznych poprawiało jakość odżywczą roślin, zwiększając zawartość cukru w chili oraz poziom manganu w batatach (Romano i in., 2022).

5.1.9 Larwy jako pokarm

Do tej pory larwy *H. illucens* nie są dopuszczone do spożycia przez ludzi w Unii Europejskiej. Jednak wraz z rosnącą liczbą ludności, poszukiwane są nowe, alternatywne źródła białka, dlatego *H. illucens* jest również badana pod kątem bezpieczeństwa jej wykorzystywania. Mąka oraz koncentraty białkowe pozyskiwane z larw *H. illucens* charakteryzują się wysoką wartością odżywczą, zbliżoną do tradycyjnych źródeł białka. Najwyższą zawartość białka uzyskano w mące odtłuszczonej, wynoszącą ok. 50% suchej masy, natomiast w koncentracie białkowym po ekstrakcji alkaliczno-kwaśnej zawartość białka sięgała ok. 73%. Skład aminokwasowy tych produktów jest porównywalny z białkiem mleka i jaj, a rozpuszczalność białka w niskim pH (pH 2) w przypadku koncentratów wynosi 85–97%, co świadczy o ich dobrej przyswajalności (Mshayisa i in. 2022). Koncentraty białkowe wykazują również wysoką zdolność wiązania wody oraz stabilność emulsji, podczas gdy mąki charakteryzują się lepszą zdolnością wiązania oleju. Produkty te są termostabilne, co zwiększa ich przydatność technologiczno-funkcjonalną (Mshayisa i in. 2022). Zawartość tłuszczu w mące z larw *H. illucens* wynosi około 18% suchej masy, czyli jest prawie 2,3 razy niższa niż w wołowinie. Profil kwasów tłuszczowych wskazuje na wysoki udział nasyconych kwasów tłuszczowych (SFA) – ok. 61%, co wymaga dalszych badań nad bezpieczeństwem długoterminowego spożycia (Anankware i in. 2021). Pod względem bezpieczeństwa mikrobiologicznego i chemicznego najniższe stężenia bakterii *Bacillus cereus* i *E. coli* odnotowano w larwach blanszowanych, szczególnie hodowanych na paszy dla brojlerów. Blanszowanie wpływało również na zawartość pierwiastków i alergenów, przy czym obecność tropomiozyny i kinazy argininowej była podobna jak u skorupiaków. Rodzaj podłoża na którym hodowane były larwy nie wpływał na poziom alergenów, natomiast zawartość metali ciężkich zależała od podłoża i metody uśmiercania larw (Bessa i in. 2020).

5.1.10. Środki kosmetyczne i produkty higieny osobistej

H. illucens stanowi obiecujące źródło składników kosmetycznych, jednak obecnie zastosowanie to jest wskazywane raczej jako możliwość teoretyczna. Podstawą takiego założenia jest fakt, że *H. illucens* może dostarczać białka zawierającego, m.in. glicynę i argininę, cenione w kosmetyce za działanie nawilżające i antyoksydacyjne oraz udział w syntezie kolagenu. Ich zawartość zależy od diety larw i może wynosić ok. 51 $g \cdot kg^{-1}$ (arginina) i 59 $g \cdot kg^{-1}$ (glicyna) (Almeida i in. 2020). Dodatkowo peptydy przeciwdrobnoustrojowe wytwarzane przez larwy mogą znaleźć zastosowanie w kosmetykach przeznaczonych dla skóry problematycznej. Chityna, ze względu na właściwościom przeciwdrobnoustrojowe, nawilżające oraz biozgodności, może pełnić funkcję substancji aktywnej w kosmetykach i przyjmować różne formy, np. hydrożele, membrany lub nanowłókna, które mogą działać jako nośniki składników aktywnych (Triunfo i in. 2021). W kolei tłuszcz z larw jest bogaty w kwasy laurynowy, mirystynowy, palmitynowy i oleinowy, i jego profil jest podobny do oleju kokosowego czy oleju z pestek palmowych (Franco i in. 2021), co pozwala traktować go jako alternatywne źródło tych składników w kosmetyce.

5.1.11. Bioplastiki

Białka z *H. illucens* mogą być wykorzystywane jako surowiec do produkcji przyjaznych dla środowiska tworzyw i służyć m.in. do wytwarzania folii, np. do celów ściółkowania. Badania te są jednak na wstępnym etapie i wymagają dalszych prac w celu uzyskania materiału o dobrej jakości. Barbi i in. (2018) wykazali, że z izolatu białkowego larw *H. illucens,* w połączeniu z glicerolem i opcjonalnie kwasem cytrynowym, można otrzymać bioplastik o dobrej rozciągliwości, przy czym dana zawartość białka ograniczała grubość materiału. Natomiast Nuvoli i in. (2021) wykazali, że zastosowanie rozpuszczalnych frakcji białkowych pozwala uzyskać biofilmy bardziej elastyczne, wytrzymałe i przezroczyste, a dodatek kwasu cytrynowego poprawiał niektóre właściwości mechaniczne i zmniejszał absorpcję wody.

Podsumowując, powyższy przegląd publikacji naukowych wypełnił założenie celu **C1**. Dodatkowo jako ostatni rozdział przeglądu zostały wskazane perspektywy i możliwe kierunki przyszłych badań w kontekście szerszego wykorzystania zarówno owadów *H. illucens,* jak i pozostałości po ich hodowli.

5.2 Frass czarnej muchy z odpadów z nasion roślin strączkowych bogatych w azot - jak długotrwałe dojrzewanie wpływa na właściwości nawozowe? (Black soldier fly frass from seed waste of nitrogen-rich legumes - How long-term maturation affects the fertilizer properties?) – **P2**

Cele badań przedstawionych w publikacji **P2** dotyczyły: określenia właściwości nawozowych frassu uzyskanego po hodowli larw *H. illucens* na odpadach nasion roślin strączkowych bogatych w azot (**C2**) oraz ocenę, czy tlenowe dojrzewanie otrzymanego frassu wpływa korzystnie na jego właściwości jako bionawozu (**C3**). Badania bazowały się na następujących hipotezach: wykorzystanie odpadów roślinnych bogatych w azot jako substratu do skarmiania larw *H. illucens* będzie skutkowało otrzymaniem bogatego w azot frassu (**H1**) oraz że dojrzewanie frassu doprowadzi do stabilizacji jego właściwości, czego efektem będzie wzrost wartości nawozowych (**H2**).

Pierwszym krokiem doświadczenia była charakterystyka składu obu odpadów z nasion (Tab. 5).

Tab. 5. Skład substratów – odpadów poprodukcyjnych nasion fasoli i grochu.

% suchej masy	Odpady fasoli		Odpady grochu
Białko	34,37 ± 3,74	*	19,56 ± 5,78
Tłuszcze	0,53 ± 0,01	*	0,17 ± 0,01
Włókno	15,38 ± 1,41	*	38,85 ± 5,85
Popiół	4,18 ± 0,12		4,13 ± 0,11
Węglowodany	45,55 ± 3,50		37,87 ± 7,40

Gwiazdka „*" oznacza istotną różnicę ($p < 0,05$, test t-Studenta) pomiędzy danym parametrem w dwóch wariantach żywienia.

Odmienny skład obu substratów nie wpłynął na stopień ich utylizacji przez larwy, który w obu wariantach wynosił ok. 67% (Tab. 8). Istotne różnice zaobserwowano jednak w przyroście biomasy *H. illucens*, osobniki hodowane na odpadach z fasoli charakteryzowały się większą masą larw i poczwarek oraz większą długością poczwarek (długość larw również była większa w tym wariancie, ale różnice nie były istotne statystycznie) (Tab. 6). Przyczyną tych różnic mogła być wyższa zawartość składników odżywczych w odpadach fasoli, takich jak białko i tłuszcz (Tab. 5). Natomiast u larw hodowanych na odpadach z grochu odnotowano wyższą przeżywalność oraz wyższy stopień przepoczwarzenia (Tab. 6), co mogło być spowodowane większą dostępnością makro- i mikroelementów (Tab. 7). Należy jednak

zaznaczyć, że niższa przeżywalność larw hodowanych na odpadach fasoli mogła być również związana z wyższymi stężeniami jonów amonowych (Rys. 6), które mogą wykazywać działanie toksyczne wobec bezkręgowców (Zhang i in. 2023). Jony te oznaczono w surowym frassie z fasoli na wyższym poziomie niż we frassie z grochu (Rys. 6).

Tab. 6. Parametry wzrostu larw i poczwarek *H. illucens* po miesiącu hodowli na odpadach z fasoli i grochu.

Parametry	Wariant fasoli		Wariant grochu
Masa 1 larwy (g)	0,10 ± 0,00	*	0,08 ± 0,00
Długość 1 larwy (cm)	1,6 ± 0,30		1,4 ± 0,28
Stopień przepoczwarczenia (%)	10,1 ± 1,27	*	42,07 ± 7,06
Masa 1 poczwarki (g)	0,13 ± 0,00	*	0,08 ± 0,00
Długość 1 poczwarki (cm)	1,8 ± 0,03	*	1,5 ± 0,02
Stopień przeżywalności (%)	77,5 ± 7,35	*	93,2 ± 6,95

Gwiazdka „*" oznacza istotną różnicę ($p < 0,05$, test t-Studenta) pomiędzy danym parametrem w dwóch wariantach żywienia.

Tab. 7. Skład pierwiastkowy ($mg \cdot kg^{-1}$ suchej masy) larw po biokonwersji odpadów z fasoli i grochu.

		Fasola		Groch
makroelementy	Ca	11823,33 ± 581,68	*	20261,67 ± 438,79
	K	8570,00 ± 144,21	*	9796,67 ± 246,19
	Mg	4306,67 ± 347,62		4180,50 ± 127,33
	P	8529,83 ± 404,51	*	11216,67 ± 183,81
	S	2930,33 ±102,99	*	3551,00 ± 98,26
mikroelementy	B	0,24 ± 0,09		0,33 ± 0,16
	Cu	12,48 ± 1,52		12,75 ± 0,29
	Fe	140,87 ± 11,74	*	273,02 ± 17,32
	Mn	71,73 ± 4,18	*	105,28 ± 3,84
	Mo	0,82 ± 0,16		0,84 ± 0,07
	Na	157,97 ± 5,76	*	144,70 ± 9,02
	Zn	84,86 ± 4,00	*	166,02 ± 26,14
metale ciężkie	As	0,38 ± 0,17		0,24 ± 0,11
	Cd	0,14 ± 0,05	*	0,31 ± 0,03
	Hg	0,26 ± 0,03	*	0,21 ± 0,03
	Ni	1,28 ± 0,35		1,01 ± 0,09
	Pb	0,43 ± 0,16	*	0,65 ± 0,06

Gwiazdka „*" oznacza istotną różnicę ($p < 0,05$, test t-Studenta) pomiędzy danym parametrem w dwóch wariantach żywienia

Biokonwersja obu substratów odpadowych przez larw *H. illucens* spowodowała zmianę pH. W substratach odczyn był kwaśny, natomiast we frassach (świeżym i dojrzałym) alkaliczny (Tab. 8). Zmiana ta mogła być spowodowana poprzez rozkład i transformacją kwasów organicznych oraz tworzenia się jonów NH_4^+ (Azim i in. 2018) co uwidoczniono na Rys. 6, a także z wytwarzania zasad organicznych w procesie rozkładu białek (Tshepelevitsh i in. 2019).

Wartość EC w świeżych frassach w obu wariantach wzrosła średnio 2,41 razy w porównaniu z substratami (Tab. 8). W świeżym frassie z fasoli wartość EC wzrosła nawet powyżej 10 $mS \cdot cm^{-1}$ co może skutkować fitotoksycznością (Tiquia 2010). Natomiast w świeżym frassie z grochu wartość EC nie przekraczała tej wartości progowej (Tab. 8).

Wartość stosunku C/N znacząco różniła się w świeżych frassach między wariantami fasoli i grochu (Tab. 8). Różnica ta wynikała ze znacznego spadku stężenia N_{tot} we frassie z grochu przy stosunkowo stałej zawartości C_{tot}. W przypadku wariantu z fasoli stężenie N_{tot} pozostało na poziomie zbliżonym do substratu, a stężenie C_{tot}, znacznie spadło po biokonwersji przez larwy (Tab. 8). W zależności od wartości stosunku C/N, azot może ulegać mineralizacji do postaci jonów łatwo przyswajalnych przez rośliny lub immobilizacji przez mikroorganizmy glebowe. Równowaga między tymi procesami występuje przy C/N ok. 20–30, gdzie mineralizacja zachodzi poniżej tego zakresu, a unieruchomienie powyżej (Brust, 2019). Można zatem stwierdzić, że świeży frass z wariantu grochu był bardziej stabilny niż z wariantu fasoli. Wszystkie te zmiany wynikają przede wszystkim z metabolizmu żerujących larw oraz mikroorganizmów współwystępujących w podłożu. Część składników odżywczych została przez nie przyswojona, a część uległa mineralizacji i opuściła system eksperymentalny w postaci gazowej (CO_2, NH_3, lotne związki organiczne i inne) (De Cesare i in., 2011).

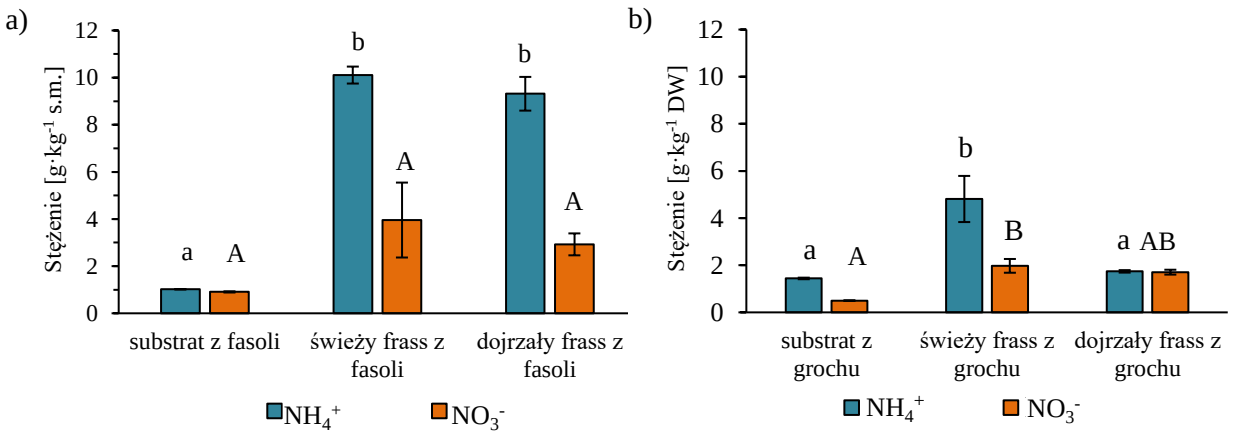
Stężenie łatwo dostępnych dla roślin jonów NH_4^+ znacząco wzrosło w świeżym frassie z fasoli (Rys. 6a). Stężenie jonów azotanowych również wzrosło w tym wariancie, jednak najprawdopodobniej ze względu na duże odchylenie wyników różnice nie były statystycznie istotne. W wariancie z grochem zmianie uległy zarówno stężenia jonów NH_4^+, jak i NO_3^- (Rys. 6b.). W tym przypadku odnotowano także znaczny spadek N_{tot} (Tab. 8). Przyczyną tej różnicy mogły być intensywniejsze procesy mineralizacji azotu oraz uwalniania amoniaku, charakterystyczne dla pierwszych tygodni kompostowania (Hao et al., 2008).

Tab. 8. Parametry substratów, świeżych frassów po biokonwersji przez larwy *H. illucens* i dojrzałych frassów z odpadów z fasoli i grochu.

Parametry	Substrat z fasoli	Świeży frass z fasoli	Dojrzały frass z fasoli	Substrat z grochu	Świeży frass z grochu	Dojrzały frass z grochu
Utylizacja (% s.m.)	nd	66,91 ± 1,69	3,18 ± 3,35†	nd	67,61 ± 1,01	40,92 ± 3,71†
Sucha masa (%)	25,63 ± 0,00a	31,19 ± 0,00a	81,51 ± 5,02b	21,79 ± 0,00A	21,53 ± 0,00A	28,33 ± 2,97B
pH	6,13 ± 0,01a	9,04 ± 0,22b	9,60 ± 0,15c	4,98 ± 0,00A	9,37 ± 0,05B	9,40 ± 0,19B
EC ($mS \cdot cm^{-1}$)	7,03 ± 0,02a	16,19 ± 0,14b	7,05 ± 0,15a	3,22 ± 0,03A	8,09 ± 0,04B	3,20 ± 0,93A
C_{tot} (% s.m.)	44,40 ± 2,05a	42,64 ± 0,88b	40,20 ± 0,78b	43,71 ± 0,43AB	44,33 ± 0,45A	39,62 ± 2,10B
N_{tot} (% s.m.)	5,50 ± 0,60a	4,65 ± 0,78a	1,52 ± 0,47b	5,32 ± 0,70A	1,46 ± 0,19B	0,96 ± 0,51B
C/N	8,15 ± 1,07a	9,33 ± 1,68a	26,55 ± 20,31b	8,30 ± 1,01A	30,68 ± 3,48AB	49,97 ± 20,67B

Skrót nd oznacza „nie dotyczy". Wartość utylizacji oznaczona symbolem „†" wskazuje na utylizację mikrobiologiczną frassów po procesie dojrzewania w stosunku do całości uzyskanej po biokonwersji (frass świeży). Litery oznaczają zmiany statystyczne (test Tukeya, $p < 0,05$): małe litery (a, b, c) odpowiadają zmianom w obrębie danej cechy w substracie, frassach surowych i dojrzałych z wariantu fasoli, a duże litery (A, B, C) oznaczają to samo w wariancie z grochu. Skrót „s.m." – sucha masa.

Rys. 6. Stężenia jonów amonowych oraz azotanowych w substratach, surowych frassach po biokonwersji przez larwy *H. illucens* i dojrzałych frassach z: a) odpadów z fasoli, b) odpadów z grochu.



Biokonwersja przez larwy przyczyniła się do zmian stężeń pierwiastków w świeżych frassach z obu wariantów (Tab. 9). Wzrost stężeń mógł wynikać z redukcji materii organicznej

jej pobierania przez larwy, jak i rozkładu mikrobiologicznego, w efekcie czego część pierwiastków uległy “zatężeniu”.

Często używany do charakterystyki nawozów stosunek pierwiastków N:P:K dla świeżego frassu z fasoli wynosił 4,7:1,8:2,3, a dla wariantu z grochu - 1,5:1,7:1,1. Wykorzystanie frassów jako dodatku do gleby wiąże się więc z dostarczaniem wszystkich trzech pierwiastków niezbędnych do rozwoju roślin.

Tab. 9. Stężenia pierwiastków (mg·kg^{-1} s.m.) w substratach, świeżych frassach po biokonwersji przez larwy *H. illucens* i dojrzałych frassach z odpadów z fasoli i grochu.

		Substrat z fasoli	Świeży frass z fasoli	Dojrzały frass z fasoli	Substrat z grochu	Świeży frass z grochu	Dojrzały frass z grochu
makroelementy	Ca	2426,67 ± 12,50a	946,22 ± 79,27b	1269,00 ± 167,35c	1357,00 ± 33,41A	538,92 ± 115,56B	859,70 ± 55,91C
	CaO (%)	*0,34 ± 0,00a*	*0,13 ± 0,01b*	*0,18 ± 0,02c*	*0,19 ± 0,00A*	*0,08 ± 0,01B*	*0,12 ± 0,01C*
	K	16220,00 ± 52,92a	18953,33 ± 1654,63b	24810,00 ± 2064,75c	8038,00 ± 184,81A	9492,17 ± 891,66A	25901,67 ± 16416,66B
	K_2O (%)	*1,96 ± 0,01a*	*2,28 ± 1,17b*	*2,99 ± 0,20c*	*0,97 ± 0,02A*	*1,14 ± 0,08A*	*3,12 ± 1,53B*
	Mg	2883,67 ± 16,17a	2785,67 ± 238,53a	3445,83 ± 27,58b	2033,67 ± 14,29A	3858,00 ± 279,07B	4688,33 ± 44,78C
	MgO (%)	*0,48 ± 0,00a*	*0,46 ± 0,02a*	*0,57 ± 0,00b*	*0,34 ± 0,00A*	*0,64 ± 0,03B*	*0,78 ± 0,00C*
	P	6872,67 ± 49,05a	7821,33 ± 472,35b	9744,17 ± 545,65c	6210,00 ± 51,29A	7443,33 ± 253,14A	12210,00 ± 2899,14B
	P_2O_5 (%)	*1,58 ± 0,01a*	*1,79 ± 0,09b*	*2,23 ± 0,10c*	*1,42 ± 0,01A*	*1,71 ± 0,05A*	*2,80 ± 0,52B*
	S	2536,00 ± 19,31a	2996,17 ± 42,19b	3763,67 ± 253,62c	1933,00 ± 16,64A	2747,83 ± 175,60B	4783,17 ± 585,72C
	SO_2 (%)	*0,51 ± 0,00a*	*0,60 ± 0,00b*	*0,75 ± 0,03c*	*0,39 ± 0,00A*	*0,55 ± 0,02B*	*0,96 ± 0,06C*
mikroelementy	B	2,77 ± 0,08a	3,61 ± 0,54b	4,56 ± 0,57c	0,98 ± 0,02A	4,99 ± 0,13B	7,26 ± 0,29C
	Cu	7,53 ± 0,14a	10,06 ± 0,18b	13,39 ± 0,30c	7,88 ± 0,18A	11,58 ± 0,26B	19,13 ± 0,34C
	Fe	122,23 ± 1,88a	128,45 ± 2,38a	165,58 ± 7,61b	58,24 ± 0,70A	420,88 ± 9,40B	447,25 ± 245,72B
	Mn	22,25 ± 0,31a	20,90 ± 1,22a	26,98 ± 2,32b	16,63 ± 0,19A	33,48 ± 2,57B	41,77 ± 2,64C
	Mo	0,69 ± 0,03a	0,98 ± 0,01b	1,31 ± 0,09c	1,09 ± 0,04A	1,66 ± 0,10B	2,18 ± 0,37C
	Na	52,07 ± 1,01a	49,38 ± 4,35a	50,11 ± 0,16a	63,36 ± 1,94A	74,40 ± 13,18A	55,68 ± 25,34A
	Zn	38,28 ± 0,32a	36,81 ± 1,32b	48,36 ± 0,41c	40,82 ± 0,36A	77,37 ± 1,69A	64,34 ± 27,95A
metale ciężkie	As	0,13 ± 0,10a	0,09 ± 0,04a	0,10 ± 0,02a	0,14 ± 0,09A	0,25 ± 0,03A	0,22 ± 0,03A
	Cd	0,04 ± 0,00a	0,05 ± 0,00a	0,08 ± 0,01b	0,04 ± 0,01A	0,18 ± 0,02B	0,20 ± 0,08B
	Hg	0,09 ± 0,03a	0,12 ± 0,04a	0,11 ± 0,01a	0,13 ± 0,03A	0,13 ± 0,00A	0,15 ± 0,03A
	Ni	5,24 ± 0,07a	6,36 ± 0,01b	8,31 ± 0,18c	3,26 ± 0,04A	3,38 ± 0,06A	8,75 ± 5,40B
	Pb	0,41 ± 0,08a	0,36 ± 0,04ab	0,52 ± 0,01b	0,09 ± 0,02A	0,82 ± 0,01B	0,89 ± 0,32B

Dane zaznaczone kursywą oznaczają procentową zawartość makroskładników po ich przekształceniu w formy tlenkowe. Różne litery oznaczają zmiany istotne statystycznie (test Tukeya, $p < 0,05$). Małe litery odpowiadają zmianom statystycznym w wariancie z fasoli, a duże – w wariancie z grochu

Proces dojrzewania frassu został przeprowadzony w warunkach tlenowych przez okres dziesięciu miesięcy. Dojrzewanie spowodowało dalszy wzrost pH w wariancie z fasoli, natomiast w wariancie z grochu pozostał na poziomie zbliżonym do świeżemu frassowi (Tab. 8). Dojrzewanie frassów w obu wariantach doprowadziło do spadku EC do poziomu równego do obserwowanego w substratach na początku eksperymentu (Tab. 8), co można wyjaśnić m.in. uwalnianiem NH_4^+ (Rys. 6) (Gondek i in., 2020). Spadek EC oznacza zmniejszenie zasolenia dojrzałych frassach, a tym samym obniżenie ich potencjalnego działania fitotoksycznego (Tiquia, 2010), zwłaszcza w wariancie z fasolą. Proces dojrzewania spowodował dalszą utratę azotu przy braku statystycznie istotnych zmian zawartości C_{tot} we frassie z fasoli (Tab. 8). W przypadku dojrzałego frassu z grochu, odnotowano sytuację odwrotną, zawartość azotu została na stałym poziomie, natomiast wartość C_{tot} spadła (Tab. 8). Zmiany te przekładały się również na wartości stosunku C/N.

Proces dojrzewania frassu z obu substratów zwiększył wartość stosunku C/N (Tab. 8), może wskazywać na ustabilizowanie się przemian azotu w materiale (Brust, 2019). Dojrzewanie frassu z fasoli nie spowodowało istotnych zmian w wartościach stężeń jonów NH_4^+ i NO_3^- w porównaniu z frassem świeżym (Rys. 6). Należy jednak zauważyć, że wyniki tych stężeń podano w przeliczeniu na suchą masę, a procentowa udział suchej masy między świeżym i dojrzałym frassem z fasoli różniła się znacząco (Tab. 8). W przypadku dojrzałego frassu z grochu stężenie NH_4^+ uległo znacznemu spadkowi (Rys. 6). Dojrzewanie mogło sprzyjać ulatnianiu amoniaku (NH_3), zwłaszcza przy zasadowym pH frassu (Tab. 8), które ułatwia ten proces (Hao i in., 2008). Najprawdopodobniej spadek stężenia NH_4^+ nie nastąpił w wyniku utlenienia do NO_3^-, ponieważ jego stężenie pozostało niemal na poziomie obserwowanym w świeżym frassie.

Zawartość metali ciężkich w świeżych i dojrzałych frassach była na ogół niska i nie przekraczała średnich górnych granic wartości dopuszczalnych dla metali ciężkich w nawozach organicznych stosowanych w krajach europejskich (Pollak i in., 2004).

Stosunek pierwiastków NPK dla dojrzałego frassu z fasoli wynosił 1,5:2,2:3,0, a dla wariantu z grochu 1,0:2,8;3,1. Sugeruje to, że dojrzałe frassy mogą nadawać się do nawożenia jesiennego ze względu na niższą zawartość azotu i wysoką zawartość potasu.

Podsumowując, wyniki opisane w pracy **P2** pozwoliły na realizację **celów C2** i **C3** i potwierdziły **hipotezę H1** oraz częściowo **H2**.

5.3. Postępująca degradacja mykotoksyn w odpadach rolniczych – wgląd z analizy bezpieczeństwa larw *Hermetia illucens* i frassów (Advancing mycotoxin degradation in agricultural waste – insights from *Hermetia illucens* larvae and frass safety analysis) – **P3**

Cel badań przedstawionych w publikacji **P3** dotyczył oceny zmian ilości i składu mykotoksyn we frassie świeżym oraz dojrzałym, po biokonwersji przez larwy *H. illucens* zanieczyszczonych przez pleśń odpadów nasion fasoli (**C4**), a opierał się na hipotezie, że biokonwersja doprowadzi do zmian, zmniejszających ich występowanie (**H3**).

Kwestia redukcji mykotoksyn jest ważna z punktu bezpieczeństwa wykorzystania frassu jako bionawozu do gleb. Mykotoksyny są nie tylko niebezpieczne dla zdrowia ludzi i zwierząt spożywających zanieczyszczoną nimi żywność (Pathre i in. 1974), ale mogą również wywoływać negatywne skutki u roślin i mikroorganizmów (Schollenberger i in. 2007). Jednakże warto zauważyć, że zagadnienie obecności mykotoksyn w glebie nadal pozostaje stosunkowo słabo zbadane. Mykotoksyny mogą w pewnej mierze ulegać rozkładowi w środowisku glebowym, istnieją jednak doniesienia, wskazujące że może dochodzić do ich sorpcji na cząstkach gleby, co może sprzyjać utrzymywaniu się zanieczyszczeń w środowisku (Juraschek i in. 2022). Dlatego, ich obecność w nawozach organicznych powinna podlegać kontroli. Do tej pory brak jest regulacji prawnych określających dopuszczalne stężenie mykotoksyn w nawozach, w tym w nawozach organicznych.

Niniejsze badanie obejmowało oznaczenie stężeń 25 różnych związków (mykotoksyn oraz ich metabolitów) w substracie, świeżym frassie oraz frassie poddanemu procesowi dojrzewania.

Badania mykologiczne substratu (nasion fasoli) naturalnie zanieczyszczonego pleśnią wykazały obecność sześciu rodzajów grzybów, z których cztery, *Penicillium*, *Fusarium*, *Alternaria* i *Aspergillus* (Tab. 10), są znane z możliwości wytwarzania metabolitów wtórnych, tj. mykotoksyn (Khan i in. 2024).

Tab. 10. Udział i rodzaj grzybów w odpadach poprodukcyjnych nasion fasoli naturalnie zanieczyszczonych pleśnią.

	Całkowita liczba grzybów	Zawartość grzybów
Poprodukcyjne odpady nasion fasoli naturalnie zanieczyszczone pleśnią	$2{,}5 \cdot 10^3$ jtk	87% *Penicillium* 5% *Fusarium* 4% *Alternaria* 2% *Mucor* 1% *Scopulariopsis* 1% *Aspergillus*

jtk – jednostka tworząca kolonię

Analizy mykotoksyn wykazały, że odpady z fasoli były zanieczyszczone niwalenolem, deoksyniwalenolem, zearalenonem, monoacetoksyscirpenolem, diacetoksyscirpenolem oraz toksynami HT-2 i T-2 (Tab. 11). Wszystkie te związki są produkowane przez grzyba z rodzaju *Fusarium*, znane z powodowania strat w plonach na całym świecie (Torbati i in. 2021).

Biokonwersja substratu przez larwy *H. illucens* doprowadziła do spadku liczby mykotoksyn. W substracie zidentyfikowano ich siedem, natomiast w przypadku świeżego frassu było ich pięć, a w dojrzałym frassie cztery (Tab. 11). Warto przy tym zauważyć, że obecne w substracie początkowym deoksyniwalenol, monoacetoksyscirpenol, diacetoksyscirpenol oraz toksyna T-2 nie zostały wykryte w żadnym frassie. W przypadku niwalenolu w układzie substrat/świeży frass/dojrzały frass jego stężenie spadało kolejno (w dojrzałym frassie już nie występował), a w przypadku zearalenonu oraz toksyny HT-2 kolejno rosło. Po biokonwersji pojawiły się nowe mykotoksyny, metabolity zearalenonu, α-zearalenonu i β-zearalenol (w obu przypadkach większe stężenie odnotowano w świeżym frassie).

Z punktu widzenia zastosowania frassów jako bionawozów wyeliminowanie, przez aktywność larw *H. illucens*, czterech wspomnianych powyżej mykotoksyn stanowi bardzo korzystny wynik. Przykładowo, obecność deoksyniwalenolu może prowadzić do inhibicji rozwoju grzybów z rodzaju *Trichoderma*, wykazujących aktywność mykopasożytniczą skierowaną przeciwko niektórym szkodliwym i uciążliwym z punktu widzenia rolnictwa grzybom, w tym *Fusarium* (Palumbo i in. 2008). Jednakże, skuteczność biodegradacji deoksyniwalenolu podczas biokonwersji zanieczyszczonego substratu przez larwy może być ograniczona, jeśli początkowe stężenie tej mykotoksyny jest wysokie (ok. 779 $\mu g \cdot kg^{-1}$ wg. Leni i in. (2019) lub ok. 4600 $\mu g \cdot kg^{-1}$ wg. Purschke i in. (2017)).

W przypadku monoacetoksyscirpenolu i diacetoksyscirpenolu obie mykotoksyny wykazują silne działanie toksyczne wobec zwierząt, roślin i mikroorganizmów, w szczególności poprzez zdolność do hamowania syntezy białek. (Schollenberger i in. 2007). W eliminację tych mykotoksyn mógł być zaangażowany cytochrom P450, o aktywności enzymatycznej, często odgrywający ważną rolę w detoksykacji ksenobiotyków (cytochrom ten został zidentyfikowany u *H. illucens* (Shah i in. 2024)). Diacetoksyscirpenol razem z toksyną T2, należą do najniebezpieczniejszych przedstawicieli trichotecenów typu A (Janik i in. 2021). Toksyna T2 również wykazała spadek stężenia w świeżym frassie do poziomu niewykrywalności. Mogło to wynikać z jej biodegradacji przez larwy bądź transformacji do toksyny HT-2 (Tab. 11). Obecność toksyny T2 w glebie może niekorzystnie oddziaływać na mikroorganizmy, np. działa hamująco na drożdże *Saccharomysces cerevisiae* (Madhyastha i in. 1994), które uczestniczą w obiegu składników odżywczych, m.in. poprzez zwiększenie ich dostępności dla roślin (Csambalik i Tóbiás, 2018). Drożdże te wykazują również zdolność do bioremediacji gleb zanieczyszczonych metalami ciężkimi (Massoud i in. 2019).

Pojawienie się nowych mykotoksyn lub wzrost stężenia związków już obecnych (Tab. 11) należy uznać za niekorzystne. Toksyna HT-2 powstaje w wyniku hydrolizy toksyny T-2 i różni się od niej grupą funkcyjną w pozycji C2 (grupa hydroksylowa, zamiast acetylowej) (Vörösházi i in., 2024). Toksyna T-2 wykazuje działanie hamujące wobec drożdży *Saccharomyces cerevisiae* (Madhyastha i in., 1994), które poprawiają dostępność składników odżywczych dla roślin (Csambalik i Tóbiás, 2018) oraz wykazują zdolność do bioremediacji gleb zanieczyszczonych metalami ciężkimi (Massoud i in., 2019). Warto dodać, że *S. cerevisiae* nie są wrażliwe na metabolit toksyny HT-2, który był obecny w świeżym frassie (Madhyastha i in., 1994). Drugą mykotoksyną, której stężenie po biokonwersji przez larwy wzrosło, był zearalenon. Dodatkowo, w świeżym frassie pojawiły się jego metabolity: α-zearalenol i β-zearalenol (Keller i in. 2015). Wzrost wartości stężenia zearalenonu najprawdopodobniej wynikał z wysokiej wilgotności frassu, wymaganej dla odpowiedniej hodowli larw *H. illucens* (60-70%). Poziom tej mykotoksyny może wzrastać m.in. podczas przechowywania materiału, jeśli wilgotność przekracza 30-40% (Tola i Kebede 2016). Obecność zearalenonu może działać hamująco na wzrost pożytecznych bakterii z rodzaju *Bacillus* (Madhyastha i in. 1994), które odgrywają istotną rolę w obiegu składników odżywczych w glebie oraz wspomagają reakcje roślin na czynniki stresowe (Saxena i in. 2020).

Metabolity α-zearalenol i β-zearalenol najprawdopodobniej pojawiły się we świeżym frassie w wyniku biotransformacji zearalenonu. Powstają one w początkowej fazie

enzymatycznej tego procesu i różnią się między sobą położeniem grupy hydroksylowej w pierścieniu cykloheksanowym. W przypadku α-zearalenolu cechuje się on wyższą toksycznością od zearalenonu, a oba te związki mogą wiązać się z receptorami estrogennymi, zaburzając prawidłowe funkcjonowanie organizmu (Keller i in. 2015).

Proces dojrzewania frassu doprowadził do spadku stężeń mykotoksyn w trzech przypadkach (Tab. 11). Eliminacja niwalenolu mogło być związana z enzymatyczna deepoksydacją. Proces ten zachodzi w trakcie metabolizmu drobnoustrojów i obniża toksyczność tej mykotoksyny (Sundstøl Eriksen i in. 2004). Możliwe, że bakterie jelitowe larw *H. illucens* również posiadały taką właściwość. W przypadku metabolitów α-zearalenolu i β-zearalenolu obserwowany spadek może wiązać się z dalszymi etapami reakcji związanych z biotransformacją zearalenonu (Gajęcka i in. 2009), w trakcie których powstają np. koniugaty glukoronidowe, wykazujące niższą aktywność estrogenną (Frizzel i in. 2015). Natomiast w przypadku zearalenonu i toksyny HT-2 dojrzewanie frassu spowodowało dalszy wzrost ich stężeń. Mogło być to spowodowane ciągłym wzrostem i rozwojem grzybów *Fusarium*, które mogą rozwijać się w szerokim zakresie temperatur, od -3 do 35 °C (Ejaz i in. 2023).

Podsumowując, wyniki opisane w pracy P3 pozwoliły na realizację **celu C4** i pomimo pojawienia się nowych metabolitów zearaleonu i wzrostu stężeń samego zearaleonu oraz toksyny HT-2 można zaryzykować stwierdzenie, że częściowo potwierdziły trafność **hipotezy H3**.

Tab. 11. Zmiany stężeń ($\mu g \cdot kg^{-1}$) mykotoksyn po biokonwersji odpadów poprodukcyjnych z nasion fasoli przez larwy *H. illucens*.

Mykotoksyna	Substrat	Świeży frass	Dojrzały frass
Niwalenol	147,0 ± 12,6a	25,0 ± 22,1b	<LOD
Deoksyniwalenol	31,8 ± 2,3	<LOD	<LOQ
3-acetyldeoksyniwalenol	<LOD	<LOD	<LOD
15-acetyldeoksyniwalenol	<LOD	<LOD	<LOD
Deepoksy-deoksyniwalenol	<LOD	<LOD	<LOD
DON-3-glukozyd	<LOD	<LOD	<LOD
Fuzarenon-X	<LOD	<LOD	<LOD
Zearalenon	75,5 ± 13,6a	88,9 ± 0,4ab	111,2 ± 14,2b
α-zearalenol	<LOD	196,8 ± 1,2a	149,9 ± 1,0b
β-zearalenol	<LOD	63,9 ± 0,3a	37,3 ± 1,1b
α -zearalanol	<LOD	<LOD	<LOD
β -zearalanol	<LOD	<LOD	<LOD
Neosolaniol	<LOD	<LOD	<LOD
Monoacetoksyscirpenol	20,0 ± 0,2	<LOQ	<LOD
Diacetoksyscirpenol	13,6 ± 0,6	<LOD	<LOD
Aflatoksyna B_1	<LOD	<LOD	<LOD
Aflatoksyna B_2	<LOD	<LOD	<LOD
Aflatoksyna G_1	<LOD	<LOD	<LOD
Aflatoksyna G_2	<LOD	<LOD	<LOD
Fumonizyna B_1	<LOD	<LOD	<LOD
Fumonizyna B_2	<LOD	<LOD	<LOD
Fumonizyna B_3	<LOQ	<LOD	<LOD
Toksyna HT-2	74,3 ± 11,3a	133,0 ± 34,4b	150,2 ± 1,6b
Toksyna T-2	12,4 ± 1,5	<LOD	<LOD
Ochratoksyna A	<LOD	<LOD	<LOD

Różnicę statystyczną pomiędzy próbkami dla danej mykotoksyny sprawdzono za pomocą analizy wariancji ANOVA oraz testu post-hoc Tukey'a ($p < 0,05$) (w tabeli oznaczono literami: a, b, c). LOD – granica wykrywalności, LOQ – granica oznaczalności.

5.4 Łącząc recykling odpadów ze zrównoważonym rolnictwem: bogaty w fitohormony bionawóz z frasu czarnej muchy (Linking waste recycling and sustainable agriculture: phytohormone-rich biofertilizer from black soldier fly frass) – **P4**

Celem badań przedstawionych w **P4** była odpowiedź na pytanie czy, a jeśli tak, to jakie fitohormony występują we frassie po biokonwersji odpadów nasion fasoli i grochu przez larwy *H. illucens* oraz w odcieku z frassu powstałym w czasie hodowli (**C5**). Hipotezą leżącą u podstaw tych badań jest stwierdzenie, że takie fitohormony występować będą (**H4**).

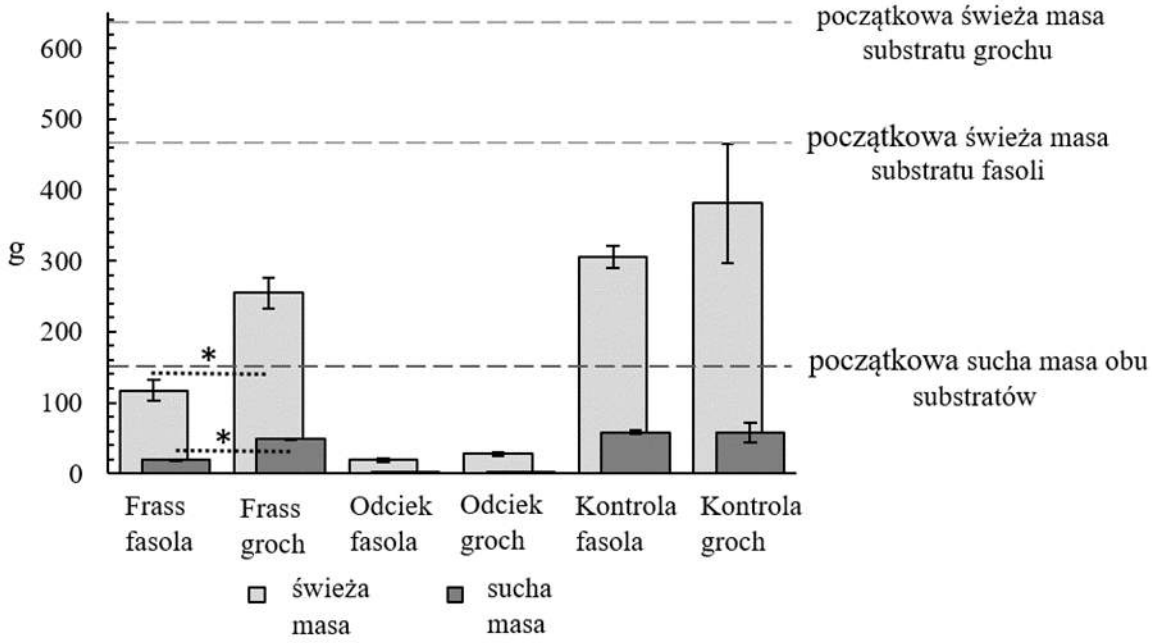
Fitohormony to związki organiczne, które wykazują aktywność w organizmie rośliny już w bardzo niskich stężeniach (Asami i Nakagawa 2018). Są one odpowiedzialne za regulację procesów morfologicznych i fizjologicznych na każdym etapie rozwoju rośliny. Fitohormony są również odpowiedzialne za zdolność adaptacyjną, umożliwiając roślinie reagowanie na zmiany warunków środowiskowych w obecności stresów abiotycznych (np. zmiany pogody) lub biotycznych (np. atak szkodników) (Bari i Jones 2009). W ostatnich latach nasilające się zmiany klimatyczne wywierają duży wpływ na roślinność. Przykłady obejmują okresy suszy lub, przeciwnie, coraz częstsze ulewne deszcze, które mogą wpływać bezpośrednio na nadziemną część rośliny, ale także przyczyniać się do zmian właściwości gleby, co niewątpliwie wpływa na wzrost i rozwój roślin. Zjawiska te wymagają od rośliny szybkiej reakcji i dostosowania się do panujących warunków (Janni i in. 2024). W związku z tym, jeśli frass z hodowli owadów zawiera fitohormony takie jak auksyny i cytokininy, mógłby on nie tylko stanowić naturalny nawóz, ale także działać jako środek biostymulujący, wspierający wzrost roślin i łagodzący skutki stresu środowiskowego.

5.4.1 Wydajność larw i produkty uboczne hodowli po biokonwersji odpadów

Biokonwersja odpadów z grochu przez larwy (przy początkowej dawce 150 mg suchej masy na jedną larwę) doprowadziła do uzyskania większej ilości frassu (o 2,17 razy więcej w przeliczeniu na świeżą masę i o 2,49 razy więcej w przeliczeniu na suchą masę) niż w wariancie z fasoli (Rys. 7). Większa redukcja odpadów z fasoli, odzwierciedlona w mniejszej ilości frassu, może świadczyć o większym poborze i lepszej strawności tego substratu, ale także o jego niższej wartości odżywczej, co może skutkować niższą zawartością białka w larwach (Tab. 12). Biomasa larw i przyrost ich długości nie różniły się istotnie między wariantami i w obu przypadkach larwy osiągnęły największą masę w drugim tygodniu doświadczenia (Rys. 7). Spadek masy po tym okresie może być związany z wejściem

w stadium prepoczwarki, w którym larwy zaprzestają pobierania pokarmu (Spranghers i in., 2018).

Rys. 7. Uzysk frassu, odcieków z frassu oraz pozostałości kontrolnych (naturalny rozkład bez larw) po jednym miesiącu doświadczenia.



Tab. 12. Wskaźniki rozwojowe i zawartość białka larw *H. illucens* po hodowli na odpadach fasoli i grochu.

(%)	**Odpady z fasoli**	**Odpady z grochu**
Stopień przepoczwarzenia	68,07 ± 2,15	70,00 ± 1,56
Stopień przeżywalności	94,40 ± 1,02	91,30 ± 2,52
Zawartość białka w larwach	54,62 ± 2,81*	61,15 ± 1,91*

Gwiazdka (*) oznacza różnice statystyczne pomiędzy wariantami grochu i fasoli (test t-Studenta, $p < 0,05$).

5.4.1 Auksyny

Analiza fitohormonów w produktach biokonwersji odpadów z fasoli i grochu przez larwy *H. illucens* obejmowała związki należące do trzech grup: auksyn, cytokinin oraz hormonów stresowych. W niniejszym badaniu, auksyny reprezentował jedynie najpowszechniejszy przedstawiciel tej grupy: kwas indolilo-3-octowy (IAA) (Korver i in. 2018), który pełni kluczową rolę w regulacji wzrostu, podziału i różnicowania komórek roślinnych, a także w formowaniu pędów i korzeni (Sabagh i in., 2022). Hormon ten uczestniczy w kształtowaniu

tropizmów roślin (Retzer i in., 2014) oraz w procesie partenokarpii, czyli tworzenia owoców bezpestkowych (Wang i in., 2021). Wymienione funkcje stanowią jedynie wybrane przykłady ich działania, natomiast szczegółowe omówienia można znaleźć w licznych przeglądach literatury (Gomes i Scortecci, 2021; Song i in., 2022; Zhang i in., 2022).

Porównując wyniki dla obu wariantów (fasola i groch), stężenia IAA znacząco różniły się zarówno w substratach, jak i we frassach (Tab. 13). Różnicę między substratami można wyjaśnić zróżnicowanymi profilami aminokwasowymi, które są zaangażowane w biosyntezie tego fitohormonu (Zhao 2012). Szczególnie ważnym aminokwasem dla tworzenia IAA jest tryptofan, który ulega dwuetapowej konwersji enzymatycznej. Comai i in. (2007) przeprowadzili analizę oznaczania tryptofanu w dziewięciu rodzajach nasion roślin strączkowych i udowodnili, że fasola zawiera około 4 razy więcej tego aminokwasu w postaci wolnej niż groch.

Stężenie IAA w obu kontrolach (fasola i groch) wzrosło w stosunku do stężenia w substratach (Tab. 13), spadło natomiast we wszystkich badanych wariantach, oprócz frassu z fasoli. W tym ostatnim przypadku stężenie wzrosło 305,46 razy w porównaniu z substratem (Tab. 13), a różnica stężeń IAA pomiędzy frassami z dwóch wariantów wynosiła pięć rzędów wielkości. Dla porównania stężenie odnotowane we frassie z grochu (Tab. 13) było zbliżone do wartości w ekstrakcie biohumusu (0,053 $ng \cdot mg^{-1}$) opisanej przez Sienkiewicz i in. (2024), natomiast poziom we frassie z fasoli (Tab. 13) odpowiadał wartościom typowym dla gleb organicznych (do 210 $ng \cdot mg^{-1}$) (Szajdak i Maryganova, 2007). Stężenie IAA we frassie *H. illucens* pochodzącym z odpadów kuchennych wynosiło 0,36 $ng \cdot ml^{-1}$ (Green 2023).

Tab. 13. Zawartość hormonu IAA (kwasu indolo-3-octowego) w substracie, kontroli bez larw, frassie, odciekach z frassu i larwach *H. illucens*.

	Stężenie IAA ($ng \cdot mg^{-1}$ na suchą masę)		
	Odpady z fasoli		**Odpady z grochu**
Substrat	1,58 ± 0,16a	*	1,00 ± 0,08a
Kontrola	18,83 ± 6,41a		22,38 ± 16,88b
Frass	482,63 ± 21,26b	*	†0,05 ± 0,01a
Odcieki z frassu	1,11 ± 0,37a		0,87 ± 0,49a
Larwy	0,97 ± 0,35a		0,43 ± 0,18a

Gwiazdka (*) oznacza różnice statystyczne między wariantami fasoli i grochu (test t-Studenta, $p < 0,05$). Różne litery oznaczają różnice statystyczne między próbkami pochodzącymi z jednego wariantu żywieniowego (test Tukey'a, $p < 0,05$). Znak (†) oznacza, że dany wynik mieści się pomiędzy limitami detekcji i kwantyfikacji (LOD < wynik < LOQ).

Przy aplikacyjnym zastosowaniu fitohormonu IAA jego dawka musi być dostosowana do gatunku rośliny. Wyższe stężenia auksyn są zwykle odpowiedniejsze dla roślin jednoliściennych, natomiast rośliny dwuliścienne wymagają mniejszych ilości tego fitohormonu (McSteen 2010).

5.4.2 Cytokininy

Cytokininy są to naturalnie syntetyzowane pochodne adeniny, posiadające łańcuch izoprenowy lub aromatyczny w pozycji N6 purynowej (Zücher i Müller, 2016). Oprócz regulacji wzrostu roślin poprzez stymulację podziałów komórkowych pełnią także inne funkcje, m.in. działanie przeciwstarzeniowe, wpływ na wydłużanie wierzchołka czy transmisję sygnałów odżywczych (Sakakibara, 2010). Ich szczegółową rolę opisano w licznych pracach przeglądowych (Argueso i Kieber, 2024; Li i in., 2021).

Wyniki niniejszego doświadczenia wskazują, że przetwarzanie odpadów nasion roślin strączkowych przez larwy *H. illucens* może doprowadzić do wzrostu stężeń wybranych cytokinin (Tab. 14), a tym samym zwiększać potencjał biostymulacyjny frassu poprzez oddziaływanie na wzrost i rozwój roślin. Analiza wykazała, że substrat fasoli był bogatszy w te fitohormony niż substrat grochu. Produkty powstałe po biokonwersji odpadów fasoli przez larwy charakteryzowały się niższymi stężeniami dihydroksyzeatyny (dh-zea), cis-zeatyny (c-zea) i rybozydu cis-zeatyny (c-zea rib) w porównaniu z substratem. Natomiast w przypadku trans-zeatyny (t-zea) zaobserwowano wzrost stężenia we frassie oraz w odcieku z fasoli (odpowiednio 6,44-krotny i 10,00-krotny w stosunku do stężenia w substracie). W przypadku rybozydu trans-zeatyny (t-zea rib) nastąpił 3,38-krotny wzrost stężenia we frassie z fasoli, podczas gdy w pozostałych próbkach (pozostałości kontrolne, odciek i larwy) stężenia spadły poniżej wartości stężenia w substracie. Podobna sytuacja miała miejsce w przypadku rybozydu dihydroksy-zeatyny (dh-zea rib), którego stężenie we frassie w porównaniu do wartości substratu było wyższe 1,31-krotnie. W przypadku IP wartości we wszystkich produktach z wariantu fasoli wzrosły, przy czym największy wzrost odnotowano we frassie (ok. 165-krotny wzrost w porównaniu z substratem). W przypadku wariantu grochu, dla c-zea, dh-zea rib i c-zea rib, najwyższe stężenia stwierdzono w substracie, a biokonwersja przez larwy spowodowała ich spadek. Dla pozostałych cytokinin, z wariantu grochu, najwyższe stężenia wykryto w odciekach. Ich wzrost stężeń w porównaniu z substratem był następujący: 56,46 razy dla t-zea, 1,74 razy dla t-zea rib i 108,82 razy dla IP, a w przypadku dh-zea z poziomu poniżej wykrywalności do ok. 4,10 $pg \cdot mg^{-1}$ suchej masy (Tab. 14). Całkowita zawartość cytokinin we frassie z fasoli była 35,56 razy wyższa niż

we frassie z grochu, a w przypadku odcieku z wariantu fasoli była 2,10-krotnie wyższa niż w odcieku z grochu. W larwach z obu wariantów określono obecność t-zea, t-zea rib, dh-zea rib, c-zea rib i IP, dodatkowo w larwach po fasoli scharakteryzowano obecność dh-zea. W przypadku IP stężenie w larwach przekroczyło stężenie w substracie, ale bez istotności statystycznej. (Tab. 14).

W porównaniu z innymi kompostami organicznymi frass z fasoli charakteryzował się szczególnie wysoką całkowitą zawartością cytokinin. Dla takich materiałów, jak kompost organiczny, pellet z kompostu organicznego, kompost z łusek gryki, kompost z plew konopnych i wytłoków jabłkowych lub kompost ogrodowy, średnia zawartość cytokinin całkowitych wahała się od 0,08 do 0,87 $pg \cdot mg^{-1}$ na suchą masę (Sienkiewicz i in. 2024). Natomiast ekstrakt z biohumusu (13,9 $pg \cdot mg^{-1}$ na suchą masę) (Sienkiewicz i in. 2024) charakteryzował się wyższą 1,5 razy zawartością cytokinin niż w przypadku frassu z grochu, a jednocześnie aż 23,0 razy niższą niż w przypadku frassu z fasoli (Tab. 14).

Tab. 14. Zawartość cytokinin (t-zea − trans-zeatyna, dh-zea − dihydrozeatyna, c-zea − cis-zeatyna, t-zea rib − rybozyd trans-zeatyny, dh-zea rib − rybozyd dihydrozeatyny, c-zea rib − rybozyd cis-zeatyny, IP − isopentenyladenina) w substracie, pozostałości kontrolnej bez larw, frassie, odcieku z frassu i larwach *H. illucens*.

		Stężenie cytokinin (pg·mg^{-1} na suchą masę)							
		t-zea	**dh-zea**	**c-zea**	**t-zea rib**	**dh-zea rib**	**c-zea rib**	**IP**	**Całkowita suma cytokinin**
Odpady fasoli	Substrat	3,58 ± 1,57a	83,70 ± 22,43c*	115,27 ± 31,56b*	0,66 ± 0,14b*	124,62 ± 21,40bc*	61,63 ± 1,99c	1,78 ± 1,15a	391,24 ± 75,96bc*
	Kontrola	†1,27 ± 0,76a*	†0,84 ± 0,73a	3,49 ± 2,73a	0,24 ± 0,10a	38,39 ± 25,72a	ND	8,59 ± 5,90a	52,88 ± 20,69a
	Frass	23,06 ± 3,47b*	57,61 ± 10,60bc*	3,79 ± 1,79a	2,23 ± 0,10c*	162,94 ± 29,12c*	†0,24 ± 0,06a	69,10 ± 28,34a	318,96 ± 46,22b*
	Odciek z frassu	35,80 ± 8,39c	47,06 ± 12,88b*	†0,65 ± 0,31a	0,32 ± 0,14a	99,50 ± 18,96bc*	3,24 ± 0,57b*	293,89 ± 54,89b*	480,47 ± 92,36c*
	Larwy	2,80 ± 0,49a	ND	ND	0,13 ± 0,03a*	2,78 ± 1,97a	†0,36 ± 0,22a	9,26 ± 7,12a	15,63 ± 4,91a
Odpady grochu	Substrat	2,25 ± 1,13a	ND	36,94 ± 7,36b*	0,23 ± 0,10ab*	0,99 ± 0,29b*	55,54 ± 7,71b	†0,79 ± 0,14a	96,72 ± 15,34a*
	Kontrola	3,98 ± 1,12a*	ND	ND	ND	ND	ND	19,45 ± 11,34a	23,43 ± 11,30a
	Frass	2,54 ± 0,14a*	ND	†0,80 ± 0,61a	†0,05 ± 0,03ab*	†0,21 ± 0,19a*	†0,33 ± 0,15a	5,04 ± 1,30a	8,97 ± 1,49a*
	Odciek z frassu	127,04 ± 48,48b	4,10 ± 3,29b*	10,74 ± 7,02a	0,40 ± 0,28b	†0,43 ± 0,44ab*	0.60 ± 0,00a	85,97 ± 11,67b*	229,29 ± 67,81b*
	Larwy	2,77 ± 0,75a	ND	ND	†0,05 ± 0,03ab*	ND	2,97 ± 1,27a	26,91 ± 18,48a	32,70 ± 19,41a

Gwiazdka (*) oznacza różnice statystyczne między wariantami fasoli i grochu (test t-Studenta, $p < 0,05$). Małe litery oznaczają różnice statystyczne między próbkami pochodzącymi z jednego wariantu żywieniowego (test Tukey'a, $p < 0,05$). Znak (†) oznacza, że dany wynik mieści się pomiędzy limitami detekcji i kwantyfikacji (LOD < wynik < LOQ). ND oznacza wynik poniżej limitu detekcji.

5.4.3 Hormony stresowe

Do trzeciej grupy – fitohormonów stresu – zalicza się kwas abscysynowy (ABA), kwas salicylowy (SA) i kwas jasmonowy (JA). Fitohormony te są produkowane w odpowiedzi na stres i regulują procesy fizjologiczne roślin. Rośliny podlegają zarówno stresom biotycznym, wywoływanym przez mikroorganizmy (bakterie, wirusy, grzyby), jak i abiotycznym, związanym z czynnikami środowiskowymi, takimi jak susza, zasolenie, skrajne temperatury czy zanieczyszczenie gleby (Ku i in., 2018). W niniejszym badaniu w wariancie

fasoli odnotowano wzrost wszystkich hormonów stresu we frassie i odciekach (Tab. 15). Ich stężenia malały w następującej kolejności: SA > JA > ABA. W wariancie grochu, wzrost ABA i SA stwierdzono tylko w odciekach, przy czym istotnie wzrósł jedynie ABA (7,63-krotnie względem substratu). Stężenie JA wzrosło z poziomu poniżej wykrywalności zarówno we frassie z grochu, jak i w odciekach (Tab. 15). Kolejność stężeń we frassie z grochu malała następująco: JA > SA > ABA. Całkowita zawartość hormonów stresu we frassie z fasoli była 67,12 razy wyższa niż we frassie z grochu. Natomiast, całkowita zawartość hormonów stresu w odciekach z fasoli była 3,47 razy wyższa niż w odciekach z grochu (Tab. 15). Żaden z roślinnych hormonów stresu nie został wykryty w larwach (Tab. 15).

Tab. 15. Zawartość hormonów stresu (ABA – kwas abscysynowy, SA – kwas salicylowy, JA – kwas jasmonowy) w substracie, pozostałościach kontrolnych bez larw, frassie, odciekach z frassu i larwach *H. illucens*.

		Stężenie hormonów stresowych (ng·mg^{-1} na suchą masę)			
		ABA	**SA**	**JA**	**Całkowita suma hormonów stresowych**
Odpady z fasoli	Substrat	†0,05 ± 0,02a	ND	ND	0,05 ± 0,01a*
	Kontrola	0,16 ± 0,11a	2,51 ± 0,56a*	1,30 ± 0,17d*	3,97 ± 0,61a*
	Frass	0,50 ± 0,14b*	10,35 ± 2,21b*	0,56 ± 0,16c*	11,41 ± 2,33b*
	Odciek z frassu	0,56 ± 0,11b	10,01 ± 2,39b*	0,41 ± 0,22bc	10,97 ± 2,63b*
	Larwy	ND	ND	ND	-
Odpady z grochu	Substrat	0,08 ± 0,01a	†1,05 ± 0,14a*	ND	1,14 ± 0,14ab*
	Kontrola	†0,02 ± 0,00a	ND	0,43 ± 0,09b*	0,45 ± 0,09ab*
	Frass	†0,03 ± 0,00a*	ND	†0,13 ± 0,04a*	0,17 ± 0,04ab*
	Odciek z frassu	0,61 ± 0,39b	†2,02 ± 1,80a*	0.53 ± 0.05b	3,16 ± 2,18b*
	Larwy	ND	ND	ND	-

Gwiazdka (*) oznacza różnice statystyczne między wariantami fasoli i grochu (test t-Studenta, $p < 0,05$). Różne litery oznaczają różnice statystyczne między próbkami pochodzącymi z jednego wariantu żywieniowego (test Tukey'a, $p < 0,05$).). Znak (†) oznacza, że dany wynik mieści się pomiędzy limitami detekcji i kwantyfikacji (LOD < wynik < LOQ). ND oznacza wynik poniżej limitu detekcji.

ABA jest syntetyzowany w warunkach stresowych, takich jak susza, zasolenie czy wahania temperatury, gdzie m.in. powoduje zamykanie aparatów szparkowych i reguluje gospodarkę wodną roślin (Kavi Kishor i in., 2022). Hormon ten jest jednak wytwarzany również podczas prawidłowego wzrostu roślin, niezależnie od czynników stresowych (Popko

i in., 2010). Wpływa wtedy na inicjację kwitnienia, krzewienie, ekspansję komórek, rozwój i funkcjonowanie chloroplastów oraz proces kiełkowania i rozwoju nasion (Kavi Kishor i in., 2022). ABA znajduje także zastosowanie jako suplement roślinny – wykazano, że może łagodzić fitotoksyczne działanie metali ciężkich poprzez ograniczenie ich akumulacji w tkankach, np. niklu w *Trigonella foenum-graecum* L. (Parwez i in., 2023) czy kadmu u *Arabidopsis* (Fan i in., 2014).

Synteza ABA w roślinach zachodzi głównie w szlaku metabolizmu karotenoidów i obejmuje m.in. reakcje hydroksylacji oraz glikozylacji, regulowane przez monooksygenazę cytochromu P450 oraz glukozylotransferazę ABA (Wu i in., 2023). Cytochrom P450 to szeroko rozpowszechniony kompleks enzymatyczny, którego aktywność wykazano również u larw *H. illucens* – m.in. w procesie detoksykacji aflatoksyny B1 w skażonym podłożu (Meijer i in., 2019). Enzymy te mogły przyczynić się do wzrostu stężeń ABA we frassie (szczególnie w wariancie z fasoli) oraz w odcieku (w obu wariantach) (Tab. 15). Frass z fasoli charakteryzował się istotnie wyższą zawartością ABA niż frass z grochu, mimo że dane literaturowe wskazują na większą zawartość karotenoidów w nasionach grochu (średnio 20,26 μg/100 g suchej masy) niż w nasionach fasoli (średnio 1,79 μg/100 g suchej masy) (Kantha i in., 1987). Różnice te mogą wynikać z faktu, że w doświadczeniu zastosowano poprodukcyjne odpady nasienne, których skład, w tym zawartość karotenoidów, mógł znacząco odbiegać od składu nasion surowych. Inną możliwą przyczyną jest stres środowiskowy podczas uprawy fasoli, prowadzący do wyższego poziomu ABA w nasionach. Nie bez znaczenia może być również odmienna mikrobiota obecna w podłożach, która mogła wpływać na biotransformację związków drobnocząsteczkowych, w tym fitohormonów.

Kwas salicylowy (SA) wpływa na wiele procesów wzrostu i rozwoju roślin, w tym kiełkowanie nasion, zamykanie aparatów szparkowych, tworzenie owoców i fotosyntezę (Sabagh i in., 2022). Bierze także udział w nabywaniu odporności na patogeny, w tym nekrotroficzne (Misra i Saxena, 2009) oraz w syntezie roślinnych metabolitów wtórnych o właściwościach antyoksydacyjnych (Sharma i in., 2023). SA jest szczególnie istotny w warunkach zasolenia gleby, gdzie może poprawiać m.in. szybkość kiełkowania (Sharma i in., 2023). Wykazano również, że ogranicza translokację metali, np. miedzi w kukurydzy, oraz stymuluje kiełkowanie nasion przy dawce 500 μM (Moravcová i in., 2018).

SA jest pochodną fenolową, a jego biosynteza może zachodzić m.in. z udziałem szlaku prowadzącego przez cynamonian, powstający w wyniku przemian fenyloalaniny (Chen i in. 2009). Aminokwas ten występuje w dużych ilościach w nasionach fasoli (Zhang i in. 2024).

Wyższa zawartość fenyloalaniny może przyczyniać się do produkcji większych ilości cynamonianu i ostatecznie SA, co może wyjaśniać różnice w stężeniach tego fitohormonu we frassie i odciekach między wariantami. Dla porównania, Sienkiewicz i in. (2024) podali zawartość SA w kompoście z łuskami gryki i w ekstrakcie biohumusu na poziomie odpowiednio 4,02 ± 0,37 i 0,28 ± 0,01 $ng \cdot g^{-1}$ suchej masy. Śladowe ilości (<0,01 $ng \cdot g^{-1}$ suchej masy) stwierdzono również w kompoście ogrodowym, kompoście z plew konopi i wytłoczyn z jabłek, kompoście organicznym oraz granulkach kompostu organicznego (Sienkiewicz i in., 2024). .

Kwas jasmonowy (JA) w największych ilościach występuje w wierzchołkach pędów, korzeniach, młodych liściach i niedojrzałych owocach. Należy do rodziny utlenionych kwasów tłuszczowych i jest syntetyzowany z oksylipin (cyklopentanów) (Ghorbel i in., 2021). Hormon ten reguluje reakcje i ruchy roślin w odpowiedzi na zmiany sezonowe i dobowe, wspierając ich adaptację do środowiska (Sabagh i in., 2022), a także uczestniczy w reakcjach odpornościowych przy uszkodzeniach i atakach patogenów (Ruan i in., 2019). Wykazano, że stosowanie JA może zmniejszać akumulację stresorów, takich jak Cd, nadtlenek wodoru czy dialdehyd malonowy, np. u bobu (*Vicia faba* L.) (Ahmad i in., 2017).

Obecność JA w nasionach fasoli (*Phaseolus vulgaris* L.) została zbadana przez Enomoto i Miyamoto (2021), którzy określili jego zawartość w poszczególnych częściach nasiona: liścieniu (0,05 $ng \cdot mg^{-1}$ świeżej masy), korzeniu zarodkowym (0,21 $ng \cdot mg^{-1}$ świeżej masy) oraz łupinie nasiennej (0,53 $ng \cdot mg^{-1}$ świeżej masy). Stężenia w substracie z obu wariantów, fasoli i grochu, były niższe niż te wartości literaturowe (Tab. 15). Mimo, że JA występuje obficie w roślinach, dane literaturowe dotyczące zawartości JA w kompostach, nawozach naturalnych, pozostałościach pofermentacyjnych i podobnych nawozach biologicznych są skąpe. Green (2023) odnotował stężenie JA na poziomie 0,18 $ng \cdot ml^{-1}$ we frassie *H. illucens* uzyskanych z odpadów spożywczych z kateringu gastronomicznego.

Podsumowując, wyniki opisane w pracy **P4** pozwoliły na realizację **celu C5** i potwierdziły trafność **hipotezy H4.**

6. Podsumowanie i wnioski

Powyższe badania potwierdziły, że odpady po hodowli *H. illucens* mogą być z powodzeniem stosowane jako bionawóz. Uszczegóławiając ten zasadniczy wniosek należy stwierdzić, że zrealizowano także wszystkie cele cząstkowe (**C1**, **C2**, **C3**, **C4** i **C5**) założone we wstępie niniejszej rozprawy.

Podsumowując najważniejsze wnioski można stwierdzić, że:

1. Frass uzyskany z hodowli larw *H. illucens* na odpadach poprodukcyjnych nasion grochu i fasoli jest bogaty w dobrze przyswajalne formy azotu (potwierdzenie hipotezy **H1**).
2. W przypadku występowania wysokiego stężenia jonów amonowych występuje konieczność dojrzewania frassu. Proces ten prowadzi do spadku stężenia potencjalnie toksycznych jonów oraz stabilizacji frassu jako bionawozu (częściowe potwierdzenie hipotezy **H2**).
3. Hodowla larw *H. illucens* na substracie zanieczyszczonym grzybami pleśniowymi może stanowić jedną z metod remediacji i utylizacji takiego materiału odpadowego. Larwy *H. illucens* nie akumulują mykotoksyn w swoim ciele. Biokonwersja przez larwy, jak i proces dojrzewania redukuje różnorodność i ilość mykotoksyn w substracie. Jednakże ze względu na pojawienie się nowych mykotoksyn (nie występujących w substracie grochowym i fasolowym) należy uznać, że hipoteza **H3** została potwierdzona jedynie częściowo.
4. Frass i odciek uzyskany po hodowli *H. illucens* zawiera fitohormony (potwierdzenie hipotezy **H4**), w tym szczególnie interesujący wydaje się być frass z fasoli, gdyż zawiera ich znaczące ilości.

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8. Publikacje

8.1. Publikacja P1

biology

MDPI

Review

The Variety of Applications of *Hermetia illucens* in Industrial and Agricultural Areas—Review

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Simple Summary: Human population growth contributes to a negative impact on the environment. In order to protect and restore nature, finding solutions and technologies in the industrial and agricultural fields that simultaneously recycle organic waste biomass, revalorize and recover nutrients and natural compounds is continuously important. The production of the insect *Hermetia illucens* (Diptera: Stratiomyidae, Linnaeus, 1758) fits well within the framework of green policy. *H. illucens* larvae fed on various biomass. The redirection of leftovers from fruit and vegetable or food processing to feed the larvae allows to produce insect proteins and fat, which can be further used in the production of animal feed. Besides, the larvae are also able to feed on manure, biogas sludge, and municipal sewage sludge, which decreases its weight and thus offers entomoremediation of the waste. Insect frass is use as an organic fertilizer. Fats and insect biomass are suitable for biodiesel production and biogas generation. From insect exoskeletons, chitin and chitosan are extracted. Thus, insect production seems to create new and unique opportunities for the environment, people, and animal nutrition, and the large and growing number of publications on *H. illucens* puts it in the center of interest of various research communities.

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Abstract: *Hermetia illucens* (Diptera: Stratiomyidae, Linnaeus, 1978), commonly known as the black soldier fly (BSF), is a saprophytic insect, which in recent years has attracted significant attention from both the scientific community and industry. The unrestrained appetite of the larvae, the ability to forage on various organic waste, and the rapid growth and low environmental impact of its breeding has made it one of the insect species bred on an industrial scale, in the hope of producing fodder or other ingredients for various animals. The variety of research related to this insect has shown that feed production is not the only benefit of its use. *H. illucens* has many features and properties that could be of interest from the point of view of many other industries. Biomass utilization, chitin and chitosan source, biogas, and biodiesel production, entomoremediation, the antimicrobial properties of its peptides, and the fertilizer potential of its wastes, are just some of its potential uses. This review brings together the work of four years of study into *H. illucens*. It summarizes the current state of knowledge and introduces the characteristics of this insect that may be helpful in managing its breeding, as well as its use in agro-industrial fields. Knowledge gaps and under-studied areas were also highlighted, which could help identify future research directions.

Keywords: black soldier fly; revalorization; circular economy; waste management; biogas; biodiesel; entomoremediation; frass; AMP; chitin

1. Introduction

Insects are one of the most diverse classes within the animal division. They occur in all climatic zones and in terrestrial and aquatic ecosystems [1]. From an anthropocentric point of view, around 5.5 million insect species worldwide [2] can be categorized as being

either useful or harmful to human interests [3]. One of the beneficial insects is *Hermetia illucens* (Linnaeus, 1758), often referred to as the black soldier fly (BSF) [4].

H. illucens belongs to the order of Diptera, in the family of Stratiomyidae. Their indigenous regions are North and South America, but nowadays this fly has also been observed on other continents in tropical, subtropical, and temperate zones [5]. The fly belongs to the holometabolous insects and its developmental cycle consists of several stages: eggs, larvae, prepupae, pupae, and adult [6]. The larvae of *H. illucens* are saprophagous, which means that they feed on various rotten organic matter. It is the only stage in the developmental cycle that can consume food. Adult *H. illucens* flies are able to intake water but it derives most of energy from reserves accumulated in the larval stage. Their only goal is to reproduce. Studies have shown that flies with access to protein (a mixture of sugar, milk protein, and bacteriological peptone) can live about 5 days longer and lay up to three times more eggs [7].

Considering the significant amount of research devoted to insects, it should be stated that *H. illucens* was rarely studied in the past. According to the Web of Science, up until 2009, this fly was the object of investigations on just 40 occasions. Only in the last 5 years has there been a significant increase in the interest in *H. illucens* (Figure 1).

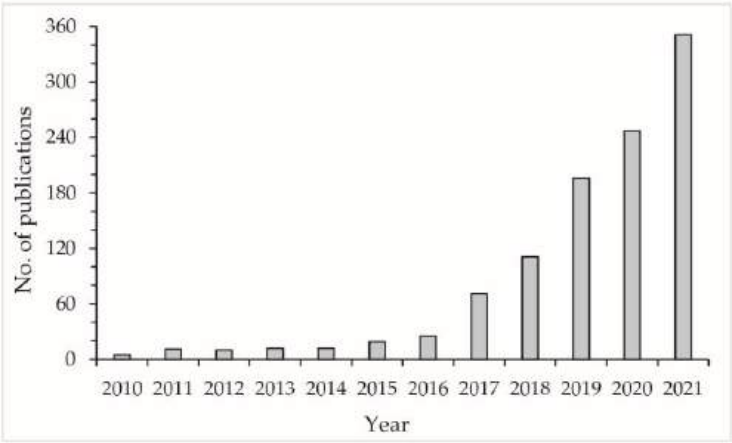

Figure 1. The dynamics of publication growth about *H. illucens*. Information from the Web of Sciences database, May 2022. For the search a combination of keywords was used: "*Hermetia illucens*" or "*H. illucens*" or "black soldier fly" and these words were entered into "all fields" in the form of core collection.

Nevertheless, until now, very few reviews describing the collective aspects of its use in industrial and agricultural applications have appeared [4,8]. The aim of this study is to gather the latest information from studies which have appeared since 2018, fill in certain information gaps, and present a broader spectrum of the industrial and agricultural applications of *H. illucens*. Moreover, on the basis of the analysis, we propose research or application areas with future potential in both scientific and industrial fields.

2. Applications of *H. illucens*

2.1. Bioconversion of Waste Biomass

Undoubtedly, interest in *H. illucens* has increased due to its ability to develop properly and increase its body mass, as well as its high survival rate when consuming different types of food. The insect's diet may include various types of organic waste, agronomic waste ranging from crop residues, such as corn straw [9], seasonal agri-food residues [10], animal

manure [11–13], municipal waste in the form of sewage sludge [14,15], as well as food waste, i.e., vegetable and fruit waste [16,17], or restaurant waste [18]. The performance of *H. illucens* larvae resemble fast and efficient bioconverters of waste biomass. Their uses, as well as those of other saprophytic insects, may be very important for bioconversion technology, due to the growth of the global population and its demand for food. This, unfortunately, means more food being discarded or becoming outdated, which is either wasted or ends up in landfill, occupying more and more land areas and contributing to uncontrolled greenhouse gas emissions. The cultivation of *H. illucens* larvae could be a possible means of utilizing waste biomass, while simultaneously obtaining larval biomass that could be a source of nutrients, such as proteins or fats [19]. In this context, the process may also be referred to as the revalorization of waste biomass, as the waste may become feed for the insect.

A compilation of data on the crude protein, the crude fat content of larvae, as well as bioconversion factors, and the dry matter reduction values of different substrates, are presented in a review article by [20]. Depending on the substrates, the protein content of the larvae ranged from 30.7–53.0% dry mass (DM), with the highest value obtained in brewery by-products. As regards larval fat, the content ranges from 8.1–47.4% DM, with the highest value obtained in fruit waste [20]. The highest biomass reduction values were obtained in substrates, such as municipal organic waste (68.0% DM) [21], chicken manure (61.7% DM) [22], a mixture of food waste and human excreta (68.4–68.8% DM) [23], a mixture of slaughterhouse waste, fruit, and vegetable waste (61.1% DM), and primary sludge (63.3% DM) [14].

Lalander et al. [14] conducted a study which evaluated the development of *H. illucens* larvae and determined the factors affecting the efficiency of bioconversion. For this purpose, several different substrates were tested: poultry feed, dog feed, food waste, fruit and vegetables, abattoir waste, fruit and vegetable waste, poultry manure, human feces, primary sludge, undigested sludge, and digested sludge. *H. illucens* larvae reared on these media contained protein in their biomass at 39.1–44.2% DM, with the highest value obtained in abattoir waste. The results of this study showed that the volatile solids (VS) content and protein content of the feed medium were the main factors affecting the timing and growth of larvae [14].

Liu et al. [24] found that a high presence of carbon component with poor digestibility, such as lignin and cellulose, had a strong negative influence on *H. illucens* larvae development. Ramzy et al. [25] also came to this conclusion by conducting an experiment with different doses of olive pomace residue, which was rich in lignin. The variant, which contained 39.54% of lignin fraction, resulted in larvae with a body weight of approximately 220 mg, which was 1.3 times lower than the control. Simultaneously, a lower larval survival rate of 75.5% was recorded, which was 1.2 times lower than the control [25].

Extensive analyses of the utilization rates of various substrates by *H. illucens* larvae have been published in recent years, among which are the following: [6,26–28].

2.2. Animal Feed

The use of *H. illucens* to feed a wide variety of animals is one of the most studied practical aspects of the use of this insect. Presently, there are review articles which focus exclusively on this use [29–31].

There are several possible ways that *H. illucens* can be used as a feed for animals. Whole larvae and pupae can be given to animals such as chickens [32–34], reptiles [35], fish/aquatic animals [36–38], pigs [39,40] or even dogs [41]. The direct use of whole and live *H. illucens* was certainly the easiest and the most practical and inexpensive way of administering food to animals [34]. Whole insects can also be dried and ground and, in this form, constitute a feed additive [39].

The nutritional components of the insect, such as fat and proteins, can also be extracted and used as feed additives or to formulate new feeds. *H. illucens* is characterized by an appropriate amino acid composition both for animal and human consumption [42]. If insect

fat is used, this can be a substitute for soybean oil [43]. It is also possible that appropriate modifications of the *H. illucens* larvae diet can improve the quality of the larvae fat, e.g., enrichment with fish residues results in an increase in the omega-3 fatty acid content in the larvae [44].

In the case of obtaining protein meal, there are three types: defatted [41], partially defatted [45] or full fat [40]. In this case, the choice of the correct type of feed should be guided by the preferences and nutritional requirements of the particular animal. This approach requires the industrial processing of raw insects, which incurs higher costs but has the advantage of creating new feed formulas, ideally suited to the nutritional requirements of a given animal.

Research has found that defatting is important when producing powdered feed from the insect, otherwise difficulties could occur during the pulverizing process, due to the high content of fatty components in the bodies of *H. illucens* [46]. Kim et al. [46] proposed two methods to remove the fat from dried and ground larvae: through the use of hot water, which reduced the fat level from the initial 30 to 16%, and the more efficient, supercritical carbon dioxide extraction method, which eventually reduced the fat level to 4.5%. This process was conducted for 6 h under an increased pressure of 350 bar and a flow rate of 26 L $CO_2 \cdot h^{-1}$ [46].

The variety of animals that have been fed by supplements produced from *H. illucens* is considerable (Table 1). *H. illucens*, as a feed, mostly had a positive effect on various animal organisms [47,48], however, in some cases, researchers reported a negative influence, manifesting, for example, in a slightly reduced body weight or the reduced digestibility of proteins and lipids [37,49]. This indicated that *H. illucens* meal is not optimal nutrient source for all animals. For instance, [37] concluded that *H. illucens* should not make up more than 17% FW of the feed composition for fish *Argyrosomus regius*, so as not to cause undesirable effects [37] (Table 1).

In addition to providing nutrients, studies have shown that feed from *H. illucens* also contained antimicrobial and antioxidant properties, as well as beneficial influence on the intestinal tract of animals (Table 1). Dabbou et al. [43] investigated the antimicrobial potential of the fat of *H. illucens*, as well as the use of insect fat as a replacement for soybean oil added to rabbit feed, at a value of 1.5%. Subsequently, an investigation was conducted to evaluate the way in which this change in diet affects the intestines of the animals.

The antimicrobial activity of the fat of *H. illucens* was tested on bacterial strains, which represent serious pathogens and are capable of causing diseases or poisoning of the digestive system. The results showed that the fat of *H. illucens* had no effect on *Salmonella* bacterial strains (*S. tiphymurium*, *S. enteritidis*). In the case of *Yersinia enterocolitica*, *Pasteurella multocida*, and *Listeria monocytogene*, bacteriostatic effects were noted [43].

Regarding the effect of the fat of *H. illucens* on the intestinal function, compared to the control, a difference was observed in the amount of volatile fatty acid (VFA) production in the cecum, which was recorded as 85.3 mmol·L^{-1} and was 1.2 times higher than the control; this could be the result of changes made in the intestinal microbiome of the rabbits. The addition of the fat of *H. illucens* increased the amount of *Ruminococcus* bacteria, which produce short chain fatty acids and have a positive effect on the microbiome of herbivores [43].

Yu et at. [39] investigated changes in the gut microbiome and the bacterial metabolites produced in female finishing pigs (crossbred Duroc, Landrace, and Large White), whose diets included 4% dried and crushed *H. illucens* prepupae. Pigs fed with a diet of *H. illucens* showed a higher concentration of beneficial *Lactobacillus* and *Pseudobutyrivibrio*, *Roseburia* and *Faecalibacterium*—butyrate-producing bacteria in their intestines, with a reduced concentration of *Streptococcus* bacteria. In addition, the inclusion of *H. illucens* in the pig diet caused the expression of pro-inflammatory genes to decrease, while the expression of anti-inflammatory genes increased [39].

Table 1. Compilation of animals as test objects and *H. illucens* as a form of feeding.

Animal	Form of *H. illucens*	Effects	References
		Positive influence	
Laying hens	Soybean meal and soybean oil + *H. illucens* pre-pupae	Increased egg weight by 1.1 times, increased SCFA concentration by 1.3 times	[32]
Laying hens	Corn-soybean meal + 25% of replaced protein by partially defatted *H. illucens*	Despite the reduced length of the intestinal villi, increased the amount of volatile fatty acids by 1.1 times and the amount of butyrate by 1.2 times in intestines	[33]
Hen broilers	Chicken feed + 5% or 10% of live *H. illucens* larvae	Decreased timidity of hens, increased activity of hens	[34]
Atlantic salmon (*Salmo salar*)	Corn protein, soybean meal + (200 g·kg^{-1}) *H. illucens* meal	No significant differences to control	[36]
European seabass (*Dicentrarchus labrax*)	Fishmeal + 50% dried *H. illucens* larvae meal	No significant differences to control	[38]
Finishing pigs	Corn, wheat bran and soybean meal + 4% dried and crushed *H. illucens* prepupea	Decreased expression of pro-inflammatory cytokines and concentrations of total amines and phenol, increased expression of anti-inflammatory cytokines, intestinal barrier genes and concentrations of short-chain fatty acids (SCFA) and butyrate (prebiotic effect)	[39]
Weanling piglets	Fishmeal + 2% full-fat *H. illucens* larvae meal	Increased lactate in *ileum* by 1.6 times, in *cecum* by 2.2 times, and SCFA by 1.2 times in *ileum* and by 1.1 in *cecum* (probiotic effect), increased anti-inflammatory protein IL-10 by 1.3 times, decreased pro-inflammatory protein TNF-α by 1.3 times	[40]
Beagle dogs	Grain-based diet + 2% defatted *H. illucens* larvae meal	Improved dry matter digestibility by 1.1 times, decreased TNF-α levels by 1.8 times (anti-inflammatory effect), increased glutathione peroxidase levels by 1.23 times (antioxidant effect)	[41]
Rabbits	Rabbits feed + 1.5% *H. illucens* fat	Inhibition of the growth of the pathogens *Pasteurella multocida* by 3.2 times, *Yersinia enterocolitica* by 2.5 times, *Listeria monocytogenes* by 2.1 times	[43]
Muscovy ducklings (*Cairina moschata domestica*)	9% partially defatted *H. illucens* meal	Decrease in uric acid by 1.2 times and creatinine by 1.2 times (improved kidney function), increase in serum iron Fe by 1.3 times	[45]
African catfish (*Clarias gariepinus*)	Fishmeal + 50% partially defatted *H. illucens* larvae meal	Increase in body weight by 1.5 times	[47]
Rainbow trout (*Oncorhynchus mykiss*)	Control diet (wheat gluten, soybean meal and hemoglobin) + 15% *H. illucens* larvae meal	Increase in the number of beneficial *Lactobacillus* and *Bacillus* bacteria, reduction in *Aeromonas* pathogens in fish gut	[48]
Female turkeys	Soybean-maize enriched with 50 g/kg *H. illucens* larvae fat (50% and 100%)	Improved intestinal digestibility of the ether extract Increase in lipase activity Reduction of *Bacteroides-Prevotella* clusters	[50]
		Negative influence	
Meagre (*Argyrosomus regius*)	Partially defatted *H. illucens* + fishmeal	Weight loss, decrease in protein efficiency	[37]
Atlantic salmon (*Salmo salar*)	Control diet with full-fat *H. illucens* larvae meal, substituting 12.5% content of protein and control diet with full-fat *H. illucens* larvae paste, substituting 6.7% of protein	Decrease in protein and lipid efficiency and protein efficiency index, decrease in phosphorus retention	[49]

In another animal study, Lei et al. [41] proved the antioxidant and anti-inflammatory properties of *H. illucens* supplementation, by studying how it affected beagle dogs. A 1% or 2% addition of defatted *H. illucens* larvae meal sample was added to the dogs' diet which consisted of cereal grains. In addition, at the end of the 6 week experiment, all dogs

were injected with *Escherichia coli* lipopolysaccharide (LPS; 100 $\mu g \cdot kg^{-1}$ body weight) to induce an inflammatory response. An analysis of serum parameters showed that the only difference between the control and the *H. illucens* larvae meal samples was the albumin concentration. With a 2% *H. illucens* larvae addition, the albumin concentration was 1.3 times higher than in the control. There was also an increase in dry matter (75.21%) and crude protein (78.51%) digestibility by 1.05 and 1.07 times, respectively, compared to the control. In a blood profile study after *E. coli* LPS injection, there was a lower tumor necrosis factor-α concentration in the blood of the dogs, which were fed the 2% *H. illucens* larvae feed; this was 1.82 times lower than the control after 6 h. Moreover, an increase in the concentration of glutathione peroxidase, which has an antioxidant function, was observed. Its activity was 53.05 $nmol \cdot min^{-1} \cdot mL^{-1}$, which was 1.26 higher than the control. In the case of superoxide dismutase, an increase in its concentration was observed in the dogs fed with *H. illucens* larvae meal 3 h after injection, with concentrations subsequently decreasing. The results maintained in this study indicate the positive effect of the addition of *H. illucens* to beagle dog food, demonstrating health-promoting activities [41].

In a study involving the replacement of 100% soybean oil with fat from *H. illucens* larvae in the diets of male broilers, Cullere et al. [51] recorded no change in the nutritional composition and sensory profile. However, a difference could be observed in the fatty acid profile, in which the amount of saturated fatty acid (SFA) increased, while the amount of polyunsaturated fatty acids (PUFA) decreased. In chicken breast, SFA increased 1.6 times more than the controls and was 45.8% of the total fatty acid methyl esters (FAME). PUFA concentration decreased 1.6 times more than the control and was recorded at 23.4%. In the leg meat, on the other hand, the SFA was 1.7 times greater than the control, being 44.2%, and the amount of PUFA decreased 1.6 times compared to the control and was recorded at 24.3% [51]. One of the reasons could be that the lipid fraction of *H. illucens* is deficient in PUFA, which are essential components for the correct development of, e.g., aquaculture animals [52,53]. An increase in the fat content of these *H. illucens* larvae is therefore a positive direction in which further research should be conducted. An interesting approach was proposed by [54], who reared *H. illucens* larvae on a diet enriched with *Schizochytrium* algae, which are rich in omega-3 PUFA. The authors found that the *H. illucens* larvae bioaccumulated this. This treatment increased the amount of PUFA in the larval biomass from 13.6% in the control to a level of 22.2% [54].

In terms of feeding, mineral composition is key. As with the basal composition of *H. illucens* larvae, the mineral composition depends on the substrate in which the larvae were bred. A review by Barragan-Fonseca et al. [55] compares the mineral values in the larvae fed with chicken feed (CF) and with poultry and swine manure. The element that was found in the greatest amounts in the body of the larvae was Ca. Its content in the larvae fed with CF was 31.4 $g \cdot kg^{-1}$ DM, while the larvae on both manures contained 50–78 $g \cdot kg^{-1}$ DM. The higher concentration of macroelements such as P, Mg, Na, and K, was obtained in larvae fed with CF, and their values were 12.8, 7.9, 2.7, and 19.6 $g \cdot kg^{-1}$ DM, respectively. A higher content of the microelements Fe, Zn, Cu and Mn were found in larvae fed on manures, and were recorded as 100–1400 $\mu g \cdot g^{-1}$ DM, 100–300 $\mu g \cdot g^{-1}$ DM, 10–30 $\mu g \cdot g^{-1}$ DM, and 200–600 $\mu g \cdot g^{-1}$ DM, respectively [55].

In a study by Surendra et al. [20], there was a compilation of the mineral compositions of *H. illucens* prepupae, reared on different types of organic wastes. The richest in mineral pupae were those fed with digestate (following an anaerobic fermentation of the mixture of vegetables) or brown algae (*Ascophyllum nodosum*). With regard to the digestate, the highest amounts were found for Ca—66.15 $g \cdot kg^{-1}$ DM, Fe—6.75 $g \cdot kg^{-1}$ DM, Mn - 0.38 $g \cdot kg^{-1}$ DM, and S—0.31 $g \cdot kg^{-1}$ DM. In the case of brown algae, the highest amounts of K—21.30 $g \cdot kg^{-1}$ DM, Mg—6.20 $g \cdot kg^{-1}$ DM, Na—12.30 $g \cdot kg^{-1}$ DM, P—12.30 $g \cdot kg^{-1}$ DM, and Zn—0.15 $g \cdot kg^{-1}$ DM were obtained [20]. The substrate supplement given to the larvae to increase the micro- and macronutrient could be fruits. Romano et al. [56] studied the mineral value of larvae fed with bananas (three variants: banana peels, banana fruit, mixture of both (1:1 *w:w*)) and orange fruit (three variants: orange peels, orange fruit,

mixture of both (1:1 *w:w*)). The Ca content in the larvae fed with banana mixture was 579 $\mu g \cdot g^{-1}$ DM. However, most of the minerals in the larvae were present when they were fed solely banana peels. In the case of the macronutrients, the P, K, and Mg contents were 13.27, 65.28, and 2.38 $\mu g \cdot g^{-1}$ DM, respectively. Regarding the micronutrients for Na, Mn, Zn, and Cu, the values were 77.4, 11.3, 17.8, 4.6 $\mu g \cdot g^{-1}$ DM. Only in larvae fed with the orange mixture was the presence of Ni recorded and it was 0.6 $\mu g \cdot g^{-1}$ DM [56].

An important context of the research is the study of *H. illucens* for elements that may be harmful to the health of the organisms. These insects have the ability to bioaccumulate elements, and attention should be paid to the composition of the substrate on which they are fed. More information on the bioaccumulation of elements and other contaminants is discussed in this review in Section 2.7.

The effect of feeding animals with *H. illucens* on properties and characteristics, such as fat profiles, meat quality, and heavy metal content in meat, was also studied. A study conducted on broilers showed that replacing 25% soybean meal with microwave-dried and press-defatted *H. illucens* meal did not harm the health of the animal or the meat quality [57]. The body weight of a chicken fed on a diet consisting of a 25% addition of *H. illucens* was 1.76 kg (live weight) compared to 1.80 kg among the control on soybean meal. However, a higher percentage addition (50%) of *H. illucens* to the broiler diet resulted in a 1.2-fold reduction in body weight gain compared to the control [57].

There are also studies which indicated that the addition of *H. illucens* to animal feed yielded neither positive effects [36,38] nor negative effects on, e.g., the weight gain of animals [37,49,58]. The negative effects should be investigated further to understand why, in some cases, *H. illucens* is not a good choice for animal nutrition. However, in the case of neither positive nor negative effects on animal growth, the addition of *H. illucens* could still, in some cases, have an economic justification and could prove to be a cheaper counterpart by comparison with other more commonly used nutritional supplements.

2.3. Chitin and Chitosan

2.3.1. Chitin

Chitin is a carbohydrate biopolymer of N-acetyl-D-glucosamine and D-glucosamine units [59]. It occurs in various living organisms, such as fungi [60], mollusks [61] and insects [62] and can have different physicochemical properties depending on its origins and internal arrangement of fibers [63]. Those arrangements differentiate chitin to α-, β-, and γ-chitin form [63]. Chitin present in *H. illucens* occurs in the α configuration [64]. The α-chitin molecule has an antiparallel arrangement of carbohydrate chains and is the most thermodynamically stable form of chitin [63]. Chitin content varies between *H. illucens* developmental stages, but unsurprisingly the highest amounts are found in the puparium (Table 2) [65]. It was also found that the chitin content in the internal organs of *H. illucens* is about 20% of the total chitin content [64,66].

Table 2. Total chitin content (%) in different developmental stages of *H. illucens* and puparia.

Extraction Methods	Insect Material	Chitin Content (%)	Crystalline Index (%)	References
Demineralization: 1 M HCl (1 h), deproteinization: 1 M NaOH (80 °C, 24 h), depigmentation: 1% $KMnO_4$	puparium	n.d.	35.0	[64]
	adults	n.d.	24.9	
Demineralization: 2 M HCl (55 °C, 1 h, 200 rpm·min^{-1}), deproteinization: 2 M NaOH (50 °C, 18 h, 200 rpm·min^{-1}), depigmentation: 3.6% HCl (0.5 h), 10-fold diluted NaClO (80 °C, 4 h, 200 rpm·min^{-1})	larvae	3.6	33.1	[65]
	prepupae	3.1	35.1	
	puparium	14.1	68.4	
	adults	2.9	87.92	

Table 2. *Cont.*

Extraction Methods	Insect Material	Chitin Content (%)	Crystalline Index (%)	References
Demineralization: 1 M HCl (1:10 (*m*:*v*), room temp., 1 h), deproteinization: 1 M NaOH (1:25, 80 °C, 1 h), depigmentation: 12-fold repetition of the deproteinization process	larvae	9.5	~88	[67]
	prepupae	9.1	~95	
	pupae	10.3	~93	
	larvae shedding	31.1	~90	
	puparium	23.8	~94	
	adults	5.6	~89	
Demineralization: 1 M HCl (100 °C, 0.5 h), deproteinization: 1 M NaOH (24 h)	puparium	25.4	74.1	[68]
	flakes after oil extraction	20.7	61.1	
	adults	7.8	77.8	
Acid detergent fiber—acid detergent lignin	puparium	21.2	70.8	
	flakes after oil extraction	26.8	50.0	
	adults	7.9	39.0	
Demineralization: 1 M HCl, 22 °C, 1 h, deproteinization: 1 M NaOH, 80 °C, 24 h, depigmentation: 9% H_2O_2, 80 °C, 2.5 h	puparium	7.0	60.0	[69]
Demineralization: 0.5 M formic acid (1:10 (*m*:*v*)), 1 h, room temperature, deproteinization: 2 M NaOH (1:10 (*m*:*v*)), 2 h, 80 °C	larvae	13.0	74.0	[70]
	puparium	31.0	78.0	
	adults	9.0	79.0	
Demineralization: 0.5 M formic acid (1:10 (*m*:*v*)), 1 h, room temperature, deproteinization: 2 M NaOH (1:10 (*m*:*v*)), 2 h, 80 °C, depigmentation: 5% H_2O_2, (1:20–30), 30–60 min, 90 °C	larvae	10.0	77.0	
	puparium	23.0	80.0	
	adults	6.0	86.0	

n.d.—no data.

To obtain this compound, the standard and most commonly used method is the sequential removal of the remaining larval fractions. Firstly, the sample is demineralized with 1 M HCl, followed by a deproteinization process in the presence of 1 M NaOH. Chitin from *H. illucens* larvae is strongly bound with melanin, therefore a depigmentation step is necessary. Chemicals such as $KMnO_4$ [64], H_2O_2 [69], and NaClO [65], can be used to remove the pigments. However, this standard approach is an environmentally unfriendly way of obtaining chitin, because it produces large amounts of highly acidic or alkaline wastewater and also uses a significant quantity of water [59].

New efficient methods for chitin extraction, such as deep eutectic solvents (DES), have been developed. They are a combination of two components that act as a hydrogen bond donor and a hydrogen bond acceptor. In addition, these solvents have low toxicity and can be reused several times [71]. Natural deep eutectic solvents (NADES), such as choline chloride, betaine, urea, glycerol, DL-lactic acid, oxalic acid, and n-butyric acid, were used to isolate the chitin from the defatted *H. illucens* prepupae [72]. The best results were obtained for choline chloride-urea and betaine-urea variants at 50 °C; the chitin yield was 26.0% and 26.7%, respectively, with a purity of 88.4% and 83.3% [72].

Another method is the extraction of chitin from dried and shredded *H. illucens* puparia by microbial fermentation using *Bacillus licheniformis* A6 [59]. For depigmentation, the chitin pellet was heated in the solution of 0.1% SDS and 0.5% $NaHCO_3$ and then left in 30% H_2O_2 The chitin content obtained by this method was 12.4% [59].

Chitin extraction studies involving organic acids, ethanol, and a bacterial enzyme (*B. licheniformis* protease) were also performed [73]. Using these reagents, four different methods of extracting chitin from the dried puparia of *H. illucens* were tested. Among these

isolation methods, *B. licheniformis* protease was the most effective, followed by degreasing with organic solvents. This removed 33.3% of the protein content and 38.5% of the lipid content compared to the untreated puparia. The percentage yield of chitin for all above-mentioned methods was 65.4–72.4%. The authors conclude that such high yields may be indicative of contamination [73]. Unfortunately, these methods, although environmentally friendly, do not yield good quality chitin [73].

Hahn et al. [74] conducted extensive research into optimizing the process of obtaining and purifying chitin from *H. illucens* puparia. They tested various chemicals for the demineralization, deproteinization and depigmentation steps. The optimum demineralization result, with an efficiency of around 90%, was obtained for 0.5 M formic acid, while a 96% of protein removal was achieved with 1.25 M NaOH. The results showed that the best method for chitin depigmentation was the use of 6% NaOCl (around 66% efficiency). Similarly, by using a mixture of 5% H_2O_2 + 1.8% NaOH + 0.2% $MgSO_4$ an efficiency value of approximately 62.2% was achieved. As the authors point out, this can minimize the use of chlorine [74].

A method that requires fewer quantities of harsh chemical solutions to isolate chitin is the use of acid detergent fiber (ADF) and acid detergent lignin (ADL) methodology. The defatted sample was added to 0.5 M H_2SO_4 with CTAB (20 $g \cdot L^{-1}$), then boiled in a reflux condenser. The ADF content was calculated from the dry matter loss. The remaining filtrate was added to 12 M H_2SO_4, mixed, percolated and after 30 min was mixed again with 25 mL H_2SO_4 for 3 h, then washed and dried [68]. The chitin content obtained by this method was 21.19%, which was 1.2 times lower for puparia, compared to the conventional method (with HCl and NaOH). However, in the case of flakes obtained after oil extraction chitin content was higher by 1.3 times (26.78%). For flies, both methods gave comparable results (7.75–7.94%) [68].

Chitin may be used in environmental applications by utilizing its molecules as a sorbent for heavy metals, dyes, or air pollutants, as well as in energy applications including biosensors, and as cells used for energy production (see review of [75]). Chitin is a polymer that may also find its place in the production of biomedical materials, such as surgical sutures, wound patches or even materials used for tissue engineering [76]. One of the applications of chitin is to obtain chitosan, which will be discussed in more detail.

2.3.2. Chitosan

Chitosan is a derivative of chitin, formed by its deacetylation. Thanks to its free amino groups and decreased molecular weight, it is more reactive and soluble in acidic conditions [77]. To characterize and distinguish a polymer molecule as a chitosan, its deacetylation degree (DD) must be higher than 50% [78].

A common deacetylation method is the use of 50% (*w:w*) NaOH, in which chitin is left for 0.5 h at room temperature, then heated to 100 °C for 2 h, followed by cooling. The resulting suspension is washed with water to neutralize the pH [79].

New methods for chitosan deacetylation were reported by [80], described as heterogeneous and homogeneous. The heterogeneous methods were conducted in a high-pressure autoclave and involved the addition of deproteinized *H. illucens* larval exoskeletons (LE) in 12 M NaOH. The headspace of the reactor was filled with nitrogen and the pressure was brought to 3 bar at 120–140 °C. After centrifugation the pellets were washed with distilled water and then added to 1% acetic acid, mixed and centrifuged again. The supernatant was brought to pH 8 and left to precipitate the chitosan [80].

The homogeneous method involved incubating a sample of deproteinized LE under cooled conditions (4 °C) in 10 M NaOH for 12 h. Then, desalinated ice was added to facilitate chitin deacetylation and stirred until the ice melted. In order to avoid the oxidation of the chitosan, the headspace was filled with nitrogen. After thawing, the solution was brought to a pH 8, incubated at 4 °C and then subjected to vacuum filtration [80]. Heterogenous conditions allowed to obtain a DD of 43–72% and a chitosan deacetylation yield of 22–47%.

In comparison, the method under homogeneous conditions recorded 38% for DD and 30% for deacetylation yield [80].

Lee et al. [81] studied the structure of chitosan from *H. illucens* larvae by spectroscopic methods, as well as the antioxidant activity of its hydrolysate, made with the use of chitosanase. The obtained chitosan had a DD of 85.7% and molecular weight of 680 kDa. A test of the hydrolysate, by analyzing the radical scavenging activity of ABTS, confirmed the antioxidant properties of the sample [81].

Recent research has focused on testing the antimicrobial properties of chitosan derived from *H. illucens*. Guarnieri et al. [82] tested the antimicrobial properties of bleached and unbleached chitosan from the larvae, as well as the insect reared residues, i.e., exuviae and dead flies on the Gram-positive *Micrococcus flavus* and the Gram-negative *E. coli* [82]. All chitosan variants showed inhibitory effects regarding the growth of these bacteria. A minimum inhibitory concentration (MIC) was determined, which ranged from 0.15–0.3 mg·mL^{-1} depending on the origin of the chitosan [82].

Alghuthaymi et al. [83] produced nanocomposite made from *H. illucens* larvae chitosan and gum Arabic, to which a conjugate with eugenol and biosynthetic selenium nanoparticles was attached. It was verified for its bactericidal activity in relation to *E. coli* and *S. aureus*. The activity was greater than that of the ampicillin by approximately 1.2 times. MICs of 15.0 µg·mL^{-1} for *E. coli* and 20.0 µg·mL^{-1} for *S. aureus* were determined [83].

Several reviews described the applications of chitosan, including its antimicrobial properties [84], characteristic features used to build nanomaterials [85], use in drug delivery [86], the food industry [87], cosmetics, medical fields [88] and even environmental protection, e.g., wastewater treatment [89]. These provide the basis for future research into the use of chitosan derived from *H. illucens*.

2.4. Antimicrobial Properties

H. illucens, whose larvae live in substrates also inhabited by diverse microflora, had to develop antimicrobial mechanisms [90]. Many research studies have shown that these resistance mechanisms are quite strong. For instance, [91] used human feces as a substrate in which to grow *H. illucens* larvae and showed a 6 $\log_{10}$ reduction (99.9999%) in *Salmonella* spp. In the leftovers from the initial amount of this bacterium. The same authors, in a continuous-flow reactor study, fed larvae an artificial, pathogen-contaminated feed mix. They reported a practically complete removal of *Salmonella* spp. From approximately 10^7 CFU·g^{-1} to below detection levels [92]. This antimicrobial effect of living larvae was related to its secretions, as well as the microflora inhabiting their entrails. Kawasaki et al. [93] found a reduction of *E. coli* in the *H. illucens* larvae frass from organic household waste.

More recent studies have investigated the effect of the addition of *H. illucens* larvae on bacterial levels in different types of manure and sewage sludge. The changes that were recorded after the application of *H. illucens* showed a reduction of pathogenic bacteria by 90–92% in chicken and cow manure, and by 86–88% in pig manure and sewage sludge. The pathogenic bacteria studied belonged mainly to the genera *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* [94].

The common way to test the antimicrobial activity of *H. illucens* is to prepare methanol extracts from the insect. Choi et al. [90] showed that methanol extracts inhibit the Gram-negative bacteria *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, and *Shigella sonnei*, with a MIC of the extracts of 44.74 mg·mL^{-1}, 43.98 mg·mL^{-1}, and 43.96 mg·mL^{-1}, respectively [90].

The acidic water-methanol extraction (AWME) method was used to obtain an larvae extract that was used for the first time against phytopathogenic bacteria: *Pantoea agglomerans*, *Xanthomonas campestris*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum*, and *Dickeya solani* [95]. The turbidimetric assay showed that for all tested pathogens the MIC was 0.78 mg·mL^{-1} and minimum bactericidal concentration (MBC) was in the range of 0.78–1.56 mg·mL^{-1} [95].

AMWE was also tested against various strains of *K. pneumoniae*. The results showed that its MIC and MBC of 250 mg·mL^{-1} showed antimicrobial activity. In addition, bacterial membrane permeability was found to be AMWE dose-dependent, and the bacteria did not gain resistance during prolonged use of the *H. illucens* extract [96].

One of the immune responses of insects attacked by pathogens is the production of antimicrobial peptides (AMPs), from which preparations can be made to eliminate or inhibit microorganisms. To increase the production of AMPs, the immunization of the larvae of *H. illucens* can be carried out with pathogenic [97], non-pathogenic or even probiotic bacterial strains [98]. These authors demonstrated the antimicrobial activity of isolated AMPs against Gram-positive pathogens such as methicillin-resistant *S. aureus* (MRSA) 40881, *S. aureus* 12256, *S. epidermidis* and *B. subtilis*, Gram-negative *K. pneumoniae* (ATCC 13883) and *Shigella dysenteriae* (ATCC 9750). A more in-depth review of the AMPs of *H. illucens* origin, including older articles, was compiled by [99].

The process of immunization of *H. illucens* larvae, with probiotic bacteria, was studied by [100] using species of the *Lactobacillus* family (*Lactobacillus acidophilus*, *L. brevis*, *L. casei*, *L. fermentum*, *L. delbrueckii*). The highest antimicrobial activity was recorded using *L. casei* as an immunizer. They obtained hemolymph extracts of *H. illucens* from the supernatant, formed after suspending the powdered larvae in 20% acetic acid dissolved in sterile water. It exhibited inhibitory activity in relation to the bacteria of the genus *Salmonella* (*S. pullorum*, *S. typhimurium* and *S. enteritidis*), *S. aureus* and *E. coli* with a MIC in the range of 100~200 µg·100 µL^{-1}, without inducing a cytotoxicity effect in animal cells as verified on CaCo-2 and L929 cell lines. In addition, the stability of the extract was found to be maintained at an elevated temperature (60–90 °C) for 24 h in a wide pH range of 2–11 [100].

Lee et al. [101] used *L. casei* immunized larvae extract. The MIC concentrations for *Enterococcus faecalis*, *Streptococcus mutans* and *Candida vaginitis* were 200, 500 and 1000 µg·100 µL^{-1}, respectively. For MRSA and multidrug-resistant *Pseudomonas aeruginosa*, the MIC was ~300 µg·100 µL^{-1}. Due to the good antimicrobial performance of the *H. illucens* extract, a mass injection system was developed to accelerate and automate the immunization process [101].

Shin et al. [102] screened the cDNA libraries from the fat bodies of *H. illucens* and noted a novel attacin-like peptide. Using a prokaryotic expression system, they produced a recombinant protein that was found to have properties resistant to *E. coli*, as well as MRSA [102]. Di Somma et al. [103] also used bioinformatics tools to target a peptide from the defensin family (C-15867) identified in *H. illucens* and carried out production of the peptide using gene expression by *E. coli*. The produced peptide showed inhibitory activity for *E.coli*, *S. aureus* and *Staphylococcus epidermidis* strains at an MIC of about 62 µM [103]. A recombinant *E. coli* expression system was also used to clone three AMPs (Hidefensin-1, Hidiptericin-1 and HiCG13551) produced by *H. illucens* immunized with *S. aureus*, *Pseudomonas aeruginosa* and *B. bombyseptieus*. The obtained products showed activity against *S. pneumoniae*, *E. coli* and *S. aureus* [104]. Proteomic and lipidomic analysis of the *H. illucens* larvae revealed the content of B3MI87 protein belonging to the hexamerin family, which registered activity similar to enzyme phenoloxidase-2, as well as resistance to some fungi and G-positive bacteria [105].

H. illucens was the first example of an insect from which a peptide with inhibitory activity against *Helicobacter pylori* was isolated [106]. Isolation has been carried out from the larvae preinoculated with *E. coli* (ATCC 25922). These peptides exhibited similar activity to chemo pharmaceutics, such as metronidazole. Their quantity was less than 10% of the extracted AMPs, and their mass ranged from 4.17–4.25 kDa [106]. The masses of this peptides were within the range of the AMP masses with anti-*H. pylori* activity extracted from other organisms (i.e., amphibians and fish with 1.99–4.40 kDa) [107].

Antimicrobial properties were also associated with larval fat. Some fatty acids, e.g., lauric acid, have antimicrobial properties by disrupting the surface of the bacteria—the cell wall and membrane [108]. This acid is a major component in the fat profile of *H. illucens* larvae.

In the case of oil extraction from *H. illucens* for antimicrobial purposes, [109] suggested that the traditional mechanical pressing of dried larvae is the most efficient method to ensure no loss of antimicrobial activity. The extracted oil did not cause changes in the growth of Gram-negative bacteria but inhibited Gram-positive bacteria, such as *B. subtilis* and *S. aureus*.

The high antimicrobial resistance of *H. illucens* may be influenced by the presence of live fungi in their digestive tract. Correa et al. [110] isolated 25 different fungal species from the gut of *H. illucens*, fed with guano from chickens. The highest antimicrobial activity (tested for MRSA ATCC 43,300 and *Salmonella typhimurium* ATCC 13311) was recorded for *Chrysosporium multifidum*. Seven antimicrobial compounds were isolated: six α-pyrone derivatives and one diketopiperazine. The α-pyrone derivative no. 4, limited the growth of MRSA at MIC 62.5 $\mu g \cdot mL^{-1}$ [110].

Other compounds with antibacterial and antifungal activities isolated from *H. illucens* are ommochromes, pigments from the flies' eyes. Dontsov et al. [111] investigated those compounds suspended in 0.1 M potassium-phosphate buffer solution. Tests were performed on *B. subtilis* ATCC 6633, *Candida albicans* ATCC 2091 and *A. niger* INA 00760, noting the zones of growth inhibition of these microorganisms [111].

Much different approach has been presented by Saadoun et al. [112]. They proposed lactic acid fermentation of the pupae, prepupae and dead flies of *H. illucens* using *Lacticaseibacillus rhamnosus* 1473 and *Lactiplantibacillus plantarum* 285 [112]. In the case of *L. monocytogenes* and *Salmonella*, the best antimicrobial results were observed for fermented puparia. A similar decrease in the growth of these bacteria, as well as *E. coli*, to near 1 log $CFU \cdot g^{-1}$ was also observed for fermented flies [112].

In recent years, research has focused on deciphering the *H. illucens* transcriptome, which may provide an interesting source of information, relevant for increasing AMP production [113]. In silico studies showed that the transcriptome in both the larvae and flies contained 68 genes encoding AMPs, 57 of which encoded putative active peptides. Peptide sequence analyses showed that some of these exhibited antimicrobial, antiviral, antitumor or antifungal activity. Among the identified peptides, defensins was the most abundant (44%), followed by cecropins (18%), lysozyme (18%), attacin (7%) and the remainder (13%). To confirm the results of the in silico studies, four putative AMPs, which should possess the highest antimicrobial activity, were selected, and synthesized to conduct laboratory trials. A reduction of *E. coli* cell growth was found at a peptide concentration of 3 μM, with rapid bacterial death observed at 12 μM [113].

2.5. Biodiesel Production

H. illucens are capable of eating a wide variety of waste biomass thus offering the possibility of their revalorization into fat. The crude fat content of *H. illucens* larvae varies depending on the type of diet and can be up to 40% DM [114,115]. It can become a substrate for biodiesel production. The developmental stages of *H. illucens*, which have the highest fat levels, are late larvae and prepupae. Interestingly, for the next stage—pupae—an approximate 2.8-fold decrease in fat content was recorded [116].

FAME are components of plant-based biodiesel and can also be synthesized by transesterification from triacylglycerols extracted from *H. illucens* larval biomass. Insect biodiesel is qualitatively comparable to that of plants, produced from rapeseed oil [117]. Biodiesel based on *H. illucens* larvae consists of a large number of saturated fatty acids, while the unsaturated fatty acid content is lower, having a positive effect on the quality of biodiesel, i.e., increasing the viscosity and decreasing the autoxidation processes [118].

The larvae of *H. illucens* reared on various substrates, such as wheat grain [119], fruit waste, sewage sludge, palm decanter [120] or exo-microbial fermented coconut endosperm waste [115] had different FAME contents, but in each of these cases, lauric acid (C12:0) was their main component (Table 3). Spranghers et al. [18] reported that the overall fatty acid composition of *H. illucens* prepupae, which were fed four different diets (restaurant waste, vegetable waste, biogas digestate and chicken feed) also remains unchanged, with

the lauric acid as the main component, even though the feed substrates contained low amounts of this acid [18].

Although [121] reported methyl oleate was the main fat component from larvae reared on solid restaurant waste, in most studies it is lauric acid. Ewald et al. [122] reared *H. illucens* larvae on 11 different diets, obtained lauric acid content 2.08 times higher than oleic acid. They found that the manipulation of fatty acid composition by changing the diet was irrelevant [122]. It has also been noted that with the increase in the weight of the larvae, there is an increasing tendency for the accumulation of saturated fatty acids [122].

Table 3. The content of the main component FAME depending on the feed on which *H. illucens* was reared.

Crude Fat Content (%)	Main Acid Residue of FAME	Content of Main Component of FAME (%)	Diet	Development Stage of *H. illucens*	References
33.6 [a]	lauric acid	57.4	chicken feed	prepupae	[18]
21.8 [a]	lauric acid	43.7	biogas digestate	prepupae	
37.1 [a]	lauric acid	60.9	vegetable waste	prepupae	
38.6 [a]	lauric acid	57.6	restaurant waste	prepupae	
35.0–40.0	lauric acid	65.7	exo-microbial fermented coconut endosperm waste	larvae	[115]
23.3	lauric acid	35.6	dairy manure	larvae	[117]
-	lauric acid	38.4	wheat grain	larvae	[119]
-	lauric acid	58.3	sewage sludge	larvae	[120]
-	lauric acid	76.1	fruit waste	larvae	
-	lauric acid	48.1	palm decanters	larvae	
39.2	oleinic acid	27.1	solid residual fraction of restaurant waste	larvae	[121]
57.8	lauric acid	51.8	bread	larvae	[122]
46.7	lauric acid	28.6	fish	larvae	
40.7	lauric acid	39.9	food waste	larvae	
33.1	lauric acid	52.1	fresh mussels	larvae	
11.2	lauric acid	13.4	ensiled mussels	larvae	
29.7	lauric acid	32.3	rotten mussels	larvae	
20.4	lauric acid	47.4	bread and mussels 10%	larvae	
19.6	lauric acid	47.6	bread and mussels 20%	larvae	
17.9	lauric acid	43.6	bread and mussels 30%	larvae	
17.9	lauric acid	42.0	bread and mussels 40%	larvae	
16.1	lauric acid	35.3	bread and mussels 50%	larvae	

Table 3. *Cont.*

Crude Fat Content (%)	Main Acid Residue of FAME	Content of Main Component of FAME (%)	Diet	Development Stage of *H. illucens*	References
35.7–39.6	lauric acid	27.8	restaurant solid waste and exo-microbial fermented rice straw	larvae	[123]
-	lauric acid	44.9	food wastes from cafeteria	prepupae	[124]
31.2	lauric acid	87.46	chicken manure mixed with rapeseed straw	larvae	[125]

[a] calculated based on data provided in the publication.

Research indicates that the method of killing the larvae is an important step when obtaining fat for the purposes of biodiesel production. It affects the quality of fat in terms of total moisture, lipid content, oxidation state, pH and the presence of microbial populations [126]. Larouche et al. [126] found that the most lipid-efficient method is asphyxiation by vacuum packing and holding in an atmosphere of 100% CO_2 or N_2.

The purpose of lipid extraction is also important. Killing the prepupae by freezing has been shown to activate the action of lipases, thus contributing to the release of fatty acids. Blanching of the prepupae resulted in a more stable lipid fraction [127]. However, ethical considerations should be kept in mind and the method used should not increase the suffering of the insects unnecessarily.

The content and quality of lipids is extremely important for efficient biodiesel production. The number of lipids extracted from *H. illucens* larvae can be increased by the addition of microorganisms and larval feed fermentation. As demonstrated by [115], inoculation of coconut endosperm waste with 0.5% DM of the commercial septic system purification powder, RID-X (Reckitt Benckiser, Parsippany, NJ, USA) containing natural bacteria and enzymes as a fermentation initiator, increased the total amount of lipids extracted from the larvae by 35–40% [115]. This was probably associated with a higher availability of nutrients, which also saved the metabolic energy of insects when digesting the feed. Another example has been reported by [125], who showed that the *H. illucens* larvae had the highest extractable lipid content (approximately 31.8% DM) when the insects were fed with chicken manure mixed with solid digestate from the methane fermentation of rapeseed straw at a ratio of 1:3.

Enzymatic treatment of the feed can be another way to improve quality of the fat. Lim et al. [128] reared *H. illucens* on pretreated palm decanter cake waste with cellulase. The highest SFA content in the fat was 82.14% under treatment with 1% cellulase for 48 h. A high SFA content characterizes good quality biodiesels, affecting oxidative stability and kinematic viscosity.

The solvent used in extraction also had a huge impact of the efficiency of the process. Ravi et al. [129] conducted the screening of different solvents used in isolation of the lipid components. Screening included n-hexane, alcohol solvents (ethanol, iso-propanol), esters (DMC, methyl acetate, ethyl acetate, ethyl lactate), ethers (2-MeO, CPME) and terpenes (α-pinene, D-limonene, p-cymene). The most efficient solvent was 2-methyloxolate (2-MeO) with a total oil yield of 35.83%. For n-hexane, a standard solvent in lipids extraction, the oil yield was 32.51% [129].

To increase the lipid extraction efficiency (up to 75.9%), it was suggested by [130] that the biomass of *H. illucens* larvae should be pre-disrupted by high-speed homogenization, followed by biphasic extraction by means of isopropanol and petroleum ether at a volume ratio of 3:5. Among the solvents tested, such as n-hexane, petroleum ether, ethanol and isopropanol and their various mixtures, the binary solvents were found to be almost twice as efficient in lipid extraction as the monophasic solvents. For example, the binary solvent consisted of isopropanol and petroleum ether in a 1:1 ratio resulted in a lipid extraction efficiency of approximately 74.2%. The use of these solvents separately resulted in efficiencies of 34.7 and 51.4% for isopropanol and petroleum ether, respectively [130].

Transesterification is a key step in the production of biodiesel, commonly carried out with a catalyst. For instance, [117] conducted a two-step lipid conversion process to produce biodiesel from *H. illucens* larvae fat, consisting of H_2SO_4-catalysed pretreatment and NaOH-catalyzed transesterification. The final yield was 15.8 g biodiesel from 70.8 g dry *H. illucens* larvae, which was 22.32% [117]. Optimizing the conditions affecting the transesterification process in overall biodiesel production can be found in a review by [131].

Transesterification can also be conducted with the use of free or immobilized lipases. The use of free lipases is more economical, however, it may give rise to various by-products [132]. He et al. [132] proposed a method to produce biodiesel from *H. illucens*, using a Lipase Eversa Transform 2.0 with Lipase SGM1. After optimizing the transesterification conditions, they obtained results showed that the best ratio of *H. illucens* larvae lipids to methanol was 1:3, and the reaction temperature should be 25 °C. They obtained a FAME yield of 98.45%, an acid value of the biodiesel of 0.10 mg $KOH \cdot g^{-1}$ and a viscosity level of 4.57 $mm^2 \cdot s^{-1}$ [132].

A more recent study produces biodiesel through non-catalytic transesterification. The process was carried out in a bulkhead reactor filled with silica, methanol, and *H. illucens* larvae lipids extract for the first run, and dried *H. illucens* larvae powder instead of lipids extract on the second run, named as the direct non-catalytic process of the reactor was heated to 390 °C for 1 min and then cooled. The biodiesel yield from the *H. illucens* larvae lipids extract and from the dried and powdered *H. illucens* larvae was 34.0% and 34.7%, respectively, similar to the common base-catalyzed reaction (33.9% yield) [133].

Regarding the practical use of *H. illucens* for the purpose of running diesel engines, two strategies can be utilized, the use of crude insect oil or the use of its methyl esters. Kamarulzaman et al. [134] conducted practical tests of using crude oil from *H. illucens* larvae and their admixtures with petroleum-derived diesel fuel in a direct injection engine and investigated the combustion, performance, and emissions characteristics. It was found that the kinematic viscosity of *H. illucens* larvae oil was 8.4 times higher than standard diesel fuel. Its density was also higher (1.1-times) but its volatility was lower. The flash point of the oil was very high (>399 °C vs. 60 °C for standard diesel fuel). Those characteristics are very important as they influenced the atomization and fuel-air mixing rate. Due to its non-optimal properties, *H. illucens* larvae oil exhibited a poorer performance as diesel fuel (e.g., the peak cylinder pressure and heat release rate was lower by 3.28% and 13.38%, respectively). As the amount of *H. illucens* larvae oil increased, the amount of CO, CO_2 and hydrocarbons produced also increased (by 17.5%, 13.6% and 474.3%, respectively). However, a reduction of NO_x and O_2 concentration was observed (by 19.6% and 1.8%, respectively) [134].

Rehman et al. [135] studied two doses (10 and 20%) of *H. illucens* larvae biodiesel as an additive to standard diesel fuel. Biodiesel from *H. illucens* larvae has more similar characteristics to diesel fuel than crude *H. illucens* larvae oil: its kinematic viscosity was only 1.3-times higher than petroleum diesel fuel, and its density was only 1.05-times higher. The researchers found that the heat release rate increased with higher *H. illucens* larvae biodiesel addition. NO_x emissions increased with the addition of *H. illucens* larvae biodiesel, as well as general fuel consumption in comparison to standard fuel. When *H. illucens* biodiesel was added in amount of 20%, smoke intensity and fuel consumption were reduced in comparison to a 10% addition [135]. Current environmental conditions and the associated

climate crisis demands for potential, alternative, and environmentally safer energy and fuel sources, thus insect oil is definitely worth future investigation.

2.6. Biogas Production

The development of the industry dealing with the breeding of insects for fodder and nutritional purposes, which has been studied in recent years, will be conducive to increasing the amount of waste generated during the breeding process [136]. Direct types of post-production waste, consisting of feed leftovers and insect excrements can easily be used as plant fertilizer [137]. Recently, the use of the generation of energy in the form of biogas through anaerobic digestion has also been explored [136,138].

Lalander et al. [138] conducted a study on producing biomethane from *H. illucens* larvae post-breeding residues in relation to food waste and human feces. Mesophilic fermentation lasted 16–22 days at 37 °C. The ratio of substrate to inoculum was 1:3 based on VS, i.e., the content of organic mass. The results of biomethane potential (BMP) are expressed as mL CH_4 g^{-1} VS in Table 4. There was a decrease in BMP on the substrates after *H. illucens* larvae culture. Larval treatment for the fecal variant reduced the result by 1.89 times and 1.25 times for food waste. These results may have been due to the low vs. content and C:N ratios in the larvae-treated materials, which was the effect of larvae feeding and assimilating the nutrients from the substrate [138].

Bulak et al. [136] performed gasification of the post-breeding residue of *H. illucens* larvae, reared on carrot-beet marc (3:1 *v:v*) with an inoculum to substrate ratio of 2:1 (VS-based) at 37 °C for 21 days. The BMP values, as well as the CH_4 concentration they obtained, were similar to the values obtained by [138] in relation to *H. illucens* larvae treated human feces (Table 4). These results approximated the BMPs of fruit and vegetable waste (153–342 mL CH_4 g^{-1} VS) and poultry manure (107–342 mL CH_4 g^{-1} VS) [139]. Compared to the above results obtained by [138,140], who also reared *H. illucens* larvae on food waste (fruit and vegetable peelings and seeds and food leftovers), obtained BMPs 1.56 times higher (Table 4). The post-breeding waste obtained by [140] had a lower TS content with a higher VS. (% of TS).

Another possibility of biogas generation is the fermentation of whole insects or their parts, e.g., a cuticule which is left after lipid extraction from the larvae [140]. The experimental variants investigated by [140] involved the use of: unchopped larvae reared on organic waste; larvae reared on chicken feed (CF) and food waste (FW) chopped, ground and sieved to achieve a particle size distribution between 90 and 250 µm; extracted *H. illucens* larvae lipids, the naturally dead flies, dried and ground larval cuticule that remained after manual extrusion of inactivated larvae, larval feeding residues. The highest results of more than 600 mL CH_4 g^{-1} VS. were obtained for larvae fed both chicken feed and food waste, which were cut and grounded (Table 4). Interestingly, the average BMP from *H. illucens* larvae was higher than that obtained from more traditional methane fermentation substrates such as energy crops. For example, the BMP from ryegrass was 140–360 mL CH_4 g^{-1} VS and from switchgrass, 122–246 mL CH_4 g^{-1} VS [139]. This observation was also supported by [141], who studied BMPs from larvae fed on plant waste (Table 4).

2.7. Entomoremediation

The idea of entomoremediation first appeared in the literature in 2013, with the theoretical publication of [142], who considered the application of collembolans, ants, beetles (e.g., dung beetles) and termites for the cleaning of degraded soil. The prefix (gr.) "entomon" means an insect, and (lat.) "remediation" is the process of cleaning or restoring the lost properties of the environment. Entomoremediation was defined as the use of specialized insects and their associated microorganisms to utilize, extract, sequester and/or detoxify pollutants from the soil, sediments, and organic biomass [143].

Table 4. Biomethane yield obtained from the fermentation of different raw substrates obtained from *H. illucens* or its wastes.

	Feedstock	Cumulative Biomethane Potential (mL CH_4 g^{-1} VS)	CH_4 Concentration (% vol.)	Reference
Whole insect or its parts	*H. illucens* larvae-food waste	675 ± 118	n.d	[140]
	H. illucens larvae-chicken feed	661 ± 29	n.d.	
	dead flies	570 ± 51	n.d.	
	lipid extracted *H. illucens* larvae-food waste	363 ± 32	n.d.	
	larval cuticule	343 ± 7	n.d.	
	lipid extracted *H. illucens* larvae-chicken feed	306 ± 23	n.d.	
	whole *H. illucens* larvae	108 ± 65	n.d.	
	whole *H. illucens* larvae	455.87 ± n.d.	64.27	[141]
H. illucens frass	*H. illucens* larvae post-breeding waste	207.9 ± 21.5	53.2 ± 3.2	[136]
	H. illucens larvae-treated human faeces	178.9 ± 7.1	55.2 ± 0.7	[138]
	H. illucens larvae-treated food waste	322.6 ± 6.4	61.4 ± 0.4	
	H. illucens residues	502 ± 9	n.d.	[140]

n.d.—no data.

Entomoremediation is of course not exclusively connected with the *H. illucens* larvae but, as this research field is very new, most of the research directly regarding this topic was carried out on *H. illucens*. More time and research are still needed in order for this word to be accepted in the general consciousness of researchers, therefore many publications that deal with this topic do not use the term "entomoremediation" but rather more general words relating to the bioremediation *sensu lato*.

In the case of the entomoremediation of inorganic pollutants (like toxic heavy metals), which can be referred to more specifically as entomoextraction, the use of a specific insect for this purpose must be associated with its ability to bioaccumulate these pollutants, in order to ensure its transfer from the environment to the insect body. In the case of *H. illucens*, older studies have focused on the phenomenon of bioaccumulation of heavy metals in different developmental stages, in the context of insect physiology and toxic effects [144–147]. Other studies, conducted in the context of the nutritional properties of *H. illucens*, have concentrated on the content of elements, primarily in two developmental stages, which could be used for the production of insect fat and proteins for feed purposes: larvae and (pre-)pupae [18,148,149]. Regardless of the context, all of these studies provide an insight into the bioaccumulation potential of *H. illucens*.

These research studies showed that metals, in particular, Cd, Zn and Pb were bioaccumulated from optimal ("model") feed, which was chicken feed or wheat bran spiked exogenously with metals [144], even when the concentrations of those elements were lower than permissible limits [145]. Gao et al. [146] found that contrary to Cd, Cr did not accumulate into any of developmental stages of *H. illucens*. The concentrations of metals, in general, decreased with the successive development stages of the insect, which was consistent both with earlier and later studies [143,144]. Spranghers et al. [18] investigated the mineral composition of *H. illucens* prepupae on different substrates, which were chicken feed, digestate from the methane fermentation of vegetable waste, vegetable waste and restaurant waste. These studies showed a bioaccumulation of Ca, Fe, Mg, Mn, P, and Zn, however, overall concentrations in the *H. illucens* were rather low.

Currently, in laboratory experiments, a more precise measurement of bioaccumulation (BAI—bioaccumulation index, [150]) has been proposed. It should also be remembered that bioaccumulation is dependent upon the type of substrate and the initial concentration of the elements [143]. Heavy metals were mainly bioaccumulated in the larvae and pupae,

as well as in puparia, which are empty pupae shells. Empty puparia constitute very interesting materials in the context of entomoremediation, as they can contain a relatively high concentration of heavy metals, are dry and light, and can be easily obtained during *H. illucens* growth [143]. As pointed out by [146] if the concentrations of a given heavy metal is sufficiently high, puparia facilitate attempts to recover these metals.

Large quantities of heavy metal-polluted plant biomass are generated during the process of phytoextraction, i.e., the use of plants (specific hyperaccumulator plant species or fast growing species, like corn) to extract metallic pollution from the soil and sequester it in its shoots, which can then be harvested [151]. One of the major drawbacks of this method is that such polluted biomass can pose a threat and must be stored in a hazardous waste landfill (hazardous effluents) or neutralized with the use of technologies that require equipment and energy expenditure [152]. The use of saprophagous insects with bioaccumulation abilities provides a new approach for the utilization of this type of biowaste. Bulak et al. [143] proposed the use of *H. illucens* for the entomoremediation of corn leaves, polluted with Cd and Zn. During the 36 days of the experiment, DM utilization of contaminated corn leaves was 44.4% for Cd and 50.0% for the Zn variant, which was better than the standard composting of contaminated plant biomass in terms of both biomass utilization and time. A high Cd concentration has been noted in the larvae and in the puparia, while the Zn in the larvae and the imagoes of *H. illucens* indicated differences between essential and toxic element physiology [143].

When *H. illucens* larvae were fed solely on solid aquaculture, their waste contained heavy metals and trace elements at levels unacceptable for use as a feed material; the larvae showed bioaccumulation of Cd (BAF 2.5–2.7), Hg (BAF 1.6–1.9), Mn (BAF 1.3), as well as the first reported bioaccumulation of Ag (BAF 0.9–1.3) [153].

A study of the bioconversion of heavy metal-rich biosolids by *H. illucens* larvae did not report a high bioaccumulation of elements in their bodies [154]. In fact, the survival ratio was around 83%, but this was related to the low number of nutrients in the substrate. A BAF value in *H. illucens* larvae-fed biosolids that was greater than 1, was recorded for Ca (approx. 5.3), Mn (approx. 1.7), Cd (approx. 1.8) and K (approx. 3.8). A reduction in waste mass can be regarded as a positive effect [154].

Proc et al. [149] investigated the potential of *H. illucens* to bioaccumulate different elements from the optimal feed (not artificial contaminated). It was found that Ca, Cd, Ga, Mn, P and S were only bioaccumulated in certain developmental stages of *H. illucens*. Conversely, elements like Ba, Bi, Cu, Fe, Hg, Mg, Mo, Se, and Zn bioaccumulated in all stages and puparia. The study also revealed that Al, As, Co, K, Pb, and Si did not bioaccumulate at all [149].

H. illucens is known as an insect which is rather resistant to toxic heavy metals in its feed. The addition of Cu and Zn did not result in differences in larval length and the average fresh weight (FW) of the individual larvae remained within a range reported in the literature (34–315 mg FW) [143,145,147]. However, Cd can cause an increase of the DM of individual larvae as shown by [143,144,147]. Some negative effects were evident in relation to the growth performance of *H. illucens*—the addition of Cd and Pb caused a slight increase in the development time of *H. illucens* larvae as reported by [144,145]. Bulak et al. [143] demonstrated a four-time increase in the mortality of the larvae when fed on Cd or Zn polluted corn leaves.

An important aspect regarding the bioaccumulation and bioavailability of heavy metals is the larval density factor. Jiang et al. [155] optimized the inoculation density of larvae fed on swine manure, using 0.08%, 0.24% and 0.40% larvae addition in FW. It was noted that the larvae from the 0.40% variant accumulated the highest amount of metals. A particularly high result was obtained for Cd, which had a bioaccumulation factor of 23.5, an increase of 6.6 times compared with the 0.08% variant [155].

Recently, much attention has been paid to the ability of *H. illucens* to degrade organic pollutants, which can be termed entomodegradation. Regarding *H. illucens*, such

studies were conducted for contaminants like mycotoxins [156,157], hydrocarbons [158], insecticides [159] and antibiotics [160].

No mycotoxins were detected in *H. illucens* larvae reared on feed that was corn contaminated with deoxynivalenol (DON), fumonisin 1 and 2 (FB1 and FB2) and zearalenone (ZEN)[156]. This suggests the possibility of entomodegradation of plant biomass contaminated within these dangerous toxins [156]. Another study revealed that *H. illucens* larvae are capable of feeding on aflatoxin B1 contaminated biomass. Meijer et al. [157] proved that cytochrome P450 and cytoplasmic reductase were involved in the detoxification of aflatoxin B1 to aflatoxicol and aflatoxin P1, with an efficiency rate of around 60%.

H. illucens larvae can also survive on feed with added polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, flourene, phenanthrene, and pyrene [158]. Moreover, it was reported that changes in the concentrations of these PAHs showed no significant differences in *H. illucens* larvae survival, harvest, conversion and eclosion rates. The degree of PAH removal by *H. illucens* larvae depended on the concentration of PAH in the substrate (1, 10, 100 $mg \cdot kg^{-1}$). During the experiment, which lasts 18–21 days, the removal rate for naphthalene was 34.1–84.2%, for fluorene 63.9–83.1%, for phananthrene 48.0–62.9% and for pyrene 52.2–74.6% [158].

A study was conducted to check the survival parameters of *H. illucens* insects, as well as their bioaccumulation ability on food containing insecticides. Meijer et al. [159] used insecticides such as chlorpyrifos, propoxur, cypermethrin, imidacloprid, spinosad, tebufenozide, cypermethrin with and without piperone butoxide (PBO) and administered them in the feed as initial doses, corresponding to the European Union maximum residue level (MRL), to evaluate the mortality and growth of *H. illucens* larvae [159]. The insecticide concentrations were then adjusted for further testing, using higher or lower doses, based on the preliminary results of whether the product was accumulated by the larvae. The final dosages were: 0.5 $mg \cdot kg^{-1}$ chlorpyrifos, 0.5 $mg \cdot kg^{-1}$ propoxur, 1 $mg \cdot kg^{-1}$ imidacloprid, 0.2 $mg \cdot kg^{-1}$ spinosad, 0.5 $mg \cdot kg^{-1}$ tebufenozide, 0.1 $mg \cdot kg^{-1}$ cypermethrin, 2 $mg \cdot kg^{-1}$ PBO, and 0.1 $mg \cdot kg^{-1}$ + 2 $mg \cdot kg^{-1}$ cypermethrin + PBO. The survival rate of the *H. illucens* larvae compared to the control was not significantly different, except for the trials with spinosad, cypermethrin, and cypermethrin mixed with PBO. It was observed that the larvae treated with an imidacloprid diet increased their biomass relative to the control. The insecticides concentrations in *H. illucens* larvae were at such a low level, that bioaccumulation was considered not to occur [159]. The lack of insecticide accumulation on the part of the larvae is beneficial if it is to be applied as feed, as it would be free from chemical contaminants that might be present in the diet substrate given to *H. illucens* during rearing.

Antibiotics are another xenochemical studied. Liu et al. [160] used three doses of oxytetracycline: 100, 1000, and 2000 $mg \cdot kg^{-1}$ on a DM basis, and after 8 days of the experiment, its degradation efficiency after *H. illucens* larvae treatment was 82.7%, 77.6% and 19.3%, respectively. As in the previously described work, there was no significant effect on larval survival, which indicates that *H. illucens* larvae are very resistant [160].

Another example of an antibiotic biodegradable by *H. illucens* larvae is lincomycin [161]. Studies were carried out to check the degree of reduction of lincomycin fermentation residues (LFR) + wheat bran (1:1), as well as the biodegradation of lincomycin by *H. illucens* larvae (wheat bran + lincomycin hydrochloride solution (1000, 1500 and 2000 $mg \cdot kg^{-1}$ DM)). The total mass of LFR decreased by 67% DW after 12 days, while the degree of lincomycin degradation was recorded at approximately 84.9%. The presence of this antibiotic in the body of *H. illucens* larvae was also not detected [161].

2.8. Insect Frass

To ensure high soil productivity a continuous supply of mainly N, P and K have to be maintained in the form of fertilizers. In the EU, natural fertilizers in the form of cattle manure are more and more difficult to access, due to the reduction of the number of these animals and high legal requirements related to the maintenance of herds, which

particularly affects individual farmers. Increasing adverse climatic changes are likely to force a reduction or a shift away from beef or swine consumption, due to the emissions of greenhouse gases during breeding, which will further reduce herds [162]. It is at this stage that insects, such as *H. illucens*, come to the fore, as they consume their feed, which can often be waste biomass, assimilate essential elements and return it to the environment in the form of insect excrements, which may provide a new type of natural fertilizer [163].

Insect frass, as defined in European Commission Regulation 2021/1925 [164], is "a mixture of excrements derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects of not more than 5% in volume and not more than 3% in weight".

According to the Regulation, insect frass can be introduced to the market after heating it up to 70 °C for one hour, which correlates with the requirements also in place for processed manure. Such products can already be found on the market, although research into how insect frass works on plants and the environment is still ongoing. An example of a commercial fertilizer already being used is HexaFrass™ (HexaFly, County Meath, Ireland), which is produced after rearing *H. illucens* larvae mainly on brewery waste [165]. The averaged content of the main nutritional elements for plants in different *H. illucens* larvae frasses was around 3.39% DM for N, 2.85% DM for P_2O_5 and 3.47% DM for K_2O [166].

Recent studies considered the effect of the aforementioned heat treatment (70 °C for 60 min) on the microbial abundance of *H. illucens* frass [167]. There was a negligible decrease in the total abundance of viable microorganisms and bacterial endospores, however, a decrease in *Enterobacteriaceae* below the detection level was registered. In the case of frass, inoculated with foodborne bacteria like *Salmonella* spp. and *Clostridium perfringens* (5.0 log CFU·g^{-1}), heating caused its complete reduction [167]. The study proved the validity of the frass procedure of sanitization, as the pathogenic bacteria for the animals were killed.

A very new and interesting research area investigates how frass influences phytopatogenic microorganisms. Gebremikael et al. [168] showed that the addition of three different *H. illucens* frasses (without thermal treatment) reduces the number of pathogenic *Rhizoctonia solani* in the soil. They highlighted that the addition of frass from general food waste and from vegetable waste in tests on beans (*Phaseolus vulgaris*) infected with this pathogen, resulted in a decrease in disease rates by approximately 50%. The researchers speculate that the reduction of *R. solani* may have been caused by chitinase activity [168].

In contrast, [93] found the presence of bacteria from the family *Xanthomonadaceae*, in which there are some plant pathogens, when investigating *H. illucens* frass from organic household waste. Consequently, there is a potential threat to plants. More studies are needed to check whether the compliance with the recommendations contained in the Commission Regulation (EU) 2021/1925 [164] can eliminate this risk of phytopathogens, spread by the use of *H. illucens* frass.

Another recent study also considered the antifungal properties of frass extracts from *H. illucens* culture on two types of substrates: fruit/vegetable/bakery/brewery waste (FVBB) and a standard Gainesville (GV) diet. The effect of frass extract filtration (nylon filter, 0.45 µm) was also investigated. The unfiltered GV extract showed high inhibition for all tested mycelium—*Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum*, *Pythium capsici*, *R. solani*, *Sclerotinia sclerotiorum*. In contrast, the unfiltered FVBB extract inhibited solely the growth of *B. cinerea*, *S. sclerotiorum* and, to a lesser extent, *A. solani*. Filtered extracts from both frasses did not show any changes in mycelial growth [169].

It is important to note that the composition and quality of frass is shaped by the food the larvae were fed [166]. Klammsteiner et al. [170] showed that the frass C:N ratio was higher when grown on fruits and vegetables (26.6) than when grown on grass (18.2) or chicken feed (18.5). When comparing the NH_4NO_3 frass with the *H. illucens* with nitrogen-equivalent amounts, a comparable increase in the growth of perennial ryegrass was noted (*Lolium perenne*). This frass also had a high total microbial load (up to 10^9 CFU g^{-1}) [170]. Interestingly, except for the Gram-negative bacteria, the frass from the chicken feed was also abundant with *E. coli* and coliforms, while in other types of frass, only Coliforms

bacteria and Gram-negative bacteria were present. After the addition of those frasses to the soil the CFUs decreased and only Gram-negative and Coliforms bacteria were detected, with a much lower load of 10^3–10^5 CFU g^{-1}, indicating that frass fertilizers did not affect the soil in a negative way in terms of hygiene [170].

Fischer and Romano [171] tested the frass obtained from *H. illucens* larvae reared on four substrates. These were fruit mixture, vegetable mixture, starch mixture and a as a last variant, a mixture of all three substrates in equal parts. The experiments lasted two weeks in a darkened room (34 °C, 70–80% moisture content). The highest NPK result was obtained for vegetable frass, which was 4.76:0.83:5.34. This was 1.58, 2.31 and 2.36 times higher than the ratio in the substrate before the experiment [171].

The *H. illucens* larvae treatment of different types of manure was found to improve the quality and stabilize the resulting product by improving the humification processes [11]. A nine-day experiment on chicken manure (CM), pig manure (PM), and cow manure (COM) resulted in a decrease in the initial protein content and an increase in the humic matter. The results showed that the degree of humification in the product after *H. illucens* larvae treatment were 42.45% for PM, 57.07% for CM and 63.74% for COM, and were higher than the control (manures without the addition of larvae) by 1.02, 1.34, and 1.27 times, respectively [11].

H. illucens frass can also be produced from the organic municipal waste from domestic, market and restaurant waste, as investigated by [172]. They noted an increase in the N, P, and K content in the frass of 41.2%, 32.4%, and 77.1%, respectively, as compared to the initial waste. The authors also investigated the bioaccumulation of various heavy metals. They found very high removal efficiencies for As (92–98%), Cd (99.4–99.9%) and Pb (80–90%) due to the bioaccumulation in the insect, however the initial concentration of these elements was below 1 mg·kg^{-1}. With the higher initial content of Fe in the range of 3.4–5.6 mg·kg^{-1}, the removal efficiency was lower (31.1–69.1%) than for toxic heavy metals. It is important to note that *H. illucens* larvae have the ability to bioaccumulate (see Section 2.7.), therefore, care should be taken when feeding the larvae with food that may contain increased levels of toxic elements, which may end up in the frass.

Research has also been carried out on the quality and effectiveness of various substrate additives or treatments that would positively affect the composition and properties of frass. One of the examples can be the inoculation of a substrate with microbial preparation. It has been found that for more efficient substrate conversion and to obtain nutrient-rich frass, the addition of *B. subtilis*, isolated from the guts of the *H. illucens* larvae may be used [173]. In the experiment, the latter used fresh chicken manure as the food substrate for the larvae and inoculated it with *B. subtilis* inoculum (10^9 CFU·mL^{-1}). The process of *H. illucens* larvae conversion lasted 13 days, followed by 11 days of aerobic fermentation. The final product was stable and characterized by a higher maturity, as a result of the enzymatic activity change during aerobic fermentation stage. The activities of catalase and polyphenoloxidase in *H. illucens* frass with bacterial inoculum continued to increase until the middle of the fermentation period, and their values were higher than in the control (*H. illucens* frass without inoculation). The peak value of urease activities did not differ significantly between the samples, while invertase activity was higher in the control. After 12 days of fermentation, each enzyme activity was undetectable or maintained at a very low level of activity, which indicated that the microbes were in a steady state. The high germination rate of Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*) and rapeseed (*Brassica napus* subsp. *napus*) also proved the maturity of the frass, after the aerobic fermentation stage in terms of no phytotoxicity [173].

The effect of the addition of gypsum (calcium sulphate) and biochar (made from rice husk at 350 °C) to a larval substrate consisting of brewery spent grain on the composting efficiency of *H. illucens* larvae and the quality of the resulting frass has been investigated by [174]. It was shown that the addition of gypsum at 10% DM resulted in the highest increase in N content of the frass, however, with the increasing dose of the additive, larval fresh mass and DM decreased. In the case of biochar, the addition of 20% DM gave the

highest increase in N content of 21% in the frass and resulted in an increase in larval DM yield by 86%. In both cases, no significant differences were observed in the germination rate of cabbage seeds (*Brassica* sp.), which was around 90% [174]. Studies have also shown that the use of *H. illucens* larvae with the addition of biochar reduces the composting time from 8–24 weeks for conventional composting, to only 5 weeks [174].

In other research, Beesigamukama et al. [175] used sawdust from the blue gum tree (*Eucalyptus globulus*) to regulate the C:N ratio in the feed. Brewery spent grains with a C:N ratio of 15 contributed to an increased biomass conversion by *H. illucens* larvae, while at the same time increasing N (by 21%) and P (by 15%) retention in the compost. All frasses obtained in this research (with different C:N ratios) were not phytotoxic, as they had a seed germination percentage higher than 80%. Frasses with C:N 25 and 30 did not differ in comparison to the control in terms of this parameter (93.3% vs. 100% of germination in the control) [175].

The effect of *H. illucens* frass amendment on soils, e.g., nitrogen supply and its availability, was also investigated. Nitrogen release in soils fertilized with organic *H. illucens* fertilizer was slow, however, mineral nitrogen was increased, which may give a positive result of application [176]. When the *H. illucens* frass from brewery spent grains was used in a dose of 1.4 t ha^{-1}, which equals 30 kg N ha^{-1} for corn (*Zea mays* variety H513) fertilization, this was sufficient for adequate N supplementation. The results were not statistically different in comparison to higher dosages of frass. Moreover, the yield of maize grains did not differ statistically in comparison to commercial organic fertilizer (SAFI Organics, Kenya) and was 4.96 t·ha^{-1} for *H. illucens* frass and 4.49 t·ha^{-1} for SAFI [176].

The addition of *H. illucens* frass from chicken manure to the soil, at a level of 4% (*w*:*w*) increased DM content in the above-ground parts of the rice (*Oryza sativa* variety E28) by 40.2%, increased the yield by 49.6% and the height and chlorophyll value by 11.9%, compared to the control with no fertilization. However, negative effects, such as a decrease in DM of the plants or a slowdown in their growth were noted with the *H. illucens* frass dose of 8% [177]. Such results could be due to, e.g., over fertilization of the plant or phytotoxicity of the fertilizer, however, the authors did not explain this result.

Gebremikael et al. [168] conducted research on the effects of the addition of three types of *H. illucens* frass from food waste, vegetable waste and chicken feed waste on soil. *H. illucens* frass from vegetables was used at a dose of 9.0 t FW·ha^{-1} and frass from chicken feed at a dose of 7.3 t FW·ha^{-1}. These additions introduced 8% and 16% of total N into the soil, respectively, over a period of four months. In the case of *H. illucens* frass from food waste, an initial 91-day immobilization of N was noted. After that period, the presence of N increased to approximately 14%. However, these doses may be insufficient for most crops and may not bring the desired effects as a stand-alone fertilizer [168]. The researchers also noticed that the initial activity of dehydrogenase in soil with *H. illucens* food waste frass was 10 times higher than the control and decreased to 0 in 140 days. Enzymes in this group contribute to the oxidation of the organic fraction of the soil, which significantly affects soil fertilization and enhances soil quality.

Frass has also been tested as a direct cultivation substrate and as an additive for commercial peat in the cultivation of potted plants. Plants grown on a mixture of cultivation substrate and *H. illucens* larvae residue performed best with a ratio of commercial peat (Fondolinfa Universale, Linfa Spa, RE, Italy) 80% + *H. illucens* frass 20%; basil (*Ocimum basilicum* L., cv. ISI 602), tomato (*Solanum lycopersicum* L., cv. Roma V.F.) and lettuce (*Lactuca sativa* L., cv. Chiara) had higher total plant DM values by 1.19, 1.17 and 1.29 times, respectively, compared to the controls on commercial peat. A larger leaf surface and a greater number of leaves were also noted in the absence of any symptoms of abiotic stress [178].

A rather different approach to the use of frass was recently presented by [179], who investigated the fertilizing potential of a so-called compost tea, made from *H. illucens* frass. *H. illucens* larvae were bred on a mixture of coffee waste, dough, fish feeds, and fruit and vegetable waste. The frass was then mixed with water in a dose of 2.25 g·L^{-1} and used as

an additive in an aquaponic culture of chili banana peppers and sweet potato. The additive increased the total sugar in the chili plant, while in the sweet potato, the Mn level was elevated in the leaves. The authors stated that *H. illucens* frass tea enhanced the nutritional quality of the plants overall [179].

2.9. H. illucens Larvae as a Food

Hunger is one of the world's major problems. According to WHO [180] data from 2018, one in nine people are experiencing hunger. Additionally, the human population is also forecast to grow, which equates to an increasing demand for food. This will require finding an alternative, yet cheap source of protein. One of the solutions might be the consumption of insects, which is well known throughout the world. However, in developed countries, in particular, there is a stigma surrounding the consumption of insects, especially in their intact, raw forms. One of the candidate insects for human food, although less obvious, is *H. illucens*. This insect can quickly build larval biomass, rich in proteins and fats. Until now, this insect has been used for animal feed, however, research is being conducted to obtain safe food for humans.

Although *H. illucens* has not yet been approved for food in the European Union, studies have been carried out on its nutritional profile, which provide useful information on the possibility of use for this purpose. Mshayisa et al. [181] tested the nutritional properties and features of two types of *H. illucens* larvae flour: freeze-dried (BSFL-FD) and defatted (BSFL-DF), and two protein concentrate extraction methods: alkaline-acid (BSFL-PC1) (1 M NaOH, 0.5 h, 25 °C; 1 M HCl, overnight, 4 °C) and alkaline (BSFL-PC2) (1 M NaOH, 2 h, 40 °C). The highest protein content was obtained for BSFL-DF flour (50.12%), while in the case of protein concentrate, this was BSFL-PC1 (73.35%). The amino acid composition of these two variants were similar to cow's milk and egg protein. The solubility of the protein at pH 2 was clearly higher for the protein concentrates and was approximately 85–97%. Protein concentrate had the best water-binding capacity, and showed the highest emulsion stability, while both flours had the best oil-binding capacity. The results proved that this protein extraction method yields a product with improved nutritional properties, with technical and functional characteristics [181].

Anankware et al. [182] tested a meal obtained from *H. illucens* and found that it was characterized by a crude fat content of 18.03% DM, which was 2.27 times lower, when compared to beef. Crude protein was 44.82% DM, which was 1.23 times less than beef. The contents of neutral detergent fiber and acid detergent fiber were 39.94% and 15.57% DM, respectively. SFA, mono-unsaturated fatty acids and PUFA content were 61.36%, 26.36% and 9.18%, respectively. Too high a level of SFA affects blood pressure and can lead to cardiovascular disease. Therefore, more research is needed regarding the safe use of *H. illucens* for human consumption [182]. By comparison, the SFA content of beef was determined to be around 36.7–46.3% of total FA [183]. Zozo et al. [184] reported 45.82% protein and 25.78% fat content in *H. illucens* flour. In addition, they found that the process of defatting the flour with the use of hexane:isopropanol mixture (3:2 *v:v*) increased its protein content by 1.2 times, while its fat content decreased by 5.3 times. The defatting process also affected the mineral composition of the flours; it caused an increase in the content of Fe, Mg, Mn, K, and Zn. There was also a 7.5-fold decrease in the Na content of skimmed flour, which can be beneficial for a low Na diet. The content of Mg, Mn, and Zn in the defatted flour was above the recommended daily intake, but this can still be very useful in terms of microelements in fortified foods. Both types of flours showed thermal stability properties [184]. These authors confirm the feasibility of using flour from *H. illucens* larvae in human food production, while suggesting the need for further studies, such as protein digestibility and defatting methods.

Bessa et al. [185] conducted a study focusing on safety in terms of microbiology, heavy metal content and allergens, when using *H. illucens* larvae as a direct-to-human food. They tested three different food substrates on which the larvae were reared: a broiler-based diet, brewers' grain, and cereal grain. Two methods of killing larvae, namely, freezing and

blanching were also tested. The lowest concentrations of *B. cereus* and *E. coli* bacteria were recorded in blanched larvae reared on broiler-based feed; the blanched larvae also had the lowest element content. In the larvae killed by freezing, microbial concentrations were at the same level as in the feed, regardless of the variant. However, the larvae killed by blanching contained a higher content of allergens than when they were frozen. These allergens were tropomyosin and arginine kinase, which are also characteristic of crustaceans. Feeding the larvae did not affect the allergen content, only the heavy metal content [186].

Equally important in terms of sanitation and food safety was the absence of pathogenic viruses, which, to date, have not been found in any of the developmental stages of *H. illucens* [187].

2.10. H. illucens in Cosmetic, Cosmeuticals and Personal Care Products

H. illucens can be a source of components used in the production of cosmetics, as for example, proteins, chitin, chitosan, or fat. However, most of the articles on this topic are theoretical and are in the form of review suggestions, based on the characteristics of the properties of larvae [77,188,189].

In the case of proteins, it is generally known that certain amino acids, such as glycine and arginine, can be used in the formulation of cosmetics, i.e., due to their hydrating and antioxidant properties or their properties in collagen production. The content of these amino acids in *H. illucens* larvae vary with the type of diet on which they were reared, and their values can reach up to around 51 $g \cdot kg^{-1}$ of crude protein and 59.2 $g \cdot kg^{-1}$ of crude protein for arginine and glycine, respectively. In addition, the AMPs produced by *H. illucens*, due to their properties, can be used as an active ingredient in cosmetics for problematic skin [188].

Chitin, due to its antimicrobial, moisturizing and biocompatibility properties, can be used as an active compound in cosmetics. Chitin can be transformed into various forms, including, hydrogels or membranes [77] however, an interesting approach and one that has been popular in recent times is the use of nanotechnology and the production of nanofibers and nanofibrils, which can act more precisely as carriers of active agents [77].

Lipids from *H. illucens* are characterized by a fatty acid profile that is rich in lauric, myristic, palmitic and oleic acids. These FAs are also found in more conventional and cosmetically used fat sources such as coconut oil, palm oil and palm kernel oil [188,189]. It has been suggested that fatty acids from *H. illucens* can replace those from coconut oil or palm kernel oil and be used as an alternative source of these ingredients. Medium chain FAs express antimicrobial properties (see Section 2.4). The most important role here has lauric acid and its metabolite monolaurin (glycerol monolaurate), with confirmed antiviral, antibacterial and even antiprotozoal properties [188]. This compound is present naturally in fats and oils but in very low concentrations [190]. Recently Xu et al. [190] demonstrated its production via enzymatic glycerolysis from the oil of *H. illucens* larvae. It should be noticed that FAs along with monolaurin presents or produced form *H. illucens* can serve also in cosmetic formulations as emulsifiers, stabilizers, and conditioning agents [188,190].

Interestingly, Ushakova et al. [119] detected also low quantities of azelaic acids in *H. illucens* lipids. This is a well-known compound used in skin care cosmetics as it inhibits the reproduction of lipophilic microorganisms and therefore helps counteract infections [189].

Chou et al. [191] proved that nano-emulsion can be made from *H. illucens* oil. Nano-emulsion is a mixture of water, oil and emulsifiers obtained by physical homogenization. They produced nearly spherical nano-emulsion with long-lasting stability [191], which could be a base for cosmetics formulation.

In addition to studies on the FAs profile in *H. illucens*, Almeida et al. [192] conducted preliminary studies on the toxicity of FAs from *H. illucens* and its antioxidant activity, which could be also of interest from cosmetic production point of view. The results showed that the *H. illucens* lipid extract (with a concentration of 0.1 $mg \cdot mL^{-1}$) showed low antioxidant activity. The authors stated that it could be the effect of too much dilution of the extract

and propose further research on this topic. Tests carried out on the organism *Artemia salina* (L.) showed a lack of toxicity of the lipid extracts [192].

Verheyen et al. [193] used extracted and refined fat from *H. illucens* to produce hand cream formulation and compared it with cream produced form mink and plant oils. The high content of lauric acid (>60%) made insect fat it less suitable for skin care product than both other tested oils. Insect fat contained also high amounts of free FAs and phospholipids, which must be removed prior to cosmetic production. Generally, from the physicochemical point of view they stated that *H. illucens* fat was suitable for leave-on cream preparation. Research focused on different refining methods of raw *H. illucens* fat from phospholipids and to improve the color and odor should be done, if one want to use it in real application in cosmetics [188,193]. Deeper studies on the presence of contaminants such as pesticide and solvents as well as more toxicological tests of real cosmetic formulations would be necessary in the future [193].

2.11. Bioplastics

In order to improve the Earth's ecological situation and reduce the production of fossil fuels, new and more biodegradable bioplastics are constantly being researched. Recent studies showed that a type of bioplastic can be made from *H. illucens* proteins. One of the earliest works focusing on bioplastics production, derived from *H. illucens* proteins, was [194]. This study proposes the use of glycerol and citric acid as additives to *H. illucens* larvae protein isolate, which would act as a plasticizer and crosslinking agent, respectively. After optimization, the following ratio of bioplastic components was proposed: 13 g distilled water, 85% (*w:w*) glycerol and 0.5 g of *H. illucens* protein, without any use of citric acid. This dose of protein gave the best result in terms of extensibility value, but it limited the thickness of the material [194].

Nuvoli et al. [195] tested the whole protein fraction with *H. illucens* prepupae and their soluble fraction for biofilm formation. The use of soluble proteins resulted in biofilms that were more stretchable, strong, and transparent. The addition of citric acid as a crosslinking agent resulted in a decrease in tensile stress resistance by almost half; elongation at the break increased by around 1.16 times, however the citric acid reduced water absorption by the *H. illucens* biofilms. In contrast, the addition of CA improved the properties of biofilms from the whole fraction [195].

More recent work involved the practical testing of bioplastic film produced from *H. illucens* as a mulch [196]. The properties of the bioplastic film, e.g., degradability, weight and thickness of the film, water evaporation and soil microbial content (SMC) were tested and compared with commercial biodegradable film from corn starch Mater-Bi (Novamont S.p.A., Novara, Italy) and non-biodegradable polyethylene film [196]. In terms of water evaporation, the bioplastic from *H. illucens* achieved similar results to the commercial Mater-Bi film at around 0.19 g H_2O day^{-1} cm^{-2}. The film with *H. illucens* was the thickest (0.36 mm) and heaviest (0.84 g) of all the variants. Due to its completely organic composition, it exhibited the fastest degradation time and after just 10 days of mulching, the film area decreased by 1.23 times, and thickness and weight decreased by 1.03 and 1.25 times, respectively, while the control films remained intact. Analyses of SMC showed that the *H. illucens* film contributed to the increased growth of *Clostridia* spp. and restricted the growth of aerobic mesophilic bacteria and some growth of fungi. However, such results were also obtained for other films, and the reason may be the limited air flow between the atmosphere and the soil [196].

3. Future Perspectives

H. illucens is an insect that continues to capture the attention of scientists. This chapter will present the potential paths of future research on *H. illucens* in various aspects of application.

The use of *H. illucens* for the production of food and feed in western countries is faced with a major problem—it must be cost effective in comparison to other established

sources, e.g., soy protein or fishmeal, which could prove difficult in the short term. Environmental problems, like overfishing could force legislators to help create laws that will promote new sources of food and feed. This could be the case in the European Union in the years to come. The nutraceutical value of insect sources associated with the content of chitin, fat, and AMPs, could help the product to come onto the market despite its higher price. At least since 2016, the literature has indicated the need to automate the insect production process, which would enable the reduction of product costs, especially in the western economic environment [197]. Currently, research in this matter is carried out e.g., [198] with the use of artificial intelligence for image recognition, and automatic solutions are already being offered commercially, such as the *Hermetia* breeding InsectoCycle (https://www.insectocycle.com) or Entoprot Ltd. (https://www.entoprot.com).

Chitin and its derivative, chitosan, are very important for many industries and this importance continues to grow. To date, chitin has been obtained from marine crustaceans, however, this source may become less relevant over time as the oceans and seas become more polluted and climate change causes unfavorable degradation in the marine environment (e.g., acidification of marine waters). Consequently, the insects, including *H. illucens*, can become an important source of chitin, especially since the world production of insects is growing, and chitinous biomass in the form of insect exuviae is a post-production waste in this process. An important aspect is also the fact that chitin obtained from different sources has different properties and, therefore, can have different applications. The aforementioned factors are a strong argument for the intensification of research on chitin and derivatives obtained from *H. illucens*.

The broad antimicrobial properties of *H. illucens* constitute a very interesting research field. It is even more important in an era in which there is increasing resistance of pathogens to commonly used antibiotics, that studies on the extraction and properties of AMPs can potentially provide new drugs to fight diseases both in medicine and in the veterinary field. Immunization with various bacteria and the use of microbially contaminated feed are the most obvious methods of initiating AMP production in *H. illucens*. The use of the latest technologies, as presented by [113], is particularly impressive, as it facilitates the treatment of entire organisms (not just insects) as "gene libraries", worked out in silico to select the most promising peptides, which can then be synthesized completely abiotically and used in research. An innovative approach that investigated the effect of isolated AMPs on plant pathogens is also very interesting [95] and opens up a completely new field of research, with potentially important future findings. It is certain that not all such substances have already been detected and isolated. Moreover, one may ask whether changing the abiotic conditions of fly farming can affect the production and secretion of AMPs and to what extent this could be possible. The feeding of the larvae causes sanitization of the substrate from certain species of pathogenic organisms, which has been reported in some substrates, like human feces. This is particularly advantageous if such larval feeding residues are to be used later as fertilizer.

There are many reasons as to why alternative fuel (biofuel) research is still very important to economies globally, to name just a few: the zero net carbon balance in the context of climate change and sustainable development, high oil prices, steadily increasing demand for fossil fuels and increasing difficulties in accessing them. The accelerating global trend to switch energy production systems to renewable sources will not make internal combustion engines disappear overnight. The change will take years, and it should be remembered that there are many countries in the world that are not ready for such a transformation for economic reasons. In addition, internal combustion engines, especially diesel engines, may be difficult to replace with other solutions, especially in industrial applications (backup generators) as well as in ultra-heavy transport (container ships, heavy mining machinery). As an alternative fuel source, insect fat meets the same pros and cons as biodiesel produced from plant sources. However, the biggest obstacle for insect biodiesel is the scale of insect production, which is constantly increasing on a global scale, however, primary research into the use of insect fat in the near future will be in the feed, food, and

cosmetics sectors. However, certain important studies into *H. illucens* larvae fat metabolism have been carried out. Zhu et al. [116] conducted transcriptome sequencing to investigate the mechanism of crude fat accumulation and the composition of fatty acid profiles in the different stages of *H. illucens*. Enzymes involved in lipid metabolism with expression being upregulated at the earlier stages (one to four-days old larvae) of *H. illucens* larvae development (lip, LPL, CES1, UGT, GLB1) and acting during the later stages (AKR1B, ELOVL4, and HSD17B4) were identified. This study may help clarify lipid metabolism, but it may also provide a basis for research into optimizing or designing the lipid content of *H. illucens* larvae through genetic engineering methods [116].

Although *H. illucens* waste or insect biomass itself can produce biogas in promising amounts by comparison with substrates applied in biogas plants, some studies have suggested that this type of waste should be cofermented [136]. Due to its C:N ratio and high content of lipids and proteins, the waste from *H. illucens* breeding can be mixed with substrates rich in carbohydrates like corn silage, which should improve biogas production [136]. However, there is a lack of research into this area, as the use of insects for biogas production is still a new and under investigated topic. Another interesting scientific topic will be the investigation of the composition of VFAs and the rate of its release during the methane fermentation. Some research has pointed out that insect waste has a high decomposition rate and therefore, does not require a long hydraulic retention time in the bioreactor [199]. However, there is a risk of excessive acidification connected with the evolution of VFA [136], which should be investigated more in detail. The possibility of the existence of specific compounds in insects or in insect post-production waste, which may affect the methane fermentation efficiency, remains an open question. It has been proven that the addition of extracts from certain plants, e.g., goldenrod to bioreactors resulted in enhanced biomethane production [200]. It is unknown whether such enhancement could be observed in the case of extract from insect wastes. A hypothesis worthy of future testing is that insect waste in low quantities can act as an additive to accelerate the methane fermentation of common livestock. It would be important to test various known methods of intensifying biogas production into insect waste or whole insects, as the publications currently available simply focus on determining the potential of unmodified raw material.

Entomoremediation is a fairly new research area and much more should be done in order to confirm its usefulness for different environmental issues. Certain future research challenges in this field can be predicted based on much better-established phytoremediation, i.e., the use of plants for environmental clean-up. Metallophytes, i.e., plants that prefer habitats with high concentrations of selected elements, have been known for a long time and are used as bioindicators of elevated concentrations in the soil, pinpointing areas which could be of mining importance and indicating the location of metal ores. Among metallophytes, plants capable of the hyperaccumulation of some elements were discovered, even up to extremely high levels [151]. The discovery of hyperaccumulators in 1974 [201] speeds up the progress of phytoremediation. Thus, the first question should consider whether there are any hyperaccumulators among insects. The highest element concentrations were recorded for herbivore insects fed on hyperaccumulator plants, mainly Ni-dependent, however, the term hyperaccumulator has not yet been defined and used in the case of insect (i.e., from which threshold for a given element would it be considered a hyperaccumulator) [202–204]. However, much more interesting is the fact that herbivores could be saprophytic insects that could be fed on different waste biomasses and could hyperaccumulate elements in any of their developmental stages. Different saprophytic species should be tested in this context. The most impressive results have already been achieved in the field of entomodegradation. This process gives hope for the effective elimination of the environmental residues of veterinary antibiotics left in manure. The use of *H. illucens* larvae for manure treatment could partly contribute to the slowing down of the phenomenon of acquiring antibiotic resistance by pathogenic bacteria.

The search for a suitable biofertilizer that reduces the use of artificial fertilizers contributes to intensifying the development of diversified management and the recirculation

of nutrients in the environment, which has a positive impact on the climate. Insect frass is a promising fertilizer material, but since it has its own microbial pool, the relationships, and interactions of the microorganisms with the soil and plants, is very interesting for in depth studies. For instance, in what conditions (diet or parameters of breeding) can pathogenic organisms survive in the frass and be delivered to the soil? Some bacteria known as plant growth promoting bacteria (PGPR) can produce phytohormone-like substances, which promote plant growth [205]. It is possible, however, it has yet to be tested, whether insect frass can contain any substances directly influencing plant growth. If this is true, insect frass could be viewed as a functional fertilizer. A noteworthy issue concerns the chitinous residues, which are left in the frass as fragments of insect exoskeletons. In in vitro plant cultures, chitin and chitosan are well known plant elicitors, i.e., compounds which stimulate the stress resistance of plants [206]. The presence of chitin and chitin fragments cut by microbial chitinases could be a positive factor, influencing plant health during the use of insect frass as fertilizers. It is also important to optimize and standardize substrates for *H. illucens* larvae, to obtain N, P, K rich fertilizer in known ratios. Frass stability and the need for its maturation, as in the case of composts, are additional interesting issues. Does frass already contain any humic substances, which are important for soil health or does the maturation of the frass cause them to develop, making frass a more stable and valuable fertilizer? These are questions not yet answered in the literature.

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8.2. Publikacja P2

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Research article

Black soldier fly frass from seed waste of nitrogen-rich legumes – How long-term maturation affects the fertilizer properties?

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ABSTRACT

Expanded insect production represents a source of post-breeding residues (frass) that can potentially be used as a soil additive. These types of biofertilizers are carriers of recirculated nutrients, as well as organic matter. In the present study, we investigated whether the bean waste (BW) and pea waste (PW) in the form of crushed seeds and post-production leftovers, naturally rich in proteins, were suitable as a substrate for rearing black soldier fly (*Hermetia illucens*) larvae. The main objective of this study was to characterize fertilizing properties of the resulting frasses (after 1 month of larvae breeding), as well as to investigate how aerobic maturation (lasted 10 months) would affect their properties. The experiment demonstrated that larval bioconversion increased the concentrations of ammonium and nitrate ions, as well as concentrations of macro- and micronutrients in the frass compared to the substrates. Raw PW frass can already be used as a fertilizer, as indicated by the appropriate ranges of N_{NX}, EC and C/N values, while raw BW frass had low C/N and high EC, which could contribute to phytotoxic effects. Maturation of frass improved the properties of BW frass, but worsened some characteristics of PW frass, such as an excessive increase in the C/N ratio, which could result in nitrogen immobilization. At the same time, maturation caused further increase in micro- and macronutrients concentration, which meant that both variants of the mature frass were rich in elements necessary for plant development.

1. Introduction

Excessive and unsustainable fertilization with synthetic chemicals can have negative effects on the agricultural economy, as well as on the environment and climate (Kumar et al., 2022). The effects can include a reduction in soil quality by decreasing the organic matter content and quantity of microorganisms, lowering biodiversity, favoring soil acidification and causing atmospheric emissions of ammonia and water eutrophication (Kumar et al., 2022). However, the surging need for food production, increasing with the human population, is prompting a search for other external sources of plant nutrients to ensure high crop yields (Chaudhary et al., 2022). One option may be organic fertilizers, which, due to their biological origin, contain, in addition to nutrients, organic matter and a pool of microorganisms that positively influence growth and development (Vessey, 2003). Examples of such fertilizers include composts or manures, the use of which is incorporated into waste management and recycling (Bergstrand, 2022). One of the goals of the European Union management plan is to transition approximately 25% of agricultural operations to organic farming (IFOAM, 2020).

One promising alternative to fertilizers may be insect post-breeding waste, known as frass, which consists of insect excrements and feed leftovers, larval exoskeleton (containing chitin) as well as a pool of microorganisms associated with larvae (Poveda, 2021). In recent times, there has been a growth in the production of insects, due to their high protein content and ease of breeding (Lu et al., 2022; Queiroz et al., 2023). The boost in the commercial production of various types of insects will increase the generation of post-breeding residues, which can be used as a biofertilizer (Schmitt and de Vries, 2020; Lomonaco et al., 2024), or a substrate for biogas (Win et al., 2018; Bulak et al., 2023a) and biochar (Yang et al., 2019) production, as well as aquaculture feed (Yildirim-Aksoy et al., 2020; Romano et al., 2024a,b). In particular, a large amount of effort has been focused on the production of black soldier fly (*Hermetia illucens*) larvae (BSFL), which compared to other insects is characterized by its ease of breeding, rapid life cycle, waste biomass recycling capacity, and ability to survive in wide ranges of environmental conditions (Raksasat et al., 2021). Additionally, this insect is able to very quickly increase in its body mass (Rehman et al., 2023; Lalander et al., 2019). The feature proves also useful in the context of entomoremediation and disposal of waste materials, while revalorizing them into their body protein and lipids (Bulak et al., 2018; Wong et al., 2020; Liew et al., 2022). In the review conducted by

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Abbreviations

BW –	waste after the production of bean seeds
PW –	waste after the production of pea seeds
BSFL –	black soldier fly larvae
DW –	dry weight
FW –	fresh weight
EDTA –	ethylenediaminetetraacetic acid
U% –	utilization percentage
U_{mic} –	microbial utilization percentage
S% –	survival rate
P% –	pupation rate
EC –	electrolytic conductivity
NO_3^- –	nitrate ions
NH_4^+ –	ammonium ions
C_{tot} –	total carbon
N_{tot} –	total nitrogen
EHOW –	experimental household organic waste

Surendra et al. (2020), they presented data on the degree of waste reduction by BSFL, with the highest results for mixture of food waste and human feces – 68.4–68.8% DW, municipal organic waste – 68.8% DW, chicken manure – 61.7% or mixture of abattoir waste and fruit and vegetable waste – 61.1% DW. It can also become a source of chitin/-chitosan (Waśko et al., 2016; Trimnfo et al., 2022), lipids for biodiesel (Jung et al., 2022) or cosmetics production (Almeida et al., 2020; Verheyen et al., 2018), as well as antimicrobial peptides (Xia et al., 2021).

Depending on the feed given to *H. illucens* larvae, frass vary in composition, physicochemical properties and fertilizer potential, which was provided in review by Lomonaco et al. (2024). Therefore there is a need to study the largest possible spectrum of feed substrate types, whose input parameters will translate into the properties of frass as a fertilizer. Insect frass in soil can become a source of nutrients and minerals (Chirere et al., 2021; Romano et al., 2022).Their use resulted in higher seed germination, improved development, increased chlorophyll content and plant growth, demonstrating fertilizing properties (Beesigamukama et al., 2020; Wu et al., 2020; Romano et al., 2024a,b). In addition, frass can influence soil microbial activity and diversity (Watson et al., 2021). Gebremikael et al. (2020) also observed a decrease in the pathogenic fungus *Rhizoctonia solani*, which may have been caused by the presence of chitinase in frass. Another positive feature that can be achieved with frass uses is supplementation of the soil with beneficial humic substances, which are not present in the initial substrates used to feed BSFL (Wang et al., 2021).

Frass, despite their potential for use as a soil additive, may nevertheless need to undergo a composting process-similar to pig, cow or chicken manure, among others (Bernal et al., 2009). Composting is the process of decomposition and mineralization of organic matter via the microflora that grows on it. The process includes three phases: mesophilic, thermophilic and maturation. During composting the following processes occurs: hydrolysis of nutrients (sugars, fats, proteins), elimination of pathogens, reduction of ammonia ions, and even formation of precursors of humic substances (Sánchez et al., 2017). All these processes affect changes in the physicochemical properties of composts and decrease its phytotoxic potential with maturation time (Azim et al., 2018).The resulting mature product is a nutrient-rich soil additive that does not undergo any changes and does not show phytotoxic effects on plants. In fact, it enhances their development and growth and acts as a fertilizer (Amuah et al., 2022).

The aim of this study was to determine the fertilizing properties of *H. illucens* frass and the changes that occurred after their prolonged maturation process. In order to achieve a high nitrogen content in the frass biofertilizer, waste from the production of two legume seeds, beans and peas, was used as a feed substrate for the larvae. The naturally high nitrogen content of the substrate allowed for drawing a hypothesis that resulting frass will also have a high N level, which will be profitable for plants growth.

2. Materials and methods

2.1. Insects

The *H. illucens* larvae used in the experiments were from a breeding colony situated at the Institute of Agrophysics of the Polish Academy of Sciences in Lublin. The larvae were bred under constant conditions of approximately 26 ± 1 °C and substrate humidity of approximately 70 ± 10% in the dark. The larvae were fed with dry fish food (EUROECO Beszczyński, Poland), with the following composition: 24.0% crude protein, 3.5% crude fat, 6.5% saccharides, 7.0% raw fibre and 7.0% ash (according to the producer's information). One thousand four-day-old larvae were used for both feed variants. Since the mass of an individual larva at this stage of life is low, counting them in large numbers would be time-consuming and biased with a high potential error, therefore the average mass of a young larva was employed. Based on five repetitions of weighing 100 young larvae, the average weight of 1000 larvae was estimated.

2.2. Feed substrates

The experiment used two types of waste from processing bean and pea seeds for human consumption. Both were obtained from local grain and seed producer. The bean seed waste (BW) consisted of seeds that were broken and shrunken. The dry weight (DW) of the BW was 91.31%. The pea seed waste (PW) was composed of seed hulls and seeds flour. DW of the PW was 90.46 %. Tests were carried out on air-dry substrates to determine the amount of water that should be added to bring the substrates to a moisture content of about 75% after imbibition (±5%). Then, full batch of substrates were moisturized by adding the right amount of distilled water and placing the batches to fridge (4 °C) over night to allow imbibition, while inhibiting bacteria and molds growth. Next, the substrates were ground with a blender to the form of wet paste. These steps were aimed at facilitating the young larvae's access to nutrients. The final substrate moisture content was 74.37% for BW and 78.21% for PW (determined gravimetrically 105 °C/24 h).

2.3. Experimental design

The substrate dose used in the experiment was 1 g fresh weight (FW) per larvae. Experimental breeding was carried out in sets of plastic containers (30 x 19 × 15 cm). The boxes were closed with plastic lids to avoid the larvae escaping, but they had secured vents for gas exchange. Air circulation was forced with the use of an air pump (Oxyboost APR 300, Aquael, Poland). The breeding of larvae on experimental feeds lasted for 30 days at a temperature of 26 ± 1 °C. The appearance of raw frass immediately after the experiment was completed can be seen in Fig. S1. During this period, appearing prepupae were pulled out and rinsed with distilled water, than with 1 mM ethylenediaminetetraacetic acid (EDTA) solution to remove metal ions bound on the surface, then rinsed once again with distilled water. Prepupae were counted, measured with a ruler and weighed on a laboratory scale (OHAUS Explorer Precision EX623, USA), and then frozen at −20 °C. The same procedure was carried out with the pupae and larvae at the end of the experiment. However, before measuring the larvae, they were left in empty plastic containers for 24 h to defecate. After that, they were rinsed in distilled water and EDTA once again. All the insect samples were frozen at −20 °C. The raw frass obtained after the bioconversion of the larvae was divided into two portions: one was utilized for the analysis of raw frass, while the other portion was dedicated to maturation. This maturation was carried out under aerobic conditions at 30 ± 1 °C for 10

months in a laboratory incubator. During this period, the frasses were stirred with a glass baguette every two days. At the end of experiment, the matured frasses were subjected to the same analysis as for raw ones.

2.4. Analysis

The dry weight (DW) of the substrates, insects and post-breeding residue was determined after drying the samples at 105 °C per 24 h. The following formula (Eq. (1)) was used:

$$DW\% = \frac{m_d \cdot 100}{m_f} \quad \text{(Eq. 1)}$$

where m_d – weight of dry sample (g) and m_f – weight of fresh sample (g).

Protein content in both substrates BW and PW was calculated by multiplying the nitrogen content by 6.25 (Weththasinghe et al., 2021). The method according to Santos Filipe et al. (2024) was used to analyze the lipids content by treating the sample with n-hexane with the sample to solvent ratio of 1:10 (m/v). The crude fibre content was determined according standard method ISO 5498-1981. The ash content was determined by incineration of the sample in an oven at 550 °C for 2h (EN 12879). The carbohydrates content was calculated according to the formula:

Carbohydrates (% DW) = 100% - (protein (% DW) + lipids (% DW) + fibre (% DW) + ash (% DW)) (Eq. 2)

The utilization percentage (U) of the DW of the waste substrate by *H. illucens* larvae was calculated according to the formula:

$$U\% = \frac{(DW_{init} - DW_{raw}) \bullet 100}{DW_{init}} \quad \text{(Eq. 3)}$$

where DW_{init} – DW of the substrate used at the beginning of the experiment and, DW_{raw} – DW of the raw frass after the bioconversion of *H. illucens* (Norgren et al., 2020).

The percentage of microbial utilization (U_{mic}) that followed the maturation of frass from bioconversion of waste was calculated using the following formula:

$$U_{mic}\% = \frac{(DW_{raw} - DW_{mature}) \bullet 100}{DW_{raw}} \quad \text{(Eq. 4)}$$

where DW_{raw} – DW of the raw frass after the bioconversion by *H. illucens* and, DW_{mature} – DW of the frass after maturation process.

The survival rate (S) of *H. illucens* was calculated according to the formula:

$$S\% = \frac{N_{live} \bullet 100}{N_{init}} \quad \text{(Eq. 5)}$$

where N_{live} – the sum of live pupae and live larvae after the experiment and, N_{init} – number of initial larvae.

The pupation rate (P) of *H. illucens* was calculated after one month of breeding, according to the formula:

$$P\% = \frac{N_p \bullet 100}{N_{init}} \quad \text{(Eq. 6)}$$

where N_p – the number of pupae and, N_{init} – the number of initial larvae.

Measurements of pH, electrolytic conductivity (EC) and nitrate (NO_3^-) and ammonium (NH_4^+) ions were carried out with the multifunction meter HQ400 (Hach Lange, Düsseldorf, Germany), utilizing the CDC40104, ISENO318101 and ISENH318101 electrodes, respectively, with a sample:distilled water ratio of 1:10 (w/v). For the measurement of NO_3^- and NH_4^+, an ionic strength adjuster (cat. 2984799 and cat. 4447169, respectively, Permachem Reagents, HACH) was added according to the producer's recommendations. A Thermo Scientific Flash 2000 Organic Elemental Analyzer was used to analyze total carbon (C_{tot}) and total nitrogen (N_{tot}) content. The elemental contents in the substrates, frasses and larvae were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES) (Thermo Scientific iCAP Series 6500) following (Bulak et al., 2023b). The recalculation of macronutrients into their oxide forms was performed based on the conversion factors (Uddin et al., 2020).

2.5. Statistics

The experiments were conducted in three independent biological repetitions. The statistical analysis of the obtained results was performed using Statistica 13. The Student's t-test ($p < 0.05$) and ANOVA post-hoc Tukey's test were utilized to establish the statistical significance. Data on the physicochemical properties and elemental content of the frass are based on means (± standard deviation (SD)) of three analytical replicates as well as three biological replicates ($n = 9$).

3. Results

3.1. Feed substrate and larval characteristics

BW substrate was characterized by significantly higher amounts of protein (by 1.76 times) and lipids (by 3.12 times) than in PW substrate (Table 1). Nevertheless, both substrates were poor in lipids. The PW substrate was richer in fibre than BW substrate by 2.53 times. The ash and carbohydrates contents were on similar levels in both substrates (Table 1).

The larval growth and survival parameters of *H. illucens* fed with both wastes are shown in Table 2. The average weights of one larva and pupa reared on BW were higher in comparison to those fed with PW by 1.25 and 1.63 times, respectively (Table 2). There was no significant difference in larval length between the two variants, while the average length of one pupa after BW was approximately 1.2 times greater than those after PW (Table 2). The survival rate of *H. illucens* for the two substrates differs significantly (Table 2). However, despite the better growth parameters on BW, there was a significantly higher percentage of pupation in the PW variant, which yielded 4.17 times more pupae after one month than the BW variant.

3.2. Physicochemical characteristics of raw frass

The utilization of the DW by the *H. illucens* larvae in both feeding variants was approximately 67% (Table 3). The bioconversion performed by the larvae caused an increase in pH to alkaline (approximately 9) regardless of the initial value of both substrates. The EC of the raw frass from the BW and PW increased by 2.30 times and 2.51 times respectively, compared to the initial substrates. In the BW variant, C_{tot} content decreased in the raw frass compared to the substrate. In the case of PW, there was a slight increase in the content of C_{tot} in the raw frass compared to the substrate (Table 3). In the case of N_{tot}, its initial values for both substrates were at a similar level of approximately 5.41%. After the bioconversion by *H. illucens*, N_{tot} did not change significantly in the BW variant, while in the PW variant the reduction was significant – approximately 3.64 times. The bioconversion by BSFL in the BW variant resulted in a C/N ratio in the substrate and raw frass that was not

Table 1
Proximate analysis of initial substrates of bean seeds (BW) and pea seeds (PW) waste (Student's t-test, $p < 0.05$, $n = 3$).

(% of dry weight)	BW	PW	
Protein	34.37 ± 3.74	*	19.56 ± 5.78
Lipids	0.53 ± 0.01	*	0.17 ± 0.01
Fibre	15.38 ± 1.41	*	38.85 ± 5.85
Ash	4.18 ± 0.12		4.13 ± 0.11
Carbohydrate	45.55 ± 3.50		37.87 ± 7.40

An asteriks "*" indicates a significant difference ($p < 0.05$, Student's t-test) between a given parameter in two feeding variants.

Table 2
Growth parameters of *H. illucens* larvae and pupae after one month of breeding on bean waste (BW) and bean waste (PW) (Student's t-test, $p < 0.05$, $n = 3$).

Final insects parameters	BW	PW	
Weight of 1 larva (g FW)	0.10 ± 0.00	*	0.08 ± 0.00
Length of 1 larva (cm)	1.6 ± 0.30		1.4 ± 0.28
Pupation rate (%)	10.1 ± 1.27	*	42.07 ± 7.06
Weight of 1 pupa (g FW)	0.13 ± 0.00	*	0.08 ± 0.00
Length of 1 pupa (cm)	1.8 ± 0.03	*	1.5 ± 0.02
Survival rate (%)	77.5 ± 7.354	*	93.2 ± 6.95

An asteriks "*" indicates a significant difference ($p < 0.05$, Student's t-test) between a given parameter in two feeding variants.

statistically different, while it increased by about 3.70 times in the PW (Table 3).

In the initial BW substrate, the NH_4^+ and NO_3^- concentrations were approximately 1 g kg^{-1} DW. In the raw frass, the concentrations of these ions increased significantly for NH_4^+ (approximately 9.92 times) and insignificantly for NO_3^- compared to the initial waste (Fig. 1a). The initial PW substrate contained NH_4^+ ion concentrations of approximately 1.44 g kg^{-1} DW, and NO_3^- ions of approximately 0.50 g kg^{-1} DW (Fig. 1b). After the bioconversion by *H. illucens* larvae, the NH_4^+ content significantly increased by 3.34 times, and NO_3^- ions by 3.96 times (Fig. 1b).

The raw BW frass had a higher macronutrient content compared to the substrate, with the exception of Ca and Mg (Table 4). However, there was only a significant difference for K, P and S (Table 4). The concentrations of microelements such as B, Cu, Mo and Zn grew considerably in the raw BW frass compared to the substrate. The only heavy metal whose content rose notably after BW bioconversion in comparison to BW substrate was Ni.

In case of the raw PW frass the contents of Mg and S increased significantly, while the concentration of Ca decreased ($p < 0.05$). Changes in other macroelements contents were insignificant. The raw PW frass was characterized by higher contents of almost all microelements than in the substrate – only that of Zn did not change. Among the heavy metals, only the concentration of Cd and Pb grew in raw PW frass (Table 4).

In most cases, the raw BW frass had a higher content of macroelements than the raw PW variant, with Mg the only exception. The concentrations of the microelements in the raw frass were higher in the PW variant than in the BW variant, similarly for heavy metals, with the exception of Ni (Table 4).

The N:P:K (NPK) ratio was reported as the weight percentage ratio of P and K in oxide forms (P_2O_5 and K_2O) and N_{tot} (Uddin et al., 2020). In the BW variant, the NPK ratio for the raw frass was 4.7:1.8:2.3, and for the mature frass it was 1.5:2.2:3.0 (Tables 3 and 4). In the PW variant, the NPK ratio for the raw frass was 1.5:1.7:1.1, and for the mature frass it was 1.0:2.8:3.1 (Tables 3 and 4).

3.3. Physicochemical characteristics of mature frass

The maturation process has resulted in further decrease in frass biomass. The maturation of the BW variant resulted in a slight DW utilization of raw frass (3.2%), while in the PW variant the change was as high as 40.9% (Table 3). Maturation caused a further significant rise in pH in the BW variant, while in the PW variant the pH remained at an even level (Table 2). The EC values decreased significantly after the maturation process compared to the raw frass and returned to the initial substrate values (Table 3). This situation occurred in both substrate variants (BW and PW). Matured frass BW was not characterised by a significant change in C_{tot} values compared to raw frass (Table 3). While, in the case of PW frass, maturation resulted in a decrease in C_{tot}. The maturation process caused futher loss of nitrogen compared to the raw frass by 3.06 times for BW, and by 1.52 times for PW (Table 3). In the BW

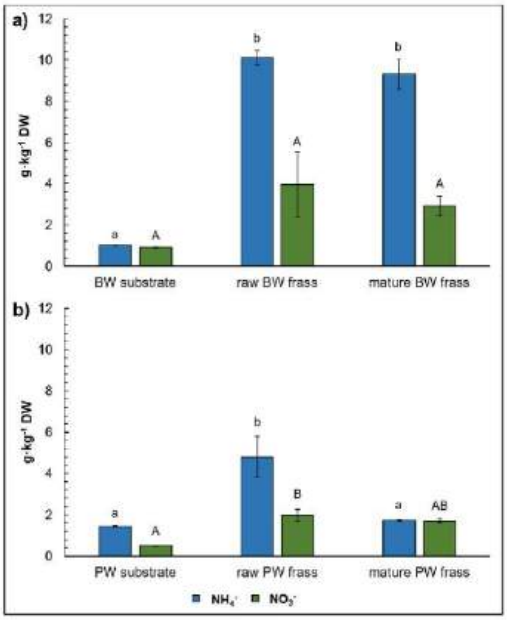

Fig. 1. Concentrations of ammonium and nitrate ions in the substrate, raw frass after bioconversion and in mature frass: a) bean seed waste (BW) b) pea seed waste (PW). Error bars represent standard deviation. Letters indicate statistical differences (Tukey test, $p < 0.05$), lowercase letters correspond to statistical changes in NH_4^+ content, while uppercase letters correspond to statistical changes in NO_3^-.

Table 3
Parameters of the initial substrates and raw and matured frasses after the bioconversion of bean waste (BW) and pea waste (PW) by *H. illucens*.

Parameter	BW substrate	BW raw frass	BW mature frass	PW substrate	PW raw frass	PW mature frass
Utilization (% DW)	NA	66.91 ± 1.69	3.18 ± 3.35†	NA	67.61 ± 1.01	40.92 ± 3.71†
DW (% FW)	25.63 ± 0.00a	31.19 ± 0.00a	81.51 ± 5.02b	21.79 ± 0.00A	21.53 ± 0.00A	28.33 ± 2.97B
pH	6.13 ± 0.01a	9.04 ± 0.22b	9.60 ± 0.15c	4.98 ± 0.00A	9.37 ± 0.05B	9.40 ± 0.19B
EC ($mS \cdot cm^{-1}$)	7.03 ± 0.02a	16.19 ± 0.14b	7.05 ± 0.15a	3.22 ± 0.03A	8.09 ± 0.04B	3.20 ± 0.93A
C_{tot} (% DW)	44.40 ± 2.05a	42.64 ± 0.88b	40.20 ± 0.78b	43.71 ± 0.43AB	44.33 ± 0.45A	39.62 ± 2.10B
N_{tot} (% DW)	5.50 ± 0.60a	4.65 ± 0.78a	1.52 ± 0.47b	5.32 ± 0.70A	1.46 ± 0.19B	0.96 ± 0.51B
C/N	8.15 ± 1.07a	9.33 ± 1.68a	26.55 ± 20.31b	8.30 ± 1.01A	30.68 ± 3.48AB	49.97 ± 20.67B

The abbreviation NA means no applicable. The utilization value with a "†" symbol indicates the utilization of frass by microorganisms after the maturation process. Letters denote statistical changes (Tukey test, $p < 0.05$): lowercase letters (a, b, c) correspond to those within a given feature in the substrate, raw and mature fractions of the BW variant, and uppercase letters (A, B, C) represent the same in the PW variant.

Table 4
Elemental concentrations (mg·kg^{-1} DW) of substrates (BW – bean waste, PW – pea waste), raw frasses after bioconversion by *H. illucens* larvae, and frasses after their maturation.

		BW substrate	BW raw frass	BW mature frass	PW substrate	PW raw frass	PW mature frass
macroelements	Ca	2426.67 ± 12.50a	946.22 ± 79.27b	1269.00 ± 167.35c	1357.00 ± 33.41A	538.92 ± 115.56B	859.70 ± 55.91C
	CaO (%)	*0.34 ± 0.00a*	*0.13 ± 0.01b*	*0.18 ± 0.02c*	*0.19 ± 0.00A*	*0.08 ± 0.01B*	*0.12 ± 0.01C*
	K	16,220.00 ± 52.92a	18,953.33 ± 1654.63b	24,810.00 ± 2064.75c	8038.00 ± 184.81A	9492.17 ± 891.66A	25,901.67 ± 16,416.66B
	K_2O (%)	*1.96 ± 0.01a*	*2.28 ± 1.17b*	*2.99 ± 0.20c*	*0.97 ± 0.02A*	*1.14 ± 0.08A*	*3.12 ± 1.53B*
	Mg	2883.67 ± 16.17a	2785.67 ± 238.53a	3445.83 ± 27.58b	2033.67 ± 14.29A	3858.00 ± 279.07B	4688.33 ± 44.78C
	MgO (%)	*0.48 ± 0.00a*	*0.46 ± 0.02a*	*0.57 ± 0.00b*	*0.34 ± 0.00A*	*0.64 ± 0.03B*	*0.78 ± 0.00C*
	P	6872.67 ± 49.05a	7821.33 ± 472.35b	9744.17 ± 545.65c	6210.00 ± 51.29A	7443.33 ± 253.14A	12,210.00 ± 2899.14B
	P_2O_5 (%)	*1.58 ± 0.01a*	*1.79 ± 0.09b*	*2.23 ± 0.10c*	*1.42 ± 0.01A*	*1.71 ± 0.05A*	*2.80 ± 0.52B*
	S	2536.00 ± 19.31a	2996.17 ± 42.19b	3763.67 ± 253.62c	1933.00 ± 16.64A	2747.83 ± 175.60B	4783.17 ± 585.72C
	SO_2 (%)	*0.51 ± 0.00a*	*0.60 ± 0.00b*	*0.75 ± 0.03c*	*0.39 ± 0.00A*	*0.55 ± 0.02B*	*0.96 ± 0.06C*
microelements	B	2.77 ± 0.08a	3.61 ± 0.54b	4.56 ± 0.57c	0.98 ± 0.02A	4.99 ± 0.13B	7.26 ± 0.29C
	Cu	7.53 ± 0.14a	10.06 ± 0.18b	13.39 ± 0.30c	7.88 ± 0.18A	11.58 ± 0.26B	19.13 ± 0.34C
	Fe	122.23 ± 1.88a	128.45 ± 2.38a	165.58 ± 7.61b	58.24 ± 0.70A	420.88 ± 9.40B	447.25 ± 245.72B
	Mn	22.25 ± 0.31a	20.90 ± 1.22a	26.98 ± 2.32b	16.63 ± 0.19A	33.48 ± 2.57B	41.77 ± 2.64C
	Mo	0.69 ± 0.03a	0.98 ± 0.01b	1.31 ± 0.09c	1.09 ± 0.04A	1.66 ± 0.10B	2.18 ± 0.37C
	Na	52.07 ± 1.01a	49.38 ± 4.35a	50.11 ± 0.16a	63.36 ± 1.94A	74.40 ± 13.18A	55.68 ± 25.34A
	Zn	38.28 ± 0.32a	36.81 ± 1.32b	48.36 ± 0.41c	40.82 ± 0.36A	77.37 ± 1.69A	64.34 ± 27.95A
heavy metals	As	0.13 ± 0.10a	0.09 ± 0.04a	0.10 ± 0.02a	0.14 ± 0.09A	0.25 ± 0.03A	0.22 ± 0.03A
	Cd	0.04 ± 0.00a	0.05 ± 0.00a	0.08 ± 0.01b	0.04 ± 0.01A	0.18 ± 0.02B	0.20 ± 0.08B
	Hg	0.09 ± 0.03a	0.12 ± 0.04a	0.11 ± 0.01a	0.13 ± 0.03A	0.13 ± 0.00A	0.15 ± 0.03A
	Ni	5.24 ± 0.07a	6.36 ± 0.01b	8.31 ± 0.18c	3.26 ± 0.04A	3.38 ± 0.06A	8.75 ± 5.40B
	Pb	0.41 ± 0.08a	0.36 ± 0.04 ab	0.52 ± 0.01b	0.09 ± 0.02A	0.82 ± 0.01B	0.89 ± 0.32B

The data in italics denote the percentage content of macronutrients after their conversion to oxide forms. Different letters indicate statistically significant changes (Tukey's test, $p < 0.05$). Lowercase letters correspond to statistical changes in the BW variant and, uppercase letters to those in the PW variant.

variant, the C/N ratio in the substrate and raw frass was not statistically different, while in the PW variant it increased by about 3.70 times (Table 3). Maturation of the BW frass led to an increase in C/N by about 3.26 times. The C/N value in the matured PW frass also increased and was almost 50.

Maturation of BW frass did not result in significant changes in the values for NH_4^+ as well as NO_3^- ion concentrations compared to the raw frass (Fig. 1a). In the case of mature PW frass, the concentrations of both these ions decreased significantly to an equal level of about 1.7 g kg^{-1} DW (Fig. 1b).

Mature frass BW had contents of all macroelements at significantly higher levels than in the raw frass (Table 4). The same situation occurred for micronutrients in mature BW frass. For heavy metals, a significant increase was recorded for Cd and Ni concentrations (Table 4). However, the concentrations of elements from this group, except Ni, were very low and ranged well below 1 mg kg^{-1} DW. In the mature PW frass, concentrations of all macronutrients increased significantly compared to the raw frass (Table 4). Most micronutrients in mature PW frass also increased – the exceptions were Fe and Zn. Maturation did not significantly affect the heavy metal content of the PW frass, with the only exception of Ni (Table 4). A comparison between the mature BW and PW frasses showed that the content of all macronutrients, except Ca, was higher in the PW frass (Table 4). Concentrations of micronutrients and heavy metals were also higher in mature PW frass than in mature BW frass (Table 4).

4. Discussion

4.1. Comparison of PW and BW as *H. illucens* larvae feed

The one-month rearing of BSFL on the BW substrate resulted in a significantly higher average weight for both larvae and pupae compared to the larvae reared on the PW substrate (Table 2). This difference might be explained by higher content of proteins and lipids in BW substrate (Table 1). For larval length, there was no statistical difference between the variants, while pupae from BW-fed larvae were significantly longer.

The larvae fed on the PW substrate showed a significantly higher survival rate than larvae after BW. Interestingly, larvae growing on PW also exhibited a significantly higher pupation rate. The reason for these differences may be the availability and uptake of nutrients by the larvae as well as the elemental composition of their bodies. PW-fed larvae had higher amounts of macronutrients such as Ca, K, P and S than the BW larvae (Table S1), which in turn may have contributed to increased transition to the pupal stage due to better nutrition and overall improvement in larvae condition. A similar situation was reported by (Bava et al., 2019), who reared BSFL on brewer's grain and on maize distillers' grain. Bava et al. (2019) obtained larval survival rates of 95.9% for brewers' grain and 73.0% for maize distillers' grain, while larvae raised on the latter were heavier than those breed on the former. The survival rates observed in our experiment were also similar to those obtained for larvae reared on various types of organic waste such as food waste (87.2%), poultry manure (92.7%), undigested sewage sludge (76.2%) and fruits and vegetables (90.7%) (Lalander et al., 2019). In addition to the type of feed substrate and its composition, the survival rate of BSFL is significantly influenced by rearing conditions, such as temperature, which can reduce this rate even within a narrow range of change of 27–30 °C (Tomberlin et al., 2009). However, in the case of the present study, it is possible that the lower survival rate of larvae on BW substrate was influenced by ammonium ions, whose concentration was higher than in the PW variant (Fig. 1ab), which may have shown toxic effects on invertebrates (Zhang et al., 2023).

The larvae of BSF contributed to a decrease of the waste substrates in both variants at a similar level (~67% DW on average) (Table 3). A comparable result of the utilization rate was obtained for municipal organic waste (68.0% DW) (Diener et al., 2011) or primary sludge (63.3% DW) (Lalander et al., 2019). For substrates of plant origin, such as soybean curd residues (Somroo et al., 2019) or corn straw (Wang et al., 2017), the values were slightly lower at 49.0% and 39.9% DW, respectively. Global production of legumes is more than 92 million tons (Dutta et al., 2022) during which leftover in form of seed wastes are generated. The revalorization of these waste stream through the use of BSFL could be a new management method, allowing to produce frass

with interesting properties.

4.2. The properties of raw frasses in the context of their use as a biofertilizer

The percentage of DW frass has not changed statistically after BSFL bioconversion of both substrates. The feature may depend on the substrate on which the BSFLwere reared. For example, frass obtained from experimental household organic waste (EHOW) had 44.4% DW (Kawasaki et al., 2020), that from chicken feed ranged from 70.6% to 90.0 % DW (Gebremikael et al., 2020, Klammsteiner et al., 2020), fruit/vegetable frass approximately 60%–90% DW (Gebremikael et al., 2020, Klammsteiner et al., 2020) and that from grass cuttings frass approximately 89.9% DW (Klammsteiner et al., 2020).

The pH values of raw frass after bioconversion by BSF were alkaline in both the PW and BW variants, despite the initial acidic pH of the substrates. These results are consistent with a study by (Meneguz et al., 2018),which reported that BSFL change the pH of the substrate to values close to 9, regardless of the initial pH. The increase in pH is caused by the decomposition and transformation of organic acids formed from proteins and the formation of NH_4^+ ions (Azim et al., 2018), and may be due to the generation of organic bases, such as aliphatic or aromatic amines in the process of protein decomposition (Tshepelevitsh et al., 2019). The results shown in Fig. 1 demonstrate an actual rise in NH_4^+ ions in the raw BW and PW frasses, which may have caused a rise in pH. High pH may be an advantage, especially if these biofertilizers would be used in acidic soils. Therefore, a decision was made to carry out a maturation process, which should ensure a more stable final product with a lower NH_4^+ content due to its transformation to NO_3^-.

The EC of the raw BW frass was higher than that of the PW variant, which was primarily due to its initially higher value in the substrate (Table 3). It is accepted that EC values above 10 $mS{\cdot}cm^{-1}$ may contribute to the phytotoxicity of the tested compost (Tiquia, 2010), caused by impeding water uptake by the plant or due to excessive salt accumulation in the plants (Gondek et al., 2020). The safe limit of EC values was exceeded in the raw frass BW amounting to 16.2 $mS{\cdot}cm^{-1}$. The EC of the raw PW frass indicated a minimal possibility of a toxic effect on plants.

The C/N ratio represents an indicator allowing researchers to assess the "activity" of nitrogen. Depending on its value, nitrogen is mineralized to ions that are readily available to plants, or immobilized, i.e., retained by soil microorganisms. The equilibrium state between the two lies in the C/N range of approximately 20–30, where mineralization occurs below this range, and immobilization above it (Burst, 2019). The value of the C/N ratio differed significantly for the raw frasses between the BW and PW variants (Table 3). This difference was due to a significant decrease in N_{tot} concentration in the PW frass, with a relatively constant C_{tot} content. In the case of BW, the N_{tot} concentration remained unchanged relative to the initial substrate, and the C_{tot} concentration, although significantly decreased after bioconversion by BSFL, the change was relatively minor (Table 3). The high value of C/N obtained in the PW frass (30.68) was comparable to the C/N ratio obtained by Klammsteiner et al. (2020) in frass from fruits and vegetables, which was 26.6. The C/N ratio in frasses from chicken feed and grass cuttings was 18.3 (Klammsteiner et al., 2020). In the case of raw BW frass the result was comparable to the C/N for frass from okara and wheat bran (9.6) (Song et al., 2021) or spent brewery grain (10.7) (Anyega et al., 2021), and to examples of other types of organic fertilizers such as poultry manure (10.3) or vermicompost (11.1) (Geisseler et al., 2021). All of these changes result primarily from the metabolism of both the feeding larvae and the microorganisms that live with them in the substrate. Part of the nutrients were assimilated by them, while part were mineralized and leave the experimental system in a gaseous form (CO_2, NH_3, volatile organic compounds and others) (De Cesare et al., 2011)

The value of N_{tot} in the raw BW frass compared to the substrate was not statistically changed. The high N_{tot} content argues for the possibility of using this type of fertilizer during the growing season rather than for autumn fertilization, due to the risk of nitrogen leaching (Engström et al., 2014). At the same time, the concentrations of NH_4^+ ions increased significantly in the raw BW frass, which are readily available to plants. Due to this feature, it may be recommended for plants with high nitrogen needs or that are tolerant of NH_4^+, such as Alliaceae, Ericaceae or Myrtaceae (Britto and Kronzucker, 2002). Similar trends in changes in both NH_4^+ and NO_3^- ions were also noted in the PW variant. However, in the PW variant, a significant decrease in N_{tot} was recorded. The reason for this difference may lie in faster processes of nitrogen mineralization and ammonia release, which are characteristic for the first weeks of composting (Hao et al., 2008). It can be concluded that the raw PW frass was more stable than that of the BW variant, which was also supported by the higher C/N ratio (Table 3).

The bioconversion by BSFL resulted in changes in the elemental profile contents in the frasses relative to the input substrate (Table 3). An increase in most element concentrations after bioconversion related to a high decrease in organic matter and its mineralization due to larvae feeding and microbial decomposition (Azim et al., 2018). On the other hand, there were also cases where the concentration decreased. For the BW variant, the highest significant decline was observed for Ca, although Mg, Mn, Na and Zn also showed a tendency to decrease. In contrast, for the PW variant, only Ca demonstrated a decline in concentrations (Table 3). These changes were connected with the nutritional demand of the larvae. The situation that occurred for Mg, B, Fe and Pb is also noteworthy – the concentrations of these elements were higher in frasses than in the substrates and raw PW frass contained them in significantly (Student's t-test, $p < 0.05$) higher concentrations than BW raw frass (Table 4).

Nevertheless, the elemental composition of frass depends on the properties of the feed substrate, on which the BSFL were reared, so studies that use a similar diet are a good choice for discussion. The presented macronutrients in fresh frass from the okara/wheat bran mixture (okara is a product of processing soybeans, which together with peas and beans belong to the Fabaceae family) consisted of 985.00 mg kg^{-1} K, 1341.00 mg kg^{-1} Ca and 11.78 mg kg^{-1} Mg (Song et al., 2021). This frass was characterized only by higher Ca concentrations in comparison with PW and BW frasses. In the case of K and Mg, frass from BW and PW had concentrations higher than frass from okara/wheat bran by approximately two orders of magnitude. The frass after bioconversion of distillers' dried grain by BSF was characterized by significantly higher concentrations of Ca (13,000 mg kg^{-1}) and Na (5000 mg kg^{-1}) than raw PW and BW frass, while the Mg concentrations were at a similar level of 3000 mg kg^{-1} (Yildirim-Aksoy et al., 2020).

For micronutrients, okara/wheat bran frass was characterized by Fe at 26.55 mg kg^{-1}, Mn at 4.21 mg kg^{-1}, Zn at 0.10 mg kg^{-1}, B at 2.77 mg kg^{-1} and Mo at 2.77 mg kg^{-1} (Song et al., 2021). Comparing these results to BW and PW frass, okara/wheet bran frass was richer only in Mo. The concentrations of micronutrients such as Cu, Fe, Mn and Zn in frass from distillers' dried grain were 15 mg kg^{-1}, 125 mg kg^{-1}, 45 mg kg^{-1} and 90 mg kg^{-1}, respectively (Yildirim-Aksoy et al., 2020) and referring to both frasses studied in our work only the Cu content was at a similar level. The Fe content was very close to the value from raw BW frass, and lower than in raw PW frass. In contrast, Mn and Zn contents were higher than in the BW and PW variants.

The BW and PW substrates were characterized by low concentrations of heavy metals such as As, Cd and Pb, not exceeding the thresholds regulated by Directive (2002)/32/EC of the (European Parliament, 2002) on undesirable substances in animal feed to allow the material to be used for feed purposes. In the case of Hg, for which the maximum amount is 0.1 mg kg^{-1} DW, the BW substrate was within the considered standard, while the PW slightly exceeded it (0.13 mg kg^{-1} DW). The Directive does not regulate the allowed content of Ni, but it can be stated that the amounts in PW and BW substrates were at a low level. After bioconversion by BSF, the values of Ni concentration increased in the BW variant, while the PW one showed a statistical increase in Cd and Pb.

Concentrations of heavy metals in both variants of raw frass did not exceed the average limit of values in biowaste compost for European countries. The maximum values of these elements in biowaste compost are as follows: for Cd-1.4 mg kg^{-1}, for Hg-1.0 mg kg^{-1}, for Ni-47 mg kg^{-1} and for Pb-121 mg kg^{-1}, but there is no information on the limit for As (Amlinger et al., 2004). In comparison, the residue from rearing BSFL on optimal feed - dry fish feed had much higher concentrations of heavy metals than in the frasses in our study, amounting to 15.22 mg kg^{-1} for As, 0.44 mg kg^{-1} for Cd, 0.22 mg kg^{-1} for Hg and 1.84 mg kg^{-1} for Pb (Proc et al., 2020), and were higher than the raw BW and PW frasses.

The NPK ratio was significantly higher in the BW raw frass than in the PW variant (Student's t-test, $p < 0.05$) (Tables 3 and 4). Gärttling and Schulz (2021) reported BSFL raw frass characteristic compiled from the literature – average NPK ratio was 3.35:1.5:2.99. It is worth to noticed, that both BW and PW raw frass had higher P content than described in above mentioned publication. Compared to the okara/-wheat bran frass, where the N value was slightly higher even than in BW frass, the NPK ratio was 5.2:0.1:0.2 (Chiam et al., 2021). In consideration of the diminishing and thus exhausting sources and reserves of P, it is necessary to look for recycled sources of this element to supply to soil and plants, and insect frass may become one of the options (Chowdhury et al., 2017).

4.3. *The properties of mature frasses in the context of their use as a biofertilizer*

As already mentioned, the frass maturation process was undertaken due to the high concentration of NH_4^+ ions in raw frass, as this situation may indicate a toxic effect (Jian et al., 2018). One of the commonly used methods to reduce the toxicity of biofertilizers obtained from animal excrements (as well as those rich in ammonium nitrogen) is composting (Dadrasnia et al., 2021). What seems most important after the analysis of Fig. 1 is the fact that the frass maturing process using PW was effective, while in the case of BW frass the concentration of NH_4^+ ions did not significantly decrease even after 10 months. The experiment was stopped after 10 months because a longer maturation period did not make practical sense (Chen et al., 2011).

The maturation of the PW and BW frasses led to a decrease in EC to the level of the initial substrates. This can be explained, among other things, by the release of NH_4^+ (Gondek et al., 2020). A drop in EC means a reduction in the salinity of the frass and a lowering of the potential for phytotoxic effects (Tiquia, 2010).

The maturation process of BW frass increased the C/N ratio (to 26.6) to a level that may indicate stabilization of rapid nitrogen release (Brust, 2019). In the case of PW frass, maturation also further raised the C/N ratio, which could result in the microbial immobilization of nitrogen (Brust, 2019). Rynk et al. (2022) investigated the relationship between the moisture content and the C/N ratio in composted material to improve this ongoing process, showing an ideal balance of these two indicators at a 50%–65% moisture content and a C/N ratio of approximately 25–40. Following these guidelines, our mature BW frass was characterized by excessive dryness and nitrogen content. The matured PW frass, on the other hand, was too wet and had an overly low nitrogen content. However, these properties can be easily improved by adding water or drying the frass. In the frass studied by (Song et al., 2021), resulting from the bioconversion of okara/wheat bran, composting for eight weeks expanded the C/N ratio from 7.76 to 9.61, which compared to frass from the BW and PW variants was a rather minor change. In case of EHOW after a 15-day bioconversion by BSFL was 16.6 (Kawasaki et al., 2020), and for frass after a two-week treatment by BSFL of brewery spent grain/potato peelings it was 13.2 (Beesigamukama et al., 2022).

The maturation process of frass BW resulted in a significant decrease in N_{tot}, which may be due to its use by microorganisms as well as its release and loss under the higher temperature accompanying the process than during the bioconversion (Zhou et al., 2023). In this variant as well, it was observed that there were no significant changes in the concentrations of NH_4^+ and NO_3^- ions, but it should be noted that the results of these concentrations are given in the unit of g kg^{-1} DW, and the percentage of DW between raw and mature BW frass differs significantly. This leads to the fact that, for example, when there is a need for a certain dose of NH_4^+, less FW mature frass than raw will be used. On the other hand, such a large drop in the moisture content between raw and mature frass may be associated with the inhibition of microbial activities. This, in turn, could suggest that maturation was not completed.

In the case of mature PW frass, there was a decrease in N_{tot}, with a simultaneous drop in NH_4^+ and a slight one in NO_3^- ions. The decline in NH_4^+ may have been caused by its dissociation and volatilization as ammonia (NH_3), especially since the pH of the frass was alkaline, which favors this type of process (Hao et al., 2008). It is also possible that the decline in NH_4^+ concentration may have been partly due to its transformation to volatile N_2. However, such a transformation, briefly called anammox, takes place under anaerobic conditions (Weralupitiya et al., 2021), which would suggest that the process of aerobic maturation did not proceed uniformly throughout the material, even despite stirring. Such cases can arise under humid conditions. The drop in NH_4^+ concentration did not occur due to conversion to NO_3^-, as its concentration remained almost equal with the raw frass. Nevertheless, is a sign of the maturation and stabilization of the material (Antil et al., 2014).

The activity of microbial organisms living in the matured material leads to a loss in organic matter content (Table 2). This can explain the increase in most elements in mature BW and PW frass compared to raw ones. However, there were exceptions in which there were no statistical changes in concentration, such as Na, As and Hg for the BW variant, and Fe, Na, Zn, As, Cd, Hg and Pb for the PW variant (Table 3). Nevertheless, in contrast to the results in our work (Song et al., 2021), showed a tendency for elemental concentrations to decrease after eight-weeks of composting okara/wheat bran frass. Their examples include K, Ca, Mn, Cu, B and Mo. However, the authors did not explain this observation. Another change that occurs in BW and PW frass that is worth noting concerned the elements K, P, S and Ni, whose concentrations in the raw BW frass were higher than in the PW one, while after maturation the PW frass was richer in them. The contents of heavy metals in both BW and PW raw and mature frass were generally at low levels and did not exceed the average upper limit for heavy metals content for European countries (Pollak et al., 2004).

The maturation process changed the NPK ratio, increasing the P and K values per part of N in both frass variants. Despite a slightly lower amount of N, maturation made PW frass richer in P and K, compared to mature BW frass. In the study of (Song et al., 2021), an eight-week maturation of okara/wheat bran frass yielded an NPK ratio equal to 3.2:0.2:0.1 (after the conversion of K and P to oxide forms). This suggested that our matured frasses may be suitable as an autumn fertilizer because of their low N and high K content.

5. Conclusions

Both substrates, BW and PW, can become good sources of nutrition for BSFL. The larvae and pupae after BW built up a higher body mass, but the PW provided higher survival rates, pupation and macro- and micronutrient content of their bodies. Breeding BSFL on this type of waste resulted in their revalorization, contributing to larval biomass as well as frass. The raw BW frass was rich in plant-available NH_4^+ ions and high total N content, as well as macro- and micronutrients, but showed too high EC and low C/N. Maturation of frass BW led to a positive change in parameters. Raw BW frass due to its high N content can be recommended as spring fertilizer (taking EC values into account) and mature frass BW as autumn fertilizer due to its higher P and K content. The raw PW frass had a lower nitrogen content than the BW variant, but already had better EC and C/N levels in the optimum range at this stage. The maturation of the PW frass resulted in the C/N ratio being too high, which may indicate nitrogen immobilization. Raw PW frass can also be

used as a spring fertilizer (despite lower N content than in raw BW frass) due to high micronutrient levels. Mature PW can be a good autumn fertilizer, similarly to mature BW frass. The use of insect frass for fertilizer purposes is an interesting area of research. Proposed research directions could include the following research questions: how will the activity and biodiversity of microorganisms in different soil types change after the application of frass? Is there a chance that frass will stimulate naturally occurring plant growth-promoting bacteria in the soil? How does the maturation of frass affect the formation of humic substances?

CRediT authorship contribution statement

Monika Kaczor: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Andrzej Bieganowski:** Conceptualization, Methodology, Validation, Writing – review & editing. **Dariusz Wiącek:** Investigation. **Piotr Bulak:** Conceptualization, Methodology, Formal analysis, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.123752.

Data availability

Data will be made available on request.

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8.3. Publikacja P3

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RESEARCH ARTICLE

Advancing mycotoxin degradation in agricultural waste: insights from *Hermetia illucens* larvae and frass safety analysis

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Abstract

The use of insects to transform low-value organic waste into high-protein products is an innovative approach to addressing agricultural waste challenges. This study explored the bioconversion of naturally mycotoxin-contaminated bean seed residues by *Hermetia illucens* larvae, emphasizing the quantification of mycotoxin (entomo)degradation and the safety evaluation of frass as an organic fertilizer. UHPLC-MS/MS was employed to measure changes in mycotoxin concentrations during larval bioconversion and subsequent aerobic maturation of frass. The larvae demonstrated efficient utilization of dry bean seed biomass (approx. 67%) and were found to completely degrade deoxynivalenol, monoacetoxyscirpenol, diacetoxyscirpenol, and T-2 toxin, alongside a measurable reduction in nivalenol. Accurate quantification revealed increased levels of HT-2 toxin, zearalenone, and its metabolites (α-zearalenone and β-zearalenone) in raw frass, which were not present in the original substrate. Further measurement of matured frass indicated elevated concentrations of HT-2 toxin and zearalenone, coupled with a reduction in α- and β-zearalenone. No bioaccumulation of any tested mycotoxins was detected in larvae or pupae, as confirmed through measurement protocols. This research highlights the ability of *H. illucens* to process hazardous agricultural residues into safer, resource-efficient organic fertilizers, aligning with global efforts to enhance the utilization of unique abilities of insects in not only sustainable food and feed systems but also circular biowaste management.

Keywords

black soldier fly – circular agriculture – entomoremediation – entomodegradation – organic frass fertilizer

1 Introduction

The threat of the emergence of toxigenic fungi increases with climate change, as environmental factors like temperature, CO_2 concentration and humidity promote their growth (Chhaya *et al.*, 2022). These fungi produce mycotoxins – secondary metabolites, which can pose a danger to the health and even life of humans and animals and make mycotoxin-contaminated material impossible to process for feed and food production, contributing to significant economic losses (Moretti *et al.*, 2019). According to FAO, up to 25% of global food production is contaminated with mycotoxins, posing a substantial challenge to the agricultural and processing industries (Eskola *et al.*, 2020). In certain regions where environmental conditions favour the proliferation of

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mould fungi, the percentage of contaminated food can be even higher (Gruber-Dorninger *et al.*, 2019; Kosicki *et al.*, 2016). The estimate suggests that about 60-80% of the crop is contaminated with mycotoxins, with 20% exceeding permitted limits (Johns *et al.*, 2022). This contamination necessitates proper disposal or treatment to prevent further spread.

Legumes are a good source of protein alternative to animal sources. Global production of legumes is estimated to be around 92 million tonnes per year and has an upward trend. This family of crops includes beans, which are one of the most widely grown legumes and account for 33.04% of their total production volume (Dutta *et al.*, 2022). With such large-scale production, it is natural that waste is generated, and one of the main contaminants of this waste are mycotoxins (Pavicich *et al.*, 2024).

In both agriculture and the food industry, mycotoxins represent a serious economic issue, as their chemical stability and resistance to environmental factors complicate effective neutralization (Kępińska-Pacelik and Biel, 2021a). There is growing demand for effective technologies to reduce mycotoxin content or alternative strategies for utilizing contaminated biomass to minimize the risk of further contamination. The problem of managing biomass contaminated with mycotoxins is still unresolved. Common processing methods include physical or chemical treatment (Shanakhat *et al.*, 2018; Sipos *et al.*, 2021). However, biological methods are also used, involving microorganisms or enzymes (Petruzzi *et al.*, 2014; Saladino *et al.*, 2016; Zhu *et al.*, 2016), anaerobic digestion (Ferrara *et al.*, 2021) and composting (Cucina and Tacconi, 2022). While anaerobic digestion and composting generate energy and biofertilizer, respectively, they have disadvantages – composting is time-consuming and can contribute to mycotoxin spread (Chen *et al.*, 2021), in case of biogas production it requires costly infrastructure.

Mycotoxins are well known to negatively affect animals, particularly farm animal (Kępińska-Pacelik and Biel, 2021b). However, their new representatives – insects, such as *Alphitobius diaperinus*, *Hermetia illucens* and *Tenebrio molitor*, show some reduced sensitivity or even insensitivity to certain concentrations of mycotoxins, as shown by studies on their rearing on mycotoxin-contaminated substrates. The bioaccumulation capacity of some mycotoxins by *H. illucens* was investigated by Purschke *et al.* (2017), that reared larvae on corn flour contaminated with aflatoxin B1 (88 μg/kg), aflatoxin B2 (17 μg/kg), aflatoxin G2 (46 μg/kg), ochratoxin A (260 μg/kg) and zearalenone (about 860 μg/kg). Studies on *A. diaperinus* and *H. illucens* were based on determining the ability to accumulate aflatoxin B_1, deoxynivalenol, ochratoxin A and zearalonone, whose initial feed concentrations lay in the ranges 0.02-0.5 mg/kg, 5-125 mg/kg, 0.1-2.5 mg/kg and 0.5-12.5 mg/kg, respectively (Camenzuli *et al.*, 2018). Leni *et al.* (2019) compared intake and excretion of some mycotoxins in *A. diaperinus* and *H. illucens*, fed wheat middlings contaminated with deoxynivalenol (938 μg/kg), corn distillation residues contaminated with deoxynivalenol (779 μg/kg), fumonisin 1 (573 μg/kg) and 2 (441 μg/kg), as well as corn gluten feed contaminated with deoxynivalenol (1207 μg/kg), fumonisin 1 (727 μg/kg) and 2 (294 μg/kg) and zearalenone (173 μg/kg). The effect of patulin (40 mg/kg in blended apple peelings), on the development, growth and nutritional composition of the *H. illucens* larvae was also determined, which indicated a reduction in growth rate as well as fat content, while increasing protein content (Xiao *et al.*, 2025). Additionally, *H. illucens* larvae reared on *Fusarium*-damaged kernels, containing 0.63 μg/kg ofdeoxynivalenol were tested for its accumulation and their effect on substrate conversion (Gulsunoglu *et al.*, 2019). Bosch *et al.* (2017) tested *T. molitor* larvae for tolerance and accumulation of aflatoxin B_1 occurring over a wide range from 0.01 to 0.5 mg/kg in the poultry feed. *T. molitor* larvae were also tested for the degradation and excretion of deoxynivalenol contained in wheat flour at a concentration of 8 mg/kg (Van Broekhoven *et al.*, 2017). Additionally, biological changes that may occur in the presence of zearalenone for the metabolic conversion of *T. molitor* reared on seven different substrates with different concentrations were also determined (Niermans *et al.*, 2019). The larvae of *T. molitor* reared on deoxynivalenol-contaminated wheat (30.73 and 630 μg/kg) have been investigated for safety and for use as an additional protein source for poultry (Duhra *et al.*, 2022). All these studies demonstrated a high survival rate of the insects, indicating that they were highly resistant to mycotoxins. In most cases, these insects did not exhibit bioaccumulation of mycotoxins. These studies primarily focused on the presence of mycotoxins in the larvae because the main objective of these studies was to evaluate the potential of using these insects as feed for higher animals.

Due to the increasing interest in the use of insect frass as a soil fertilizer, it is essential to examine it in terms of changes in mycotoxin concentrations. Gold *et al.* (2024) evaluated *H. illucens* frass from mycotoxin contaminated substrate as plant fertilizer. Depending on the mycotoxin, various changes in concentration were

observed, e.g. a decrease in aflatoxin concentration, no change in deoxynivalenol content, and an increase in sterigmatocystin, alternariol-monomethylether, alternariol and three types of fumonisin. The authors stated that frass can be used as a fertilizer, as frass comes rich in macro- and micronutrients, provides a green alternative to synthetic fertilizers (Gold *et al.*, 2024). However, the topic of mycotoxins in soil is still little under studied. There is information that mycotoxins degrade in soil, but they can also absorb into soil particles and thus become a source of contamination (Juraschek *et al.*, 2022). Therefore, although there are not yet any standards for acceptable concentrations of mycotoxins in fertilizers and soil additives, it is important that they contain as low a contaminant as possible.

Despite various studies, there are still many gaps in the knowledge. One of important one is the fate of mycotoxin in composted frass. This study continues research on the safety of *H. illucens* frass as soil fertilizer. The frass has been obtained from low quality beans seeds waste naturally polluted with mycotoxins, which is crucial, due to the fact that in nature mycotoxins co-occurred most often (Gold *et al.* 2024). In the contrast to previous publications, not only raw frass has been tested, but wanting to fill the knowledge gap, we performed also its composting (aerobic maturation), which stabilized it and created more suitable fertilizer for plants. This study examined the concentrations changes of 25 total different compounds (mycotoxins and metabolites) in the substrate, residue (frass), matured frass. The potential bioaccumulation of various mycotoxins in the larvae bodies was also investigated. Among these compounds, changes in the concentration of monoacetoxyscirpenol were determined for the first time, according to our knowledge. In the valorisation studies of waste contaminated with mycotoxins, those from corn or other cereals predominate. However, in the processing of legume seeds, which constitute an important segment of the agricultural market, contamination with mycotoxins is also common and is an equally important and pressing problem (Pavicich *et al.*, 2024). We therefore believe that by using for this experiment low-quality, real waste from the production of bean seeds with native levels of contamination with various mycotoxins, we will draw attention to this problem.

2 Materials and methods

Insects

The *H. illucens* larvae used in the experiment were from a colony maintained at the Institute of Agrophysics of the Polish Academy of Sciences in Lublin (Poland). The stock larvae breeding was conducted in a larval chamber on coconut fiber at 26 ± 1 °C and 70 ± 10% moisture content in the dark. Fish food (FloraZoo, Chełmża, Poland) (with composition: carbohydrates, 54.8%; protein, 25.0%; fat, 5.0%; crude fibre, 5.8%; ash, 5.7%; others, 3.7%) was used as the standard feed to provide optimal nutrients for growth and development for the larvae (Bulak *et al.*, 2023). The experiment used about 1000 four-day-old larvae per replication. Since four-day-old larvae were very small and it was difficult to count such a large number, the weight method was used. One hundred larvae were selected and weighed in three replications and on the basis of its average weight the number of larvae needed for the replication has been determined.

Substrate

The feed material used in the experiment was post-production bean seed waste (BW), obtained from a local seed producer. This material was not suitable for further use for e.g. farm animal due to its mould contamination. The dry weight (DW) of the bean seeds was 91.31% (24 h/105 °C). In order to allow the *H. illucens* larvae access to nutrients, these seeds were flooded with water and allowed to soak for 24 h. The seeds were then ground with a laboratory blender. The substrate prepared in this way was characterized by a DW of 25.63%.

Experimental design

The dosage used was 1 g fresh weight of substrate per larva. The experimental breeding was carried out in plastic containers of 16 × 24 × 14 cm. The containers were tightly closed with lids, in which ventilation holes were installed, to which air pumps (Oxyboost APR 300, Aquael, Poland) were connected to force air flow and exchange (duration of work: 4 times for 15 min/24 h). The experiment lasted for 30 days. After this time, the larvae and pupae were separated from the substrate, rinsed in distilled water and set aside in an empty container for 24 h to defecate. Then, they were washed in distilled water, counted, weighed on a laboratory balance (Ohaus Explorer Precision), measured with a ruler and finally frozen at −20 °C.

The raw frass was divided into two parts, which one was frozen and the other was intended for maturation

process. The maturation process lasted for 10 months and took place under aerobic conditions, at temperature of and 30 °C in the dark in laboratory incubator (TS 9135 Termaks). Every two days, the ripened frasses were stirred using a laboratory glass baguette. At the end of the process, the matured frasses were frozen and then analyzed.

Analysis

The larvae were weighed on a laboratory scale (Ohaus Explorer Precision EX623) and measured with a ruler. The survival rate (*S*%) of the larvae was calculated based on the formula:

$$S\% = (\text{sum of live pupae and live larvae after the experiment} \cdot 100) / (\text{number of larvae before experiment}) \quad (1)$$

Factors relating to substrate exploitation capacity were also calculated, which included: mass reduction (MR) refers to the ability of *H. illucens* larvae to utilize BW biomass (equation (2)) (Norgren *et al.*, 2020), efficiency of conversion of ingested feed (ECI) (equation (3)) and feed conversion ratio (FCR) (equation (4)) (Bordiean *et al.*, 2022). The following equations were used for their calculation:

$$MR\% = (\text{weight of ingested feed by } \textit{H.illucens} \text{ larvae} \cdot 100) / (\text{initial weight of substrate}) \quad (2)$$

$$ECI\% = \frac{\text{weight gain of } \textit{H.illucens} \text{ larvae} \cdot 100}{\text{weight of ingested feed}} \quad (3)$$

$$FCR = \frac{\text{weight of ingested feed}}{\text{weight gain of } \textit{H.illucens} \text{ larvae}} \quad (4)$$

Identification of total number of fungi: Initial substrate of 10 g was placed in sterile bags and homogenized in a Stomacher-type homogenizer (BagMixer 400, Interscience). Subsequently, 90 ml of sterile diluting fluid (containing 1 g enzymatic hydrolysate of casein, 8.5 g sodium chloride, and 1000 ml distilled water, adjusted to pH 7.0 ± 0.2) was added, and the material was homogenized for 90 s. To determine the total number of fungi, a modified spread-plate inoculation technique was employed. A series of decimal dilutions were prepared from the homogenized suspension (1:10 dilution of the study material). Then, 1 ml and 0.1 ml of the prepared dilutions were inoculated onto YGC agar (yeast extract glucose chloramphenicol) in triplicate. The inoculated plates were incubated at 25 ± 1 °C for 5-7 days. After incubation, plates containing 10-100 colonies were counted. The results were expressed as the number of colony-forming units (CFU) per gram of the sample (CFU/g). Dominant types of anamorphic fungi were determined based on colony morphology and the type of sporulation.

Analyses for the presence of mycotoxins: Standards of mycotoxins, including both unlabelled, were obtained from Romer Labs (Tulln, Austria). Solvents and salts used in extraction and analysis, including acetonitrile (gradient grade), methanol (LC-MS grade), ammonium acetate (LC-MS grade), and acetic acid (LC-MS grade), were purchased from Merck (Darmstadt, Germany). Analytical-grade water was purified using Elix 3 and Simplicity UV systems (Merck, Darmstadt, Germany).

Sample preparation was adapted from protocols with minor modifications (Sulyok *et al.*, 2006; Varga *et al.*, 2012). Briefly, 1 g of the sample was extracted with 4 ml of acetonitrile/water/acetic acid (79:20:1, v/v/v) using a Multi Reax shaker (Heidolph) for 90 minutes at room temperature. The extract was centrifuged at 7000 rpm for 10 min using a 5430 R centrifuge (Eppendorf). A 0.5-ml aliquot of the supernatant was diluted with an equal volume of methanol/water (2:8, v/v), mixed thoroughly, and centrifuged again at 14 500 rpm for 10 minutes. Subsequently, an 80-µl aliquot of the supernatant was transferred to an HPLC vial containing a microinsert, and 20 µl of the U-^{13}C-labelled internal standard solution was added. After mixing, 5 µl of the final extract was injected into the LC-MS/MS system.

Analytes were quantified using an ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) system, which included an UHPLC Nexera (Shimadzu, Tokyo, Japan) coupled to a QTRAP 5500 mass spectrometer (Sciex, Foster City, CA, USA) with a TurboIonSpray electrospray ionization (ESI) source. Chromatographic separation was performed on a Gemini C18 column (150 × 4.6 mm, 5 µm) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of two eluents: eluent A (methanol/water/acetic acid, 10:89:1, v/v/v) and eluent B (methanol/water/acetic acid, 97:2:1, v/v/v), both containing 5 mM ammonium acetate. A gradient elution was applied as follow: the elution started with 100% of A and these conditions were maintained for 2 min, then the percentage of B was increased linearly to reach 50% in 5 min and later 100% in 14 min of run, followed by a hold time of 4 min and 3.5 min re-equilibration at 100% of A. During the analyses the column temperature was maintained at 25 °C and the flow rate was 1 ml/min.

ESI-MS/MS was performed in scheduled multiple reaction monitoring (sMRM) mode, detecting both negative and positive ionization polarities in a single chromatographic run. Optimized ESI source parameters were as follows: source temperature 550 °C, curtain gas 30 psi, sheath gas 80 psi, drying gas 80 psi, collision gas medium, ion-spray voltage −4500 V and +5500 V. Summarized the optimized ESI parameters for all analysed mycotoxins are presented in Table S1.

The method validation (Table S2) involved assessing the limits of detection (LOD) and quantification (LOQ), recovery rates, and precision (as repeatability). The LOD and LOQ values were determined using a signal-to-noise ratio of 3 and 10, respectively, with a script in Analyst 1.6.3 software (Sciex). Recovery values were evaluated by spiking a mycotoxin-free substrate with mycotoxins at two different concentration levels. Precision was determined by performing three independent replicates for each concentration level.

Statistics

The experiment was conducted in three independent biological replications. Data on larval performance and mycotoxin concentrations were the mean ± SD of the three analytical replicates (n = 3). Statistica 13 software was used to perform statistical analyses. A Student *t*-test ($p < 0.05$) was used to test for a significant difference in the data for larvae parameters. Statistical differences of mycotoxin concentrations between variants of samples were tested by analysis of variance ANOVA and post-hoc Tukey's test ($p < 0.05$).

3 Results

Total number of fungi identification

Mycological examination revealed the presence of fungi in BW substrate, which are known to produce secondary metabolites (Table 1). The largest proportion of these was for molds of the *Penicillium* genus.

TABLE 1 Contribution and the type of fungi in post-production bean seed waste

	Total number of fungi	Content of fungi (%)
Natural mould-contaminated post-production bean seed waste	2500 CFU	87% *Penicillium* 5% *Fusarium* 4% *Alternaria* 2% *Mucor* 1% *Scopulariopsis* 1% *Aspergillus*

CFU, colony-forming unit.

Larval performance

The larvae, after BW bioconversion, increased their body weight up to 370.37 times compared to their initial weight at the start of the experiment. The pupae weighed 481.48 times more than the initial larvae (Figure 1a). In the case of *H. illucens* growth in length, the larvae after the experiment were 8 times longer and the pupae 9 times longer than the initial larvae (Figure 1b). Despite being reared on a nuisance substrate, the survival rate of the larvae was at a fairly high level (Figure 1c).

Breeding *H. illucens* larvae led to a significant reduction in the mycotoxin-contaminated BW substrate. The MR parameter for fresh weight (FW) and dry weight (DW) showed statistical difference, with a higher value observed for fresh matter (Table 2). However, the ECI coefficient did not differ significantly when calculated to either dry or fresh sample mass (Table 2). Similarly, no significant difference was observed between dry and fresh weight for the FCR index (Table 2).

Mycotoxins changes

Mycotoxicological studies presented in Table 3 indicate that the substrate was contaminated with various types of mycotoxins, with the highest concentration observed for nivalenol, whose level was an order of magnitude higher than that of other mycotoxins present in the substrate. Furthermore, lower concentrations of zearalenone, deoxynivalenol, di- and monoacetoxyscirpenol, as well as T-2 and HT-2 toxins, were detected in the substrate. The concentrations of other toxins analysed were below detectable levels.

Bioconversion by larvae resulted in distinct differences in the concentrations of each type of mycotoxins. The raw frass generated after *H. illucens* breeding was characterized by complete elimination of mycotoxins such as deoxynivalenol, monoacetoxyscirpenol, diacetoxyscirpenol and T-2 toxin. A decrease in concentration in raw frass was also observed for nivalenol (by about 5.88 times compared to the substrate). However, for HT-2 toxin and zearalenone, their concentrations increased by 1.79 times and 1.18 times, respectively (compared to the substrate). Additionally, the appearance of toxic compounds that were not initially present in the substrate was recorded in raw frass, these were α-zearalenol and β-zearalenol (Table 3).

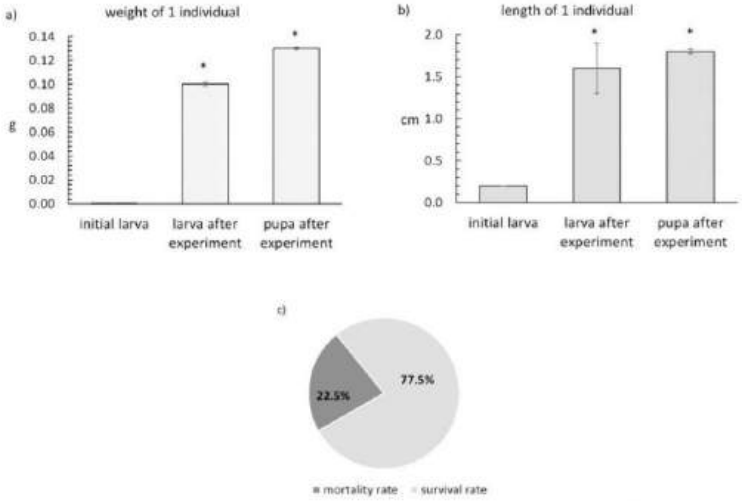

FIGURE 1 (a) Average biomass of *H. illucens* larvae and pupae after the experiment. (b) Changes in length of *H. illucens* after the experiment (c) Survival rate of *H. illucens* on mycotoxin-contaminated bean waste. Means ± SD, n = 3, Student *t*-test, $p < 0.05$. The asterisk (*) indicates statistical differences between initial larvae and individuals after the experiment.

TABLE 2 Parameters of exploitation of mycotoxin-contaminated bean waste by *H. illucens* larvae

	On FW	On DW
MR (%)	72.97 ± 1.37*	66.91 ± 1.70
ECI (%)	11.37 ± 0.95	13.76 ± 1.23
FCR	8.86 ± 0.74	7.33 ± 0.65

Means ± SD, n = 3, Student *t*-test, $p < 0.05$. The asterisk (*) indicates statistical differences between initial larvae and individuals after the experiment.MR, substrate mass reduction; ECI, efficiency of conversion of ingested feed; FCR, feed conversion ratio; FW, fresh weight; DW, dry weight.

Maturation of frass also led to varying changes. In the case of nivalenol, this resulted in a further decrease in its concentration to undetectable levels. Mycotoxins, which appeared later in the raw frass, also decreased in concentration in the mature frass. In the case of α-zearalenol, its concentration decreased by 1.31 times, and in the case of β-zearalenol by 1.71 times. In contrast, for HT-2 toxin and zearalenone, their concentrations in mature frass continued to increase by 2.02 times and 1.47 times, respectively, compared to the substrate (Table 3).

Interestingly, *H. illucens* larvae that were reared on the contaminated substrate turned out not to show the bioaccumulation of any of the mycotoxins. Similarly, in the case of pupae, the content of all mycotoxins was at undetectable levels (Table 3).

4 Discussion

Larval performance

Mycotoxins in the feed substrate could have influenced the growth of *H. illucens* larvae. Despite the significant rise in average body weight (Figure 1a,b), it was lower than the average weight reported in the literature (0.158 ± 0.02 g FW), for larvae bred on optimal substrates (Barragan-Fonseca *et al.*, 2017). However, in the case of *H. illucens* reared on naturally contaminated corn semolina, the average larval weight was lower (0.065 g) than in the present study (Figure 1) (Purschke *et al.*, 2017). The lengths of the larvae and pupae in the present experiment were also lower (Table 1), compared to the larvae reared on the optimum feed (2.0 cm and 2.2 cm, respectively) (Proc *et al.*, 2020). However, it should be noted that in this research, the larvae were used to treat and utilize waste bean biomass above all. The survival rate of larvae in this study was lower than that in studies testing the effects of aflatoxin B_1 (Bosch *et al.*, 2017; Meijer *et al.*, 2019) or zearalenone, deoxynivalenol, and ochratoxin (Camenzuli *et al.*, 2018) where *H. illucens* survival ranged between 92.7 and 97.3%. In those studies, however, the larvae were bred on optimal

TABLE 3 Changes in concentrations (μg/kg) of mycotoxins after bean waste (BW) bioconversion by *H. illucens* larvae (means ± SD, n = 3)

No.	Mycotoxin	Bean waste	Raw frass	Mature frass	Larvae	Pupae
1	Nivalenol	147 ± 12.6a	25 ± 22.1b	<LOD	<LOD	<LOD
2	Deoxynivalenol	31.8 ± 2.3	<LOD	<LOQ	<LOD	<LOD
3	3-acetyldeoxynivalenol	<LOD	<LOD	<LOD	<LOD	<LOD
4	15-acetyldeoxynivalenol	<LOD	<LOD	<LOD	<LOD	<LOD
5	Deepoxy-deoxynivalenol	<LOD	<LOD	<LOD	<LOD	<LOD
6	DON-3-glucosid	<LOD	<LOD	<LOD	<LOD	<LOD
7	Fusarenon-X	<LOD	<LOD	<LOD	<LOD	<LOD
8	Zearalenon	75.5 ± 13.6a	88.9 ± 0.4ab	111.2 ± 14.2b	<LOD	<LOD
9	α-Zearalenol	<LOD	196.8 ± 1.2a	149.9 ± 1.0b	<LOD	<LOD
10	β-Zearalenol	<LOD	63.9 ± 0.3a	37.3 ± 1.1b	<LOD	<LOD
11	α-Zearalanol	<LOD	<LOD	<LOD	<LOD	<LOD
12	β-Zearalanol	<LOD	<LOD	<LOD	<LOD	<LOD
13	Neosolaniol	<LOD	<LOD	<LOD	<LOD	<LOD
14	Monoacetoxyscirpenol	20.0 ± 0.2	<LOQ	<LOD	<LOD	<LOD
15	Diacetoxyscirpenol	13.6 ± 0.6	<LOD	<LOD	<LOD	<LOD
16	Aflatoxin B_1	<LOD	<LOD	<LOD	<LOD	<LOD
17	Aflatoxin B_2	<LOD	<LOD	<LOD	<LOD	<LOD
18	Aflatoxin G_1	<LOD	<LOD	<LOD	<LOD	<LOD
19	Aflatoxin G_2	<LOD	<LOD	<LOD	<LOD	<LOD
20	Fumonisin B_1	<LOD	<LOD	<LOD	<LOD	<LOD
21	Fumonisin B_2	<LOD	<LOD	<LOD	<LOD	<LOD
22	Fumonisin B_3	<LOQ	<LOD	<LOD	<LOD	<LOD
23	HT-2 toxin	74.3 ± 11.3a	133 ± 34.4b	150.2 ± 1.6b	<LOD	<LOD
24	T-2 toxin	12.4 ± 1.5	<LOD	<LOD	<LOD	<LOD
25	Ochratoxin A	<LOD	<LOD	<LOD	<LOD	<LOD

The statistical difference between samples for a given mycotoxin was checked using ANOVA and post-hoc Tukey's test ($p < 0.05$) (in the table marked with different letter: a,b,c). LOD, limit of detection; LOQ, limit of quantification.

substrate spiked with exogenously added mycotoxins. The increased mortality in this study may therefore be related to the presence of mycotoxins such as, nivalenol, monoacetoxyscrirpenol, diacetoxyscirpenol, T-2 toxin or HT-2 toxin. Nevertheless, the *H. illucens* showed a high survival rate, indicating some resistance to mycotoxins tested in this study, especially T-2 toxin and HT-2 toxin whose sum of concentrations exceeded the maximum allowable limit for unprocessed cereal grains in food which is 50 μg/kg (European Commission 2024), and nivalenol, which according to the European Food Safety Authority its temporary tolerated daily intake is 1.2 μg/kg BW per day (EFSA Panel, 2013).

The rearing of *H. illucens* larvae on BW biomass appears to be a suitable method for managing this type of waste, with a high utilization rate (66.91 ± 1.70% DW, Table 2). Surendra *et al.* (2020) reviewed waste biomass reduction by *H. illucens* larvae, reporting the lowest for cattle manure (12.7%) and highest for a mixture of food waste and human faeces (68.4 68.8%). Therefore, the results obtained in this study can be considered high.

ECI, commonly used in entomological studies, refers to the percentage of substrate mass converted to insect biomass. In this study, ECI for BW was lower (Table 2) than for optimal feeds (like chicken feed, which reached approx. 17.6%) (Barragan-Fonseca *et al.*, 2017). Regarding FCR, which measures the mass of substrate required to produce one kilogram of larval biomass, the value obtained in this experiment was higher than that for optimal feeds (average FCR approx. 1.8; Barragan-Fonseca *et al.*, 2017). Lower ECI and higher FCR obtained for BW suggest reduced larval productivity, which may be negative for breeding programs focused on maximizing larval biomass. This findings indicates that for efficient larval growth on this substrate, it should be mixed with other feed (or different biowastes) with a more bal-

anced nutrient composition for *H. illucens*. However, in entomoremediation, high FCR and MR are desirable for waste disposal.

Characterization of fungi in the substrate

The fungi *Penicillium*, *Fusarium*, *Alternaria* and *Aspergillus*, identified in the substrate used (Table 1), are common in bean seeds and can produce mycotoxins (Duarte Santos and Badiale Furlong, 2021, Chen *et al.*, 2021; Khan *et al.*, 2024). However, *Scopulariopsis* and *Mucor* fungi (present in the smallest amounts in the BW substrate; Table 1) are not significant mycotoxin producers, but can cause infections in humans and animals. *Scopulariopsis* fungi, which are keratinophilic, can cause pathogenic infections or allergies (Viegas *et al.*, 2013), while *Mucor* can lead to mucormycosis of the blood, intestines, skin, nose or brain (Morin-Sardin *et al.*, 2017).

All the mycotoxins detected in the substrate and frass appeared to be produced by *Fusarium* spp. (Table S3), a Nectriaceae family member, known for worldwide crop losses (Torbati *et al.*, 2021). It can attack entire plants as well as seedlings and seeds, as was the case with the tested BW substrate (Qu *et al.*, 2024). Some toxigenic *Fusarium* species can produce zearalenone and other mycotoxins belonging to the trichothecenes groups. The mycotoxins identified in BW (Table 3) belong to this groups (Chen *et al.*, 2020). Trichothecenes shares a tetracyclic sesquiterpenoid core with various functional groups which are attached (Qu *et al.*, 2024).

Mycotoxins in insect samples

This study is the first to investigate the presence and changes of monoacetoxyscirpenol in the substrate, residue and in *H. illucens* larvae (Table 3). The results revealed no contamination of larvae or pupae with any of the tested mycotoxins (Table 3), aligning with findings by Leni *et al.* (2019) and Purschke *et al.* (2017). This suggests mycotoxins were either not bioavailable or, most probably, efficiently detoxified. Camenzuli *et al.* (2018) and Gulsunoglu *et al.* (2019) detected deoxynivalenol in *H. illucens* larvae, but at a low levels. Nevertheless, most studies conclude that mycotoxin contamination is absent in *H. illucens* larvae.

Mycotoxins in raw frass

Raw frass from BW was characterized by a reduced concentration of deoxynivalenol to undetectable levels (Table 3). In contrast, Leni *et al.* (2019) and Purschke *et al.* (2017), recorded higher amounts of this mycotoxin in *H. illucens* frass than in the initial substrate, probably due to the significantly higher initial contamination in their studies. Lower amounts of the mycotoxin are easier to biodegrade by the larvae and associated microorganisms and their enzymatic apparatus. Gold *et al.* (2024) reported no change in mycotoxin concentrations in frass. Nevertheless, it should be noted that the concentration of nivalenol, which belongs to the same group of mycotoxins – trichothecenes type B – also decreased in frass in this study. So far, to the best of the authors' knowledge, it has not been studied how *H. illucens* affects the presence of nivalenol. In the context of the agronomic effects of deoxynivalenol, its presence may reduce the mycoparasitic activity of the commonly occurring *Trichoderma* fungus, which can inhibit the growth of some *Fusarium spp.*, and this occurs by limiting the expression of the nag1 chitinase gene (Palumbo *et al.*, 2008).

Monoacetoxyscirpenol and diacetoxyscirpenol, type A trichothecenes, were not detected in frass (Table 3). These mycotoxins are highly toxic to animals, causing severe health issues or death Pathre *et al.* (1974). They are also dangerous to plants and microorganisms (Schollenberger *et al.*, 2007). The rearing of *H. illucens* larvae resulted in the biodegradation of this mycotoxin may be related to the CYP450 gene, which is implicated in the detoxification of xenobiotics and which has been found in the *H. illucens* genome (Meijer *et al.*, 2019; Shah *et al.*, 2024). T-2 toxin ranks among the most dangerous trichothecenes type A produced by *Fusarium* (Janik *et al.*, 2021). Its presence was not detected in raw BW frass despite its occurrence in the substrate (Table 3), which may be related to degradation after bioconversion by the larvae. A second possibility could be biotransformation into the HT-2 toxin metabolite, whose concentration in raw frass increased compared to the substrate. HT-2 toxin is formed by hydrolysis of T-2 toxin and differs from it by a functional group at the C2 position. However, like its parental compound, it can produce equally negative health effects (Vörösházi *et al.*, 2024). The T-2 toxin has an inhibitory effect on the yeast *Saccharomyces cerevisiae* (Madhyastha *et al.*, 1994), which is known for increasing nutrient availability for plants (Csambalik and Tóbiás, 2018) and their ability to bioremediate soil contaminated with heavy metals (Massoud *et al.*, 2019). Thus, the reduction of T-2 toxin (Table 3) in frass, when used as a soil additive, is a positive outcome, as it limits the inhibitory potential for this type of yeast. Additionally, *S. cerevisiae* does not show sensitivity to the metabolite HT-2 toxin (Madhyastha *et al.*, 1994).

Zearalenone levels increased in raw frass (Table 3), possibly due to the high moisture content (60-70%) required for *H. illucens* (Hoc *et al.*, 2019). The content

of this mycotoxin during storage already increases if the moisture content exceeds 30-40% (Tola and Kebede, 2016). An increase in zearalenone in *H. illucens* breeding residues was also observed in studies by Leni *et al.* (2019) and Camenzuli *et al.* (2018). Leni *et al.* (2019) noted the presence of zearalenone in the frass despite the fact that its concentration in the substrate (corn distillation residues) was below the detection limit, and explained this as a result of hydrolytic activity of the larvae. The presence of zearalenone can inhibit the beneficial *Bacillus* bacteria (Madhyastha *et al.*, 1994), which aid soil nutrient cycling and plant stress responses (Saxena *et al.*, 2020). In turn, α-zearalenol and β-zearalenol, metabolites of zearalenone, appeared in the raw BW frass. They were most likely formed during the first phase of enzyme-catalyzed biotransformation of zearalenone and differ in the position of the hydroxyl group in the cyclohexane ring. The α-zearalenol are even more toxic than parent zearalenone, however both of them have estrogenic activity and may interact with oestrogen receptors (Keller *et al.*, 2015).

Mycotoxins in mature frass

Matured frass was characterized by a decrease in nivalenol concentration to undetectable levels (Table 3). This could be related to enzymatic de-epoxidation, which reduces toxicity of this compound, as it is associated with the presence of epoxy group. Such process can occur during microbial metabolism (Sundstøl Eriksen *et al.*, 2004), including the *H. illucens* gut microbe. De-epoxidation is a reductive chemical process and can occur under both aerobic and anaerobic conditions, its occurance during frass maturation is desirable (Islam *et al.*, 2012).

The frass maturation caused increase in the concentrations of zearalenone and HT-2 toxin (Table 3). This may be due to the continued proliferation of *Fusarium* fungi, which develop in humid environments and a temperature range of −3 to 35 °C (Ejaz *et al.*, 2023). The decrease in α-zearalenol and β-zearalenol it may be due to further reactions associated with zearalenone biotransformation (Gajęcka *et al.*, 2009). During this type of decomposition, glucuronide conjugates are produced, which strongly differ in estrogenic activity, showing lower intensity (Frizzell *et al.*, 2015).

Frasses from *H. illucens* bioconversion, both raw and matured, had reduced mycotoxin diversity. The total number of detected mycotoxins decreased from seven in the substrate to five in the case of raw frass and four after maturation of the frass. There are no legal regulations on maximum limits for mycotoxin concentrations in organic fertilizers, composts or soil. Such regulations are established only for food and feed products. The fate of mycotoxins introduced into soil is also still a poorly researched topic, as highlighted in the review by Juraschek *et al.* (2022). A few articles stated that some mycotoxins can be absorbed, degraded or run off with leachate (Juraschek *et al.*, 2022). However, an important factor influencing the content of such contaminants is the soil itself, including its type and structural properties (Schrader *et al.*, 2013). The mycotoxin content in soil can also be significantly affected by the presence of different types of organisms that can degrade toxigenic fungi (such as *Fusarium*) or directly mycotoxins, that have already been produced (Schrader *et al.*, 2013). These organisms can include soil macrofauna, such as earthworms (e.g. *Lumbricus terrestris*), collembolans (e.g. *Folsomia candida*) or nematodes (e.g. *Aphelenchoides saprophilus*), as well as associated microorganisms (Schrader *et al.*, 2013). Various types of microorganisms, including those inhabiting the soil environment, can also possess mycotoxin-degrading properties, contributing to soil detoxification (Vanhoutte *et al.*, 2016). An important aspect of agricultural management is the reduction of introduced mycotoxins, which may be contained in natural fertilizers, such as manure, or in straw or post-harvest plant residues. Particularly, biowastes of plant origin can introduce mycotoxins at concentrations as high as 6-25 g/ha (Juraschek *et al.*, 2022). It has been proven that some microorganisms have the ability to degrade mycotoxins (Ji *et al.*, 2016), but it is also possible to encounter situations where mycotoxins inhibit certain bacteria or yeasts also found in the soil (Madhyastha *et al.*, 1994).Therefore, *H. illucens* frass appears to be safer soil additive than untreated biowaste, due to its lower mycotoxins content.

5 Conclusion

This study confirms that rearing *H. illucens* larvae effectively reduces bean seed biowaste contaminated with mycotoxin, as indicated by the significant dry mass reduction. No tested mycotoxins were detected in larvae or pupae, ensuring their safe use. Bioconversion reduced five mycotoxins, with four to reaching undetectable levels, though HT-2 toxin and zearalenone increased, along with two zearalenone metabolites in raw frass. Frass maturation did not reduced HT-2 toxin and zearalenone, but lowered the concentrations of zearalenone metabolites. The obtained frasses were characterized by

a lower diversity of mycotoxins, suggesting that they may be safer for use in soil than bean waste biomass. Further research should be conducted, e.g. to determine the microbiological composition after long-term use of frass after bioconversion of a substrate contaminated with mycotoxins, or to study the growth, development and overall plant health in soil fertilized with this type of frass.

Supplementary materials

Data is available on https://doi.org/10.1163/23524588-bja10296 under Supplementary Materials.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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8.4. Publikacja P4

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Linking waste recycling and sustainable agriculture: Phytohormone-rich biofertilizer from black soldier fly frass

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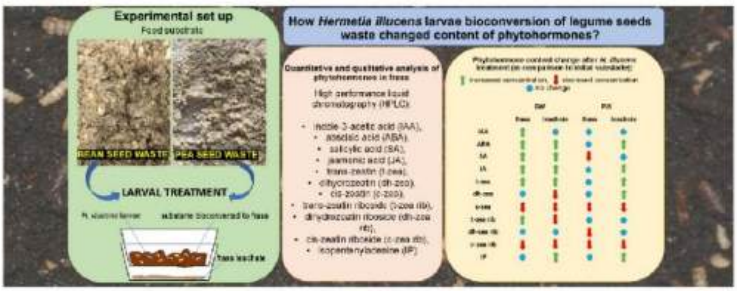
HIGHLIGHTS

- Bioconversion enriches frass with auxins, cytokinins and plant stress hormones.
- Frass from bean waste had higher auxin and cytokinin concentrations.
- Pea waste leachates were richer in trans-zeatin, cis-zeatin and IP.
- Results showed frass potential as biofertilizer beyond nutrient supply.
- Insect-mediated waste recycling supports sustainable resource management.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic fertilizers obtained through bioconversion processes represent a key component of sustainable and cleaner agricultural production systems. Among these, insect frass has emerged as a promising biofertilizer due to its nutrient richness, beneficial microbiota, and plant growth-promoting properties. However, the phytohormonal composition of frass produced by *Hermetia illucens* (black soldier fly, BSF) larvae remains insufficiently characterized. This study provides a comprehensive assessment of 3 major phytohormone group in BSF frass and frass leachates derived from the bioconversion of pea (PW) and bean (BW) seed waste - representative agri-food by-products from legumes. Both substrates supported efficient larval development, with BW frass exhibiting higher concentrations of indole-3-acetic acid, stress-related hormones, and total sum of cytokinins, while PW leachates contained elevated levels of trans-zeatin, cis-zeatin and IP. IAA and some cytokinines were also detected in the larvae, suggesting metabolic interactions between insects, microbiota, and substrates. Overall, our findings reveal that BSF-mediated waste bioconversion enhances the phytohormonal composition of frass more than its decomposition with only microbiota, thereby increasing its potential as a multifunctional, eco-friendly biofertilizer. This approach illustrates how insect bioconversion can add functional value to agri-food waste within sustainable production systems.

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Abbreviations:

BW –	bean seed waste
PW –	pea seed waste
IAA –	indole-3-acetic acid
ABA –	abscisic acid
SA –	salicylic acid
JA –	jasmonic acid
t-zea –	trans-zeatin
dh-zea –	dihydrozeatin
c-zea –	cis-zeatin
t-zea rib –	trans-zeatin riboside
dh-zea rib –	dihydrozeatin riboside
cis-zea rib –	cis-zeatin riboside
IP –	isopentenyladenine
DW –	dry weight
FW –	fresh weight

1. Introduction

The insect breeding has been stimulated in recent years due to the constant increase in protein demand for feed and food purposes, associated with the growth of the human population (van Huis et al., 2025). Insects are a great source of proteins, which are primarily used to feed livestock, aquaculture, or some domestic pets (Zou et al., 2024). One example of insects reared at industrial scales is *Hermetia illucens* (Diptera: Stratiomyidae, Linnaeus 1758), also known as a black soldier fly. Insect rearing has many advantages from an economical and environmental point of view: they generates fewer greenhouse gases than farming other livestock (van Huis and Oonincx, 2017), they need less water, their food substrate may be a biowaste, and they require a much smaller area to breed them successfully (as the larvae crates are stacked in heights) (Spykman et al., 2021). Furthermore, with proper maintenance and management, the production of *H. illucens* can become highly sustainable and almost free of by-products (Purkayastha and Khanal, 2024). This insect can be used for other technological applications at any stage of its development: larvae and pupae primarily as feed for animals and a source of protein and fats (Kawasaki et al., 2019), flies as well as pupal remains (puparia) can be used to extract chitin (Triunfo et al., 2022) or in pristine form as biosorbent (Bąk et al., 2024). Puparia also can be used as a substrate for biochar production (Bulak et al., 2023). In addition, such rearing also produces frass, i.e., the remains of the substrate the larvae were fed, their droppings, and dead individuals, as well as the entire pool of microorganisms living in and on the body of the larvae (Schmitt and de Vries, 2020). Frass, similarly to manure, is a type of natural fertilizer and therefore could be used as a soil additive, also due to its high macro- and micronutrient content (Kaczor et al., 2025; Lomonaco et al., 2024) and a positive microbiota load (Nurfikari et al., 2024). Although the interest in research on frass for fertilization purposes is increasing, frass has not been tested widely so far for its phytohormone content, and the knowledge about the occurrence of these compounds remains very limited (Lopes et al., 2025).Phytohormones are organic compounds that express activities on the plant already at very low concentrations (Asami and Nakagawa, 2018). They are competent to regulate the morphological and physiological processes of the plant at each stage of its development. Phytohormones are also responsible for adaptability, allowing the plant to react to changes in environmental conditions in the presence of abiotic (e.g., weather changes) or biotic (e.g., pest attack) stresses (Bari and Jones, 2009). Recently, intensifying climate change has had an extraordinary impact on flora. Examples include periods of drought or, conversely, increasingly frequent heavy rains, which can directly affect the aboveground part of the plant but also contribute to changes in soil properties, which undoubtedly affect the whole plant. All this requires the plant to react quickly to adapt and adjust to the prevailing conditions (Janni et al., 2023). The effects of climate change must also be faced by agriculture, which is forced to respond to the changes taking place to obtain the greatest and most fertile yield and minimize possible damage and losses. Therefore, if insect frass could contain stimulating plant phytohormones, such as auxins and cytokinins, it could not only be a natural fertilizer but also act as a stimulant, improving plant growth and mitigating environmental stresses.

Legumes, such as beans, peas, lentils, and chickpeas, are important annual agronomic crops grown primarily for protein-rich seeds (20 - 40%) (Haque et al., 2016) and provide additional nutrients, including fiber, B vitamins, minerals, and antioxidants (Maphosa and Jideani, 2017). Their consumption can reduce saturated fat intake, which is linked to elevated blood cholesterol and lifestyle disease risk (Mitchell et al., 2009). Legumes improve soil fertility via symbiosis with nitrogen-fixing nodule bacteria - especially *Rhizobium* spp. - that convert atmospheric N_2 into plant-available forms (Kebede, 2021), supporting sustainable agriculture and their use in crop rotations to enhance soil fertility and structure (Graham and Vance, 2003).

According to the FAO, global production of legumes in 2022 was approximately 9.6 billion tons, and it is estimated that production will increase to 125 million tons by 2032 (FAO, 2023). However, during processing, primarily during cleaning, sorting, and grinding, waste is generated, which can constitute from 5 to as much as 25% of the total weight (Karaca and Nickerson, 2022). These low-quality wastes, especially when contaminated with mycotoxins, must be withdrawn from the production cycle and disposed of e.g., by incineration under controlled conditions, generating economic losses and additional costs (Chen et al., 2020). From the other side, nutritionally rich waste should be managed in a more environmentally-friendly way, reducing its biomass and revaluing it. One method may be bioconversion using *H. illucens* larva.

Despite the increasing interest in *H. illucens* frass as a soil amendment, its phytohormone composition remains largely uncharacterized (Barragan- Fonseca et al., 2022; Lopes et al., 2025). To our knowledge, only one study has directly quantified phytohormones in *H. illucens* frass (Green, 2023). This gap is important because phytohormones can act at trace concentrations to modulate plant growth and stress responses, meaning that frass may influence crops not only through nutrients and microbial inputs but also via hormone-mediated, biostimulant-like effects.

Therefore, the study is projected to address this gap by profiling major phytohormone classes in frass produced from larval bioconversion of pea and bean seed waste. We also analyze frass leachate and larvae and include substrates without larvae as controls. In order to provide a better assessment of the impact of the larvae on their content in waste, analyses for phytohormones were also carried out in the substrates without the larvae. In this study we analyzed indole-3-acetic acid (IAA), abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), trans-zeatin (t-zea), dihydrozeatin (dh-zea), cis-zeatin (c-zea), trans-zeatin riboside (t-zea rib), dihydrozeatin riboside (dh-zea rib), cis-zeatin riboside (cis-zea rib), isopentenyladenine (IP), kinetin, kinetin riboside, and ortho- and meta-topolins. This research provides new knowledge about the presence of a wide amount of phytohormones from different classes in the frass, frass leachate, and larvae of *H. illucens*.

2. Materials and methods

2.1. Experimental set-up

Experiments were performed using *H. illucens* larvae obtained from a breeding conducted at the Institute of Agrophysics of the Polish Academy of Sciences in Lublin (Poland). In order to standardize the size of the larvae used in the experiment, they were sieved with a mesh diameter of 1 mm. Before transferring the larvae to the experimental substrates, they

were set aside in an empty box for 24 h to clean the intestines.

Experimental substrates were peas (PW – pea waste) and beans (BW – bean waste) seed waste obtained from a local seed producer. To allow the larvae to consume feed, both variants of the waste were flooded with distilled water and left for 24 h to soak. After this time, they were blended to a pulpy consistency. As prepared substrates had a dry weight (DW) of 32.32% for BW and 23.53% for PW.

The experiment used 1000 larvae and a substrate dose of 150 mg DW per larvae, therefore 464.12 g FW of initial substrate for BW and 637.62 g FW of PW were used. Rearing was done in an air-conditioned room with a temperature 26 ± 2 °C. To determine how the presence of feeding *H. illucens* larvae affects the concentrations of phytohormones, control PW and BW substrates without the addition of the larvae were also included by leaving them at the same conditions for the whole length of the experiment. To minimize edge effects during the experiment, containers with larvae and with substrates without larvae were regularly swapped every 3 days in a random manner. The experiment lasted 30 days and was conducted in plastic boxes (16 x 24 × 14 cm) with closed lids. The lids had mesh-protected vents connected to a pump (Oxyboost APR 300, Aquael, Poland) allowing airflow (15 min aeration, 4 times a day), which also prevented the larvae from escaping. This method of aeration had no significant effect on the moisture content of the substrate. In addition, the containers had holes in the bottom (smaller than larvae size) through which the resulting leachate dripped into a second box underneath. This leachate was taken for the analysis at the end of the experiment (water was not added during the test).

2.2. Analysis

Chemicals used for sample preparation and high-performance liquid chromatography (HPLC) analysis were obtained from Sigma-Aldrich Sp. z o.o. (Poznań, Poland). Analytical standards, including both unlabeled and stable isotope-labeled compounds, were purchased from Olchemim (Olomouc, Czech Republic).

During the experiment, the weight and length of the randomly selected the larvae (n = 20) were measured weekly. At the end of the experiment, all the larvae were taken from the resulting frass, rinsed with distilled water, and left in the empty container for 24 h to clean the intestines from a feed remains. Samples of frass, leachate, and control residue were weighed and frozen (−20 °C). The dry weight of frass, frass leachate, and control residues was determined after drying the samples in a laboratory oven at 105 °C for 24 h. Survival rate and larval pupation at the 30-day stage of their rearing (the duration of the experiment) were calculated based on the formulas used by (Cho et al., 2020). The protein content of the larvae was determined by converting the total nitrogen obtained with the Thermo Scientific Flash 2000 Organic Elemental Analyzer (multiplication of nitrogen content and 6.25 (Weththasinghe et al., 2021)).

For phytohormone analysis, seed waste material, insect samples, frass, and frass leachate were lyophilized. From a well-mixed pool of each sample type, smaller parts were randomly taken, homogenized, and an internal standard mixture was added to each sample. This mixture included deuterium-labeled indoleacetic acid, abscisic acid, salicylic acid, and jasmonic acid, as well as ^{15}N-labeled trans-zeatin. Phytohormones were extracted twice using a methanol:water:formic acid solution (15:4:1, v/v/v), according to the protocol described by (Ivanov Dobrev and Kamínek, 2002), with modifications after (Štefancič et al., 2007). The combined extracts were evaporated to dryness and reconstituted in 1 M formic acid.

Extracts were fractionated using Oasis MCX 30 mg solid-phase extraction (SPE) columns (Waters). Acidic phytohormones (auxins and stress phytohormones) were eluted with methanol, whereas basic phytohormones (cytokinins) were eluted with 0.35 M ammonia in 60% methanol. Both eluates were evaporated to dryness and reconstituted in 100 µL of methanol.

Chromatographic separation was performed using a Supelco Ascentis RP-Amide HPLC column (75 mm × 4.6 mm, 2.7 µm particle size). For acidic phytohormones (including auxins and stress-related hormones), the mobile phase consisted of 0.1% formic acid in water (solvent A) and a 1:1 (v/v) acetonitrile:methanol mixture (solvent B). For basic phytohormones (cytokinins), the mobile phase consisted of 0.001% acetic acid in water (solvent A) and the same 1:1 acetonitrile:methanol mixture (solvent B). In both cases, gradient elution was applied at a flow rate of 0.5 mL/min.

Analysis was carried out using an Agilent Technologies 1260 HPLC system coupled to an Agilent Technologies 6410 Triple Quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Detection was performed in multiple reaction monitoring (MRM) mode, monitoring the two most abundant product ions for each analyte. Calibration curves were constructed for each compound using the corresponding analytical standards.

2.3. Statistical analysis

The experiment was conducted in three independent biological replications. The number of technical replicates for each instrumental analysis (HPLC and protein content) for each sample was 3-5. All phytohormones were measured in all samples. The results presented below are the mean ± standard deviation. Statistica 13 software was used to perform statistical analyses. For each hormone, differences among sample types were assessed separately within each feed variant (BW and PW) using one-way ANOVA followed by Tukey's HSD post hoc test. To account for multiple testing across hormones, the ANOVA p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure, applied separately within BW and within PW (Tab. 1S). Effect sizes were quantified using Cohen's d (Tab. 2S, 3S, 4S). Student's t-test ($p < 0.05$) was used to check for differences in concentrations of a given phytohormone within a given sample between the BW and PW variants. Limit of detection (LOD) and limit of quantification (LOQ) for each analyzed compound are reported in Tab. 5S.

3. Results

3.1. Yield of larvae and rearing by-products after waste bioconversion

Frass yields differed significantly between BW and PW on both fresh weight (FW) and dry weight (DW) bases (Fig. 1). PW frass weighed 254.41 g FW and 48.62 g DW - 2.17-fold and 2.49-fold higher than BW, respectively. Controls (microbial decomposition without larvae) showed similar residual masses between variants, averaging 343.33 g FW and 58.08 g DW with no statistical differences (Fig. 1). Frass leachate did not differ between BW and PW, averaging 46.83 g FW and 1.32 g DW

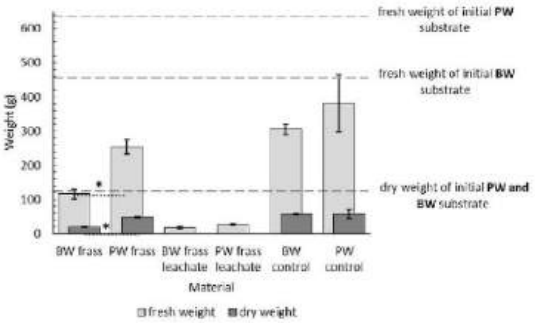

Fig. 1. The yield of frass, frass leachate, and control residues (decomposition without the larvae) after one month of experiment (mean ± SD). The asterisk (*) indicates statistical differences between BW and PW variants (t-Student test, $p < 0.05$).

(Fig. 1).

Larval fresh weight did not differ statistically between diets and peaked in the second week and then declined (Fig. 2a). Larval lengths were also similar between variants, peaking in the first week (Fig. 2b).

Survival averaged 92.85% and pupation 69.04% in both treatments, with no significant differences (Table 1). Protein content differed, with PW-fed larvae containing 1.12-fold more protein than BW-fed larvae (Table 1).

3.2. Auxin

IAA concentrations differed significantly between BW and PW in the initial substrates and frass (Table 2). BW frass showed an exceptionally high IAA level, rising 305.46-fold versus the BW substrate. In contrast, BW frass leachate and larvae contained IAA levels similar to the substrate. In PW, IAA decreased in frass, leachate, and larvae relative to the PW substrate. After one month of microbial decomposition, IAA increased in both controls: 11.92-fold for BW and 22.38-fold for PW (Table 2).

3.3. Plant stress hormones

The BW variant showed the largest increases in abscisic acid (ABA) in frass and frass leachate, rising 10.0-fold and 11.2-fold over the BW substrate, respectively. Salicylic acid (SA) followed a similar pattern, increasing from below the limit of detection in the initial substrate to approximately 10 ng mg^{-1} DW frass and frass leachate after larval rearing. An increase in SA concentration was also observed for the control residue, but when compared to the frass, the SA concentration in the control was 4.12 times lower. Jasmonic acid (JA) also increased in the control residues, frass and leachates, but in this case the contents in the frass and leachates were lower than in the control, by 2.32 times and 3.17 times, respectively. All three stress hormones were not detected in *H. illucens* larvae after BW feeding (Table 3).

In the PW variant, increases in ABA and SA concentrations were observed only in leachate after larval rearing; only ABA rose significantly, by 7.63-fold versus the PW substrate. Concentration of JA increased in the control, frass, and leachate, with the highest concentration determined for both frass leachate and control residues (Table 3). As in the BW variant, stress hormone content was also not recorded for larvae reared on PW (Table 3).

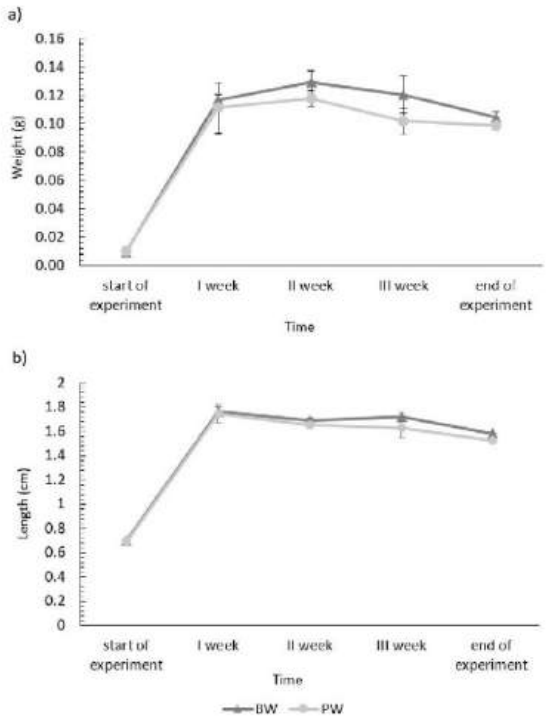

Fig. 2. Growth of larval fresh biomass during the course of the experiment: (a) average weight increase per one larva, (b) average length increase per one larva (mean ± SD).

Table 1
Developmental indices and protein content of *H. illucens* larvae after rearing on bean waste and pea waste (mean ± SD). The asterisk (*) indicates statistical differences between BW and PW variants (t-Student test, $p < 0.05$).

Parametr	Bean waste (BW)	Pea waste (PW)
Pupation rate (%)	68.07 ± 2.15	70.00 ± 1.56
Survival rate (%)	94.40 ± 1.02	91.30 ± 2.52
Protein content in larvae (%)	54.62 ± 2.81*	61.15 ± 1.91*

Table 2
The content of IAA (indole-3-acetic acid) hormone in the substrates, controls without larvae, frass, leachates, and *H. illucens* larvae. The asterisk (*) indicates statistical differences between BW and PW variants (t-Student test, $p < 0.05$). Different letters indicate statistical differences between samples from one feed variant (Tukey's test, $p < 0.05$).

	Concentrations of IAA (ng·mg^{-1} DW)	
	Bean waste	Pea waste
Initial substrate	1.58 ± 0.16a[a]	1. 00 ± 0.08a[a]
Control	18.83 ± 6.41a	22.38 ± 16.88b
Frass	482.63 ± 21.26b*	[a]0.05 ± 0.01a*
Frass leachate	1.11 ± 0.37a	0.87 ± 0.49a
Larvae	0.97 ± 0.35a	0.43 ± 0.18 a

[a] LOD < result ≤ LOQ.

Table 3
The content of stress hormones (ABA-abscisic acid, SA-salicylic acid, JA-jasmonic acid) in the substrate, control residue without larvae, frass, leachate, and *H. illucens* larvae. The asterisk (*) indicates statistical differences between BW and PW variants (t-Student test, $p < 0.05$). Different letters indicate statistical differences between samples from one feed variant (Tukey's test, $p < 0.05$).

	Concentrations of stress hormones (ng·mg^{-1} DW)			
Bean waste (BW)	ABA	SA	JA	Total sum of stress hormones
Initial substrate	[a]0.05 ± 0.01a	ND	ND	0.05 ± 0.01a*
Control	0.16 ± 0.11a	2.51 ± 0.56a*	1.30 ± 0.17d*	3.97 ± 0.61a*
Frass	0.50 ± 0.14b*	10. 35 ± 2.21b*	0.56 ± 0.16c*	11.41 ± 2.33b*
Frass leachate	0.56 ± 0.11b	10.01 ± 2.39b*	0.41 ± 0.22bc	10.97 ± 2.63b*
Larvae	ND	ND	ND	-
Pea waste (PW)				
Initial substrate	0.08 ± 0.01a	[a]1.05 ± 0.14a*	ND	1.14 ± 0.14 ab*
Control	[a]0.02 ± 0.00a	ND	0.43 ± 0.09b*	0.45 ± 0.09*ab*
Frass	[a]0.03 ± 0.00a*	ND	[a]0.13 ± 0.04a*	0.17 ± 0.04 ab*
Frass leachate	0.61 ± 0.39b	[a]2.02 ± 1.80a*	0.53 ± 0.05b	3.16 ± 2.18b*
Larvae	ND	ND	ND	-

ND result < LOD.
[a] LOD < result ≤ LOQ.

Stress-phytohormone concentrations ranked in BW frass as SA > JA > ABA and in PW frass as JA > SA > ABA. Total stress hormones were 67.12-fold higher in BW than PW frass. In leachate, for both variants (BW and PW), the decrease in phytohormone concentrations was SA > ABA > JA; total stress hormones were 3.47-fold higher in BW than PW leachate (Table 3).

3.4. Cytokinins

The initial BW substrate was richer in the total sum of cytokinins than PW (Table 4). In BW frass, the contents of dh-zea, c-zea, and c-zea rib were lower versus the substrate, while t-zea rose 6.44-fold in frass and 10.00-fold in leachate. T-zea rib increased 3.38-fold in BW frass but declined below the substrate in the control residue, leachate, and larvae. Dh-zea rib showed a similar pattern, with a 1.31-fold rise in frass. IP increased across all BW products, with a 165.11-fold maximum in leachate. In the larvae after BW, the presence of t-zea, t-zea rib, dh-zea rib, c-zea rib and IP was determined, and only in the case of IP the concentration in the larvae exceed the concentration in the substrate (but without statistical significance) (Table 4).

In the PW variant, c-zea, dh-zea rib, and c-zea rib were highest in the substrate and decreased after bioconversion. Other cytokinins peaked in leachate, increasing versus the substrate by 56.46-fold (t-zea), 1.74-fold (t-zea rib), and 108.82-fold (IP), and in the case of dh-zea, from a level below detection to approximately 4.10 pg mg^{-1} DW (Table 4). Total content was 35.56-fold higher in BW than PW frass and 2.10-fold higher in BW than PW leachate (Table 4). The content of t-zea, t-zea rib, c-zea rib and IP was determined in PW larvae, and, similarly to BW larvae, IP was the hormone whose concentration in larvae exceeded that in the substrate, but without statistical significance (Table 4).

Samples from both BW and PW variants were also tested for the presence of kinetin, kinetin riboside, and ortho- and meta-topolins, but their presence was not detected (data not shown).

4. Discussion

4.1. Yield of larvae and rearing by-products after waste bioconversion

Bioconversion by the larvae resulted in higher amounts of PW frass than in the BW variant (both dry and fresh weight) (Fig. 1), which is also reflected in the lower ability of *H. illucens* larvae to utilize PW. The higher reduction in BW waste, which is reflected in the lower amount of frass left behind after breeding, may indicate a better uptake and digestibility of this type of substrate by the larvae. This may suggest a lower nutritional value of BW for the larvae, which may result in a lower protein content in the larvae (Table 1). However, larval biomass and length gain were not significantly different between the variants (Fig. 2). In both BW and PW variants, larvae had their highest weight during the second week of the experiment (Fig. 2a). The decrease in larval weight after the second week may be due to the transition from larvae to prepupae, which represents a state where larvae stop consuming feed (Spranghers et al., 2018). Furthermore, the high survival rate (Table 1) confirmed that BW as well as PW waste were suitable for larval rearing. In comparison, the average survival rate of larvae reared on different substrates described as optimal for the growth and development of *H. illucens* was approximately 89.4% (Barragan-Fonseca et al., 2017).

4.2. Auxin

Auxins comprise five compounds, with indole-3-acetic acid (IAA) the most prevalent (Korver et al., 2018). They regulate cell growth, division, and differentiation, drive shoot and root formation, underlie plant tropisms, and promote parthenocarpy (Retzer et al., 2014; El Sabagh et al., 2022; Wang et al., 2021). Broader roles have been reviewed elsewhere (Gomes and Scortecci, 2021; Song et al., 2023; Zhang et al., 2022).

IAA content differed significantly between BW and PW substrates (Table 2). This likely reflects contrasting amino acid profiles, particularly tryptophan, a key IAA precursor formed via a two-step enzymatic pathway (Zhao, 2012). Comai et al. (2007) reported that beans contain ~4.03-fold more free tryptophan than peas, which may also explain the large BW-PW differences observed in frass. IAA in BW frass exceeded PW by five orders of magnitude (Table 2). Microbiota may further

Table 4
The content of cytokinins (t-zea-trans-zeatin, dh-zea -dihydrozeatin, c-zea-cis-zeatin, t-zea rib-trans-zeatin riboside, dh-zea rib-dihydrozeatin riboside, c-zea rib-cis-zeatin riboside, IP-isopentenyladenine) in the substrate, control residue without larvae, frass, leachate, and *H. illucens* larvae. The asterisk (*) indicates statistical differences between BW and PW variants (t-Student test, $p < 0.05$). Different letters indicate statistical differences between samples from one feed variant (Tukey's test, $p < 0.05$).

	Concentrations of cytokinins (pg·mg^{-1} DW)							
Bean waste (BW)	t-zea	dh-zea	c-zea	t-zea rib	dh-zea rib	c-zea rib	IP	Total sum of cytokinins
Initial substrate	3.58 ± 1.57a	83.70 ± 22.43c*	115.27 ± 31.56b*	0.66 ± 0.14b*	124.62 ± 21.40bc*	61.63 ± 1.99c	1.78 ± 1.15a	391.24 ± 75.96bc*
Control	[a]1.27 ± 0.76a*	[a]0.84 ± 0.73a	3.49 ± 2.73a	0.24 ± 0.10a	38.39 ± 25.72a	ND	8.59 ± 5.90a	52.82 ± 20.70a
Frass	23.06 ± 3.47b*	57.61 ± 10.60bc*	3.79 ± 1.79a	2.23 ± 0.10c*	162.94 ± 29.12c*	[a]0.24 ± 0.06a	69.10 ± 28.34a	318.96 ± 46.22b*
Frass leachate	35.80 ± 8.39c	47.06 ± 12.88b*	[a]0.65 ± 0.31a	0.32 ± 0.14a	99.50 ± 18.96bc*	3.24 ± 0.57b*	293.89 ± 54.89b*	480.47 ± 92.36c*
Larvae	2.80 ± 0.49a	ND	ND	0.13 ± 0.03a*	2.78 ± 1.97a	[a]0.36 ± 0.22a	9.26 ± 7.12a	15.32 ± 4.91a
Pea waste (PW)								
Initial substrate	2.25 ± 1.13a	ND	36.94 ± 7.36b*	0.23 ± 0.10 ab*	0.99 ± 0.29b*	55.54 ± 7.71b	[a]0.79 ± 0.14a	96.72 ± 15.37a*
Control	3.98 ± 1.12a*	ND	ND	ND	ND	ND	19.45 ± 11.34a	23.43 ± 11.30a
Frass	2.54 ± 0.14a*	ND	[a]0.80 ± 0.61a	[a]0.05 ± 0.03 ab*	[a]0.21 ± 0.19a*	[a]0.33 ± 0.15a	5.04 ± 1.30a	8.97 ± 1.49a*
Frass leachate	127.04 ± 48.48b	4.10 ± 3.29b*	10.74 ± 7.02a	0.40 ± 0.28b	[a]0.43 ± 0.44 ab*	0.60 ± 0.00a*	85.97 ± 11.67b*	229.29 ± 67.81b*
Larvae	2.77 ± 0.75a	ND	ND	[a]0.05 ± 0.03 ab*	ND	2.97 ± 1.27a	26.91 ± 18.48a	32.70 ± 19.41a

ND result < LOD.
[a] LOD < result ≤ LOQ.

contribute, as several genera produce auxins, including *Agrobacterium*, *Azospirillum*, *Bradyrhizobium*, *Pseudomonas*, and *Rhizobium* (Costacurta and Vanderleyden, 1995). Knowledge and information on the phytohormonal characteristics of organic fertilizers or similar biomass is limited. IAA in PW frass was comparable to biohumus extract (0.053 ng mg^{-1}) (Sienkiewicz et al., 2024, Table 2), whereas the much higher BW frass level matched concentrations typical of organic soils such as peat (Szajdak and Maryganova, 2007). In *H. illucens* frass from catering waste, Green (2023) found 0.36 ng ml^{-1} IAA, with higher levels in the corresponding leachate (50.4 ng ml^{-1}).

During IAA application, the dose of this phytohormone should be adjusted according to the plant type. Monocots generally tolerate or benefit from higher auxin levels than dicots (McSteen, 2010). For maize, 0.01 mmol L^{-1} IAA improved Cd tolerance, biomass, and mineral uptake (Hu et al., 2022). This concentration corresponds to the IAA contained in 3.63 g DW of BW frass (where IAA was highest; Table 2). For dicots such as potato, 17.14 μM IAA enhanced growth, protein content, and antioxidant enzyme activity under normal and saline conditions (Gull et al., 2023). An equivalent dose is present in 6.22 g DW of BW frass.

4.3. Plant stress hormones

Plants face biotic stresses from microbes (bacteria, viruses, fungi) and abiotic stresses from environmental factors such as drought, salinity, temperature, and pollution (Ku et al., 2018) and they respond via stress hormone production (Sonkar et al., 2021). Abscisic acid (ABA) accumulates under drought, salinity, and heat, promoting stomatal closure and water conservation (Kavi Kishor et al., 2022). ABA is also present under non-stress conditions and influences flowering, tillering, cell expansion, chloroplast function, and seed germination and development (Popko et al., 2010; Kavi Kishor et al., 2022). In angiosperms, ABA is synthesized mainly via carotenoid metabolism, including hydroxylation and glycosylation steps regulated by cytochrome P450 monooxygenases and ABA glucosyltransferases (Wu et al., 2023).

Initial ABA levels in both seed-waste substrates were similarly low, but concentrations increased by the end of the experiment when larvae were present. This effect was strongest in BW, reaching ~0.5 ng mg^{-1} DW in frass and frass leachate (Table 3). In PW, a comparable ABA level occurred only in leachate, not in frass. Cytochrome P450 is a common enzyme complex conserved across taxa. Their activity in *H. illucens* larvae has been shown to detoxify aflatoxin B_1 in contaminated substrate (Meijer et al., 2019) and may partly underlie the elevated ABA in larval treatments (Table 3). Differences in microbial diversity and activity between substrates likely also influenced the biotransformation of low molecular-weight compounds such as plant hormones.

ABA content in organic fertilizer materials is generally low relative to other phytohormones. LC-MS analyses of agri-food waste composts identified ABA as one of the least abundant compounds, averaging ~1.29 ng g^{-1} DW, with frequent cases of undetectability and individual samples in the range of 0.09–1.20 ng g^{-1} (from compost and biohumus) (Sienkiewicz et al., 2024). In compost extracts ("compost teas"), ABA was often undetectable, consistent with its very low levels in the solid phase (Pant et al., 2012). For example, Pant et al. (2012) detected 41.6 ng L^{-1} ABA only in green-waste thermophilic compost; it was absent in fresh or aged chicken-manure vermicompost, chicken-manure thermophilic compost, and food-waste vermicompost.

On the other hand, anaerobic digestates also can be a source of plant phytohormones (Li et al., 2022). found ABA content in anaerobically digested slurries from chicken manure, dairy manure, and pig manure that was 3.58 ± 0.50, 5.15 ± 1.21, and 5.21 ± 1.68 μg mL^{-1} respectively. Li et al. (2016) analyzed raw digestate originating from a large-scale biogas plant at a pig breeding farm and found ABA in the concentration of 34.8 ± 0.7 mg L^{-1} (Wu and Dong, 2020). reported ABA content in different digestates from chicken manure, pig manure, cattle manure, and maize silage in the rage of 5.23 – 35.59 mg L^{-1}.

Examples of the use of ABA as a plant supplement include studies leading to mitigation of the phytotoxic effects of heavy metals by hindering their storage in plant tissues, e.g. Ni in *Trigonella foenum-graecum* (L.) (80 mg Ni · kg^{-1} soil, 40 μM of ABA; Parwez et al., 2023)) or Cd in *Arabidopsis* plants (10 μM Cd in hydroponics, 0.0 - 0.5 μM ABA; Fan et al., 2014)). The presence of heavy metals in soils is an increasing problem, mainly through industrialization of increasingly large areas or through the use of sewage sludge as a fertilizer, which can negatively affect plant growth and development (Angon et al., 2024).

Salicylic acid (SA) influences seed germination, stomatal closure, fruit formation, and photosynthesis, and it mediates resistance to pathogens - including necrotrophs - and the synthesis of antioxidant secondary metabolites (Misra and Saxena, 2009; El Sabagh et al., 2022; Sharma et al., 2023). Its effects are especially important under soil salinity, where SA correlates positively with germination rate (Sharma et al., 2023).

SA was much higher in BW frass and leachate (~10.2 ng mg^{-1} DW) than in PW, despite higher initial SA in the PW substrate. In PW, leachate contained approximately 1.92 times more SA than the substrate, but the result was not significant. In frass, SA did not exceed the detection limit (Table 3). SA is a phenolic derivative produced from cinnamate arising from phenylalanine catabolism (Chen et al., 2009). Because *Fabaceae* seeds contain phenylalanine (Köse et al., 2024), higher phenylalanine availability may increase cinnamate and ultimately SA, helping explain the BW-PW differences in frass and leachate (Table 3).

Sienkiewicz et al. (2024) reported SA content in compost with buckwheat husk and in biohumus extract at the levels of 4.02 ± 0.37 and 0.28 ± 0.01 ng g^{-1} DW, respectively. Traces (<0.01 ng g^{-1} DW) were also stated in garden compost, compost hemp chaff and apple pomace, organic compost, and organic compost pellets (Sienkiewicz et al., 2024). Morales-Corts et al. (2018) found SA in garden compost and vermicompost at 5.85 ± 1.23 and 1.33 ± 0.24 mg L^{-1}. Some amount of SA can also be found in the anaerobic digestate liquid fraction. Proskynitopoulou et al. (2024) investigated this type of waste using selective electrodialysis (SED) to increase its cleaning in order to produce clean water and nutrient recovery. SA has been found in all investigated samples and achieved 3.4 μg L^{-1} in reverse osmosis permeate.

SA has also been demonstrated to have the inhibitory effect on translocation of metals from roots in the case of Cu in maize and stimulate seed germination when using dosage of 500 μM of SA (Moravcová et al., 2018).

Jasmonic acid (JA) accumulates in shoot apices, roots, young leaves, and immature fruit. It is an oxidized fatty acid derived from oxylipins (cyclopentanes) and mediates responses to biotic stress, daily/seasonal movements, and wound- or pathogen-induced immunity (Ruan et al., 2019; Ghorbel et al., 2021; El Sabagh et al., 2022).

The concentration of JA in both BW and PW substrates was below LOD, which increased during the experiment (Table 3). It is worth to notice that in control residues JA had higher concentrations than in frass and in leachate from BW. The presence of larvae increases its content only in PW frass leachate as compared to the control. This enrichment may reflect microbiological and enzymatic processes in the liquid phase that hydrolyze esters or conjugates to release free acids. Physicochemical factors (pH, ionic strength, adsorption/desorption) can shift equilibria toward the aqueous phase, and percolating water may wash weakly bound, mobile compounds from the solid matrix, concentrating them in the effluent.

JA is abundant in plant tissues; for instance the presence of JA in bean (*Phaseolus vulgaris* L.) seeds was investigated by Enomoto and Miyamoto (2021), who determined its content in cotyledon at 0.05 ng mg^{-1} FW, in radicle at 0.21 ng mg^{-1} FW, and in seed coat at 0.53 ng mg^{-1} FW. Concentrations in the initial BW substrate, as in PW, were lower (even undetected) (Table 3) than these literature data, however this changes depending on the plant genus, environmental condition, and metabolic status of the tissue (age and growth rate) (Enomoto and Miyamoto, 2021). Green (2023) recorded JA concentration at 0.18 ng ml^{-1} and 0.02 ng ml^{-1} methyl jasmonate in *H. illucens* frass obtained

from catering food waste. Although JA is abundant in plants literature, data about JA content in composts, manures, digestates and similar biofertilizers are scarce. Benazzouk et al. (2020) researched the impact of vermicompost leachate on salt-stressed (125 mM NaCl) tomatoes (*Solanum lycopersicum* L.) in hydroponics. They found only 0.06 pmol mL^{-1} (~0.0126 ng mL^{-1}) of JA in the investigated leachate. The use of exogenous JA may reduce plant stress such as salinity (Benazzouk et al., 2020) or heavy metals (Ahmad et al., 2017).

4.4. Cytokinins

Naturally occurring cytokinins are adenine derivatives with isoprenoid or aromatic side chains at the N6 position (Zürcher and Müller, 2016). Beyond promoting cell division and development, they delay senescence, influence apical growth, and mediate nutrient signaling (Davies, 2010). Their roles in plants were extensively reviewed (Li et al., 2021; Argueso and Kieber, 2024). Once thought to be root-derived and xylem-transported, cytokinins are now known to be synthesized throughout the plant (e.g., Arabidopsis) (Kieber and Schaller, 2014).

After bioconversion, significant increases were observed for t-zea, t-zea rib, and IP in BW frass and leachate. In PW significant increases were observed for t-zea and IP only in frass leachate (Table 4). These hormones are dominant, more abundant, and generally more active than, for example, their cis counterparts (Frébort et al., 2011). IP and t-zea can arise via pathways from dimethylallyl diphosphate (DMAPP) and adenosine 5′-monophosphate (AMP), or via tRNA post-translational modification that introduces isopentenyladenosine (Hošek et al., 2020). The rise in t-zea may reflect conversion from IP, potentially mediated by cytochrome P450 monooxygenases, which are also implicated in ABA metabolism (Zürcher and Müller, 2016).

The total sum of cytokinins in the BW variant was higher than in the PW variant both in frass and in leachate (Table 4), which could be related to the substrate composition as well as the microbial biodiversity. Compared to other organic fertilizers, frass from BW in particular was characterized by high total cytokinin content. For substrates such as organic compost, organic compost pellets, compost with buckwheat husk, compost hemp chaff and apple pomace, or garden compost, the average contents of total cytokinins ranged from 0.08 to 0.87 pg mg^{-1} DW (Sienkiewicz et al., 2024). In contrast, biohumus extract was characterized by a higher dose of cytokinins of 13.9 pg mg^{-1} DW (Sienkiewicz et al., 2024).

Characterization of cytokinins in larval samples also showed interesting results, mainly in the context of rearing *H. illucens* larvae for feed or for protein extraction. In particular, IP was the phytohormone found in *H. illucens* larvae at elevated levels. Based on studies in rat L6 myoblasts, it has been shown that IP can affect animal cell proliferation and protein synthesis, contributing to their growth (Yagasaki et al., 1986). In the present study, IP concentrations were higher (however, without a statistical change) in larvae from the PW variant, whose larval protein content was also statistically higher (Table 1) than that of BW larvae.

The present experiment shows that the conversion of legume seed waste by *H. illucens* larvae can lead to an increase in the concentrations of certain cytokinins (Table 4). The frass leachate for which the total sum of all cytokinins increased from the initial substrates may find application in the production of preparations for use on the leaf surface of plants. Cytokinins applied in this way are known to have a positive effect on the regulation of the stomatal apparatus and transpiration process during stress conditions (Jini and Joseph, 2018). Foliar application of cytokinins, particularly zeatin, allows wheat plants subjected to drought stress to increase tolerance through more efficient uptake of macro- and micronutrients as well as water, an increase in chlorophyll concentration, or an increase in yield (Zaheer et al., 2019). Exogenous treatment of plants with cytokinins also contributes to the regulation of plant hormones, balancing their concentrations and also affecting the expression of their synthesis (e.g., in the case of SA and JA) (Kosakivska et al., 2022).

5. Conclusion

Bean (BW) and pea (PW) seed wastes supported normal larval development and growth, indicating both are suitable substrates for *H. illucens*. Larval residues - frass and leachate - were characterized by elevated concentrations of various phytohormones. The BW variant generally contained higher concentrations than PW, although PW leachate was comparatively rich in certain plant stress hormones and cytokinins. These results point out the potential of *H. illucens* frass not only as a nutrient-rich organic fertilizer but also as a source of natural biostimulants that can enhance crop performance and resilience to environmental stresses. Valorizing agricultural waste into multifunctional soil amendments will help contribute to sustainable resource management and the circular bioeconomy. Future work should include pot and field trials across crop species and should link phytohormone profiles with biostimulant endpoints, such as nutrient use efficiency, abiotic stress tolerance, and quality traits. Studies should also test frass from other insect species, gained from a wider variety of feeding substrates. Evaluation of storage and processing effects, like the thermal treatment at 70 °C for 1 h, on phytohormone stability would be important. From a policy perspective, frass can already be used as fertilizer in organic agriculture in EU countries (EU Regulation 2021/1165, 2021), but there is still a need to provide data, shedding new light on the potential benefits and safety requirements resulting from the agricultural use of insect frass. Our data therefore complement the evidence base that could be helpful for the development of guidelines and future standardization.

CRediT authorship contribution statement

Monika Kaczor: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Piotr Bulak:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **Piotr Waligórski:** Methodology, Investigation. **Andrzej Bieganowski:** Writing – review & editing, Validation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2026.147855.

Data availability

Data will be made available on request.

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8.4.1. Suplement do **P4**

Supplementary information

Linking waste recycling and sustainable agriculture: Phytohormone-rich biofertilizer from black soldier fly frass

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Tab. 1S. Benjamini–Hochberg false discovery rate (FDR)-adjusted p-values (q-values) for one-way ANOVA tests performed separately for each hormone within BW and within PW feed variants. For each hormone, the table reports the raw ANOVA p-value and the corresponding BH-FDR q-value; significance was assessed at $q < 0.05$

BW		
Phytohormone	**p (ANOVA)**	**q (BH FDR)**
c-zea rib	5.33E-15	5.86E-14
IAA	1.15E-13	6.33E-13
t-zea rib	2.10E-09	7.69E-09
IP	6.13E-07	1.69E-06
t-zea	3.35E-06	7.38E-06
c-zea	4.94E-06	8.08E-06
JA	5.14E-06	8.08E-06
SA	6.50E-06	8.93E-06
dh-zea rib	2.44E-05	2.98E-05
dh-zea	3.00E-05	3.30E-05
ABA	5.34E-05	5.34E-05
PW		
Phytohormone	**p (ANOVA)**	**q (BH FDR)**
c-zea rib	7.89E-09	8.68E-08
JA	3.65E-07	2.01E-06
c-zea	6.44E-06	2.36E-05
IP	1.76E-05	4.85E-05
t-zea	9.94E-05	2.19E-04
dh-zea rib	5.18E-03	9.50E-03
ABA	6.10E-03	9.59E-03
IAA	1.78E-02	2.45E-02
t-zea rib	2.47E-02	3.02E-02
dh-zea	2.75E-02	3.02E-02
SA	4.77E-02	4.77E-02

Tab. 2S. Effect sizes for **IAA concentrations** for BW vs PW comparisons reported as percent fold-change and Cohen's d for each hormone (within each sample type, if applicable). Percent fold-change was calculated as [(BW−PW)/PW] × 100%. Cohen's d was computed as the standardized mean difference (BW−PW)/SDpooled. Positive values indicate higher concentrations in BW, and negative values indicate higher concentrations in PW.

BW vs PW	Cohen's d and percent change for f IAA
Initial substrate	5.62 58%
Control	0.34 -16%
Frass	39.32 965160%
Frass leachate	0.68 28%
Larvae	2.38 126%

Tab. 3S. Effect sizes for **stress phytohormones concentrations** for BW vs PW comparisons reported as percent fold-change and Cohen's d for each hormone (within each sample type, if applicable). Percent fold-change was calculated as [(BW−PW)/PW] × 100%. Cohen's d was computed as the standardized mean difference (BW−PW)/SDpooled. Positive values indicate higher concentrations in BW, and negative values indicate higher concentrations in PW.

BW vs PW	ABA	SA	JA	Total sum
Initial substrate	3.67 -38%	12.99 -100%	0 n.d.	13.45 -96%
Control	2.20 700%	7.76 n.d.	8.86 202%	9.89 782%
Frass	5.81 1567%	8.11 n.d.	4.52 331%	8.35 6612%
Frass leachate	0.21 -8%	4.63 369%	0.92 -23%	3.96 247%
Larvae	n.d.	n.d.	n.d.	n.d.

n.d. – no data due to concentration < LOD

Tab. 4S. Effect sizes for **cytokinines concentrations** for BW vs PW comparisons reported as percent fold-change and Cohen's d for each hormone (within each sample type, if applicable). Percent fold-change was calculated as [(BW−PW)/PW] × 100%. Cohen's d was computed as the standardized mean difference (BW−PW)/SDpooled. Positive values indicate higher concentrations in BW, and negative values indicate higher concentrations in PW.

BW vs PW	t-zea	dh-zea	c-zea	t-zea rib	dh-zea rib	c-zea rib	IP	Total sum
Initial substrate	1.19 59%	6.46 n.d.	4.19 212%	4.33 187%	10.01 12488%	1.32 11%	1.48 125%	6.58 304%
Control	3.47 -68%	1.99 n.d.	2.21 n.d.	4.16 n.d.	2.59 n.d.	0 n.d.	1.47 -56%	2.16 125%
Frass	10.23 808%	9.41 n.d.	2.74 374%	36.17 4360%	9.68 77490%	0.96 -27%	3.91 1271%	11.61 3456%
Frass leachate	3.21 -72%	5.60 1048%	2.49 -94%	0.44 -20%	9.05 23040%	8.02 440%	6.42 242%	3.80 110%
Larvae	0.06 1%	0 n.d.	0 n.d.	3.27 160%	2.42 n.d.	3.51 -88%	1.54 -66%	1.49 -53%

n.d. – no data due to concentration < LOD

Tab. 5S. Limits of detections (LOD) and limits of quantifications (LOQ) for analyzed phytohormones. For LOD S:N ratio was 3 and for LOQ was 10.

	IAA	ABA	SA	JA	t-zea	dh-zea	c-zea	t-zea rib	dh-zea rib	c-zea rib	IPA
	ng·mg^{-1}				pg·mg^{-1}						
LOD	0.039	0.018	0.670	0.042	0.402	0.493	0.521	0.032	0.164	0.140	0.267
LOQ	0.132	0.059	2.233	0.139	1.342	1.644	1.739	0.108	0.548	0.468	0.891

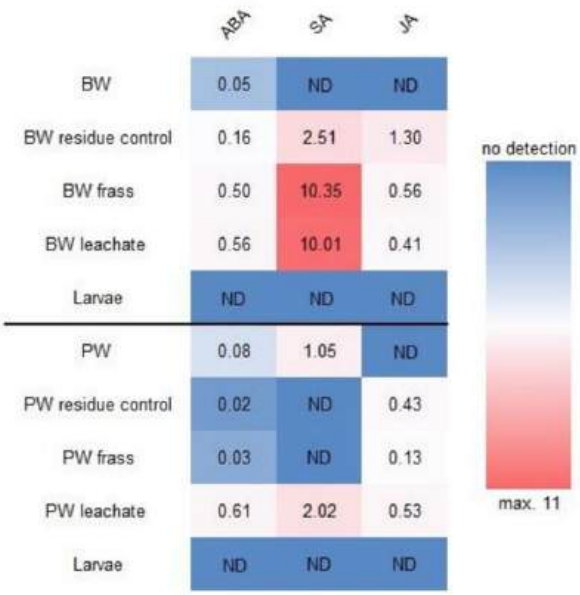

Fig. 1S. Heatmap presenting changes in concentrations of **stress phytohormones** across different samples for both BW and PW variants.

Fig. 2S. Heatmap presenting changes in concentrations of **cytokinines** across different samples for both BW and PW variants.

9. Oświadczenia współautorów

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Oświadczenie

Niniejszym oświadczam, że mój udział w publikacjach:

P.1: Kaczor M., Bulak P., Proc-Pietrycha K., Kirichneko-Babko M., Bieganowski A., 2023. **The variety of applications of *Hermetia illucens* in industrial and agricultural areas – review.** Biology 12, 25. DOI: 10.3390/biology12010025.

P.2: Kaczor M., Bieganowski A., Wiącek D., Bulak P., 2025. **Black soldier fly frass from seed waste of nitrogen-rich legumes – How long-term maturation affects the fertilizer properties?** Journal of Environmental Management 373, 123752. DOI: 10.1016/j.jenvman.2024.123752.

P.3: Kaczor M., Bulak P., Kosicki R., Twarużek M., Bieganowski A., 2025. **Advancing mycotoxin degradation in agricultural waste – insights from *Hermetia illucens* larvae and frass safety analysis.** Journal of Insects as Food and Feed. https://doi.org/10.1163/23524588-bja10296.

P4: Kaczor M., Bulak P., Waligórski P., Bieganowski A., 2026. **Linking waste recycling and sustainable agriculture: Phytohormone-rich biofertilizer from black soldier fly frass.** Journal of Cleaner Production 548, 147855. DOI: 10.1016/j.jclepro.2026.147855.

obejmował:

- współudział w tworzeniu koncepcji badań,
- samodzielne przeprowadzenie większości pomiarów oraz przeglądu literaturowego
- opracowanie wyników,
- przygotowanie pierwszej wersji manuskryptów i naniesienie uwag od współautorów
- przygotowanie pierwszej wersji odpowiedzi na recenzje
- przygotowanie finalnej wersji manuskryptu do publikacji.

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Oświadczenie

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P4: Kaczor M., Bulak P., Waligórski P., Bieganowski A., 2026. **Linking waste recycling and sustainable agriculture: Phytohormone-rich biofertilizer from black soldier fly frass.** Journal of Cleaner Production 548, 147855. DOI: 10.1016/j.jclepro.2026.147855.

obejmował:

- współudział w tworzeniu koncepcji badań,
- udzielanie konsultacji i wsparcia Doktorantce na wszystkich etapach realizacji badań,
- korekta przygotowywanych manuskryptów.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr Moniki Kaczor.

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obejmował:

- pomoc w przeglądzie literatury.

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obejmował:

- pomoc w przeglądzie literatury.

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P.2: Kaczor M., Bieganowski A., Wiącek D., Bulak P., 2025. **Black soldier fly frass from seed waste of nitrogen-rich legumes – How long-term maturation affects the fertilizer properties?** Journal of Environmental Management 373, 123752. DOI: 10.1016/j.jenvman.2024.123752.

obejmował:

- przeprowadzenie analizy stężeń pierwiastków metodą ICP.

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Oświadczenie

Niniejszym oświadczam, że mój udział w publikacji:

P.3: Kaczor M., Bulak P., Kosicki R., Twarużek M., Bieganowski A., 2025. **Advancing mycotoxin degradation in agricultural waste – insights from *Hermetia illucens* larvae and frass safety analysis.** Journal of Insects as Food and Feed. https://doi.org/10.1163/23524588-bja10296.

obejmował:

- przeprowadzenie analizy stężeń mykotoksyn,
- konsultacje merytoryczne z zakresu obecności i aktywności mykotoksyn,
- korekta manuskryptu oraz współudział w odpowiedzi na recenzje.

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obejmował:

- przeprowadzenie analizy stężeń mykotoksyn,
- konsultacje merytoryczne,
- korekta manuskryptu i współudział w odpowiedzi na recenzje.

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P4.: Kaczor M., Bulak P., Waligórski P., Bieganowski A., 2026. **Linking waste recycling and sustainable agriculture: Phytohormone-rich biofertilizer from black soldier fly frass.** Journal of Cleaner Production 548, 147855. DOI: 10.1016/j.jclepro.2026.147855.

obejmował:

- przeprowadzenie analizy stężeń fitohormonów,
- konsultacje merytoryczne i korekta manuskryptu.

Jednocześnie wyrażam zgodę, aby praca została wykorzystana w rozprawie doktorskiej mgr Moniki Kaczor.

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