Black soldier fly larvae and *T. reesei* treatment – exploring the fate of the mycotoxin patulin

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Påverkan av fluglarv-, och T. reesei behandling på svampgiftet patulin

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Abstract

In order to transition our food system from a linear to a circular one we need more tools to recycle, not only plant nutrients, but proteins and fats from all types of waste streams. Black Solider Fly Larvae (BSFL) composting offers a good solution both as waste management strategy and for replacing other unsustainable protein sources. Mycotoxins are secondary metabolites produced by several molds (fungi) that can cause serious harm to humans and animals. In order to safely rear BSFL on food waste or other type of waste streams it is important that associated risks are investigated. This study was conducted in two parts: a Literature review and an experimental trial. This study begins with literature review to identify the types of mycotoxins previously investigated in the context of BSFL. The experimental trial evaluated three methods for inactivating the mycotoxins patulin. One treatment with *T. reesei*, one with BSFL composting and one combined treatment. The literature review revealed that either no or only low concentrations of studied mycotoxins have been detected in the larvae. The low concentrations observed in the larvae have been linked to very high concentrations in the input material; yet the concentrations in the larvae have been below EU regulatory limits. The result of the experimental trial showed that larval weight gain where not affected by patulin. Combined treatments showed the highest larval survival and the highest loss of volatile solids (VS). In terms of patulin inactivation, samples were sent to two different commercial laboratories, while a control sample was sent to a third for verification. The results obtained where not reliable enough to draw any conclusions. The key take-away message from the patulin analysis were that the methodology must be further developed to obtain trustworthy results. Trustworthy mycotoxin analyses is a crucial part of getting a safe and circular food system in the future.

Keywords: Black solider fly composting, *Hermetia illucens*, Mycotoxin, Patulin, *T. reesei*, waste management,

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1. Introduction

The world today is facing major challenges regarding the amount of waste we create through our way of life (Salvia et al. 2021). In 2016, the world generated 2.01 billion tons of solid waste and the amount is projected to grow to 3.40 billion tons by 2050 (The World Bank 2018). Approximately 70% of the waste ends up in landfills or open dumps (Kaza et al. 2018). The solid waste in the world comprises of > 50% biodegradable waste (ibid). Biodegradable waste (biowaste) that ends up in landfills and open dumps is a major source of pollution to the atmosphere (*e.g.* methane), water bodies (eutrophication and toxic compounds) and contribute to spreading of diseases (Al-Wabel et al. 2022). Two common ways to treat organic waste used today is traditional composting and anaerobic digestion (Lin et al. 2018). In these technologies, products that can be used in agriculture (compost and digestate) and/or as vehicle gas (biogas) are generated. However, according to Hogg et al. (2003) and Lohri et al. (2017) these treatments are costly to municipalities, and creates little economic value.

Today, the production of animal protein is associated with unsustainable methods. For instance, cattle ranching is a major driver for deforestation in the world (Ritchie & Roser 2024) and productions of soyabean is a source of biodiversity loss (Green et al. 2019). To feed the livestock for our increasing meat consumption, the production of soyabean has now 10-folded in the past 50 years (Ritchie & Roser 2024). As much as 76% of the global soy production is used for animal feed and the largest share goes to poultry and pigs (ibid).

According to updated research on planetary boundaries in 2023, 6 out of the 9 boundaries have now been transgressed (Richardson et al. 2023), of which the biogeochemical flows of nitrogen (N) and phosphorus (P) is one. The excesses use of synthetic fertilizer, combined with the reliance on virgin materials in agriculture, is largely responsible for this (ibid). In addition, modern agricultural practices have a significantly negative effect on biodiversity (ibid). However, according to the recent work by Schlesier et al. (2024), a good life is possible for all within the planetary boundaries. For that to happen, we need to improve agricultural practices and increase circularity.

The larvae of black solider fly (BSFL), *Hermetia illucens* (L.) (Diptera: Stratiomyidae) offers a good alternative to conventional treatment of food waste (Singh & Kumari 2019). The larvae can be used as a high-quality feed source, in

terms of protein and fats, for livestock, chicken, pigs and fish (Chia et al. 2020). The treatment residuals, called frass, can be used as an organic fertilizer (Lopes et al. 2022) or be used as feedstock in anaerobic digestion (Lalander et al. 2018). According to Smetana et al. (2016), the environmental impact of insect protein are lower than fish meal and poultry feed, when waste substrate such as municipal food waste is used. One major benefit is that BSFL can be reared on almost any type of biowaste, *e.g.* food waste, manure, agricultural residues, and even human feces (Naser El Deen et al. 2023)

Insect-derived proteins and oils have been authorized for use in aquaculture and pet food in the EU, with the condition that it has to comply with the Feed Hygiene Regulation (European Parliament 2005). However, there are numerous restrictions on rearing insects on food waste, along with various EU regulations, hindering the development of insect farming and the effective marketing of insect-based foods and feeds in Europe (Żuk-Gołaszewska et al. 2022). For example, it has been estimated that food safety regulations prevented the use of approximately 70% of available food waste in EU that could have been used as insect feed (Lalander & Vinnerås 2022).

It is important that rearing insects on food waste is safe and poses no risk to health or environment when transitioning to a circular economy (European Commission 2020a). The United Nation Policy Analysis Branch states that the key challenges to rearing insects on food waste, are food safety and legislation (Behre et al. 2023). One potential risk of rearing larva on food waste is the presence of mycotoxins in discarded food and other food wastes that never reaches consumers (Swedish Food Agency 2023). Mycotoxins are secondary metabolites produced by molds (fungi) that can cause serious harm to humans and animals (WHO 2023). Mycotoxins are naturally occurring in many foods and feeds and can either start to be produced in the fields and/or in storage (Kosicki et al. 2016). Mycotoxins can be found in most agricultural crops but are most commonly found in cereals, apples and other dried fruits and nuts. Reddy et al. (2010) found that the potential health risks of consuming mycotoxin contaminated food and feed range from nausea, vomiting, headaches, to liver lesions, impact on immune system, infertility and various types of cancers. Additionally, mycotoxins can cause health risks when inhaling or by dermal contact.

Several approaches to treat and inactivate mycotoxins have been investigated, *e.g.* using microorganisms, biofilms and enzymes (Nahle et al. 2022). One approach that have been investigated is using *T. reesei,* which is a mesophilic and filamentous fungus commonly found in soil and root ecosystems (Suo et al. 2023). *T. reesei* is used in industrial processes as production of cellulases due to its ability to produce enzymes that can break down plant biomass (Geng 2014), by transforming lignocellulosic biomass into soluble and fermentable sugars. Suo et al. (2023) investigated the impact on the degradation of mycotoxins on various strains of *T.*

reesei. Several strains demonstrated potential as biological detoxifiers on mycotoxins in various foods and feeds. *T. reesei* has also been shown to enhance the biomass conversion efficiency in BSFL composting by increasing the digestibility of the substrate for the larvae (Isibika et al. 2019).

Only rather few studies have examined the impact of BSFL composting on mycotoxin inactivation. These studies suggest that rearing insects on mycotoxin-contaminated substrates could reduce the concentration of several mycotoxins (Niermans et al. 2021; Bisconsin-Junior et al. 2023). Even though selected mycotoxins regulated in EU are represented in these few studies, experimental studies on the mycotoxin patulin are still lacking. Recommendations from UN, for successful implementations of BSFL as feed for livestock, include filling the research gap on risks associated with BSFL treatment of food waste. Providing this information can help governments address regulatory amendments to create good conditions for industries to produce safe and trustworthy products (United Nations 2023).

1.1 Aim and objective

The aim of this study was to assess the potential safety risks, regarding feeding potentially mycotoxin-contaminated food waste to BSF larvae intended as feed for livestock. This was investigated by reviewing the current state of knowledge, regarding fate of mycotoxins in fly larvae composting, to identify research gaps and to examine the impact of treatment with *T. reesei* and BSFL composting, alone and in combination, on the mycotoxin patulin.

1.2 Research questions

A. What impact does *T. reesei* treatment and BSFL composting have on the mycotoxin patulin?

B. How does mycotoxin impact on the BSFL composting process in terms of larval survival and process efficiency?

C. How can *T. reesei* impact on the BSFL composting process in terms of larva survival, process efficiency and concentration of mycotoxins?

2. Background

Environmental impacts from food waste and food losses

Food production accounts for approximately 26 % of total global greenhouse gas emissions according to Poore & Nemecek (2018). A large proportion of these emissions originates from food waste, which is estimated to account for 6 % of total global emissions and include both losses in the supply chain and waste produced in the consumer stage (Figure 1). This number is likely underestimated since it does not account for food losses occurring on farms during production and harvesting (Ritchie & Roser 2023).

Figure 1. Schematic view of the share of total food production emissions that comes from food that is never eaten worldwide. Out of emissions coming from food production (26 %), were 15 % comes from food losses in supply chain and 9 % comes from consumer waste (Poore & Nemecek 2018)

Where the losses occur in the food production system varies greatly in different parts of the world (World resources report 2018). In Sub-Saharan Africa the share of losses in the consumption step is small (5 %), while it is high in production, handling, and storage (76 %). In Europe, the losses in consumption step is large (52 %), while the losses in production, handling and storage are smaller (35 %) (ibid). At the same time, feeding the world's population is a major challenge and is expected to become more difficult due to climate change where extreme heat and drought will be more common (ibid).

The current linear food system must become more circular, primarily by reducing food waste, in all parts of the food productions system, but also by recycling resources (energy, complex molecules such as amino-, and fatty acids, plant nutrients) in the biowaste (Net Zero Cities 2023).

Pathways for Europe

In 2015, the European commission launched the first Circular Economy Action plan, titled "Closing the Loop", which introduced specific measures addressing the entire life-cycle; from production and consumption to waste management (European Commission 2015). In 2020, the European Commission adopted a new Circular Economy Action Plan as one of the key components of the European Green Deal, which aims to transitioning the EU economy into a climate-neutral one, while ensuring that no one is left behind (European Commission 2020b). The foundation of EU waste management is the "waste hierarchy" (Figure 2b), established in the Waste Framework Directive in 2008. It sets a preferred order for how waste should be managed, where reducing waste is the top priority, while landfill is the least preferred and to be avoided (European Commission 2008). Open dumps and landfills, however, are still a major problem from a global perspective (Figure 2a). To deal with this problem, the European Commission (2008) launched the Landfill Directive. The aim was to elevate solid waste management higher up the waste hierarchy and transition away from landfilling. In 2014, the commission proposed several new targets specifically related to biodegradable waste as part of the upcoming revision of the Waste Framework Directive. One of the new target was to increase *recycling* and *preparation for re-use* of municipal waste to 70 % by 2030 (European Commission 2014).

a) *b)*

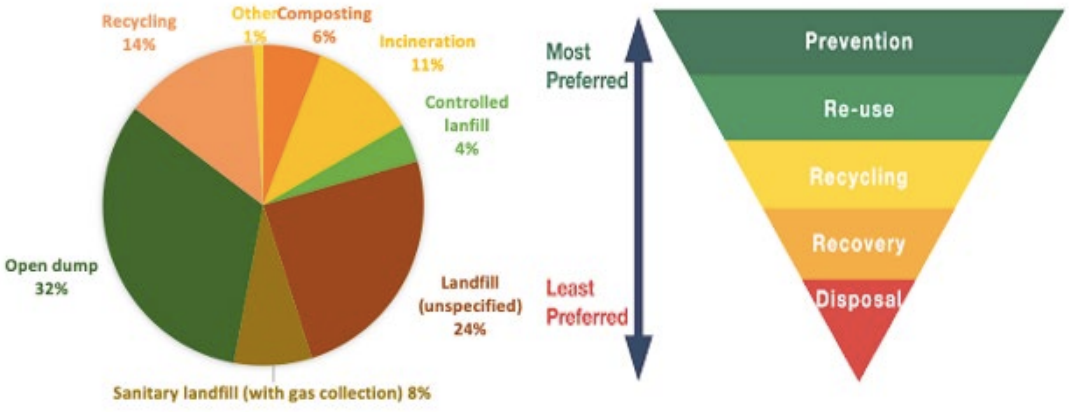

Figure 2. a) Pie chart of Global solid waste treatment and disposal diagram in percent (Kaza et al. 2018) and b) a schematic view of the waste hierarchy (European Commission 2008))

One of the corner stones of the European Green Deal is the *Farm to Fork* strategy, aimed at transforming the EU food system into a fair, healthy and environmentally-friendly system (European Commission 2020a). One issue addressed in the strategy is the excess use of plant nutrients and the use of virgin materials in agriculture. One of the goals of the EU commission is to develop an Integrated Nutrient

Management Plan, with the purpose of ensuring more sustainable use of plant nutrients, reduce pollution caused by nitrogen and phosphorus from fertilizers and stimulate the development of a market for recovered nutrients. The commission also emphasizes the need for research and innovation to develop alternative protein sources to substitute meat, from plant, microbial, marine, and insect-based sources (ibid). The demand for globally traded commodities such as meat products and soy, are significant drivers of deforestation (Ritchie & Roser 2024) and loss of biodiversity (Green et al. 2019). The vast majority of all the produced soy products is consumed by livestock (Figure 3), especially for poultry and pig production (Ritchie & Roser 2024).

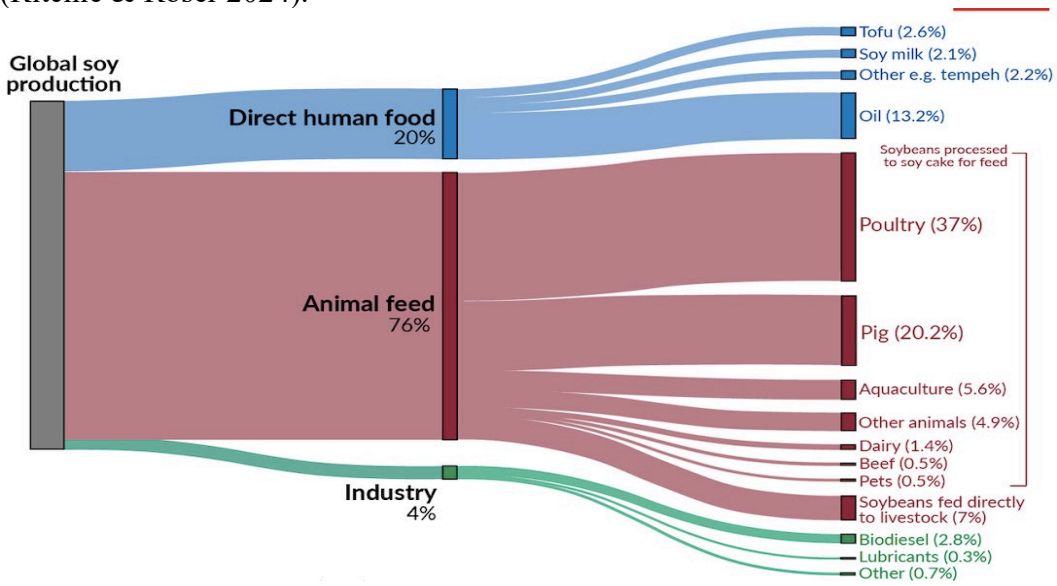

Figure 3. Allocation of global soy production to its end uses by weight. Based on data from 2017-2019 (Ritchie & Roser 2024).

In 2023, the European Commission launched a new regulation (2023/1115) preventing the European Union from contributing to deforestation through consumption of specific products. Targeted products are cattle, cocoa, coffee, oil palm, rubber, soy and wood (European Commission 2023a). All operators in the food and/or industry sectors must have a business strategy that includes risk assessment and mitigation measures for each targeted product within the scope.

Mycotoxins in biodegradable waste

Mycotoxins are secondary metabolites produced by several molds (fungi) that can cause serious harm to humans and animals (WHO 2023). They can be produced before, during or after harvest, or at any stage during the food chain (Reddy et al. 2010). Several mycotoxins are found in humid and warm conditions, but some also grow in colder climates. They can be found in most agricultural crops but are most common in cereals, fresh fruit, like apples, and other dried fruits and nuts. EU regulation No 2023/915 has set maximum levels for certain foodstuffs for seven

mycotoxins (Table 1) considered the most harmful (European Commission 2023b). The regulations for food intended for human consumption are the strictest, but there are also regulations for animal feed. According to the Swedish Board of Agriculture (2023), there are no restrictions or maximum levels for mycotoxins in fertilizer. Although there are strict regulations for mycotoxins in food to enter the food market, the occurrence of mycotoxins in food waste remains a concern (Kosicki et al. 2016).

Table 1. Occurrence, health risks, exposure route and maximum levels for food of all mycotoxins regulated in Commission Regulation 2023/915 (Speijers & Speijers 2004; Reddy et al. 2010; Wright 2015; Kosicki et al. 2016; Kamle et al. 2022; WHO 2023; European Commission 2023b).

Mycotoxin	Fungal species	Occurrence in food/feed	Health risks	Exposure route	Max. level EU (µg/kg)
Aflatoxins (AF)	*Aspergillus flavus, Aspergillus parasiticus*	Maize, wheat, rice, sorghum, ground nuts, tree nuts, figs. Occurred in 5 % of samples in a 4-year study. Arise in both field and storage	Liver lesions, cirrhosis, primary hepatocellular carcinoma, Kwashiorkor, Reye's syndrome, genotoxic, liver cancer	Ingestion inhalation, and dermal contact	0,025 - 15
Ochratoxin A (OTA)	*Aspergillus* spp., *Penicillium* spp.	Cereals, dried vine fruit, wine, coffee. Occurred in half of samples in a 4-year study. Arise in high humid storage	Endemic nephropathy, urothelial tumors, development, effect immune system, kidney damage	Ingestion	0,5 - 10
Patulin (PAT)	*Aspergillus* spp., *Penicillium expansum, Byssochlamys* spp.	Apples, Apple juice mainly, grapes, other fruits and berries. Arise during fruit growth, harvest, or processing.	Damage of gastrointestinal and respiratory systems, nausea. Genotoxic, increased risk of cancer. Acute symptoms: vomiting, diarrhea	Ingestion, dermal contact	10 - 50
Deoxynivalenol (DON)	*Fusarium garminearum, Fusarium culmorum*	Cereals, cereal products. Most common in a 4-year study. Arise in fields at cool, high humidity.	Nausea, vomiting, abdominal pain, diarrhea, dizziness, headache	Ingestion	200 - 1750
Zearalenone (ZEN)	*Fusarium* spp., *Gibberella* spp.	Cereals, cereal products Most common in a 4-year study. Arise in fields.	Premature puberty in girls, cervical cancer, hormonal disturbance, infertility	Ingestion	20 - 200
Fumonisins (FM)	*Fusarium* spp.	Maize, maize products, sorghum. B1 most common. Occurred in half of samples in a 4-year study.	Esophageal carcinoma in humans, liver, and kidney toxicity in animals	Ingestion	200 - 2000
Citrinin (CIT)	*Aspergillus* spp.,	Cereals, Beans, fruit, vegetable juice, herbs	Respiratory damage, dysfunction of	Ingestion	100 (only food

Penicillium spp., *Monascus* spp.	and spices. Monascus in red mold rice. Arise during storage. Commonly found in combination with Patulin and Ochratoxin	mitochondria, liver and kidney damage.	supplements based on rice fermented with red yeast are regulated)

The losses of food commodities is estimated to range from 30 to 50 % of total crop-productions worldwide, and occur during pre-harvest and/or post-harvest (Pandey et al. 2023) In tropical and humid climates, fungal contamination when storing food commodities is a major problem. This is not only a threat to global food security, but it is also estimated that 1.47 – 1.96 Gha of arable land is essentially wasted (ibid). The Food and Agriculture Organization has estimated that 25% of the world's crops are lost to mycotoxins each year (Eskola et al. 2020).

Patulin is a mycotoxin produced by toxigenic strains of the fungi *Penicillium expansum*, *Aspergillus* spp. and *Byssochlamys* spp. (Reddy et al. 2010). Patulin is most commonly found in fresh fruit, especially in apples and grapes, but also in processed products such as jam or juice. Patulin can occur in all stages of the of the cultivations process, but most commonly found after harvest and during storage, due to injuries sustained during harvest (ibid). Hussain et al. (2020) analyzed 133 apple samples from a market in Pakistan and found that 27 % of them had concentrations exceeding the regulatory limit of 50 µg/kg. Patulin is toxic and can cause damage to the gastrointestinal and raspatory system, can increase the risk of cancer, cause internal bleeding and act as an immunotoxin (Kosicki et al. 2016).

Black soldier fly lifecycle

The BSF is a tropical fly originated from the American continent. Today, the species can be found in most temperate and tropical regions (Surendra et al. 2020).

The BSF lifecycle is short (Figure 4) compared to other insect species commonly used as protein source, such as crickets and mealworms (Oonincx et al. 2015). The lifecycle starts when the female lays her eggs. A female fly can lay between 400 to 800 eggs and dies shortly after (Chia et al. 2020). The eggs hatch after about four days and the hatch larvae then enters the larval stage. The larval stage lasts for approximately two to three weeks, during which the larvae consume organic material. During this feeding stage, the larvae grow from a few millimeters in size to approximately 2.5 cm (Dortmans et al. 2021) and their weight increase 200 times (Lalander et al. 2019). After the larval stage they escape from the food source to pupate (Surendra et al. 2020). From this stage onward, the larvae stop consuming food, and the mouth transform into a hook-like feature to help them retreat to a dry and dark place. The prepupal stage lasts approximately 7 – 10 days, until they find a suitable place to pupate (Dortmans et al. 2021). The pupation process lasts

between 10 days to a month, upon which the flies emerge from the pupa shell. The flies mates and a new cycle begins (ibid).

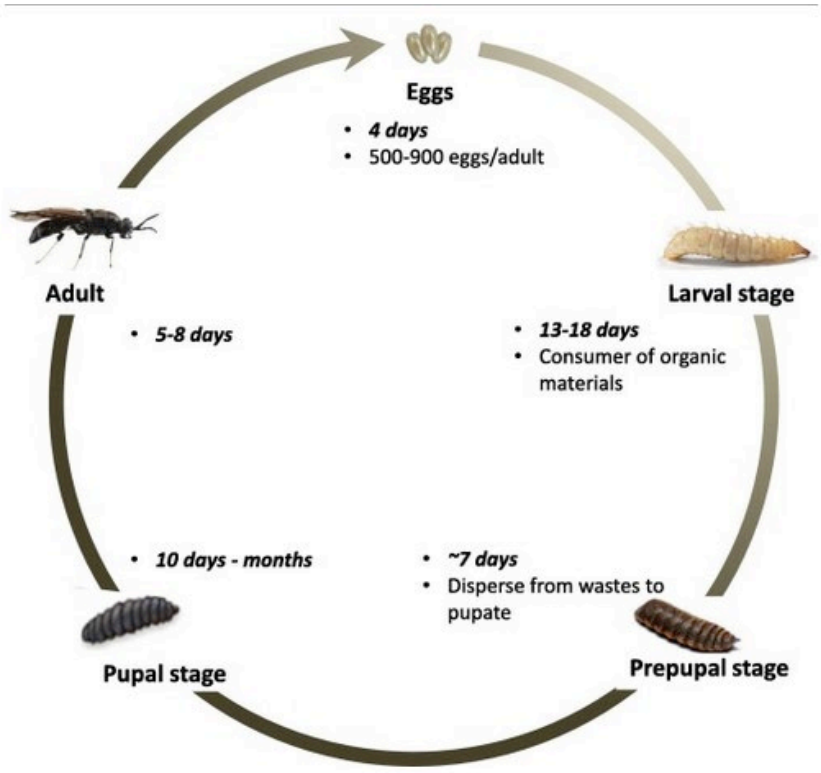

Figure 4. Black solider fly life-cycle from egg, larva, prepupal, pupal to fly (Surendra et al. 2020).

BSFL Composting and products

BSFL composting offers an effective solution to many problems associated with linear food production. The products generated in BSFL composting can replace unsustainable sources of plant nutrients and animal feed used in conventional farming (Singh & Kumari 2019). The products generated from BSFL composting are larval biomass and a processing residue, called frass.

The larvae are rich in proteins and fats, making them a high quality feed source for livestock such as poultry and pigs (Lalander & Lopes 2024).

After the treatment is finished, the larvae and frass are separated. The frass from the BSFL composting can then be used directly as fertilizer (Lopes et al. 2022), but if it is instead anaerobically digested more value can be added as both organic fertilizer and vehicle fuel is generated (Lalander et al. 2018). Salomone et al. (2017) argues that the frass produced by BSFL is one of the main outputs of this process and could replace some more unsustainable fertilizers, such as conventional nitrogen fertilizers.

BSFL composting offers numerous advantages as a waste management solution. As BSFL can be reared on almost any type of organic waste streams, including food

waste, manure, agriculture residue and even human feces (Surendra et al. 2020). In the BSFL composting process, waste volumes are significantly reduced, with reductions up to 87% on wet weight basis is reported for municipal organic solid waste (Lindberg et al. 2022). Another advantage is that the flies do not feed, as they rely on the fats stored in the larval stage, they are less likely to spread diseases compare to other fly-species (Naser El Deen et al. 2023).

The quality of the larvae and frass and the environmental impact will vary depending on the substrate the larvae were reared on (Smetana et al. 2016). Also, other abiotic factors, such as humidity and temperature will affect the growth of the larva and how they thrive (ibid). According to Dortmans et al. (2021), optimal conditions for BSFL composting is temperature between 24 and 30°C and to keep the humidity low during the rearing stage. The moisture content of the substrate also affects the biomass conversion efficiency and larval survival. Lalander et al. (2020) demonstrated that at a moisture content of 76% larval survival was 97.2%, while survival at a moisture content of 97.5% dropped to 19.3%. In addition, active or passive ventilation affects the larval process efficiency, and it is an important factor to consider when setting up a BSFL treatment facility (ibid).

BSFL in regulation; risks and opportunities.

To legalize the rearing of BSFL on food waste, the process must be proven safe and to pose no risk to human health or environment (Behre et al. 2023). The United Nation Policy Analysis Branch (2023) state that the key challenges to overcome in order to legalize the use food waste in BSFL composting, is food safety and legislation. In Europe, BSFL are classified as farmed animals and therefore only allowed a strictly vegetable-based diet (Regulation (EC) 1069/2009) (European Commission 2009). This regulation was partly enforced due to the outbreak of Bovine spongiform encephalopathy (BSE), commonly known as “mad cow disease”, that reached its peak in 1992 in the United Kingdom. This resulted in regulations that forbid the usage of animal-by products in animal-feed for farmed animals (ibid). Since food waste can contain animal by-products, the regulation essentially is making it impossible to rear BSFL on post-consumer food waste (Lalander et al. 2020). Insect-derived proteins and oils have been authorized for use in aquaculture and pet food (Commission regulation (EU) 2017/893) and, since 2021, in feed for poultry and pigs (Commission regulation (EU) 2021/1372). All insect-feed must comply with the Feed Hygiene Regulation (EC) 183/2005 and be registered as “feed business operators” (IPIFF 2022). According to Lalander & Vinnerås (2022) insects could play a vital role in achieving true circularity in the food productions system in the EU if insects could be reared on food waste. Therefore, more research needs to address uncertainties related to risks (ibid). The same authors stipulate that rearing BSF larvae on food waste to replace other protein

sources should be placed higher up than, for example composting and digestion, in the waste hierarchy.

BSFL effect on mycotoxins

Since food waste and other biowaste streams may contain mycotoxins, there is a safety issue when rearing BSFL on food waste that will depend on the larvae's ability to metabolize and/or accumulate mycotoxins (Niermans et al. 2021). Berenbaum et al. (2021), suggested that specific enzymes (cytochrome P450) are responsible for degrading mycotoxins and converting them to excretable metabolites. The authors also present several examples of insect-species that seem to have the ability to metabolize mycotoxins using this specific enzyme, of which BSFL is one of them.

The physiological and morphological features of the BSFL mid-gut enables them to digest a diverse range of organic recourses (Surendra et al. 2020). The mid-gut of the BSFL is divided into three regions with distinctly different pH: 2, 6 and 8.5. This variation in pH plays an important role in enzyme activity, nutrient solubility and is believed to be one of the reasons for BSFL ability to inactivate toxins (ibid). According to Xia et al. (2021), BSFL is a promising sources of functional antimicrobial peptides, which works as immune defense to inactivate toxins and pathogens.

Pre-treatment with T. reesei; improvements in BSFL composting

T. reesei is a mesophilic and filamentous fungus commonly found in soil and root ecosystems. It is used in industrial processes, such as the production of cellulases, due to its ability to produce enzymes that break down plant biomass, by converting lignocellulosic biomass into soluble and fermentable sugars (Suo et al. 2023). Mustafa et al. (2016) found that rice straw pre-treated with *T. reesei* resulted in a 23% lignin reduction. Subsequently, when digesting the pre-treated substrate, the methane yield was increased by 120 % compared to non-pre-treated rice straw. Rearing BSFL on fruit and vegetables has been shown to result in a lower process efficiency, in terms of biomass conversion efficiency, than mixed food waste (Lalander et al. 2019). This is likely due to the high content of lignin and hemicellulose in relation to its low protein content. Also, the higher moisture content in fruit and vegetable also contribute to a lower process efficiency. Lindberg et al. (2022) found that pre-treatment with *T. reesei* increased the availability to the larvae, while also drying out the substrate. Isibika et al. (2019) found that pre-treating banana peels with *T. reesei* enhanced the biomass conversion efficiency in BSFL larvae composting.

T. reesei as degrader of mycotoxins

Trichoderma spp. is commonly used to control soil-borne, leaf and panicle diseases in agriculture (Yao et al. 2023). Because of the secondary metabolites and cell wall-degrading enzymes; *T. reesei* can improve plant growth, the plant utilization of nutrients and plant resistance (Kubicek et al. 2019). Yue et al. (2022) found that a specific strain of *T. reesei (*CGMCC3.5218) could degrade 100% of 50 ng/kg aflatoxin B_1 within 3 days and 87.6% of 10 μg/kg aflatoxin B_1 within 5 days. A study by Dini et al. (2022) on *Trichoderma* spp., also demonstrated promising results in degradation when aflatoxin B1 and ochratoxin A were in combination.

3. Material and Method

This work was done in two parts: a qualitative part through literature review and an experimental trial. The aim of the literature overview was to identify mycotoxins that had already been studied in the context of BSFL composting and to determine if any mycotoxins listed in EU regulations had not yet been investigated. In addition, the methodologies utilized in the identified studies were evaluated.

The specific objectives of the experimental trial were to assess the impact of *Trichoderma* spp. treatment and BSFL composting, alone and in combination, on different concentrations of a selected mycotoxin. In addition, the bioaccumulation factor of selected mycotoxin in larvae consuming these substrates was assessed. In addition, the impact of mycotoxin addition on the BSFL composting efficiency, in terms of larval survival, biomass conversion efficiency (BCE), larval yield and material reduction, was assessed.

3.1 Part 1 – setting the boundaries

Part one of the study entailed a literature overview was conducted in two rounds using the Primo database. In the first round, the search word was *Hermetia illucens* + mycotoxin. This resulted in 24 hits, of which 11 was considered relevant based on method and aim of the study. If the methodology in the identified article was similar to the approach conducted in this study, it was considered relevant. Two of the articles were literature overviews on mycotoxin exposure in BSF larvae composting and became the basis of the subsequent search round.

Second search round, the search was done by combining the Latin name for BSF (*Hermetia illucens)* and the specific mycotoxin regulated in EU regulation (2023/915) (Table 2). The search excluded articles that had not been peer-reviewed. Articles were selected based on the methodology utilized in the respective studies. It was decided that the methodologies employed in the respective studies should have been conducted in such a way that would allow for a comparison of the results among them. Articles were considered relevant if their objective was to evaluate the impact of specific mycotoxins on both the larvae and the frass from the BSFL treatment. The selected articles were then compared with the ones referred to in two

review-articles, in order to identify additional resources not already listed. Only one additional article was added in the second round.

Table 2. All search words used for the second search round, Showing the result of number of hits and number of articles considered relevant. Database used: Primo

Search words	Hits	Relevant
Hermetia illucens + *Aflatoxin*	13	5
Hermetia illucens + *Ochratoxin A*	4	2
Hermetia illucens + *Patulin*	0	0
Hermetia illucens + *Deoxynivalenol*	6	4
Hermetia illucens + *Zearalenone*	6	3
Hermetia illucens + *Fumonisins*	3	1
Hermetia illucens + *Citrinin*	1	1

Eight unique articles were selected. Several of the studies were done on two or more mycotoxins in the same article. Out of those eight, five studies were identified based on their experimental set up and laid the foundations for the continued work. Parameters considered were number of larvae used, chosen concentrations and the reasoning behind, length of experiment and the method for applying the mycotoxins.

3.2 Part 2 – Preforming the trial

3.2.1 Materials

The substrate used in the experiments was commercial poultry feed, containing cereal and maize, that were soaked in water to reach a dry mater content of 29.6 ± 1,2%. The BSFL used in the study was provided by the BSF colony at the Department of Energy and Technology, SLU Uppsala. The mycotoxin patulin was purchased from the manufacturer Sigma-Aldrich Solutions in powder form at 98 % purity where deionized water was used as solvent. *T. reesei* was used in fungi treatments and were grown on malt extract agar (MEA) plates (SVA, Uppsala). Physiological (0,9% NaCl) solution (SVA, Uppsala) was used as buffer, 10-uL inoculation loop and 50 ml centrifuge tubes were used to harvest the *T. reesei*.

Tiny tag-sensors to record the relative humidity (RH) and temperature with +-3% and 0.45°C accuracy, respectively. The resolution was <0.3% for RH 0.01°C for temperature. A WIFI Thermometer was also used to monitor the real-time temperature curve through a phone application (Govee Home), allowing to track and control the temperature during treatment. All the treatments were performed in a ventilated tent with a radiator inside, to create a stable environment at 28°C +/-3°C and to avoid contamination to and from surroundings. Plastic crates (boxes)

with inner dimensions of 18,5x16x10 (LxWxD) were used, as treatment unit, for each replicate. Aluminum cups were used for TS/VS sampling.

3.2.2 Experimental trial

The experimental trial was divided up in three treatment strategies: one BSFL treatment, one *T. reesei* treatment and one combined treatment (Table 3). Within these treatment strategies, a control group without toxin and a low patulin toxin concentration (100 µg/kg on TS basis) scenario was included. The BSFL treatment and combined treatments also included a high toxin concentration (1000 µg/kg on TS basis) scenario. One toxin control was also set up to see if time alone could have an impact on patulin.

Table 3. Experimental setup. L = Low toxins = 100 µg/kg (TS basis), H = High toxin = 1000 µg/kg (TS basis). Treatments whit and without Trichoderma were conducted separately.

Treatment name	Substrate	Patulin	*T. reesei*	Larvae
Toxin control	x	L		
BSFL treatment	x	L/H		x
BSFL control	x			x
T. reesei treatment	x	L	x	
T. reesei control	x		x	
Combined treatment	x	L/H	x	x
Combined control	x		x	x

The low toxin concentration scenario was based on the concentration commonly found in fruits (Hussain et al. 2020)., while still being higher than the maximum level of patulin in EU regulations (Table 1). The high concentration scenario was set to ten times the low concentration. Treatments with *T. reesei* and treatments without were performed separately to avoid cross contamination. The ones without toxin were conducted first. All treatments were performed in triplicate.

Substrate preparation

The substrate was mixed to a 1:2 ratio of poultry feed and water. The ratio was defined based on a pre-trial conducted using a feeding protocol developed by the Department of Energy and Technology. The goal was to achieve a substrate with 33% total solids (TS)

The patulin powder was diluted in deionized water to a concentration of 10 mg/mL in the vial. Using a syringe, the content of the vial was emptied into a glass bottle and further diluted in deionized water to a concentration of 100 ug/mL (Figure 5). The substrate was inoculated with two different concentrations of

patulin, 100 µg/kg and 1000 µg/kg on a TS basis, approximately 33 µg/kg and 330 µg/kg on a wet weight basis. The purity of the patulin used was 98%. Patulin was added to the substrate by weighing the amount of toxins, dissolve it in water and then pouring the inoculated water into the dry poultry feed and allow it to soak. The mixture was stirred for 3 min in order to evenly distribute the toxins. The substrate was then put in plastic bags with the respective feeding amount and stored in the freezer -18°C until day before feeding.

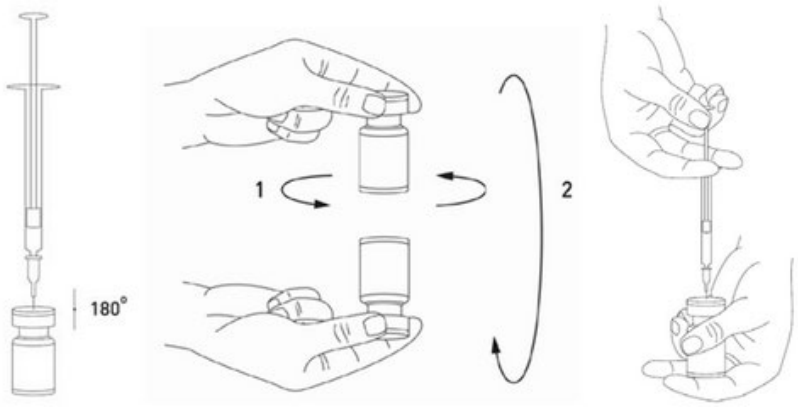

Figure 5. Descriptive picture on how patulin extraction were preformed (FASS 2022)

T. reesei was pre-cultured on malt extract agar plates at 27 – 30°C in an incubator for 7 d before adding it to the substrate. The fungi were harvested by adding 0.9% NaCl solution directly onto the agar plat and then gently scraping the fungi off with a 10-uL inoculation loop. The solution was poured into a 50 mL centrifuge-tube. This was repeated until the centrifuge tube was full, one tube per plate. Then, 0.5 % of the *T. reesei* solution was added to the thawed substrate and thoroughly mixed to evenly distribute the fungal cells into the substrate. The substrate and fungi mixture were kept for 7 d in an incubator at 27 – 30°C to allow for the fungi to colonies the substrate. This procedure, pre-culturing, harvest and adding to substrate, was conducted three times, once for each feeding occasion, to ensure uniformity across all larval treatments containing *T. reesei*.

To estimate concentration of added *T. reesei*, fungal cells were enumerated using a *Bürker chamber* under a microscope. The camber depth was 0.1 mm, and the chosen squares had a side length of 0.05 mm, resulting in a volume of $2.5 \times 10^{-7} c\mathrm{m}^3$. Concentration was calculated by taking the number of cells and dividing it by number of squares plus the volume. Since the pre-culturing of *T. reesei* was preformed three times; one counting was done for each centrifuge tube. The following concentrations was achieved:

Batch 1: 5.79×10^6 cells/cm^3
Batch 2: 4.89×10^6 cells/cm^3
Batch 3: 13.96×10^6 cells/cm^3

BSFL composting

All treatments were performed in a ventilated tent with a radiator inside, to create a stable environment at 28°C +/- 3°C and to avoid contamination to surroundings. Plastic crates (boxes) with inner dimensions of 18,5x16x10 (LxWxD) were used for each replicate. All trials were conducted in the same way in terms of number of feedings and feeding schedule. The trial started at day 0 and ended at day 16 (Figure 6), In all larval treatments, 1.7 - 2.3 grams of larvae (1155 larvae with an average weight 1.5-2 mg per larva) were added, while 876 g of substrate was added to each replicate, divided into three feeding occasions (day 0, 3 and 6) of each 292 g. The amount of substrate was based on pre-test of substrate with the aim to reach a feeding dose of 0,2 g of volatile solids (VS) per larva. Water was added when needed throughout the experiment.

At the end of the experiment, larvae were harvested and separated from the frass and their weight was recorded. The larvae were counted to get an approximate weight of each larva. Larvae and frass were placed in plastic bags and stored at -18°C until further analysis.

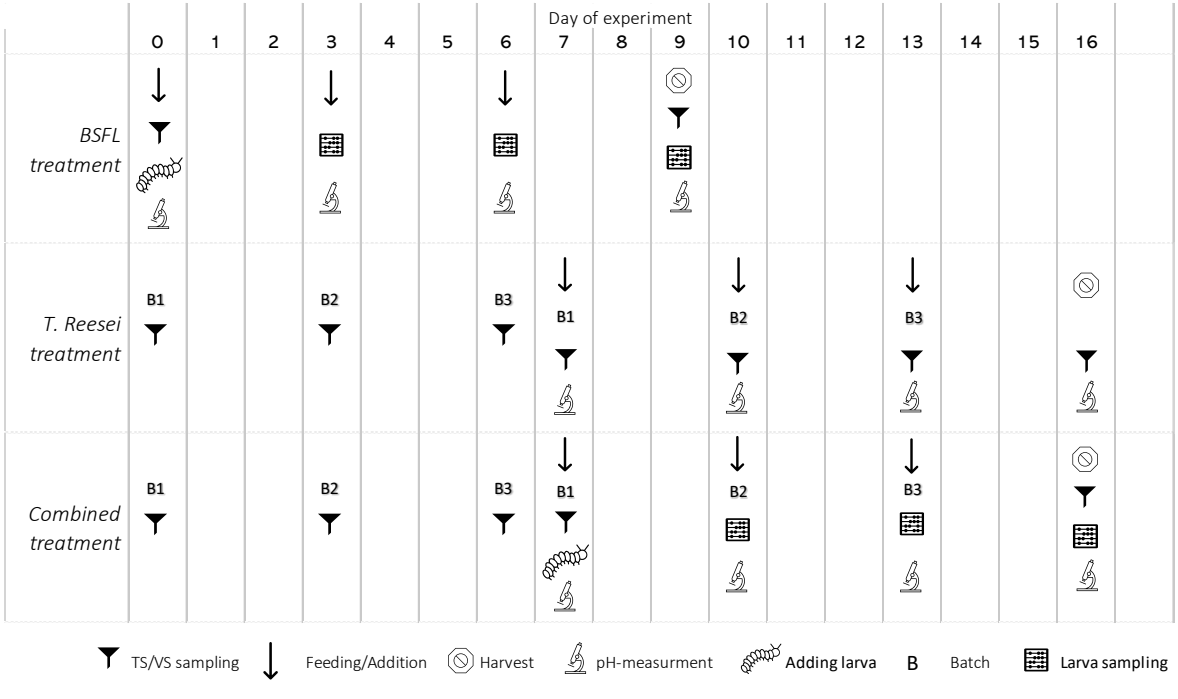

Figure 6. Schematic representation of sampling and feeding scheme. Inoculating the substrate with pre-cultured T. reesei was done in three batches (B1, B2, B3) for seven days.

Sampling

The larvae were sampled before every feeding event (day 3 and 6) to assess larval growth. The initial larval count was done by taking three samples of larvae and enumerate manually. Subsampling during the trial were done by taking five small samples of larvae from each corner and in the middle according to subsampling protocol from the Department of Energy and Technology (Figure 7). The larvae were manually enumerated and weighed. This was done three times for each replicate to get an average weight. The same procedure was used at harvest. The frass and larvae were separated using a sieve with different sizes and weighed separately.

In BSFL treatments, TS/VS samplings were taken at the start on the feeding material and at harvest on larva and the frass. In *T. reesei* treatments, TS/VS were, in addition, taken at every feeding event. pH was measured on day 0, 3, 6 and 9.

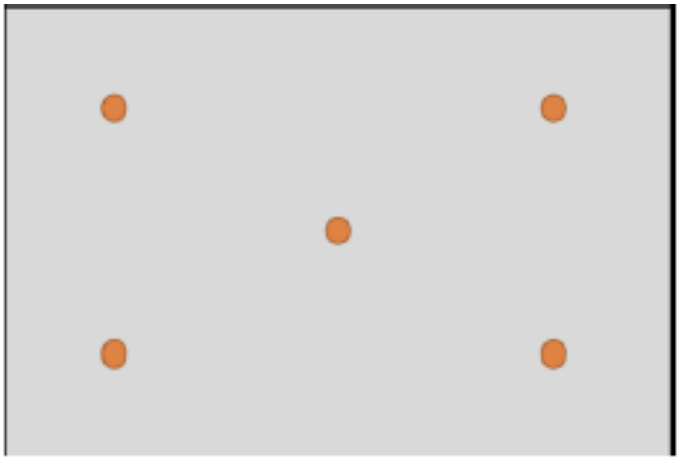

Figure 7. Larval count illustration. Subsampling protocol (SLU 2023)

Physio-chemical analysis

Total solids (TS) analysis was conducted by weighing an aluminum cup, adding approximately 10 grams of material (substrate, frass, larvae) and then drying it in an oven at 60°C for at least 48 h. For total volatile solids analysis (VS), the dried sample was burned in a furnace firstly at 250 °C for 2 h and then at 550 °C for 4 h.

Measurement of pH was done by placing the pH-meter directly in the material. When the larvae grew larger, the pH was measured by taking a sample of substrate/frass in a cup in which the pH-measurements were performed.

Patulin analyses

Patulin analyses were performed using AOAC (analyzing various food components) method and LC-MS/MS (liquid chromatography-mass spectrometry) and were performed at three different accredited laboratories.

Calculations

The concentration of *T. reesei* was calculated in three steps:

$$V_{small\ square} = 10^{-2} \times (5 \times 10^{-3}) \times (5 \times 10^{-3})$$

$$n_{small\ squares} = 16\ small\ squares \times 7$$

$$Concentration_{cell/mL} = \left(\frac{n_{cells}}{n_{squares} \times V_{small\ squares}}\right)$$

where the $V_{small\ square}$ is the Volume of the chosen squares in the *Bürker chamber* and $n_{small\ squares}$ is the number of squares.

The concentration of Patulin to add to substrate was calculated as:

$$m_{TS\ CF} = m_{ww\ CF} \times TS\%_{CF}$$

$$V_{Patulin} = CF_{TS} \times \left(\frac{mass_{Patulin}}{\%purity_{Patulin}}\right)$$

where $m_{ww\ CF}$ and $TS\%_{CF}$ is the mass of poultry feed on a wet weight basis and total solids in poultry feed, respectively. The purity of the patulin should be accounted for when adding it to a substrate, to calculate the actual concentration in the substrate (poultry feed).

The percentage material reduction (MR) on a VS basis was calculated as:

$$MR_{VS} = \left(1 - \frac{mVS_{res}}{mVS_{inital}}\right) \times 100$$

where mVS_{res} and mVS_{inital} in BSFL composting is the total mass of volatile solids in residue (frass) and initial substrate, respectively. For material reduction of the *T. reesei* material, before feeding to larvae, mVS_{res} and mVS_{inital} is the total mass of volatile solids in treated material and initial material, respectively.

To evaluate the efficiency of the trials with BSFL, substrate to biomass conversions efficiency was calculated as:

$$BCE_{VS} = \left(\frac{mVS_{larvae}}{mVS_{inital}}\right) \times 100$$

where mVS_{larvae} and mVS_{inital} is the total mass of volatile solids in larvae and initial substrate, respectively.

Total VS loss was calculated as:

$$Total\ loss_{VS} = \left(\frac{mVS_{inital} - mVS_{larvae} - mVS_{res}}{mVS_{inital}} \right) \times 100$$

where mVS_{inital}, mVS_{larvae} and mVS_{res} is the total mass of volatile solids in initial substrate, larvae and residue (frass), respectively.

Percentage of larval survival in each BSFL treatment was calculated as:

$$Survival\ _{\%} = \frac{n_{LV-end}}{n_{LV-start}}$$

where $n_{LV-start}$ is the number of larvae at start and n_{LV-end} is the number of larvae at end of treatment.

Bioaccumulation factor (BAF) was calculated as:

$$BAF = \frac{C[Patulin]_{larvae}}{C[Patulin]_{substrate}}$$

where BAF > 1 means that accumulation is occurring.

Statistical analysis

Analysis of variance (ANOVA) with 5 % significance level, was used to evaluate whether the outcome of the treatments was significantly different. If statistical difference was found, Tukey's honestly significant difference (HSD) *post hoc* test with 5% significance level was performed, in order to identify significant differences between the treatments. The data were analyzed in Minitab software.
The data was checked for nonequal variances, no significant difference between the variances was found. All values were normally distributed.

4. Results

Part 1 - Literature overview

To enable comparison, the five selected studies were conducted with set-ups within the same range as used in the experimental trial of this study. Treatment times were around 12 to 15 days, and the number of larvae ranged from 100 to 7500. The number of feeding events varied between 1-2, of which two of them did the last feeding on substrate without toxins to clear the gut of the larvae before analysis. Two studies used only naturally contaminated substrate, and one used partly naturally contaminated substrate (Table 4). Across all five studies combined, all mycotoxins listed in EU regulation No. 1881/2006 were represented, except patulin. Since patulin was the only mycotoxin not investigated in any previous studies, it was chosen as the focus of this study.

Table 4. Summary of the different set-ups used in terms of number of larvae, time of exposure to mycotoxins, number of feedings, substrate, naturally or artificially contaminated substrates and type of mycotoxins analyzed. [a]Not specified

Ref.	No. of larvae	Time expos (d)	Number of feedings	Substrate	Natural/ artificial cont.	Mycotoxin analyzed
Bosch et al. (2017)	100	12	One feeding with toxins for 10 days, last feeding without toxins for 1 d to clear gut.	Poultry feed	Spiked feed artificially	AFB1 B1
Camenzuli et al. (2018)	100	12	One feeding with toxins for 10 d, last feeding without toxins for 1 d to clear gut.	Wheat base and water.	Spiked feed artificially	AFB1, DON, OTA, ZEN and one mix of all.
Purschke et al. (2017)	700	1 3	2 feedings	Substrates based on corn semolina + contaminated corn grains.	Spiked feed AF. DON and ZEN naturally cont.	AFB1, AFB2, AFG2, DON, OTA, ZEN
Leni et al. (2019)	NS[a]	15	1 feeding	byproducts of corn, wheat, rice, rapeseed, apple, olive and carrots.	Naturally contaminated.	DON, FB1, FB2, ZEN.
Gold *et al.,* (2023)	7500	12	1 feeding	Maize and agri-food byproducts.	Natural contaminated.	AFB1, AFB2, AFG2, DON, FB1, FB2, CIT, ZEN, OTA in mix

In the five studies in which the larval survival was monitored, none of the studies showed any significant impact on larvae survival. Furthermore, all but one study showed that weight gain was not impacted. Gold et al (2023) showed a small decrease in weight gain when feeding larvae high concentration of contaminated maize. For aflatoxins (AF) (Table 5), the highest initial concentration investigated was 500 µg/kg (Bosch et al. 2017), which is 30 to 50 times higher than upper level of allowed concentration in food in EU regulations (Table 1). All studies conducted on aflatoxins and its metabolites reported concentrations below the limit of detection in the larvae,; except Gold et al. (2023), which found concentration up to 11 µg/kg in the larval biomass when feeding high concentrations of aflatoxins to the larvae.

For deoxynivalenol (DON), the highest concentration investigated was 112,000 µg/kg (Table 5), which is 64 times the regulatory upper limit of allowed concentrations in the EU. There were three studies investigating DON and all showed a decrease to below EU limits or below limit of detection in larvae.

In the case of ochratoxin A (OTA), the highest concentrations investigated was 1700 µg/kg, 20 times the upper regulatory limit allowed in the EU. In the one study that investigated OTA, a significant decrease in concentration in larvae was demonstrated. The highest concentration detected in the larvae was 2.6 µg/kg. The same trends as for AF, DON and OTA was demonstrated for fumonisins (FB), zearalenone (ZEN), citrinin (CIT) and mycotoxins in mix. In several trials, a reduction of mycotoxins was observed when the larvae were allowed to empty their gut in different ways prior to analysis; *e.g.* by feeding non- contaminated substrate or by subjecting the larvae to a fasting period of 24 h. When investigating the concentration of mycotoxins in the frass (Table 5), all studies reported a reduction.

Camenzuli et al. (2018) observed an increase in concentration in the frass for DON, OTA and ZEN. However, the concentration in larvae and frass are both expressed in dry weight (Table 5; column 3 and 4), while the initial concentration (Tabel 5; column 2) is expressed on a wet weight basis. If the initial concentration were to be expressed in dry weight, like the larvae and frass, the initial concentration would be higher and thereby show a decrease in larvae and frass.

Table 5. Summary of findings in selected studies on mycotoxins in BSFL feeding trials. LOD=Limit of detection, LOQ= Limit of quantification. Concentrations in dry weight.

Ref.	Initial conc. (µg/kg)	Conc. In larvae post treatment (µg/kg)	Conc. In frass post treatment (µg/kg)	Analyzing method	Limit of detection/ quantific.
Bosch et al. (2017)	AFB1: 10, 25, 50, 100, 250 and 500	<LOD	AFB1: 0,010 - 1,3	HPLC w Fluorescence Detector	0.10 µg/kg
Camenzuli et al. (2018)	**In wet weight (WW)** AFB1: 8, 70, 390	**In dry weight (DW)** AFB1: <LOQ	**In dry weight (DW)** AFB1: <LOQ, 5.5, 303.3.	LC-MS/MS analyses	1 µg/kg

	AFmix: 18, 180, 430. DON: 3900, 38000, 112000 DONmix: 4100, 41000, 100000 OTA: 170 1300, 1700. OTAmix: 80, 800, 2000. ZEN: 280, 2500, 13000. ZENmix: 400, 3800, 9400	AFmix: <LOQ, DON: <LOQ, 129,3, 256,7 DONmix: <LOQ, 109.5 176.7. OTA: <LOQ, 2.2, 2.6 OTAmix: <LOQ, 1.8, 3.9 ZEN: <LOQ, <LOQ, 27.5 ZENmix: <LOQ.	AFB1mix: <LOQ 13.7, 353.3 DON: <LOQ, 7700, 316700. DONmix: <LOQ, 15700, 296700. OTA: <LOQ, 400, 5100. OTAmix: <LOQ 200, 5000. ZEN: <LOQ, 700, 35000. ZENmix: <LOQ, 990, 22700.		
Purschke et al. (2017)	AFB1: 88, DON: 697, OTA: 39.4, ZEN: 160	<LOD	AFB1: 10.9. DON: 1135. OTA:<LOQ. ZEN: 103.	HPLC-MS/MS QTRAP	4 – 20 µg/kg
Leni et al. (2019)	DON: 779 FB1: 573 FB2: 441 ZEN: <LOD.	<LOD	DON: 1473 FB1: 951 FB2: 344 ZEN: 334	HPLC w Fluorescence Detector, UHPLCMS/MS	Not listed
Gold et al. (2023)	AFB1:16.2-99.4 AFB2: 2.5-12.2 AFG2: 0.5-2.7 DON: 130.7-142.1 FB1: 347-1,035 FB2: 122-379 ZEN: <6.8-15.9 OTA: < 0.54 CIT: 0.9-23.2	AFB1: <0.3-11.4 AFB2: <0.1-1.4 AFG2: < 0.5 DON: < 29.8 ZEN: < 7.1 FB1: 85-296 FB2: 29-114 OTA: < 0.5 CIT <0.5	AFB1: 14.4-62.1 AFB2: 1.7-9.8 AFG2: 1.7-7.8 DON: 88.9-126.6 FB1: 89-1068 FB2: 151-365 ZEN: <6.7-24.0 OTA: < 0.5 CIT <0.5	LC-MS/MS analyses	0.07 – 32 µg/kg

Most of the studies did not examine the effects of process efficiency in BSFL composting in terms of material reduction and/or bioconversion efficiency. Purschke et al. (2017) did compare the feed conversion ratio – which is the consumed feed in gram divided by the larval growth in gram –and reported no significant difference between the group with mycotoxin contaminated substate and the control group.

4.1 Part 2 - Experimental trial

4.1.1 Process efficiency

The temperature and relative humidity in the treatment tent was on average 27.8 ± 1.9 °C and 18.0 ± 3.8%, respectively, during *T. reesei* treatments and combined treatments and 27.1 ± 2.3 °C and 42.0 ± 4.5% during BSFL treatments and control treatment. All treatments had similar initial total solids (TS) and volatile solids (VS), which varied between 30.1% to 35.8% and 86.0% to 87.8%, respectively (Table 6). There was a significant difference in the TS and VS in the harvested larvae between the BSFL treatments and the combined treatments. A significant difference between treatments was observed for TS and VS in the treatment residues. In all treatments, a reduction in moisture content by the increasing percentage of TS was demonstrated. The highest reduction in moisture content was observed in the combined treatments and the BSFL treatments. VS in the treatment residue varied greatly between treatments, from 66.9% in combined treatments to 82.4% in *T. reesei* treatments. *T. reesei* treatments and BSFL treatments ended up with treatment residue of similar VS content (Table 6).

Table 6. Mass balance of trial process. Total solids (TS), total volatile solids (VS) of all substrates measured at start of the trial, larvae, and residues at end in the different treatments. Treatments with T. reesei is presented as mean of the three feedings. Values are presented as mean ± SD (n=3). Initial feed before T. reesei is results if sampling before adding T. reesei and initial feed before BSFL is when adding the larva (except for T. reesei treatment that did not receive larva). Different superscript letters within columns indicate significant difference ($p < 0.05$).

	Initial feed *(Before adding T. reesei)*		Initial feed *(Before adding BSFL)*		Larvae out		Residue out	
	TS%	VS%	TS%	VS%	TS%	VS%	TS%	VS%
Toxin control								
Control			31.3 ± 0.30^{cd}	86.5 ± 0.25^{b}	–	–	50.8 ± 1.05^{c}	86.5 ± 0.21^{a}
BSFL Treatment								
Control			$30.8 \pm 0{,}20^{d}$	88.4 ± 0.40^{ab}	37.6 ± 0.99^{a}	91.3 ± 1.22^{a}	62.5 ± 4.0^{b}	71.4 ± 3.76^{c}
Low toxin			31.3 ± 0.82^{cd}	87.4 ± 0.83^{ab}	36.9 ± 0.37^{a}	92.8 ± 0.22^{a}	66.5 ± 2.74^{ab}	70.9 ± 1.80^{c}
High toxin			29.7 ± 0.82^{d}	89.9 ± 2.39^{a}	37.5 ± 0.21^{a}	91.5 ± 0.39^{a}	49.4 ± 4.27^{c}	70.5 ± 1.41^{c}
T. reesei treatment								
Control	30.6 ± 0.27^{a}	86.3 ± 0.49^{a}	33.9 ± 0.92^{b}	86.0 ± 0.60^{b}	–	–	45.0 ± 3.55^{c}	82.4 ± 1.25^{ab}
Low toxin	30.5 ± 2.11^{b}	87.3 ± 1.18^{b}	32.5 ± 0.25^{bc}	86.5 ± 0.42^{b}	–	–	42.6 ± 2.74^{c}	77.9 ± 3.43^{b}
Combined treatment								
Control	30.6 ± 0.27^{a}	86.3 ± 0.49^{a}	32.9 ± 0.68^{b}	86.0 ± 0.60^{b}	33.2 ± 0.21^{b}	87.5 ± 0.39^{bc}	61.1 ± 3.31^{b}	67.4 ± 1.28^{c}
Low toxin	30.5 ± 2.11^{b}	87.3 ± 1.18^{b}	32.4 ± 0.28^{bc}	86.5 ± 0.42^{b}	32.8 ± 0.64^{b}	86.7 ± 0.69^{c}	67.9 ± 1.40^{ab}	66.9 ± 1.43^{c}
High toxin	30.3 ± 0.18^{a}	86.9 ± 0.71^{a}	35.8 ± 0.54^{a}	86.2 ± 0.20^{b}	33.9 ± 0.21^{b}	88.7 ± 0.12^{b}	72.0 ± 1.63^{a}	66.9 ± 1.68^{e}

There was a significant difference in pH between the initial feeding of the treatments with and without *T. reesei* treatment, where the *T. reesei* treatment and combined treatment started at lower pH (4.7) than the BSFL (6.8) and Control Treatment (5.9) (Figure 8). At harvest, the BSFL treatments (6.2) and *T. reesei* treatments (6.0) were significantly different from the combined treatment (8.0). The combined treatments had a significantly higher pH at harvest. The BSFL treatments reached the highest peak in pH among the treatments (8.7), while the *T. reesei* treatment reached the lowest peak (4.7), with the exception of the Toxin control (4.4). In the combined treatment the pH steadily increased throughout the treatment, while the pH in BSFL treatment decrease at the beginning of the trial, reaching a peak at pH around 8.5 before the third feeding and decreased again to around pH 6 at the end.

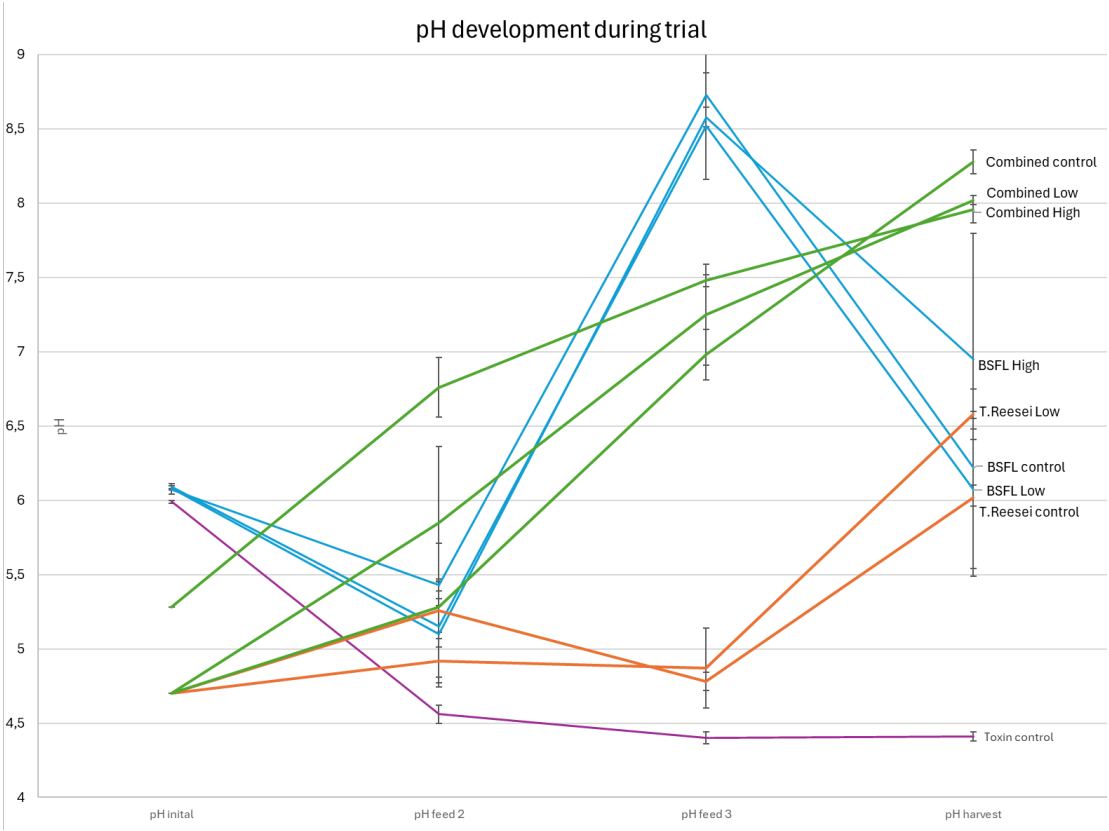

Figure 8. pH in substate at start and in treatments before each feeding and at harvest.

Bio conversion efficiency (BCE) on both TS and VS basis differed significantly between treatments. The highest BCE on VS basis was obtained in BSFL high toxin treatment (28.8%) and the lowest on VS basis in BSFL control treatment (17.6%). Similar material reduction, on a TS and a VS basis, were obtained in BSFL treatment (59.3% to 62.2%) and combined treatment (61.1% to 63%). The material reduction and the loss of VS in *T. reesei* treatments were significantly different between the control and the low toxin within the treatment group (Table 7).

The combined treatments had the highest material reduction on a VS basis (72.0%) and the highest total loss of VS (48.3%).

Table 7. Bioconversion efficiency and material reduction on a TS and VS basis for all treatments. Total loss of VS for all treatments in percent. Values presented are mean ± SD (n=3) Different superscript letters within columns indicate significant difference ($p < 0.05$).

	$BCE_{TS}[\%]$	$MR_{TS}[\%]$	$BCE_{VS}[\%]$	$MR_{VS}[\%]$	$Total\ loss\ of\ VS[\%]$
Toxin control					
Control	–	3.6 ± 2.87^{d}	–	4.4 ± 3.10^{d}	–
BSFL Treatment					
Control	17.1 ± 0.88^{d}	62.2 ± 0.63^{a}	17.6 ± 0.85^{d}	69.6 ± 1.89^{a}	38.0 ± 1.73^{d}
Low toxin	25.1 ± 1.12^{bc}	59.7 ± 0.92^{a}	26.7 ± 1.20^{ab}	67.3 ± 1.20^{a}	39.7 ± 0.56^{cd}
High toxin	27.7 ± 0.91^{a}	59.3 ± 3.16^{a}	28.2 ± 0.85^{a}	67.5 ± 1.96^{a}	40.3 ± 2.52^{bcd}
T. reesei treatment					
Control	–	14.0 ± 6.56^{c}	–	17.7 ± 4.73^{c}	–
Low toxin	–	25.3 ± 4.93^{b}	–	33.0 ± 5.57^{b}	–
Combined treatment					
Control	$26{,}3 \pm 0.56^{ab}$	61.0 ± 3.46^{a}	27.0 ± 0.01^{ab}	71.7 ± 3.51^{a}	44.7 ± 3.51^{abc}
Low toxin	23.3 ± 1.16^{c}	63.0 ± 1.00^{a}	22.7 ± 1.53^{c}	72.0 ± 1.00^{a}	48.3 ± 1.53^{a}
High toxin	24.7 ± 0.58^{bc}	61.3 ± 0.58^{a}	25.3 ± 0.58^{b}	70.3 ± 0.58^{a}	45.3 ± 0.58^{ab}

The total BCE (%) showed similar results for both BSFL treatments and combined treatments. The larvae out in grams were higher in combined treatments (655.99 to 692.67) than in BSFL treatments (558.56 to 579.27) (Table 8).

Table 8. Illustrative table of total feed going into each treatment and total mass of larvae out for all treatments. Feed load in grams as sum of all replicates for each treatment receiving larva (BSFL treatments and combined treatments). Calculation of BCE in wet weight. For BSFL treatments: larvae out/initial feed before adding BSFL, for combined treatments: larvae out/initial feed before adding T. reesei.

	Initial feed (g) *(Before adding T. reesei)*	Initial feed (g) *(Before adding BSFL)*	Larvae out (g)	Total BCE (%WW)
BSFL Treatment				
Control		2629	563	21.4
Low toxin		2630	558	21.2
High toxin		2630	579	22.0
Combined treatment				
Control	3281	2646	692	21.0
Low toxin	3302	2654	656	19.9
High toxin	3676	2646	693	18.8

Feed load in grams per larva were significantly different between treatments; the combined treatment received 5-15 % more feed than the other BSFL treatments (Table 9). The survival rate where higher in the combined treatments (90.3% – 102%) and the lowest survival was found in the BSFL high toxin treatment (64%). Overall, BSFL treatment showed a low survival rate. The average weight per larva differed significantly between treatments. The high toxin, combined treatment had the lowest weight (0.20 g/larva), while larvae in BSFL high toxin treatment had the highest weight (0.26 g/larva).

Table 9. Feed load in grams of VS per larvae. Larvae survival throughout the BSFL composting process in percent of numbers out divided with number of larvae in. The average weight per larva at the end of the trial. All values were normally distributed. Values presented are mean ±SD (n=3) Different superscript letters within columns indicate significant difference ($p < 0.05$).

	$Feed\ load$ $[g_{VS}/larvae]$	$Survival[\%]$	$Weight/$ $larva_{WW}[g]$
Toxin control			
Control	–	–	–
BSFL Treatment			
Control	0.21 ± 0.001^{d}	69.9 ± 3.41^{cd}	0.23 ± 0.005^{b}
Low toxin	0.21 ± 0.00^{d}	71.7 ± 1.53^{c}	0.23 ± 0.004^{b}
High toxin	0.20 ± 0.00^{e}	64.3 ± 3.06^{d}	0.26 ± 0.004^{a}
T. reesei treatment			
Control	–	–	–
Low toxin	–	–	–
Combined treatment			
Control	0.22 ± 0.001^{c}	90.3 ± 2.52^{b}	0.22 ± 0.005^{b}
Low toxin	0.23 ± 0.001^{b}	91.7 ± 0.58^{b}	0.21 ± 0.006^{c}
High toxin	0.23 ± 0.001^{a}	102.0 ± 2.65^{a}	0.20 ± 0.003^{c}

The larval weight gain development followed the same pattern in all treatments throughout the BSFL treatments and combined treatments (Figure 9).

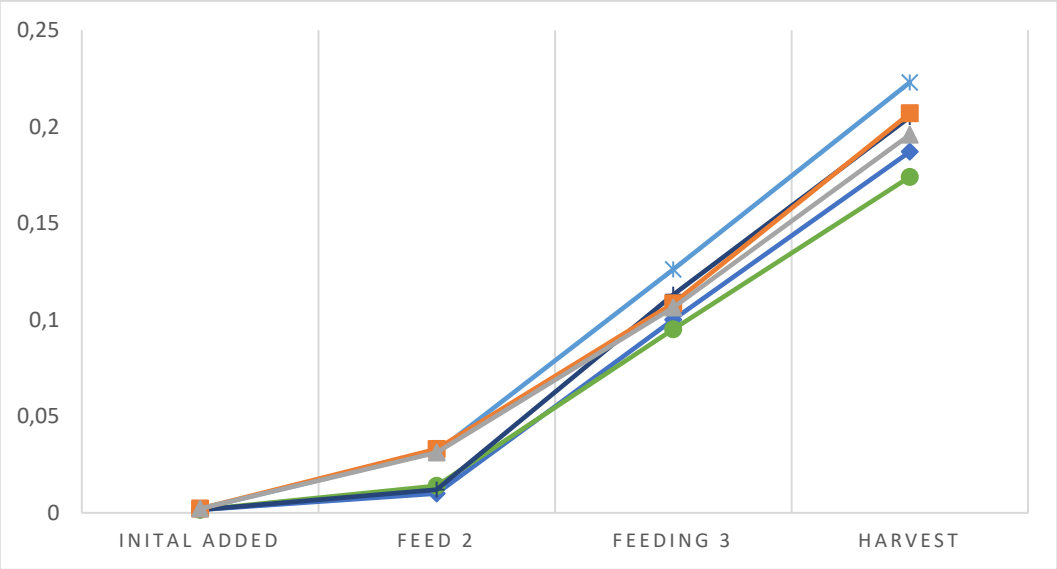

Figure 9. Larval weight development throughout the BSFL composting in grams per larvae measured at each feeding event.

When examining the material/frass at harvest a few things were noted. The frass of the combined treatments had a much finer texture compared to the frass obtained in BSFL treatments. In the BSFL treatment, a hard crust was formed on top of the material/frass. The frass of the combined treatments felt moister throughout the material/frass and had less of a smell than the BSFL treatments.

4.1.2 Patulin analysis

The patulin analysis was performed for all samples at two different accredited laboratories and one control group was sent to a third laboratory.

The first group of samples were sent to Trilogy Analytical Laboratory in Washington, USA. The method used was HPLC with reference Journal of AOAC method #995.10. The detection limit for the analysis was 10 ppb. Second round of samples were sent to SGS Analytics, Linköping, Sweden. The method used was LC-MS/MS. Detection limit for the analysis was 5 µg/kg. The third lab used was Eurofins food and feed testing, Linköping, Sweden. The method used was LC-MS/MS. Detection limit for the analysis was 5 µg/kg.

Table 10. Results from Triology analytical laboratory in ppb and from SGS analytics, to compare with the properties of the samples sent. The results presented in wet weight and the substrate concentration in dry weight. The dry mater content was 30%.

Sample Identification	Sample Description	Results Triology lab [ppb]	Results SGS analytics [µg/kg]	Substrate toxin concentration [µg/kg]	Added fungi	Added larvae
C1R1-F	Frass	143,5	<5			x
C1R2-F	Frass	835,7	<5			x
C1R3-F	Frass	58,7	<5			x

C1R1-L	Larvae	<RL	<5			x
C1R2-L	Larvae	37,3	<5			x
C1R3-L	Larvae	21,2	<5			x
C2R1-F	Frass	<RL	<5	100		no
C2R2-F	Frass	<RL	<5	100		no
C2R3-F	Frass	25,5	<5	100		no
C3R1-F	Frass	59,3	<5	100	x	no
C3R2-F	Frass	113,4	<5	100	x	no
C3R3-F	Frass	43,2	<5	100	x	no
C4R1-F	Frass	64,5	<5		x	no
C4R2-F	Frass	66,3	<5		x	no
C4R3-F	Frass	<RL	<5		x	no
C5R1-F	Frass	404,8	<5		x	x
C5R2-F	Frass	480,1	<5		x	x
C5R3-F	Frass	637,1	<5		x	x
C5R1-L	Larvae	23,4	<5		x	x
C5R2-L	Larvae	39,2	<5		x	x
C5R3-L	Larvae	42	<5		x	x
T1R1-F-LOW	Frass	1383,2	<5	100		x
T1R2-F-LOW	Frass	928,1	<5	100		x
T1R3-F-LOW	Frass	1374,7	<5	100		x
T1R1-L-LOW	Larvae	37,3	<5	100		x
T1R2-L-LOW	Larvae	56,9	<5	100		x
T1R3-L-LOW	Larvae	28,6	<5	100		x
T1R1-F-HIGH	Frass	969,8	<5	1000		x
T1R2-F-HIGH	Frass	1108,3	<5	1000		x
T1R3-F-HIGH	Frass	334,2	<5	1000		x
T1R1-L-HIGH	Larvae	18,9	<5	1000		x
T1R2-L-HIGH	Larvae	<RL	<5	1000		x
T1R3-L-HIGH	Larvae	16,2	<5	1000		x
T1PL-HIGH	Purge Larvae	<RL	<5	1000		x
T1P-HIGH	Purge Water	10,7	<5	1000		x
T2R1-F-LOW	Frass	300,8	<5	100	x	x
T2R2-F-LOW	Frass	397,7	<5	100	x	x
T2R3-F-LOW	Frass	572,7	<5	100	x	x
T2R1-L-LOW	Larvae	27,9	<5	100	x	x
T2R2-L-LOW	Larvae	14,4	<5	100	x	x
T2R3-L-LOW	Larvae	15,6	<5	100	x	x
T2R1-F-HIGH	Frass	151,6	<5	1000	x	x
T2R2-F-HIGH	Frass	232,9	<5	1000	x	x
T2R3-F-HIGH	Frass	46,5	<5	1000	x	x
T2R1-L-HIGH	Larvae	<RL	<5	1000	x	x
T2R2-L-HIGH	Larvae	<RL	<5	1000	x	x
T2R3-L-HIGH	Larvae	<RL	<5	1000	x	x
T2PL-HIGH	Purge Larvae	<RL	<5	1000	x	x
T2P-HIGH	Purge Water	0	<5	1000	x	x
ing. mat no toxin	Substrate	-	<5			-
ing. mat no toxin	Substrate	-	<5			-
ing. mat no toxin	Substrate	-	<5			-
ing. mat low tox	Substrate	-	<5	100		-
ing. mat low tox	Substrate	-	<5	100		-
ing. mat low toxin	Substrate	-	<5	100		-
ing. mat high tox	Substrate	-	<5	1000		-
ing. mat high tox	Substrate	-	<5	1000		-
ing. mat high tox	Substrate	-	<5	1000		-

The results from Triology analytics lab showed an inconsistency in patulin concentrations when comparing to added patulin (Table 10). For samples where no patulin was added – as in all control groups for all treatments –very high concentrations on a wet weight basis were measured. For the toxin control, that did not undergo any treatment and was inoculated with low patulin concentrations (100µg/kg), the result showed that the toxin concentration was below detections limit. For several samples, a higher concentration of patulin was reported than what had been added.

SGS analytics reported that the patulin concentration in all samples were below the detection limit. In response to that, an extra set of samples with ingoing material, that was used in the different treatments (*e.g.*, ing. mat. low toxin) was sent to SGS Analytics. All these samples were reported to be below the detection limit.

In order to rule out whether our matrix (poultry feed mixed with water) was the critical flaw in our experimental design, samples with a different kind of matrix (bread mixed with water, inoculated with patulin) were sent to SGS Analytics. The results obtained were much more in line with what was expected.

To rule out that there was something wrong with the added patulin, an extra set of samples with ingoing material inoculated with high concentrations of patulin was sent to Eurofins. Additionally, a sample of the patulin inoculum (the pure patulin sample diluted in water) was sent for analysis. Eurofins reported a concentration of >10 000µg/kg (or >10ug/mL) in the inoculum and a concentration of 72µg/kg on a wet weight basis in the ingoing material. This corresponded to approximately 218µg/kg on a dry weight basis (targeted concentration was 300µg/kg on a dry weight basis).

5. Discussion

5.1 Literature overview

Niermans et al. (2021), who conducted a systematic review of the impact of mycotoxins on insects, came to the conclusions that mycotoxin does not have a negative effect on BSFL in terms of mortality and larva growth. Also, accumulation was low in the insect body. This is in accordance with the conclusions of the five assessed studies in the literature overview.

Camenzuli et al. (2018) found that, even at very high concentrations, the effect on survival and the development of the larvae seems small (Table 4). Gold et al. (2023) showed a small decrease in weight gain when BSFL received high concentrations on contaminated maize. However, the authors stress that this small change must be re-exanimated again before drawing any conclusions.

A large portion of The safety issue regarding mycotoxins mainly lies within the risk of bioaccumulation in BSFL (Niermans et al. 2021). All five studies assessed demonstrated a small or negligible bioaccumulation in BSFL. Both Bosch et al. (2017) and Camenzuli et al. (2018) did one feeding without toxin, to empty the gut, and reported no accumulation, except for very high concentrations. This indicates that the mycotoxins found in the larvae, in the other three studies, could be what is left in the intestinal system. Since the frass contained less mycotoxins than what was initially added it seems some degree if inactivation of the toxins occurred. Since there are no legal limit for mycotoxins in fertilizer in Swedish regulation (Swedish Board of Agriculture 2023) and *T. reesei* can improve the plant utilization of plant nutrients (Kubicek et al. 2019) and reduce the occurrence of mycotoxins (Yue et al. 2022), the frass could create a sustainable source of plant nutrients.

When comparing the studies where the mycotoxins were artificially added (Bosch et al. 2017; Camenzuli et al. 2018) with the studies were the mycotoxins were naturally occurring (Leni et al. 2019; Gold et al. 2023), it also seems as if the inactivation that can be expected will be enough to meet the regulatory demands, especially since the concentrations in the artificially added toxins where very high. In fact, the concentration of toxins in the artificial trials were much higher than is commonly found in nature (Juraschek et al. 2022). Moreover, the added

concentrations were many times higher (30 to 40 times) than the regulatory limits for food (European Commission 2023b).

5.2 Experimental design

In the experiments, 100 µg/kg and 1000 µg/kg of patulin were used in low and high concentration scenarios, respectively. This was calculated on a total solid (TS) basis. The analysis was done on a wet weight (WW) basis, and with the water content at approximately 66 %, resulting in a concentration on wet weight of 30 µg/kg and 300 µg/kg, respectively. Since the limit of detection for the analysis ranged from 5 to 10 µg/kg, one way to ensure that the concentration of patulin would be above detection limit in the ingoing material is to increase concentration of added patulin in the inflow substrate.

To more accurately determine how much patulin is added to the chosen matrix/substrate one improvement of design could be to purchase patulin in liquid form instead of a powder that needs to be diluted. The patulin inoculated solution was determined to have a concentration of >10 000 µg/kg by Eurofins, which makes it hard to know exactly how much have been added to the substrate. The downside of purchasing patulin in liquid form is that it is much more expensive. This is not necessarily a fault in the experimental design, rather an analytical limitation.

Gold et al. (2023) reported that a difference in result, in terms of reduction, was obtained if the substrate had been naturally contaminated instead of inoculated. The authors argue that this could be due to the fact that, in substrates with inoculated mycotoxin, the fungal species producing the mycotoxin is not necessarily present. One way to improve the experimental design is to use naturally contaminated sources as substrates to ensure the presence of toxin-producing fungi and to achieve a more real-life scenario. The downside of using naturally contaminated substrate is that ingoing concentration is difficult to control and get uniformity in trails. However, with the right design, this could be managed.

According to Lalander et al. (2020), ventilation settings are a factor that affect the outcome of BSFL composting, *e.g.* larval survival. The ventilation in the tent was high, creating a strong draft. Since the survival rate in the BSFL treatment (Table 8) was much lower than what has been previously reported – for example Lalander et al. (2020) reported 97.2 % survival in 76 % moisture content, but only 56.6 % survival in the treatment with more active ventilation – it can be stipulated that the ventilation could have contributed to the outcome of the BSFL treatment.

5.3 Process efficiency

One interesting aspect of the trial was that the pH development in the combined treatments, BSFL treatments and *T. reesei* treatments, differed significantly between each treatment (Figure 8) and had its own unique development curve. Even though the pH in the *T. reesei* treatments and combined treatment was lower at the start of the trial, pH was still higher at the end of the trial. Examining the larva weight development (Figure 9) the higher pH did not seem to affect the BSF larvae in a negative way. However, a higher pH could perhaps enhanced the inactivation of the mycotoxins since the variation of pH in the larval gut is one detoxifying property according to (Surendra et al. 2020). Also, studies where aflatoxin were treated with different pH, the highest reduction of aflatoxin were obtained at pH 11.8 and 12.5 (Moreno-Pedraza et al. 2015)

The larval weight development followed the same pattern in all treatments (Figure 9). The final weight of the larvae the was the same, while the larval survival was higher in the combined treatments than the BSFL treatments (Table 9). The larval survival in the BSFL treatments was low even compared to other studies (Lalander et al. 2020), as discussed in the experimental design when discussing the choice of ventilation. In the combined treatments the frass had a finer texture, and the larvae were dryer and had no frass stuck on them after harvesting. In the BSFL treatments, the larvae were "sticky" and there was a hard crust on top of the frass at harvest. *T. reesei* ability to break down lignin into soluble sugars (Suo et al. 2023; Mustafa et al. 2016) and making the nutrients more accessible to the larvae (Isibika et al. 2019) could possibly have contributed to the higher survival, but it also seems to have created a more suitable environment.

The material reduction was higher in the combined treatments, but not significantly. Furthermore, the total loss of VS was significantly higher in the combined treatment. This also indicate that *T. reesei* helps break up organics and making them more easily accessible to the larvae (Lindberg et al. 2022). In addition, treating the substrate with *T. reesei* got the same total BCE in both the BSFL treatment and the combined treatment (Table 8), even though the combined treatment got a larger amount of initial substrate. This indicates that, in this trial, the same number of larvae could convert a larger amount of substrate when pre-treating the substrate with *T. reesei.* However, the lower survival rate in the BSFL treatment could also contribute to these results, so this must be further investigated before drawing any conclusion.

In terms of the impact of patulin on the BSFL, the toxins did not seem to affect the larval survival or weight development, since there was no significant difference between no, low or high toxins in the different treatments (Table 8-9). This is in accordance with the studies included in the literature overview, in which other types of mycotoxins were investigated. However, the highest weight per larva was obtained in the BSFL treatment with high toxin. This could be due to the Hormesis

effect, which is a response by a low dose of a potentially harmful stressor, such as a toxin (Calabrese E. J. 2014). But, instead of harming the organism, it stimulates beneficial adaptive system in the larvae.

For moisture content, the highest reduction was obtained in combined treatments and in BSFL treatments. However, *T. reesei* treatments did also show a reduction in moisture content (from approximately 66% to 55%). *T. reesei* could be useful when working with substrate with high moisture content. When pre-treating orange peel with *T. reesei*; Lindberg et al. (2022) showed a reduction in moisture content from approximately 79% to 70%.

Since the results from the patulin analysis could not be obtained, it is not possible to draw any conclusions on the effects of BSFL composting on patulin degradation. However, all the mycotoxins listed in the literature study shows the same pattern in terms of reduction through BSFL composting (Table 5). Also, several studies (Yue et al. 2022; Dini et al. 2022) has shown significant reduction of mycotoxins though *T. reesei* treatment. Although, to make any conclusions, laboratory testing must confirm this statement.

5.4 Patulin analysis methodology

When testing for mycotoxin, the laboratory is accredited on a matrix in which the toxin is commonly found. In the case of patulin, common matrices are apple juice, fruit puree or other fruits or foods in which it could be expected to be found naturally. So, when testing for something other than that, the laboratory must choose a matrix that is similar in terms of density, moisture etc. Although the laboratories to which the samples were sent guaranteed that the analyses could be done for the matrix used in this study (poultry feed), it does not appear to have been the case. The results, particularly for the ingoing material, would likely have been more reliable if a source where patulin is more commonly found would have been used. It thus appear that for the analysis of mycotoxins in the larvae and frass to be reliable, the matrices for these materials must be developed.

The different laboratories used in this study did use different analyzing methodologies. Triology Lab used HPLC and SGS Lab used LC-MS/MS which, according to both these laboratories, is a more specific and reliable method.
Therefore, to effectively research and mitigate risks associated with mycotoxins, and thereby support the transition to a truly circular food system - where not just plant nutrient but also proteins, fibers and, fats are recycled back into the food chain (Lalander & Vinnerås 2022) - reliable analytic methodologies must be developed.

6. Conclusion

To be able to reach a safe and circular food system it is at most important that lab results can be trusted, both in terms of food entering the food market and to be able to use food waste as a resource. The development of methods for analyzing more types of matrixes or methods that are not dependent on specifying matrix is crucial. The gaps in research (Behre et al. 2023) is a contributing factor that hinder the transition to a circular food system.

Since losses of food commodities due to mycotoxins is 30-50% worldwide (Pandey et al. 2023), it is important to prove that it is safe to use these waste streams in BSFL composting. If these losses could be used in BSFL composting, the larvae could replace some of the soy produced (Figure 3) as a protein source for poultry and pigs. Also, since emissions from food that is never eaten stand for 6% (Figure 1), being able to use all available waste streams, together with replacing some soy production, would have a great impact on global greenhouse gas emissions.

6.1 Further studies

Other than designing a new study to make conclusions about the effect of BSFL on Patulin, since many other pathogens is sensitive to high pH it would be interesting to study the combined effect of BSFL and *T. reesei* on other pathogens or toxins. According to (Kubicek et al. 2019), *T. reesei* is used to improve plant growth etc. A study on how Trichoderma could enhance the frass as a nutrient resource would be interesting.

7. References

Al-Wabel, M.I., Ahmad, M., Rasheed, H., Rafique, M.I., Ahmad, J. & Usman, A.R.A. (2022). Environmental Issues Due to Open Dumping and Landfilling. In: Pathak, P. & Palani, S.G. (eds) *Circular Economy in Municipal Solid Waste Landfilling: Biomining & Leachate Treatment : Sustainable Solid Waste Management: Waste to Wealth*. Springer International Publishing. 65–93. https://doi.org/10.1007/978-3-031-07785-2_4

Behre, E., Heukels, B., Merino Mayayo, A. & Verschuur, X. (2023). Policy brief insects as livestock feed UN policy Analysis Bransh, Division for Sustainable Development

Berenbaum, M.R., Bush, D.S. & Liao, L.-H. (2021). Cytochrome P450-mediated mycotoxin metabolism by plant-feeding insects. *Current Opinion in Insect Science*, 43, 85–91. https://doi.org/10.1016/j.cois.2020.11.007

Bisconsin-Junior, A., Feitosa, B.F., Silva, F.L. & Mariutti, L.R.B. (2023). Mycotoxins on edible insects: Should we be worried? *Food and Chemical Toxicology*, 177, 113845. https://doi.org/10.1016/j.fct.2023.113845

Bosch, G., Fels-Klerx, H.J. van der, Rijk, T.C. de & Oonincx, D.G.A.B. (2017). Aflatoxin B1 Tolerance and Accumulation in Black Soldier Fly Larvae (Hermetia illucens) and Yellow Mealworms (Tenebrio molitor). *Toxins*, 9 (6), 185. https://doi.org/10.3390/toxins9060185

Calabrese E. J. (2014). Hormesis: a fundamental concept in biology. Microbial cell (Graz, Austria), 1(5), 145–149. https://doi.org/10.15698/mic2014.05.145

Camenzuli, L., Van Dam, R., De Rijk, T., Andriessen, R., Van Schelt, J. & Van der Fels-Klerx, H.J. (Ine) (2018). Tolerance and Excretion of the Mycotoxins Aflatoxin B1, Zearalenone, Deoxynivalenol, and Ochratoxin A by Alphitobius diaperinus and Hermetia illucens from Contaminated Substrates. *Toxins*, 10 (2), 91. https://doi.org/10.3390/toxins10020091

Chia, S.Y., Tanga, C.M., Osuga, I.M., Cheseto, X., Ekesi, S., Dicke, M. & van Loon, J.J.A. (2020). Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products. *Entomologia Experimentalis et Applicata*, 168 (6–7), 472–481. https://doi.org/10.1111/eea.12940

Dini, I., Alborino, V., Lanzuise, S., Lombardi, N., Marra, R., Balestrieri, A., Ritieni, A., Woo, S.L. & Vinale, F. (2022). Trichoderma Enzymes for Degradation of Aflatoxin B1 and Ochratoxin A. *Molecules*, 27 (12), 3959. https://doi.org/10.3390/molecules27123959

Dortmans, B., Diener, S., Egger, J. & Zurbrügg, C. (2021). *Black Soldier Fly Biowaste Processing - A Step-by-Step Guide, 2nd Edition*.

Eskola M, Kos G, Elliott CT, Hajšlová J, Mayar S, Krska R. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25. Crit Rev Food Sci Nutr. 2020;60(16):2773-2789. doi: 10.1080/10408398.2019.1658570. Epub 2019 Sep 3. PMID: 31478403.

European Commission (2008). *Waste Framework Directive - European Commission*. https://environment.ec.europa.eu/topics/waste-and-recycling/waste-framework-directive_en [2024-02-24]

European Commission (2009). Regulation - 1069/2009 - EN - EUR-Lex. https://eur-lex.europa.eu/eli/reg/2009/1069/oj [2024-03-11]

European Commission (2014). *Biodegradable waste - European Commission*. https://environment.ec.europa.eu/topics/waste-and-recycling/biodegradable-waste_en [2024-03-04]

European Commission (2015). *First circular economy action plan - European Commission*. https://environment.ec.europa.eu/topics/circular-economy/first-circular-economy-action-plan_en [2024-03-04]

European Commission (2020b). *Circular economy action plan - European Commission*. https://environment.ec.europa.eu/strategy/circular-economy-action-plan_en [2024-02-16]

European Commission (2023b). *Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance)*. *OJ L*. http://data.europa.eu/eli/reg/2023/915/oj/eng [2024-02-22]

European Commission (2020a). *Farm to Fork Strategy - European Commission*. https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en [2024-02-16]

European Commission (2023a). *Regulation (EU) 2023/1115 on the making available on the Union market and the export from the Union of certain commodities and products associated with deforestation and forest degradation | Knowledge for policy*. https://knowledge4policy.ec.europa.eu/publication/regulation-eu-20231115-making-available-union-market-export-union-certain-commodities_en [2024-02-16]

European Parliament (2005). *Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene (Text with EEA relevance)*. *OJ L*. http://data.europa.eu/eli/reg/2005/183/oj/eng [2024-02-21]

FASS (2022). *Xeomin - FASS Vårdpersonal*. https://www.fass.se/LIF/product?userType=0&nplId=20140702000039#handling-life-shelf-storage [2024-02-26]

Geng, A. (2014). Genetic Transformation and Engineering of *T. reesei* for Enhanced Enzyme Production. In: Gupta, V.K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R.S., Druzhinina, I., & Tuohy, M.G. (eds) *Biotechnology and Biology of Trichoderma*. Elsevier. 193–200. https://doi.org/10.1016/B978-0-444-59576-8.00013-8

Gold, M., Niermans, K., Jooste, F., Stanford, L., Uwamahoro, F., Wanja, M., Veldkamp, T., Sanderson, A., Nunes, V., Mathys, A., Van der Fels-Klerx, H. (Ine), Hoek-

van den Hil, E. & Nishimwe, K. (2023). Conversion of mycotoxin-contaminated maize by black soldier fly larvae into feed and fertilizer. *Journal of Insects as Food and Feed*, 1–14. https://doi.org/10.1163/23524588-00001006

Green, J.M.H., Croft, S.A., Durán, A.P., Balmford, A.P., Burgess, N.D., Fick, S., Gardner, T.A., Godar, J., Suavet, C., Virah-Sawmy, M., Young, L.E. & West, C.D. (2019). Linking global drivers of agricultural trade to on-the-ground impacts on biodiversity. *Proceedings of the National Academy of Sciences*, 116 (46), 23202–23208. https://doi.org/10.1073/pnas.1905618116

Hogg, D., Favoino, E. & Nielsen, N. (2003). *Economic analysis of options for managing biodegradable municipal waste – Final report to the European Commission.* Eunomia Research & Consulting.

Hussain, S., Asi, M.R., Iqbal, M., Akhtar, M., Imran, M. & Ariño, A. (2020). Surveillance of Patulin in Apple, Grapes, Juices and Value-Added Products for Sale in Pakistan. *Foods*, 9 (12), 1744. https://doi.org/10.3390/foods9121744

IPIFF (2022). Insects As Feed EU Legislation – Aquaculture, Poultry & Pig Species. *International Platform of Insects for Food and Feed, Brussels*. https://ipiff.org/insects-eu-legislation-general/ [2024-03-11]

Isibika, A., Vinnerås, B., Kibazohi, O., Zurbrügg, C. & Lalander, C. (2019). Pre-treatment of banana peel to improve composting by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae. *Waste Management*, 100, 151–160. https://doi.org/10.1016/j.wasman.2019.09.017

Juraschek, L.M., Kappenberg, A. & Amelung, W. (2022). Mycotoxins in soil and environment. *Science of The Total Environment*, 814, 152425. https://doi.org/10.1016/j.scitotenv.2021.152425

Kamle, M., Mahato, D.K., Gupta, A., Pandhi, S., Sharma, N., Sharma, B., Mishra, S., Arora, S., Selvakumar, R., Saurabh, V., Dhakane-Lad, J., Kumar, M., Barua, S., Kumar, A., Gamlath, S. & Kumar, P. (2022). Citrinin Mycotoxin Contamination in Food and Feed: Impact on Agriculture, Human Health, and Detection and Management Strategies. *Toxins*, 14 (2), 85. https://doi.org/10.3390/toxins14020085

Kaza, S., Yao, L.C., Bhada-Tata, P. & Van Woerden, F. (2018). *What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050*. Washington, DC: World Bank. https://doi.org/10.1596/978-1-4648-1329-0

Kosicki, R., Błajet-Kosicka, A., Grajewski, J. & Twarużek, M. (2016). Multiannual mycotoxin survey in feed materials and feedingstuffs. *Animal Feed Science and Technology*, 215, 165–180. https://doi.org/10.1016/j.anifeedsci.2016.03.012

Kubicek, C.P., Steindorff, A.S., Chenthamara, K., Manganiello, G., Henrissat, B., Zhang, J., Cai, F., Kopchinskiy, A.G., Kubicek, E.M., Kuo, A., Baroncelli, R., Sarrocco, S., Noronha, E.F., Vannacci, G., Shen, Q., Grigoriev, I.V. & Druzhinina, I.S. (2019). Evolution and comparative genomics of the most common Trichoderma species. *BMC Genomics*, 20, 485. https://doi.org/10.1186/s12864-019-5680-7

Lalander, C., Diener, S., Zurbrügg, C. & Vinnerås, B. (2019). Effects of feedstock on larval development and process efficiency in waste treatment with black soldier

fly (*Hermetia illucens*). *Journal of Cleaner Production*, 208, 211–219. https://doi.org/10.1016/j.jclepro.2018.10.017

Lalander, C., Ermolaev, E., Wiklicky, V. & Vinnerås, B. (2020). Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content. *Science of The Total Environment*, 729, 138968. https://doi.org/10.1016/j.scitotenv.2020.138968

Lalander, C. & Lopes, I.G. (2024). Advances in substrate source composition for rearing black soldier fly larvae as a protein source. https://shop.bdspublishing.com/store/bds/detail/product/3-190-9781801469579 [2024-02-16]

Lalander, C., Nordberg, Å. & Vinnerås, B. (2018). A comparison in product-value potential in four treatment strategies for food waste and faeces – assessing composting, fly larvae composting and anaerobic digestion. *GCB Bioenergy*, 10 (2), 84–91. https://doi.org/10.1111/gcbb.12470

Lalander, C. & Vinnerås, B. (2022). Actions needed before insects can contribute to a real closed-loop circular economy in the EU. *Journal of insects as food and feed*, 8 (4), 337–342. https://doi.org/10.3920/JIFF2022.x003

Leni, G., Cirlini, M., Jacobs, J., Depraetere, S., Gianotten, N., Sforza, S. & Dall'Asta, C. (2019). Impact of Naturally Contaminated Substrates on Alphitobius diaperinus and Hermetia illucens: Uptake and Excretion of Mycotoxins. *Toxins*, 11 (8), 476. https://doi.org/10.3390/toxins11080476

Lin, L., Xu, F., Ge, X. & Li, Y. (2018). Improving the sustainability of organic waste management practices in the food-energy-water nexus: A comparative review of anaerobic digestion and composting. *Renewable and Sustainable Energy Reviews*, 89, 151–167. https://doi.org/10.1016/j.rser.2018.03.025

Lindberg, L., Ermolaev, E., Vinnerås, B. & Lalander, C. (2022). Process efficiency and greenhouse gas emissions in black soldier fly larvae composting of fruit and vegetable waste with and without pre-treatment. *Journal of Cleaner Production*, 338, 130552. https://doi.org/10.1016/j.jclepro.2022.130552

Lohri, C., Diener, S., Zabaleta, I., Mertenat, A. & Zurbrügg, C. (2017). Treatment technologies for urban solid biowaste to create value products: a review with focus on low- and middle- income settings. *Reviews in Environmental Science and Bio/Technology*, 16. https://doi.org/10.1007/s11157-017-9422-5

Lopes, I.G., Yong, J.W. & Lalander, C. (2022). Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives. *Waste Management*, 142, 65–76. https://doi.org/10.1016/j.wasman.2022.02.007

Moreno-Pedraza A, Valde´s-Santiago L, Herna´ndez-Valadez LJ,Rodrı´guez-Sixtos HA, Winkler R, Guzma´n-de Pen˜a DL (2015)Reduction of aflatoxin B1 during tortilla production and theidentification of degradation by-products by direct-injectionelectrospray mass spectrometry (DIESI-MS). Salud Publica Mex57:50–57.

Mustafa, A.M., Poulsen, T.G. & Sheng, K. (2016). Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *T. reesei* to enhance methane production under solid-

state anaerobic digestion. *Applied Energy*, 180, 661–671. https://doi.org/10.1016/j.apenergy.2016.07.135

Nahle, S., El Khoury, A., Savvaidis, I., Chokr, A., Louka, N. & Atoui, A. (2022). Detoxification approaches of mycotoxins: by microorganisms, biofilms and enzymes. *International Journal of Food Contamination*, 9 (1), 3. https://doi.org/10.1186/s40550-022-00089-2

Naser El Deen, S., van Rozen, K., Elissen, H., van Wikselaar, P., Fodor, I., van der Weide, R., Hoek-van den Hil, E.F., Rezaei Far, A. & Veldkamp, T. (2023). Bioconversion of Different Waste Streams of Animal and Vegetal Origin and Manure by Black Soldier Fly Larvae Hermetia illucens L. (Diptera: Stratiomyidae). *Insects*, 14 (2), 204. https://doi.org/10.3390/insects14020204

Net Zero Cities (2023). *Food and Nutrient Recycling*. https://netzerocities.app/ [2024-03-03]

Niermans, K., Meyer, A.M., den Hil, E.F.H., van Loon, J.J.A. & van der Fels-Klerx, H.J. (2021). A systematic literature review on the effects of mycotoxin exposure on insects and on mycotoxin accumulation and biotransformation. *Mycotoxin Research*, 37 (4), 279–295. https://doi.org/10.1007/s12550-021-00441-z

Oonincx, D.G.A.B., Broekhoven, S. van, Huis, A. van & Loon, J.J.A. van (2015). Feed Conversion, Survival and Development, and Composition of Four Insect Species on Diets Composed of Food By-Products. *PLOS ONE*, 10 (12), e0144601. https://doi.org/10.1371/journal.pone.0144601

Pandey, A.K., Samota, M.K., Kumar, A., Silva, A.S. & Dubey, N.K. (2023). Fungal mycotoxins in food commodities: present status and future concerns. *Frontiers in Sustainable Food Systems*, 7. https://doi.org/10.3389/fsufs.2023.1162595

Poore, J. & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science*,. https://doi.org/10.1126/science.aaq0216

Purschke, B., Scheibelberger, R., Axmann, S., Adler, A. & Jäger, H. (2017). Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (Hermetia illucens) for use in the feed and food value chain. *Food Additives & Contaminants: Part A*, 34 (8), 1410–1420. https://doi.org/10.1080/19440049.2017.1299946

Reddy, K., Salleh, B., Saad, B., Abbas, H., Abel, C. & Shier, W. (2010). An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Reviews*, 29 (1), 3–26. https://doi.org/10.3109/15569541003598553

Richardson, K., Steffen, W., Lucht, W., Bendtsen, J., Cornell, S.E., Donges, J.F., Drüke, M., Fetzer, I., Bala, G., von Bloh, W., Feulner, G., Fiedler, S., Gerten, D., Gleeson, T., Hofmann, M., Huiskamp, W., Kummu, M., Mohan, C., Nogués-Bravo, D., Petri, S., Porkka, M., Rahmstorf, S., Schaphoff, S., Thonicke, K., Tobian, A., Virkki, V., Wang-Erlandsson, L., Weber, L. & Rockström, J. (2023). Earth beyond six of nine planetary boundaries. *Science Advances*, 9 (37), eadh2458. https://doi.org/10.1126/sciadv.adh2458

Ritchie, H. & Roser, M. (2021). Is our appetite for soy driving deforestation in the Amazon? *Our World in Data*,. https://ourworldindata.org/soy [2024-03-04]

Ritchie, H. & Roser, M. (2023). Food waste is responsible for 6% of global greenhouse gas emissions. *Our World in Data*,. https://ourworldindata.org/food-waste-emissions [2024-02-16]

Ritchie, H. & Roser, M. (2024). Is our appetite for soy driving deforestation in the Amazon? *Our World in Data*,. https://ourworldindata.org/soy [2024-02-25]

Schlesier, H., Schäfer, M. & Desing, H. (2024). Measuring the Doughnut: A good life for all is possible within planetary boundaries. *Journal of Cleaner Production*, 141447. https://doi.org/10.1016/j.jclepro.2024.141447

Development and Sustainability,. https://doi.org/10.1007/s10668-023-04306-6

Singh, A., & Kumari, K. (2019). An inclusive approach for organic waste treatment and valorisation using Black Soldier Fly larvae: A review. *Journal of Environmental Management*, *251*, 109569. https://doi.org/https://doi.org/10.1016/j.jenvman.2019.109569

Smetana, S., Palanisamy, M., Mathys, A. & Heinz, V. (2016). Sustainability of insect use for feed and food: Life Cycle Assessment perspective. *Journal of Cleaner Production*, 137, 741–751. https://doi.org/10.1016/j.jclepro.2016.07.148

Speijers, G.J.A. & Speijers, M.H.M. (2004). Combined toxic effects of mycotoxins. *Toxicology Letters*, 153 (1), 91–98. https://doi.org/10.1016/j.toxlet.2004.04.046

Suo, J., Liang, T., Zhang, H., Liu, K., Li, X., Xu, K., Guo, J., Luo, Q. & Yang, S. (2023). Characteristics of Aflatoxin B1 Degradation by Stenotrophomonas acidaminiphila and It's Combination with Black Soldier Fly Larvae. *Life*, 13 (1), 234. https://doi.org/10.3390/life13010234

Surendra, K.C., Tomberlin, J.K., van Huis, A., Cammack, J.A., Heckmann, L.-H.L. & Khanal, S.K. (2020). Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). *Waste Management*, 117, 58–80. https://doi.org/10.1016/j.wasman.2020.07.050

Swedish Board of Agriculture (2023). Regulation on mycotoxins in fertilizer

Swedish Food Agency (2023). *Mögelgifter*. *Mögelgifter*. https://www.livsmedelsverket.se/livsmedel-och-innehall/oonskade-amnen/mogelgifter [2023-11-08]

The World Bank (2018). *Trends in Solid Waste Management*. https://datatopics.worldbank.org/what-a-waste/trends_in_solid_waste_management.html [2024-03-03]

United Nations (2023). *Brief for GSDR - Brief submitted in support of sci | Department of Economic and Social Affairs*. https://sdgs.un.org/documents/brief-gsdr-brief-submitted-support-sci-22256 [2024-02-21]

WHO (2023). *Mycotoxins*. https://www.who.int/news-room/fact-sheets/detail/mycotoxins [2023-11-14]

World resources report (2018). *Creating a Sustainable Food Future | World Resources Report*. https://research.wri.org/wrr-food [2024-03-03]

Wright, S.A. (2015). Patulin in food. *Current Opinion in Food Science*, 5, 105–109. https://doi.org/10.1016/j.cofs.2015.10.003

Xia, J., Ge, C. & Yao, H. (2021). Antimicrobial Peptides from Black Soldier Fly (Hermetia illucens) as Potential Antimicrobial Factors Representing an Alternative to Antibiotics in Livestock Farming. *Animals*, 11, 1937. https://doi.org/10.3390/ani11071937

Yao, X., Guo, H., Zhang, K., Zhao, M., Ruan, J. & Chen, J. (2023). Trichoderma and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology*, 14, 1160551. https://doi.org/10.3389/fmicb.2023.1160551

Yue, X., Ren, X., Fu, J., Altomare, C., Haidukowski, M., Logrieco, A.F., Zhang, Q. & Li, P. (2022). Characterization and mechanism of aflatoxin degradation by a novel strain of *T. reesei* CGMCC3.5218. *Frontiers in Microbiology*, 13, 1003039. https://doi.org/10.3389/fmicb.2022.1003039

Żuk-Gołaszewska, K., Gałęcki, R., Obremski, K., Smetana, S., Figiel, S. & Gołaszewski, J. (2022). Edible Insect Farming in the Context of the EU Regulations and Marketing—An Overview. *Insects*, 13 (5), 446. https://doi.org/10.3390/insects13050446

Popular science summary

In order to transition our food system from a linear to a circular one we need more tools to recycle, not only plant nutrients, but proteins and fats from all types of waste streams. Mycotoxins are secondary metabolites produced by several molds (fungi) that can cause serious harm to humans and animals. There are many species of mycotoxins, but seven, that are considered highly toxic, have maximum levels in food regulated in EU. Losses of food commodities due to mycotoxins are 30-50% worldwide.

Black Solider Fly Larvae (BSFL) composting offers a good solution both as waste management strategy and for replacing other unsustainable protein sources. In order to safely rear BSFL on food waste or other type of waste streams it is important that associated risks are investigated. *T. reesei* is a mesophilic and filamentous fungus commonly found in soil and root ecosystems. It is commonly used for its ability to break down plant biomass into soluble fermentable sugars.

This study was conducted in two parts: a Literature review and an experimental trial. This study begins with literature review to identify the types of mycotoxins previously investigated in the context of BSFL, where Patulin was the only one previously not examined. The experimental trial evaluated three methods for inactivating the mycotoxins patulin. One treatment with *T. reesei*, one with BSFL composting and one combined treatment.

The literature review revealed that either no or only low concentrations was detected in the larvae. The low concentrations observed were linked to very high concentrations in the input material, yet the levels in the larvae stayed below EU regulatory limits. The result of the experimental trial showed that larva weight gain development where not affected by Patulin. Combined treatments showed the highest larva survival and the highest loss of volatile solids (VS). For the Patulin inactivation, two different laboratories were used and a third as a control. The study was not able to obtain any results that where reliable enough to make any conclusions.

The key message to take away from the result of the Patulin analysis where that theses analysis must be future developed to obtain trustworthy results to get a safe and circular food system in the future.

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