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RESEARCH ARTICLE

Conversion of mycotoxin-contaminated maize by black soldier fly larvae into feed and fertilizer

M. Gold[1,#,], K. Niermans[2,3,#], F. Jooste[4], L. Stanford[4], F. Uwamahoro[5], M. Wanja[4], T. Veldkamp[6], A. Sanderson[7], V. Dos Santos Nunes[7], A. Mathys[1], H.J. van der Fels-Klerx[3], E.F. Hoek-van den Hil[3] and K. Nishimwe[8]*

[1]ETH Zurich, Sustainable Food Processing, 8092 Zurich, Switzerland; [2]Department of Plant Sciences, Laboratory of Entomology, Wageningen University & Research, 6708 PB Wageningen, The Netherlands; [3]Wageningen Food Safety Research, Wageningen University & Research, 6708 PB Wageningen, The Netherlands; [4]The Bug Picture, Kigali, Rwanda; [5]Rwanda Agriculture and Animal Resources Development Board, Rubona, Rwanda; [6]Wageningen University & Research, Livestock Research, 6708 WD Wageningen, The Netherlands; [7]The International Finance Corporation, a member of the World Bank Group, Rwanda; [8]School of Agriculture and Food Sciences, College of Agriculture, Animal Sciences and Veterinary Medicine-University of Rwanda, Musanze, Rwanda; *moritz.gold@hest.ethz.ch

[#]Both authors contributed equally to this work.

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Abstract

Globally, large amounts of various crops such as cereals, oilseeds, nuts and spices are contaminated with mycotoxins during pre-harvest, postharvest handling, processing and/or storage. Mycotoxin contamination results into economic and health issues, and valorisation options of contaminated crops are urgently needed. The aim of this research was to evaluate whether quality feed and fertilizer can be safely produced from naturally mycotoxin contaminated crops using black soldier fly larvae (BSFL, *Hermetia illucens L.*) under realistic field conditions in East Africa. Naturally mycotoxin contaminated maize (corn; *Zea mays L.*) was used as a model due its prevalence as food and feed and utilized by BSFL together with local agri-food by-products at a research facility in Rwanda. To assess the influence of the initial maize mycotoxin contamination and maize inclusion, larval diets with three mycotoxin contamination levels and two maize inclusion levels were tested. BSFL were tolerant against the high mycotoxin concentrations (e.g. 99.4 μg aflatoxin B1 kg dry mass^{-1}) as the presence of mycotoxins in the substrate did not affect BSFL mass at harvest. Product safety was assessed by quantifying the presence of 38 common and emerging mycotoxins and metabolites in the maize, substrates and BSFL products (e.g. larvae and frass). The results show that it is possible to produce feed and fertilizer with BSFL considered safe within the European Union and East African legal limits with maize contaminated with mycotoxins typical for East Africa. Thereby, this research works towards the safe recycling of nutrients from mycotoxin contaminated maize within the food system in East Africa and beyond.

Keywords

Hermetia illucens – bioconversion – aflatoxin – safety – circular economy – Rwanda

1 Introduction

Mycotoxins are secondary metabolites produced by fungi. Mycotoxins count in hundreds and exposure to them can affect human and animal health (Zain, 2011). However, the type of toxicity depends on the chemical structure, the dose and duration of exposure (Bennett and Klich, 2003; Chilaka *et al.*, 2022). In general, mycotoxins can cause both acute toxicity and chronic carcinogenicity and are known to be carcinogenic, mutagenic, teratogenic, and immunosuppressive amongst others. Furthermore, exposure may lead to gastrointestinal symptoms e.g. feed refusal and vomiting which affects productivity in livestock (Fink-Gremmels *et al.*, 1999; Prelusky *et al.*, 1994). Amongst the mycotoxins, the aflatoxins are considered as the most toxic human carcinogens (IARC, 2012). It is worth noting that there are hundreds of different types of mycotoxins; most concerning are aflatoxins (e.g. aflatoxin B1 (AFB1)), fumonisins (e.g. fumonisin B1 (FB1)), zearalenone (ZEN), and deoxynivalenol (DON), mainly produced by *Aspergillus*, *Fusarium*, and *Penicillium* (Ankwasa *et al.*, 2021).

Mycotoxin contamination is especially a challenge in tropical countries due to favorable fungi growth conditions (i.e. warm and humid environmental conditions), poor crop management practices, and non-existing standard enforcement (Chilaka *et al.*, 2022; Matumba *et al.*, 2017). In addition, mycotoxin contamination causes economic impact due to low market value of contaminated products, or their rejection from high-value markets (Adeyeye *et al.*, 2022). It is expected that climate change will worsen the current situation (Medina *et al.*, 2015). Different management strategies are used to mitigate mycotoxin contamination, along the value chain. Pre-harvest measurements include agronomic (e.g. planting and harvesting timing, crop rotation), biological (e.g. fungal antagonists) and chemical (i.e. fungicides and insecticides) means (Conte *et al.*, 2020). Post-harvest strategies include a good storage management (e.g. control of temperature and humidity), and biological (e.g. bacteria, fungi, enzymes), physical (e.g. sorting, drying, thermal inactivation, irradiation) and chemical (e.g. ozone, organic acids) methods (Peng *et al.*, 2018). Although there are strategies in place, the issue of mycotoxin contamination along crop value chains remains significant. Contaminated batches of crops are regularly present in food systems; therefore, more solutions are needed to manage contaminated crops.

Black soldier fly larvae (BSFL; *Hermetia illucens L.*) have emerged as a promising approach because they can grow on a variety of low value and mycotoxin-contaminated substrates (Camenzuli *et al.*, 2018; Gold *et al.*, 2018; Niermans *et al.*, 2021). Growing BSFL on mycotoxin-contaminated food and by-products is different from using pre- and post-harvest technologies that protect agricultural products from mycotoxin contamination. Instead, BSFL feed on mycotoxin contaminated products and thereby convert it into nutrient-rich larval biomass and compost-like frass (i.e. BSFL excreta and undigested substrate). The larval biomass has particular value as animal feed (e.g. pets, poultry, pigs) (Barragán-Fonseca *et al.*, 2017) or food but also for biotechnological, cosmetic and pharmaceutical applications. In low-income countries, larval biomass (e.g. live, dried or defatted) can increase the availability, quality and affordability of animal feeds important for food security and livelihoods. In addition, the frass remaining from larval bioconversion has value as organic fertilizer (Fuhrmann *et al.*, 2022).

Interestingly, research to date has shown that BSFL are highly tolerant and do not bioaccumulate AFB1, DON, FB1, OTA and ZEN present in the substrate (Bosch *et al.*, 2017; Camenzuli *et al.*, 2018; Leni *et al.*, 2019; Niermans *et al.*, under review; Purschke *et al.*, 2017). Only Heuel *et al.* (2023) has observed a slower larval development in comparison to an uncontaminated control after spiking food waste with 800 μg AFB1 kg DM^{-1}. Similar to the other studies, even at these high concentrations, only small amounts of AFB1, below legal limits for feed in the European Union (EU) were found in the larvae and frequently attributed to undigested substrate in the larval digestive tract. Even though considerable amounts of mycotoxins and decomposition metabolites are found in the frass, concentrations are frequently less than in the initial substrate suggesting that similar to other insects, BSFL and associated microbes have mycotoxin metabolization pathways (Evans and Shao, 2022; Suo *et al.*, 2023; Meijer *et al.*, 2019). These results are promising but evidence is lacking on the utilization of naturally contaminated crops and foods in subtropical and tropical regions. To date, most studies on the utilization of mycotoxin-contaminated substrates with BSFL were completed at a laboratory scale, with high quality substrates and/or one or several artificially spiked mycotoxin contaminations (Bosch *et al.*, 2017; Camenzuli *et al.*, 2018; Heuel *et al.*, 2023). This is a shortcoming considering that in reality, mycotoxins frequently co-occur (Ankwasa *et al.*, 2021). In addition, artificially spiking with one or several mycotoxins may produce different results than naturally contaminated materials. Purscke *et al.* (2017) and Leni *et al.* (2019) studied naturally contaminated crops but only at relatively low mycotoxin

TABLE 1 Summary of substrates assessed in this study, their proportion of maize, agri-food byproducts and clean and contaminated maize

Substrate	Abbreviation	Substrate composition (% DM)		Maize composition (%)		
		Maize	Agri-food byproduct	Mycotoxin level	Clean	Contaminated
1	CM50	50%	50%	Control	100%	0%
2	LM50	50%	50%	Low	75%	25%
3	HM50	50%	50%	High	0%	100%
4	CM80	80%	20%	Control	100%	0%
5	LM80	80%	20%	Low	75%	25%
6	HM80	80%	20%	High	0%	100%

DM = dry matter; CM50 = with control maize, 50% DM maize; LM50 = low mycotoxin contaminated maize, 50% DM maize; HM50 = high mycotoxin contaminated maize, 50% DM maize; CM80 = with control maize, 80% DM maize; LM80 = low mycotoxin contaminated maize; 80% DM maize, HM80 = high mycotoxin contaminated maize, 80% DM maize.

concentration relative to peak concentrations found in crops in subtropical and tropical regions.

The objective of this study was to assess the viability of producing high-quality feed and fertilizer using BSFL from crops contaminated with mycotoxins, in real-world field conditions in Rwanda, Africa. By doing so, this research aims to promote the safe recycling of nutrients from mycotoxin-contaminated maize in the food system of East Africa and other regions.

2 Materials and methods

Experimental design and substrates

The study consisted of maize and BSFL substrate analyses, larval feeding trials and post-harvest analyses of products (i.e. larvae and frass). Six different substrates (Table 1) were used to test the influence of maize inclusion and substrate maize mycotoxin contamination levels on product mycotoxin concentrations and BSFL growth. Clean maize (4.8 μg kg^{-1} total aflatoxins, AgraStrip Pro Total Aflatoxin WATEX, Rober Labs, Getzendorf, Austria) was mixed with contaminated maize (>460 μg kg^{-1} total aflatoxins) at increasing ratios starting from the clean maize to produce maize with three mycotoxin contamination levels: control maize (CM, 0% mycotoxin contaminated maize), low mycotoxin contaminated maize (LM, 25% mycotoxin contaminated maize) and high mycotoxin contaminated maize (HM, 100% mycotoxin contaminated maize) (Table 1). Maize with the three mycotoxin contaminated levels (CM, LM and HM) was then mixed at two levels with local agri-food by-products resulting in 50% and 80% of maize (based on dry mass (DM) of substrate, Table 1).

Clean maize was provided by a maize handling company (AflaSight, Kigali, Rwanda) following automatic sorting (SORTEX A LumoVision, Bühler, Uzwil, Switzerland) and unprocessed contaminated maize was purchased from a local food market (Nyabugogo, Kigali, Rwanda). Both clean and contaminated maize were milled to 1.2 mm maize flour (Supplementary Figure S1) and classified as clean and contaminated based on limits for whole grains in the East African Community (EAC) (10 μg kg DM^{-1}; Supplementary Table S15). The agri-food byproducts (see Supplementary Figure S2) consisted of homogenized brewers spent grain and fresh fruits stored for four days to mimic typical decomposition of agri-food byproducts.

Black soldier fly larvae feeding trials

Young BSFL from a colony at The Bug Picture at Bishenyi, Rwanda, were reared on pig feed for seven days according to procedures described in the Supplementary Material to around 0.3 mg $larva^{-1}$. Thereafter, the larvae were separated from the pig feed residue and reared for twelve days in plastic trays (56 cm × 38 cm) containing 6,705 g substrate (70% moisture content, Supplementary Table S1) and 7,500 larvae (3.5 larvae per cm^{-2}, 74.5 mg (larva × $day)^{-1}$) according to Gold (2020). Temperature and relative humidity measured daily (9 am, 1 pm and 5 pm) with a handheld thermometer and humidity meter during the trial are summarized in the Supplementary Tables S2 and S3. Following twelve days, larval mass was determined, and residue and larval samples were collected by manual separation. No specific procedure allowing larvae to empty their gut was applied. Larvae and residue were placed in plastic zip lock bags and stored at –18 °C until further analyses.

Substrate and product nutrient and chemical composition

One pooled sample of the four biological replicates for the substrates and larvae were analysed for moisture content and gross nutrient composition (i.e. crude protein from total nitrogen, crude fat, ash) and the frass was analysed for moisture content, typical physicochemical (see Table 4) metrics for compost quality and *Aspergillus*. Analyses were done by an external certified laboratory (Cropnuts, Nairobi, Kenya) according to standard procedures (ISO, 2022, 2009, 2008, 1999a,b). One soybean and fishmeal sample from Nyabugogo market in Kigali was analysed in parallel to BSFL using the same procedures as a benchmark for the animal feed quality in Rwanda. Crude protein was estimated by multiplying the nitrogen value with sample-specific factors: 5.6 for substrates (based on maize) (Mariotti *et al.*, 2008), 4.7 for larvae (Janssen *et al.*, 2017), 5.7 for soybean meal (Mariotti *et al.*, 2008) and 6.25 for fishmeal. In parallel, the true protein content was also analysed as the sum of all amino acids by external laboratories for the larvae fed on CM50 (Agrolab, Kiel, Germany), and soybean and fishmeal (Groen Agro Control, Delfgauw, The Netherlands) according to EC (2009).

Mycotoxin analyses

The maize, substrates, larvae and frass were analysed in quadruplicate for 38 common and emerging mycotoxins and metabolites (Table 2, Supplementary Table S4) with an in-house method validated according to EC (2019) at Wageningen Food Safety Research. Samples were ground under liquid nitrogen and then stored at –80 °C until analysis. Sample extraction, quantification of the mycotoxin concentration and LC-MS/MS analyses were mostly done according to Camenzuli *et al.* (2018). In the current study a smaller sample mass (1.0 g) of the substrate, larvae and frass samples was used as mentioned in the original method (2.5 g). The used reagents were adjusted accordingly. Mycotoxin concentration in the sample was quantified by means of standard addition, and the mycotoxin concentrations were analysed with LC-MS/MS. A detailed description of the extraction procedure, method of quantification, LC-MS/MS analyses and the parameters used can be found in the Supplementary Material.

Data analyses

LC-MS/MS data was analysed with SCIEX OS-MQ v2.1.6 software (Sciex, Framingham, MA USA). Peak identification was performed according to criteria set in an in-house validated method at Wageningen Food Safety Research and identification and recovery criteria outlined in EC (2019). A detailed description of the methods utilized to determine a Limit of Detection (LOD) and Limit of Quantification (LOQ) of the LC-MS/MS method, final LOD and LOQ concentrations, as well as the formula used for quantification of the mycotoxin concentration in the samples can be found in the Supplementary Tables S5-S8. For statistical analyses all mycotoxins below the LOQ were considered to be of lower analysis quality and set to 0. Mycotoxin concentrations were analyzed together with the maize, substrate and frass compositional data, larval mass as well as substrate/frass temperature from feeding trials in R software and RStudio version version 3.6.3 (RStudio Inc., Boston, MA, USA). Mean and standard deviation of results between replicates were calculated. Significance of differences in mean results was assessed using analysis of variance (ANOVA) followed by pairwise Tukey post-hoc comparisons. A p-value of <0.05 was chosen to declare significance.

3 Results

Maize and substrates

The contaminated maize had 16 mycotoxins (Table 2), was significantly higher in AFB1, FB1 and NPA and included five additional mycotoxins (AFG2, AFM1, ZEN, AOH, AC) than the clean maize. In the clean maize, relatively small amounts of 13 mycotoxins were detected, whereas the concentration of 15AcDON was significantly higher than that found in the contaminated maize.

The substrates used in the BSFL feeding trials had a moisture content of 65-80%, i.e. in the typical range of BSFL substrates. The substrate was low in protein (8-9% DM), fat (5% DM), ash (2-3% DM) and crude fibre (3% DM) with similar results regardless of the maize inclusion level (Supplementary Table S9).

As expected, mycotoxins found in the substrates were generally those present in the clean and contaminated maize (Table 2). Only, ENNB, ENNB1 and AME were found in small amounts in one or more of the substrates but not in the clean and contaminated maize. AFM1, 15AcDON and AC were found in very low concentrations in the maize but not in the substrates. Similarly, as expected, substrates formulated with contaminated maize were rich in mycotoxins and the composition of the substrate changed the mycotoxin concentrations in the substrate. Inclusion of a higher percentage of maize with a higher mycotoxin contami-

TABLE 2 Concentrations of all mycotoxins found in the maize and different substrates (μg kg DM^{-1})

Parameter	Clean maize	Contaminated maize	CM50	LM50	HM50	CM80	LM80	HM80
Aflatoxins[1]								
AFB1	2.3 (3.2)^{a}	172.3 (136.7)^{b}	0.4 (0.5)^{a}	16.2 (1.7)^{b}	41.0 (12.4)c	1.5 (0.2)^{a}	18.8 (3.8)^{b}	99.4 (47.5)^{c}
AFB2	0.2 (0.4)^{a}	17.5 (19.8)^{a}	<LOQ^{a}	2.5 (0.2)^{b}	7.5 (1.1)^{c}	<LOQ^{a}	2.7 (0.1)^{b}	12.2 (1.4)^{c}
AFG1	0.5 (0.5)^{a}	16.9 (28)^{a}	0.4 (0.3)^{a}	3.7 (1.2)^{b}	8.6 (0.4)^{c}	1.0 (0.1)^{a}	3.3 (0.7)^{b}	11.3 (1.6)^{c}
AFG2	<LOQ^{a}	2.3 (4.2)^{a}	<LOQ^{a}	<LOQ^{a}	2.7 (0.6)^{b}	<LOQ^{a}	<LOQ^{a}	0.5 (1.1)^{a}
AFM1	<LOQ^{a}	1.1 (1.5)^{a}	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Fusarium mycotoxins[2]								
15AcDON	38.3 (30)^{a}	<LOQ^{b}	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
DON	121.2 (33.8)^{a}	104.7 (50)^{a}	115.9 (3.6)^{a}	131.1 (6.3)^{a,b}	142.1 (19)^{b}	127.6 (7.8)^{a}	130.7 (7.5)^{a}	135.2 (17.3)^{a}
ZEN	<LOQ^{a}	3.4 (6.8)^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	15.9 (31.7)^{a}
FB1	290 (225.3)^{a}	2340.4 (1976.1)^{b}	109.7 (11.1)^{a}	347.4 (73.3)^{b}	493.3 (50.3)^{c}	215.8 (13.5)^{a}	469.2 (49.2)^{b}	1034.6 (93.5)^{c}
FB2	149.7 (110.2)^{a}	1446.3 (1780.9)^{a}	37.8 (8.9)^{a}	121.8 (34.1)^{b}	169.2 (31.4)^{b}	66.8 (5.9)^{a}	156.3 (30.7)^{a}	378.6 (104.6)^{b}
FB3	35.6 (19.2)^{a}	208.4 (140.6)^{a}	12.7 (8.6)^{a}	45.6 (12.6)^{b}	65.7 (5.3)^{c}	29.1 (1.4)^{a}	69.9 (9.2)^{a}	208.5 (55.9)^{b}
ENNB	<LOQ	<LOQ	<LOQ^{a}	<LOQ^{a}	9.2 (18.4)^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}
ENNB1	<LOQ	<LOQ	<LOQ^{a}	<LOQ^{a}	4.6 (9.3)^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}
BEA	6.9 (2.5)^{a}	69.9 (53.4)^{a}	<LOQ^{a}	3.0 (5.9)^{a}	13.5 (1.4)^{b}	<LOQ^{a}	3.7 (7.3)^{a}	28.1 (11)^{b}
MON	62.1 (33.1)^{a}	26.5 (52.9)^{a}	36.1 (4.4)^{a}	54.9 (8.4)^{b}	68.3 (5.9)^{c}	54.6 (3.7)^{a}	79.2 (12.9)^{a}	193.5 (23.1)^{b}
Aspergillus, Penicillium, and Alternaria mycotoxins[3]								
NPA	20 (28.4)^{a}	101.2 (15.2)^{b}	<LOQ^{a}	<LOQ^{a}	47.8 (4.6)^{b}	<LOQ^{a}	<LOQ^{a}	67.4 (13.9)^{b}
STER	2.1 (3.9)^{a}	4.7 (1.4)^{a}	<LOQ^{a}	0.2 (0.5)^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}
CIT	2.1 (3.9)^{a}	4.7 (1.4)^{a}	<LOQ^{a}	0.9 (1.9)^{a}	8 (1.9)^{b}	<LOQ^{a}	0.9 (1.9)^{a}	23.2 (30.8)^{a}
AME	<LOQ	<LOQ	9.9 (0.9)^{a}	12 (1)^{b}	8.4 (0.6)^{a}	4.4 (5.3)^{a}	<LOQ^{a}	13.9 (24.6)^{a}
AOH	<LOQ^{a}	1.0 (2)^{a}	12 (0.9)^{a,b}	13.9 (1.8)^{b}	10.7 (1.4)^{a}	2.2 (4.3)^{a}	<LOQ^{a}	15.2 (30.5)^{a}
AC	<LOQ^{a}	0.1 (0.2)^{a}	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Average and standard deviation in parentheses with n = 4. Results with no shared letter are significantly different from each other (comparing CM50, LM50, HM50 and CM80, LM80 and HM80 separately). LOQs, see Supplementary Table S9. DM = dry matter; CM50 = with control maize, 50% DM maize; LM50 = Low mycotoxin contaminated maize, 50% DM maize; HM50 = high mycotoxin contaminated maize, 50% DM maize; CM80 = with control maize, 80% DM maize; LM80 = Low mycotoxin contaminated maize; 80% DM maize, HM80 = high mycotoxin contaminated maize, 80% DM maize.

1 Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and aflatoxin M1 (AFM1).

2 15-acetyldeoxynivalenol (15AcDON), deoxynivalenol (DON), zearalenone (ZEN), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), enniatin B (ENNB), enniatin B1 (ENNB1), beauvericin (BEA) and moniliformin (MON).

3 nitropropionicacid (NPA), sterigmatocystin (STER), citrinin (CIT), alternariol-monomethylether (AME), alternariol (AOH) and agroclavine (AC).

nation increased the mycotoxin concentrations in the substrate. Overall, aflatoxins (AFB1, AFB2, AFG1) and fumonisins (FB1, FB2, FB3) were significantly higher in LM80 than LM50 and HM80 than HM50 (Table 2). Substrates with clean maize had low mycotoxin concentrations (Table 2) with significantly higher concentration for CM80 than CM50. CM50 and CM80 had significantly lower mycotoxin concentrations than LM50 and LM80, respectively. As expected, concentration of mycotoxins (AFB1, AFB2, AFG1, FB1, FB2, FB3, BEA, MON and NPA) were highest in HM80 as compared to all other substrates (Table 2, Supplementary Table S10 for statistical analysis). HM80 had 123 ± 17 μg kg DM^{-1} total aflatoxins (AFB1+AFB2+AFG1+AFG2), 135 ± 17 μg kg DM^{-1} DON and 1,622 ± 251 μg kg DM^{-1} total fumonisins (FB1+FB2+FB3). ZEN was only detected in HM80 and no other substrate.

Influence of maize inclusion and mycotoxin contamination on larval mass

The mean larval mass of CM50, HM50 and LM50 were not significantly different. Larval mass was also not significantly different between CM80, LM80 and HM80 (Figure 1a,b). However, these conclusions should be interpreted with caution due to the limited data per replicate (n = 4) and relatively high standard deviation of the results, and it is plausible that increasing the number of replicates and reducing the standard deviation could reveal significant differences in performance. In contrast, it could be carefully concluded that the maize inclusion level influenced larval growth (Figure 1c). Larval mass was significantly lower on the substrate containing more maize when considering all maize mycotoxin levels (i.e. CM80, LM80 and LM80 vs CM50, LM50 and HM50), however the differences were small. Mean larval mass ranged from 72.8-108.0 mg with 50% DM maize and 72.2-75.8 mg with 80% DM in the substrates. Substrate/frass temperature appeared to be similar between treatments, ranging from 21-27 °C. Over the entire feeding trial, mean temperature in the substrate/frass was 23-24 °C (Supplementary Tables S2 and S3).

Mycotoxin transfer from substrate to products

Despite the partially or very high concentration of mycotoxins in the substrate (Table 2), mycotoxins were either only found in low amounts or not detected in BSFL (Table 3). As expected, in the larvae from clean maize (CM50 and CM80) few mycotoxins (e.g. AFG1, FB1, FB2 and AME) were detected in low amounts (Table 3). However, also larvae from LM50 and LM80 were not significantly different from larvae from CM50 and CM80, respectively.

Even though mycotoxin concentrations in larvae were generally very low, mycotoxin concentration in BSFL appear to increase with higher substrate mycotoxin contamination. Larvae from the substrate with the highest mycotoxin contamination (HM80; Table 2) had significantly more AFB1, FB1, FB2, FB3 and BEA than larvae from CM80. Larvae from HM50 contained only significantly higher concentrations of FB1, FB2, FB3 and BEA than larvae from CM50.

In summary, larvae reared on HM50 and HM80 are the most relevant regarding BSFL product safety and shown in Figure 2 to illustrate the transfer and potential bioaccumulation of mycotoxins from substrates to BSFL products. Except for BEA, mycotoxins in the BSFL were much lower than in the substrates or even absent confirming that no bioaccumulation took place. BEA was the only mycotoxin that bioaccumulated with a factor of 1.6 (concentration BEA in larvae/concentration BEA in substrate) for HM50 and HM80 reared BSFL (Figure 2c).

In contrast to the larvae, aflatoxins, DON, fumonisins, BEA, CIT, AME and AOH were detected in the frass (Figure 2). Frass mycotoxin concentrations relative to the initial substrate mycotoxin concentration varied among substrates (0-3.9, frass concentration/substrate concentration) and mycotoxins (Figure 2, Supplementary Tables S10-S12). Most often, frass mycotoxins were significantly lower or not significantly different from the substrate. For example, AFB1 in frass from HM50 and HM80 was significantly lower than AFB1 in the substrate but not significantly different between frass and substrate for DON. However, frass mycotoxins could also be significantly higher from the substrate. For example, frass from HM50 was significantly higher in FB1, FB2, FB3, STER, AME and AOH than the substrate. Interestingly AFP1, a known metabolite of AFB1, was only found in the frass, but not the larvae and substrate, indicating that metabolism of AFB1 occurred (Figure 2a).

Product composition

BSFL from substrates with the two maize inclusion levels were low in ash (4 %DM) and had 26-29% DM crude protein and 23% DM crude fat (Supplementary Table S13). BSFL from 50% DM maize had 26.1% DM true protein confirming that an appropriate nitrogen to crude protein conversion factor was used. BSFL had 13-16% DM more crude protein, while having less crude fibre and a similar ash content than soybean meal. Fishmeal had 13-16% DM more crude protein than BSFL. Soybean meal and fishmeal had 10.2% DM and 45.0% DM true

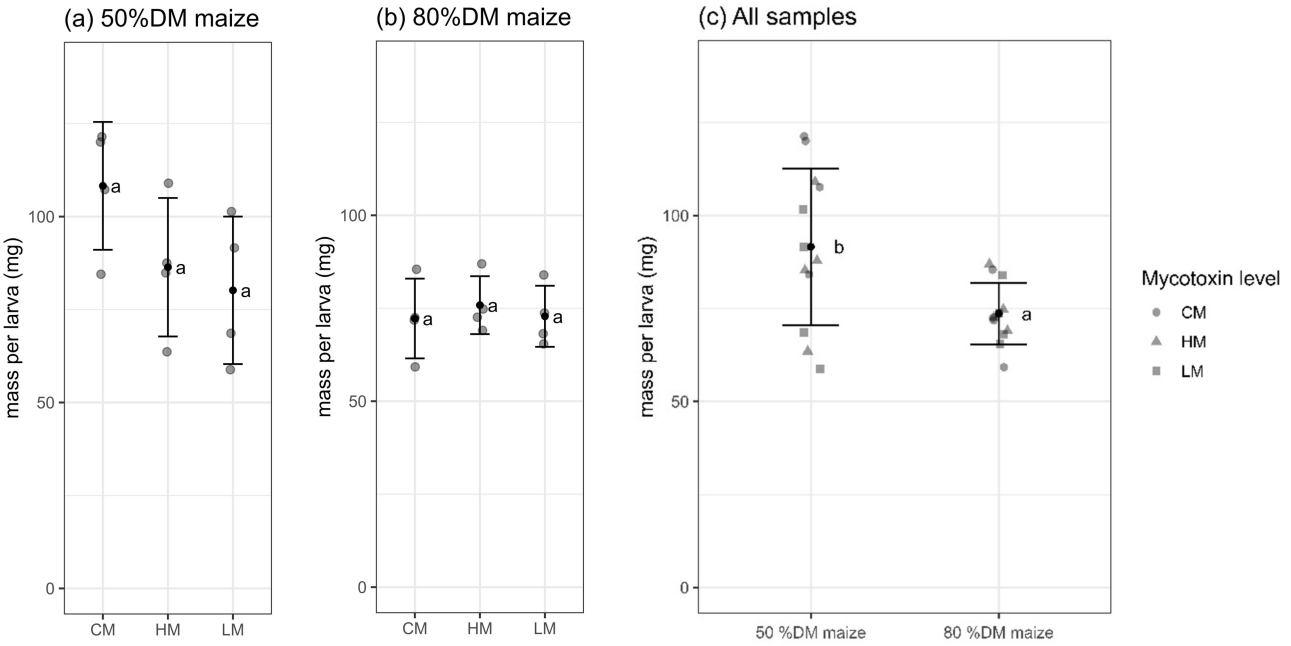

FIGURE 1 Effect of maize mycotoxin contamination on larval mass with (a) 50%DM maize and (b) 80%DM maize. (c) Effect of maize inclusion level on larval mass. CM = Control maize, LM = Low mycotoxin contaminated maize, HM = High mycotoxin contaminated maize.

TABLE 3 Mycotoxins in unprocessed larvae from different substrates containing different amounts of mycotoxin contaminated maize and maize (μg kg DM^{-1})

Parameter	CM50	LM50	HM50	CM80	LM80	HM80
AFB1	<LOQ^{a}	<LOQ^{a}	3.4 (3.2)^{a,b}	<LOQ^{a}	3.61 (1.2)^{a,b}	11.4 (11.8)^{b}
AFB2	<LOQ^{a}	<LOQ^{a}	0.4 (0.6)^{a}	<LOQ^{a}	0.4 (0.3)^{a}	1.5 (1.5)^{a}
AFG1	<LOQ^{a}	0.1 (0.2)^{a}	0.4 (0.7)^{a}	0.3 (0.3)^{a}	0.3 (0.4)^{a}	1.5 (1.4)^{a}
FB1	46.8 (9.15)^{a}	84.9 (13.4)^{a}	232.7 (99.7)^{b}	41.6 (31.0)^{a}	99.3 (30.6)^{a}	296.0 (85.2)^{b}
FB2	15.3 (2.58)^{a}	28.6 (4.9)^{a}	73.5 (30.8)^{b,c}	15.9 (11.5)^{a}	37.6 (7.4)^{a,b}	114.3 (31.1)^{c}
FB3	<LOQ^{a}	3.5 (7.0)^{a}	30.2 (12.4)^{b}	<LOQ^{a}	15.3 (3.5)^{a,b}	54.6 (16.2)^{c}
BEA	<LOQ^{a}	<LOQ^{a}	20.9 (14.3)^{b}	<LOQ^{a}	<LOQ^{a}	44.2 (15.8)^{c}
AME	1.0 (2.1)^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}

Results with no shared letter are significantly different from each other (comparing CM50, LM50, HM50 and CM80, LM80 and HM80 separately). CM50 = with control maize, 50% DM maize; LM50 = Low mycotoxin contaminated maize, 50% DM maize; HM50 = high mycotoxin contaminated maize, 50% DM maize; CM80 = with control maize, 80% DM maize; LM80 = Low mycotoxin contaminated maize; 80% DM maize, HM80 = high mycotoxin contaminated maize, 80% DM maize. Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), beauvericin (BEA) and alternariol-monomethylether (AME).

protein, respectively. *Aspergillus spp*. was not detected in any of the frass samples. The frass was high in nitrogen, phosphorus, potassium, and carbon (Table 4). Noteworthy, frass from both maize inclusion levels had an overall high electrical conductivity (EC) (4.0 and 6.0 mS cm-1) and pH was much higher with 50% than 80% DM maize.

4 Discussion

Mycotoxin contamination of crops is a global problem with enormous health and economic impacts. Particularly in tropical and subtropical low-income countries where crops are indispensable for food security and livelihoods, new approaches are needed to valorise mycotoxin contaminated crops. Given the economic importance of crops in low-income countries, these approaches do not only increase food security and safety but provide economic value to farmers pro-

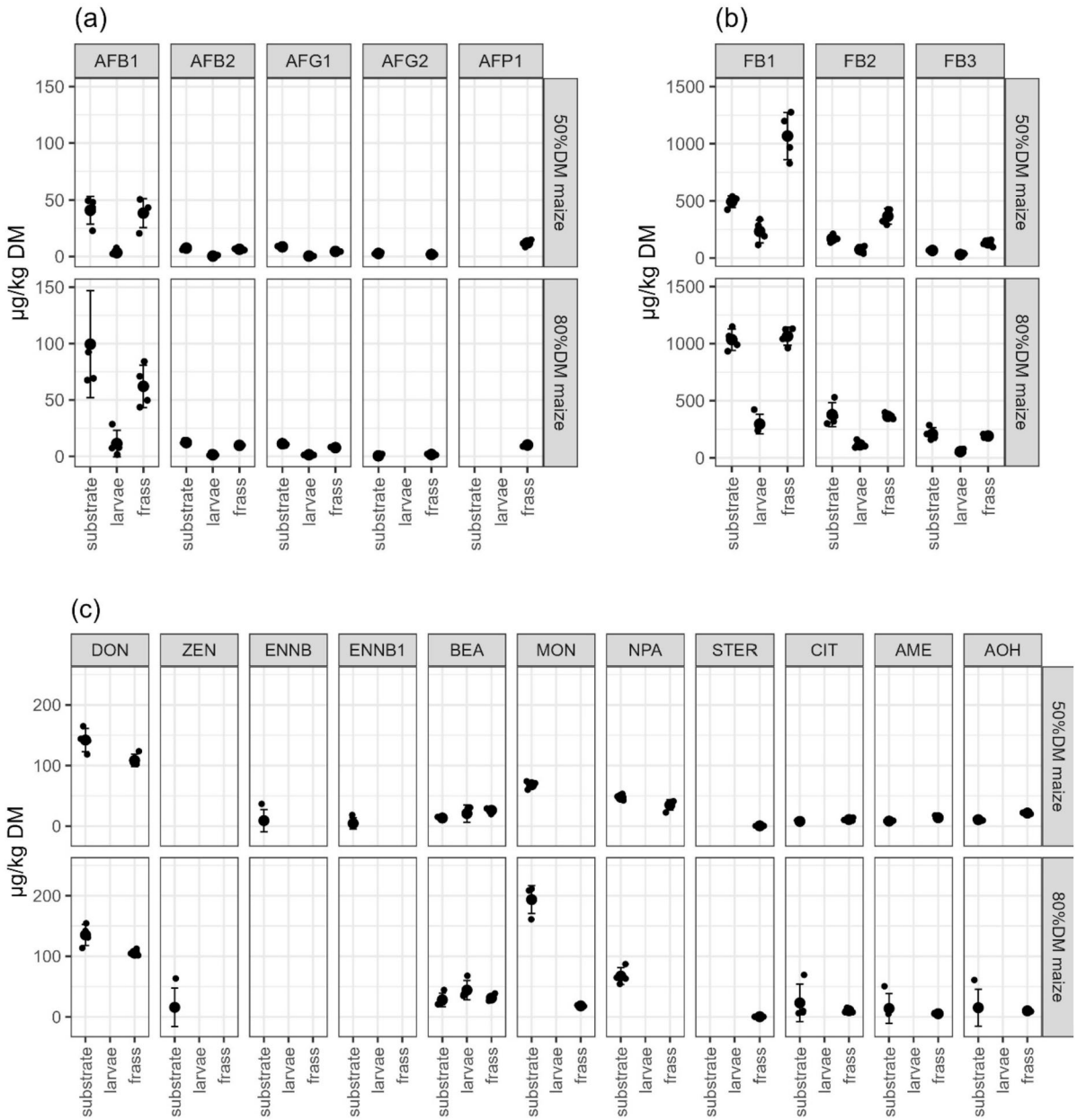

FIGURE 2 Evolution of mycotoxins between substrate and products (i.e. larvae and frass) for the highest contaminated maize inclusion level HM50 and HM80 (n = 4). (a) Aflatoxins, (b) fumonisins and (c) other mycotoxins.

ducing mycotoxin contaminated crops. Such economic value could come from producing feed and fertilizer from mycotoxin contaminated crops with BSFL.

The mycotoxin contaminated maize used in this study was representative for mycotoxin contaminated maize in Rwanda, or elsewhere. The contaminated maize was sourced from a public food market in Kigali, heavily contaminated with various mycotoxins confirming that mycotoxins co-occur (Ankwasa *et al.*, 2021). Maize was unsafe as food and feed based on EAC, and EU legal limits/guiding values (5 µg AFB1 kg^{-1}, 75 µg DON kg^{-1}, 75 µg ZEN kg^{-1}, 3 µg OTA kg^{-1}, 2,000 µg fumonisins kg^{-1}) (EC, 2002, 2006; Supplementary Table S15). The contaminated maize in this study (172 µg AFB1 kg DM^{-1}, 3,995 µg fumonisins kg DM^{-1}) exceeded EAC legal limits for food by 17-times for AFB1 (5 µg kg^{-1}) and 2-times for fumonisins (2,000 µg kg^{-1}) (Supplementary Table S15). AFB1 exceeded the EAC limits as feed (20 µg kg-1) while fumonisins, DON, ZEN and OTA were below the EU guiding values for maize as feed (Supplementary Table S15). AFB1 and fumonisin concentrations found in this study were in the range as earlier found in Rwanda (0-500 µg kg^{-1} total aflatoxins and 6,000 µg kg^{-1} fumonisins) (Nishimwe *et al.*, 2017). These results highlight the potential risks associated with mycotoxin-contaminated maize entering the food chain as food

TABLE 4 Physicochemical frass characteristics in comparison to compost and literature values (% DM)

Parameter	Unit	Frass (this study)		Fertilizer Standard Rwanda[1]	Compost Rwanda[2]	Frass typical range[3]
		50% DM maize	80% DM maize			
DM	%	41.6 (3.6)	43.9 (0.7)	≥ 70	63.4	–
EC	mS cm^{-1}	4.0 (0.09)	6.0 (0.15)	< 5.0	772	1.2-5-7
pH	–	8.0 (0.1)	5.9 (1.4)	6-9	7.5	5.4-9.0
N	g kg^{-1}	18.6 (0.2)	20 (0.3)	> 10	15.0 (1.1)	18-51
C	g kg^{-1}	517 (0)	527.7 (1.2)	–	173.5 (9.5)	268-488
C:N ratio	–	27.8 (0.3)	26.4 (0.3)	≤ 20:1	11.6 (0.3)	7-27
P	g kg^{-1}	1.9 (0.2)	2.3 (0.2)	–	2.4 (0.2)	3-52
K	g kg^{-1}	13.4 (0.5)	6.6 (0.5)	–	3.0 (0.2)	2-41
Ca	g kg^{-1}	< 5.0	< 5.0	> 10	23.7 (3.8)	0.2-45
Mg	g kg^{-1}	1.3 (0.1)	1.1 (0.1)	> 5.0	3.2 (0.5)	0.2-11
Na	mg kg^{-1}	15.8 (7.8)	17.6 (6.5)	–	0.5 (0.0)	0.3-5.0
Fe	mg kg^{-1}	590 (99.9)	337 (112)	1,000-2,500	21.0 (6.4)	4-896
Mn	mg kg^{-1}	22.4 (1)	12.8 (2.1)	200-800	0.9 (0.1)	0.2-149
Zn	mg kg^{-1}	12.7 (1.6)	18.7 (4)	40-1000	0.1 (0.0)	0.1-182
Cu	mg kg^{-1}	3.9 (0.1)	2.3 (0.7)	8-300	28.6 (5.2)	0.7-46

[1]Rwanda Standards Board (2021), [2]Fuhrmann *et al.* (2022), [3]Lopes *et al.* (2022).

DM = Dry mass, EC = Electric conductivity, N = total nitrogen, C = total carbon, P = total phosphorus, K = Total potassium, Ca = total calcium, Mg = total magnesium, Na = Total sodium, Fe = total iron, Mn = total manganese, Zn = Total zinc, Cu = total copper.

and/or feed, the potential for acute or toxic impacts to humans or animals, and the urge to find viable solutions such as recycling into feed and fertilizer by BSFL. The clean maize after sorting-out of aflatoxin-contaminated maize had AFB1 and fumonisins concentrations below EAC and EU limits.

As anticipated, formulating the mycotoxin-contaminated maize with agri-food by-products, presumably absent or low in mycotoxins, resulted in a decrease in mycotoxin concentrations in the substrate. This study aimed to utilize maize that is naturally heavily contaminated with AFB1, as other studies have only achieved high AFB1 concentration (400-800 μg kg DM^{-1}) by spiking. In comparison to previous studies, the HM80 substrate (Table 2) had the highest AFB1 (99.4 μg kg DM^{-1}) and FB1 concentration (1,035 μg kg DM^{-1}) in naturally contaminated substrates used with BSFL so far (13.3 μg AFB1 kg DM^{-1}, 727 μg FB1 kg DM^{-1}). DON, ZEN and OTA were lower in this study in comparison to previous work that used naturally contaminated maize and by-products (Bosch *et al.*, 2017; Camenzuli *et al.*, 2018; Heuel *et al.*, 2023; Supplementary Table S14).

Consistent with previous research (Supplementary Table S14), BSFL exhibited tolerance to substrate mycotoxins. To date, only Heuel *et al.* (2023) has observed a slower BSFL development with food waste containing 842 μg AFB1 kg DM^{-1}. In contrast to substrate mycotoxins, our study found that substrate composition (i.e. amount of maize and agri-food byproducts) significantly influenced larval growth. Greater inclusion of agri-food byproducts increased larval mass, likely due to changes in nutrient digestibility that affected larval growth, even though gross nutrients remained similar (Gold *et al.*, 2020). Purschke *et al.* (2017) obtained a similar larval mass (75 mg larva^{-1}) using a maize-based substrate. However, overall, larval mass in this study was low (73-108 mg larva^{-1}) in comparison to high-performing insect substrates such as restaurant food waste (200 mg larva^{-1}). This could be due to low substrate protein (8-9% DM) and fat contents fat (5% DM) crucial for larval development in comparison to higher performing food wastes (e.g. 12-32% DM protein, 29-35% DM fat; Gold *et al.*, 2020). Larval mass with maize could potentially be further improved by incorporating biowaste with higher protein and lipid content in the substrate mixture, such as animal manure, food waste, oilseed press cakes, or starch or ethanol facility byproducts. Additionally, optimizing context-specific rearing conditions (e.g. larval density, ventilation rates, substrate texture) and ambient and substrate/frass temperatures which were relatively low in this study (Harnden and Tomberlin, 2016) could result in more efficient bioconversion and larval

growth. BSFL were reared using the natural climate in Kigali, suggesting that the results could be transferrable to similar climates across Africa.

This study found that BSFL do not generally accumulate substrate mycotoxins, and that larvae from mycotoxin-contaminated substrates were safe as animal feed considering legal standards. Even larvae from substrates with the highest mycotoxin concentrations (HM80; Table 3) had AFB1 (11.4 μg kg DM^{-1}), FB1 (296 μg kg DM^{-1}), FB2 (114 μg kg DM^{-1}) and FB3 (55 μg kg DM^{-1}) concentrations below legal limits for compound animal feeds in the EU (20 μg AFB1 kg^{-1}, 5,000 μg DON kg^{-1}, 100-500 μg ZEN kg^{-1}, 10 μg OTA kg^{-1}, 5,000-20,000 μg fumonisins kg^{-1}) (EC, 2002, 2006) and dried insect products for animal feeds in the EAC (20 μg AFB1 kg DM^{-1}; Supplementary Table S15). Although larvae at the highest substrate mycotoxin contamination had more AFB1 than permitted in fish, pig and poultry starter feeds in the EAC (10 μg AFB1 kg DM^{-1}; Supplementary Table S15), the authors suggest that a starter compound feed with BSFL and clean other feed ingredients would result in a compound feed AFB1 concentration below the legal limits considering that they typically include less than 30% of a high-protein ingredient. Overall, this suggests that even at high substrate mycotoxin contamination, BSFL and BSFL-based animal feeds are safe for fish, poultry, and pigs considering current legal limits in the EU and EAC. The authors note that there is currently no legislation for BSFL for food production, but assuming that such legislation would have similar values to unprocessed grains (5 μg AFB1 kg DM^{-1}, 2,000 μg fumonisins kg DM^{-1}; Supplementary Table S15), BSFL from all substrates except for HM80 were below legal limits. This suggests that BSFL could be raised for food even on substrates contaminated with mycotoxins up to a certain level of contamination, if BSFL are to be considered as a viable food source in the future (Bessa *et al.*, 2020). However, our data confirm that metabolism of AFB1 took place, likely via the larval Cytochrome P450 enzyme system (Meijer *et al.*, 2019). It is not fully elucidated if other (unknown) toxic metabolites of mycotoxins could be formed in BSFL which should be further studied to ensure food and feed safety.

Our study confirms previous findings that AFB1, DON, ZEN, OTA and fumonisins do not bioaccumulate in BSFL, with these mycotoxins typically being below the LOQ (Supplementary Table S14). However, in studies with higher substrate mycotoxins concentrations (Camenzuli *et al.*, 2018; Heuel *et al.*, 2023), AFB1 (4.3 μg kg DM^{-1}), DON (275 μg kg DM^{-1}), ZEN (27.5 μg kg DM^{-1}) and OTA (3.9 μg kg DM^{-1}) concentration in BSFL above the LOQ were reported. Even though BSFL mycotoxin contamination levels are low, substrates with a higher mycotoxin concentration tend to result in BSFL with higher mycotoxin concentrations. This could also be due to small amounts of undigested substrate in the larval digestive tract. Even though these minor contaminations still make BSFL safe as feed according to legal limits, future research should investigate if they can be removed with egestion approaches of substrates from the BSFL digestive tract. This study is the first to identify bioaccumulation of BEA, with low amounts being detected in BSFL. High contaminated substrates with BEA could potentially result in high concentration of BEA in larvae. This could have caused by the lipophilic nature of this mycotoxin (Tran *et al.*, 2020), potentially accumulating in the larval biomass that is rich in fat.

Frass from mycotoxin contaminated substrates were high in mycotoxins. However, this does not appear to limit its application as fertilizer as mycotoxins are not regulated in compost and fertilizer. However, considering that mycotoxins in frass were partially similar or above the initial high substrate values, post-processing (e.g. composting, mixing with compost or fertilizer) and appropriate application and management practices should be considered to prevent negative health impacts following ingestion or inhalation. The contaminated maize and BSFL substrates were rich in aflatoxins produced by the mycotoxin producing fungus *Aspergillus*, suggesting that they contained this important mycotoxin production group of fungi (not quantified). However, no *Aspergillus* were detected in frass with mycotoxin-contaminated substrates suggesting that at least this group of mycotoxin-producing fungi are not propagated to soil when frass is used as fertilizer. Mycotoxins in the frass can help draw conclusion on whether they were decomposed by BSFL and/or the associated microbiome. Because BSFL and microbes reduce the substrate/frass mass during bioconversion (not quantified in this study), a lower or not significantly different frass concentration relative to the initial substrate suggests that mycotoxins were metabolized in the process. Consequently, in this study some part of the aflatoxins and DON were metabolized by BSFL and/or associated microbes. This is similar to previous studies who found 11-18% AFB1 and 39-55% of initial DON in the substrate in larvae and frass (Camenzuli *et al.*, 2018). AFP1, a typical AFB1 metabolite, was detected in frass and confirms that metabolism took place as mentioned above. In contrast, no conclusions can be drawn on other mycotoxins such as fumonisins because concentration in frass were significantly higher in the frass than

the substrate. Previous studies with spiked and naturally contaminated wheat bran and olive pomace have found that 70% of total fumonisins are found in larvae and frass (Leni *et al.*, 2019; Niermans *et al.*, under review). Different trends, meaning that some mycotoxins in frass were below and some above initial concentrations in the substrate, suggest that metabolization during the bioconversion process could vary among mycotoxins. Suo *et al.* (2023) recently concluded for AFB1, that such decomposition is partially mediated by the digestive tract microbiome which should be investigated for other mycotoxins.

Our study suggests that the frass from maize and agri-food by-products has potential as compost and/or fertilizer. Frass nutrients were comparable to a Rwandan compost (Fuhrmann *et al.*, 2022) (Table 4), indicating that frass has a similar fertilizer value. Frass contained 19-20 N, 1.9-2.3 P and 7-13 K (mg kg DM^{-1}), whereas compost had 15 N, 2.4 P and 3 K (mg kg DM^{-1}). Frass contained 3-times more carbon than the compost (520-530 vs 173 mg kg DM^{-1}). Frass varies greatly among substrates, particularly in terms of P, K and micronutrients (Lopes *et al.*, 2022), but the frass fell within the typical range (Table 4). Our frass was higher in EC (4-6 vs 1.2-5.7 mS cm^{-1}), C (517-528 vs 268-488 mg kg DM^{-1}), C:N (26-28 vs 7-24) and Na (16-18 vs 0.3-5.0 mg kg DM^{-1}) than the frass reviewed by Lopes et al. (2022). Future research should determine whether the high frass EC is due to ammonia produced by BSFL excretions, which could be a valuable nutrient for plants, or related to salt, which could be toxic to plants, especially those that are salt-sensitive (Beesigamukama *et al.*, 2021; Liu *et al.*, 2019). This research should also measure soluble nutrients (e.g. P_2O_5-K_2O), assess optimal plant applications (e.g. soil and plant type, dose), and investigate nutrient supplementation to meet crop-specific nutrient requirements and fertilizer standards in Rwanda (Table 4) where the frass from our study falls short in terms of EC, C:N ratio, and some micronutrients (Ca, Fe, Mn, Zn, Cu). As highlighted previously, such plant trials with frass could also reveal effects of additional frass compounds, such as bioactive compounds (e.g. humic substances, phytohormones, chitin), and beneficial microorganisms that could promote high plant health and yield (Fuhrmann *et al.*, 2022; Lopes *et al.*, 2022).

The unprocessed BSFL appear to have value as feed and food due to their protein and fat content. However, the protein content of the larvae (26-29% DM) was lower than the typical range for BSFL (32-58% DM), while fat content (23% DM) fell within the typical range (18-39% DM) (Gold *et al.*, 2018). The larvae's protein and fat content is influenced by substrate composition and operational parameters, such as temperature, humidity, air flow, larval densities, and feeding rate. Future research should focus on optimizing these parameters to increase larval protein content and thereby improve the economics of using BSFL to manage mycotoxin-contaminated by-products such as maize. While the larval protein content was below average, unprocessed larvae from our substrates including mycotoxin-contaminated maize had twice as much protein (26-29% DM) than soybean meal (13% DM) in Rwanda. After optimizing substrate and bioconversion conditions, BSFL could be competitive with soybean and fishmeal. Removing fat from BSFL with an oil press can increase protein content up to 59% DM (Maurer *et al.*, 2016). This would also be required to make the BSFL competitive with high-quality soybean and fishmeal other than those analysed in this study. Future research should include feeding trials with target animals to optimize larval product quality, considering ash and crude fibre contents, which are typically considered undesirable and indigestible by livestock. BSFL had similar ash (4% DM) and lower fiber content (16-17% DM) than soybean meal, and lower ash than fishmeal (4% DM vs 21% DM). Only fishmeal had a lower fiber content than BSFL (6% DM vs 16-17% DM), possibly due to chitin in the larval exoskeleton.

5 Conclusions

BSFL can be used to produce feed and fertilizer within legally safe limits from maize contaminated with mycotoxins at concentrations typical for Africa. Mycotoxins in the substrate did not affect BSFL process performance and mycotoxins in BSFL were found in low amounts, not detected and below the legal limits in the EU and EAC. BSFL grown on contaminated maize had a superior feed value as compared to locally obtained soybean meal. Frass was absent in *Aspergillus* and rich in plant nutrients. Future research should elucidate if unknown toxic metabolites of mycotoxins could be formed in BSFL and optimize the substrate composition and BSFL production system to maximize feed and fertilizer production. Given that BSFL amounts that can be included in feed are a lot lower than the initial crops, pre- and post-harvest strategies to prevent and minimize crop mycotoxin contamination should be prioritized before utilization by BSFL.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.24526774

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Authorship contribution statement

Moritz Gold: conceptualization, methodology, formal analysis, writing: original draft, visualization. Kelly Niermans: methodology, formal analysis, investigation, writing: review and editing. Frans Jooste: conceptualization, methodology, investigation, resources, writing: review and editing, project administration, funding acquisition. Laura Stanford: conceptualization, resources, writing: review and editing, project administration, funding acquisition. Florence Uwamahoro: conceptualization, resources, writing: review and editing. Maryann Wanja: investigation. Teun Veldkamp: project administration, funding acquisition, review and editing. Alexandra Sanderson: conceptualization, resources, writing: review and editing, supervision, project administration, funding acquisition. Vasco Dos Santos Nunes: conceptualization, resources, writing: review and editing, supervision, project administration, funding acquisition. Alexander Mathys: supervision, writing: review and editing. H.J. van der Fels-Klerx: conceptualization, supervision, writing: review and editing. Elise F. Hoek-van den Hil: conceptualization, methodology, resources, supervision, writing: review and editing, funding acquisition. Kizito Nishimwe: conceptualization, methodology, investigation, writing: review and editing.

Conflict of interest

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

Data availability

All data and code related to this manuscript is publicly available online at: https://github.com/MoritzGold/mycotoxins_BSFL/.

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