Agrochemical contaminants in six species of edible insects from Uganda and Kenya

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ABSTRACT

Edible insects are currently promoted worldwide as an alternative animal protein source, but they are mostly still harvested from the wild where they are predisposed to contamination with agrochemicals. This study analysed six species of edible insects (*Ruspolia differens, Rhynchophorus phoenicis, Schistocerca gregaria, Oryctes* sp, *Pachnoda ephippiata* and *Acanthoplus* sp) collected from different habitats and/or reared in the laboratory in Kenya and Uganda for safety from agrochemical contaminants using liquid chromatography tandem mass spectrometry. The residue levels were statistically compared with the Codex Alimentarius Commission maximum residue limits (MRLs). Residues of only nine agrochemicals were detected in the insects out of 374 chemicals which were screened. The detected agrochemicals include two insecticides (aminocarb and pymetrozine), three herbicides (atraton, methabenzthiazuron and metazachlor) and four fungicides (carboxin, fenpropimorph, fludioxonil and metalaxyl). *Ruspolia differens* and adult *Oryctes* sp were free from detectable levels of any agrochemical. Whereas the pesticides residue levels in most insect samples were within maximum residue limits, some of them notably *P. ephippiata* from black soldier fly larval frass, *R. phoenicis* from oil palm and *P. ephippiata* from plant compost contained 2-, 8- and 49-fold higher levels of atraton, methabenzthiazuron and metazachlor, respectively, than MRLs. These findings demonstrate that edible insects may accumulate harmful residues of agrochemicals from the environment where they breed or forage, rendering them unsafe for human consumption or feeding animals. The mechanisms for possible bioaccumulation of these agrochemicals in the insects remains to be investigated. Development of methods for farming edible insects under regulated indoor conditions to ensure their safety as sources of food or feed is recommended.

1. Introduction

Worldwide, human consumption and trade in insects has been popular in selected communities for generations, but these activities are rapidly spreading to other communities (Egonyu et al 2021; Kelemu et al 2015; Magara et al 2021; Tanga et al 2021; Verner et al 2021). Value addition through new food products development is growing as a way of improving acceptability of edible insects to consumers (Cheseto et al 2020; Maiyo et al 2022; Ssepuuya et al., 2016). The most common edible insects in East Africa and other countries in sub-Saharan Africa are long-horned grasshoppers (*Ruspolia differens*), mopane worm (*Gonimbrasia belina*), armoured bush cricket (*Acanthoplus* sp), house crickets (*Acheta* spp), palm weevil larvae (*Rhynchophorus phoenicis*), cabbage tree emperor moth (*Bunaea alcinoe*), Silkmoth (*Anaphe panda*), termites

(*Macrotermes* spp) and rhinoceros beetles (*Oryctes* spp) (Kelemu et al., 2015; Kusia et al., 2021; Mugova et al., 2018). In Uganda and Kenya, termites, grasshoppers, locusts and crickets are among the most commonly consumed insects (Egonyu et al., 2021; Kelemu et al., 2015; Kusia et al., 2021). However, these insects are still largely harvested from the wild where they are predisposed to contamination with different agrochemicals which spillover from crop farms to wild vegetation where the insects forage or breed (Belluco et al., 2013; Murefu et al., 2019; van der Fels-Klerx et al., 2018). Although edible insects are commonly heat processed (e.g., sun drying, roasting and deep frying) which may cause evaporation or degradation of pesticide residues, not all types of pesticides can be eliminated by heat (Nuran and Yakup, 2019). Also, some insects (e.g., grasshoppers and termites) are eaten in raw form in some communities in Uganda and Kenya (Agea et al., 2008; Kelemu et al., 2015;

Kusia et al., 2021), hence exposing consumers to the risk of pesticide poisoning. Whereas the risk of pesticide contaminants in edible insects and insect-derived food products have been assessed in some parts of the world e.g., Belgium and Canada (Kolakowski et al., 2021; Poma et al., 2017), and Kuwait and Saudi Arabia (Egonyu et al., 2021), information on harmful agrochemical residue levels for African edible insects is scarce.

Millions of tons of pesticides are annually used to control pests and disease-vectors worldwide, with an estimated 10,000-100,000 tons imported into East Africa in 2018 (FAO/WHO, 2018; Sarkar et al., 2021). In some African countries like Uganda, some organochlorine pesticides which were banned in the 1970's due to persistence in the environment, non-selectivity and high toxicity are still detectable in the environmental matrices at varying concentrations, suggesting that they could still be used illegally (Ntirushize et al., 2019; Mukiibi et al., 2021). Most of the agrochemicals are not judiciously used, while some expire and are inappropriately disposed of due to weak regulatory systems in most developing countries (Akpan and Olukanni, 2020; Bempah et al., 2011; FAO/WHO, 2018; Fayiga et al., 2018). This situation predisposes the environment and non-target organisms and the food chains to chemical contamination, hence posing serious health risks to consumers of wild collected foods like edible insects. Health complications associated with pesticide food poisoning range from acute symptoms like headache and stomachache to chronic impacts like cancer and endocrine disorders (Bempah et al., 2011; Chiou et al., 2015). The lipophilic nature of most pesticides favors their quick accumulation in fats of organisms like edible insects (Maitera et al., 2018). Analysis of the scale of agrochemical residue levels in edible insects is an important step in designing measures of mitigating their hazardous impacts to insect consumers.

Pesticide residues in food are monitored with reference to the Codex Alimentarius Commission maximum residue limits (MRLs) in mg/kg, determined by field trials combined with toxicological risk evaluations (Boobis et al., 2008). Maximum residue limits refer to the maximum allowable levels of pesticide residues in food products (Kolakowski et al., 2021). Although no MRLs are defined for edible insects, published values for meat/meat products have been suggested to apply (Kolakowski et al., 2021; Poma et al., 2017).

In this study, we analyzed six species of edible insects collected from different habitats or reared in the laboratory in Uganda and Kenya for 374 pesticides residues relative to MRLs in meat and meat products (FAO/WHO, 2016). For pesticides whose MRLs are not defined, 0.1 mg/kg was adopted as the MRL according to Kolakowski et al., (2021). The research questions were: (i) which pesticides are detectable in samples of the edible insects? (ii) When present, do pesticide residue levels in the different insect samples exceed the permissible MRLs?

2. Materials and methods

2.1. Insect sample collection

Details of insect samples are presented in Table S1. A total of 12 batches of whole raw and plucked deep fried wild collected *R. differens* adult samples (6 batches each) were purchased from three randomly selected traders from Katwe and Busega markets in Uganda's capital city, Kampala in December 2020. Wild collected *R. phoenicis* larvae (6 batches; 3 from oil palm and 3 from raffia palm) were sampled from infested palms in Kalangala, Uganda in June 2020. The palms were purposively selected based on symptoms of infestation with palm weevils, and cut down to extract the weevil larvae. Adult wild collected desert locust *Schistocerca gregaria* (3 batches) were sampled from swarms at Barsaloi, Maralal, Samburu, Kenya in October 2020 when the locust plague had invaded the country. Laboratory reared samples of adult *S. gregaria* (3 batches) were obtained from the colony maintained at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi since 1991 (Njagi and Torto, 2002) with periodic infusions with wild collections whenever there's an invasion. Adult *Oryctes* sp samples (3

batches) were obtained from the fourth generation of a colony at *icipe* reared on cattle dung and its larvae (3 batches) were collected on cattle dung from a farm at Kasarani, Nairobi, Kenya in February 2021. The colony at *icipe* was initiated with collections from Busia, Kenya in August 2019. *Acanthoplus* sp adults (3 batches) were collected from wild grasses at the outskirts of Kumi district headquarters, Uganda in September 2019. A total of 9 batches of *P. ephippiata* larvae were collected from cattle-dung, plant compost and black soldier fly frass (3 batches each) at the *icipe* campus in February 2021. Coordinates from sampling sites were taken using a Global Positioning System (GPS) (GARMIN eTrex 20X, Garmin Ltd, Olathe, Kansas, U.S.A.) and plotted using Arc Map QGIS 3.10.9 software (Fig. 1). Apart from *S. gregaria* which was sampled from the wild and also in the laboratory, all insects tested were unique species from either wild or laboratory. Therefore, all the insect samples were analyzed as stand-alone treatments.

Insect samples obtained from *icipe* campus were placed in sterile zip lock bags and killed by freezing at -80°C; whereas the insect samples obtained from distant places were immediately placed in sterile zip lock bags and transported in cool boxes with flaked ice to *icipe*, where they were preserved in a freezer at -80°C prior to use.

2.2. Sample preparation

Each insect sample (∼200 g) was homogenised in a blender. The samples were then freeze-dried and stored at -20 °C until chemical analysis. Modified Quick, Easy, Cheap, Effective, Rugged, Safe (QuECHERS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) (Poma et al., 2017) were used in sample preparation and analysis of pesticide residues in insect samples. Briefly, 2.0 g of each insect batch was weighed into a 15 mL Polypropylene (PP) falcon tube containing QuECHERS salts ((PSA, C18EC, Bulk Carbograph and MgSO$_4$ (Agilent Technologies, Inc. Folsom)) and 7 mL of Acetonitrile was added. The mixture was shaken for 1 min, vortexed for 1 min, sonicated for 5 min (Branson 2510, Danbury, CT, USA) and centrifuged at 4200 relative centrifugal force (rcf) for 5 min. Aliquot (2 mL) of the supernatant was transferred into a clean glass tube, where it was concentrated to dryness under a gentle nitrogen stream and reconstituted in 150 µL ACN:MilliQ (1:1 v/v). The samples were filtered through a 0.22 µm nylon membrane into 2 mL clear glass vials, each containing 250 µL conical point glass inserts (Supelco, Bellefonte, PA, USA) and immediately analyzed using LC-MS/MS.

2.3. Liquid chromatography mass spectrometry (LC-MS/MS)

The analysis of insect samples (0.2 µL), mostly in triplicates, but also in duplicates due to limited availability, was performed on a Waters Xevo TQ-S LC-MS/MS (Waters Corp., Milford, MA). The chromatographic separation was done on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) I-class system fitted with an ACE C$_{18}$ column (250 mm × 4.6 mm i.d., 5 µm) from Advance Chromatography Technologies, Aberdeen, Scotland. Mobile phase A was 10 mM ammonium acetate (pH 5) in water, whereas the mobile phase B comprised 10 mM ammonium acetate (pH 5) in methanol. The following gradient elution program was used: 0.25 min, 2% B; 0.25–12.25 min, 2–99% B;13.00 min, 99% B; 13.01–17.00 min, 2% B. The flow rate was held constant at 0.45 mLmin^{-1}. The Ultra UPLC system was coupled to a Xevo TQ-S equipped with an electrospray ionization source operated in positive mode. The LC inlet and MS acquisition methods were automatically generated from Waters® Quanpedia Database where up to three MRM transitions for each of the 374 pesticides (Table S2) in the data base were targeted. The method was optimized and validated by running a mixture of pesticides standard following LC-MS/MS criteria for residue analysis according to European Commission (2015). The calibration curves and matrix-matched calibration standards were prepared from seven calibration levels covering 0.01-100 pg/µL using the certified pesticides standard mixtures (Dr. Ehrenstorfer, Augsburg, Germany,
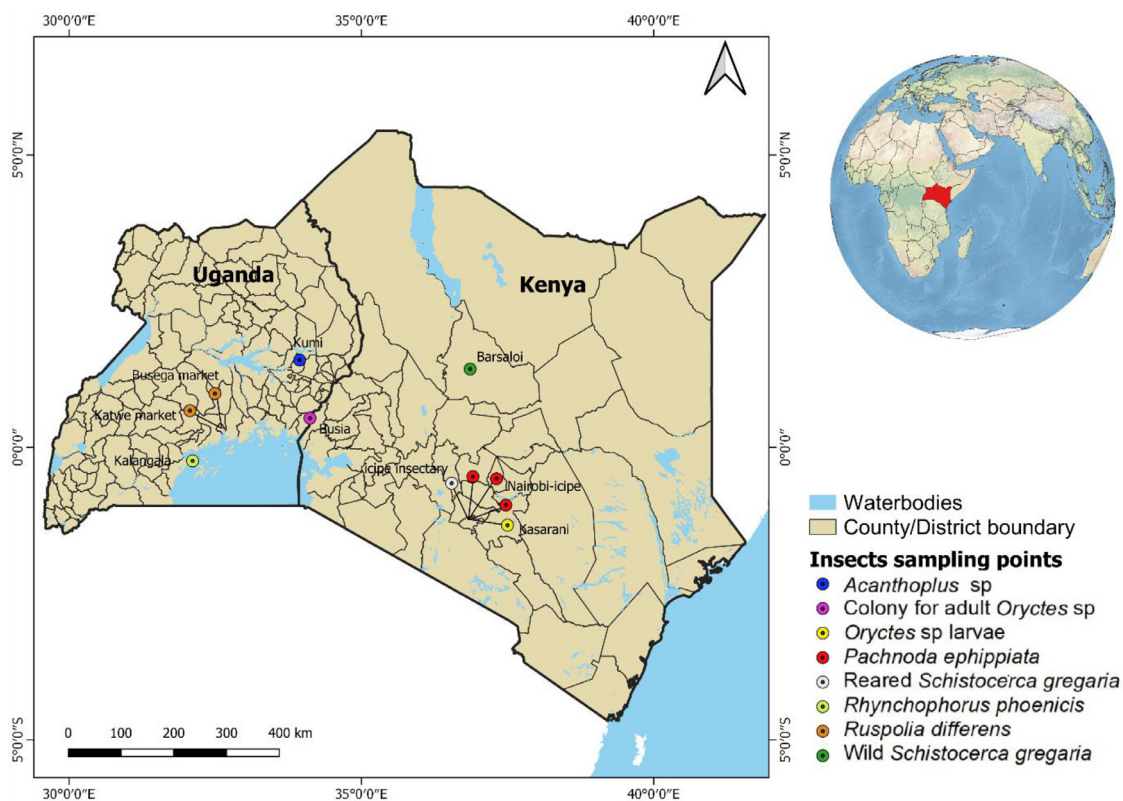
Fig. 1. Insect sampling sites in Uganda and Kenya.

> 95% purity). The analysis included the zero point in blank extracts of the respective matrices. The resultant calibration curves were used to determine the method's limit of quantification (LOQ) (minimum concentration that could be quantified with acceptable accuracy and precision), and limits of detection (LOD). Other MS parameters included, capillary voltage, 3.3 kV; desolvation gas temperature, 250°C; source temperature, 150°C; nitrogen desolvation flow rate, 600 L/h; cone gas flow rate,150 L/h; and collision gas Argon at 3.5×10^{-3} mbar. Extracting solvent and experimental blanks were assessed during the analysis; whereby no quantifiable target compounds were found in the blank samples.

2.4. Statistical analyses

Permutation-based one sample t-tests were carried out to compare pesticide residue values in the insect samples (Tables; 1-3) with MRLs using *MKinfer* package (Kohl, 2020) in R statistical software version 4.1.2 (R Development Core Team, 2021). Mean pesticide residue levels in the samples and the 95% permutation percentile confidence intervals are presented. Other pesticides with only 2 samples were not statistically analysed, but their means and standard errors of the means were computed and presented.

3. Results

3.1. Agrochemicals detected in edible insects

A total of nine pesticides were detected in the edible insect samples out of 374 which were screened. These included two insecticides [Table 1], three herbicides [Table 2] and four fungicides [Table 3]. The fungicides fenpropimorph and metalaxyl were the most detected pesticides, i.e., detected in 50 % and 33.3 % respectively of insect batches analyzed. Atraton (herbicide) and carboxin (fungicide) were the least detected pesticides (each detected in 8.3 % of the samples analyzed).

3.2. Pesticides residue levels in the edible insects relative to maximum residue limits

No pesticide residues were detected in adult *R. differens* (both raw and fried) and adult *Oryctes* sp (reared on cattle dung) (Tables 1-3). From the insecticides recorded, the mean concentration of aminocarb in wild collected *S. gregaria* and that of pymetrozine in *R. phoenicis* from oil palm were significantly lower than MRLs; whereas the residue levels of aminocarb in *R. phoenicis* from raffia palm were not significantly different from MRL.

The mean residue concentration of the herbicide atraton in *P. ephippiata* from BSF frass was statistically higher than the MRL. The residue level of the herbicide methabenzthiazuron in *R. phoenicis* from oil palm was significantly higher than MRL.

For fungicides, the mean concentration of carboxin residues in *P. ephippiata* collected from cattle dung was significantly higher than MRL. Similarly, the residue levels of fenpropimorph in wild *S. gregaria* and *P. ephippiata* from BSF frass were significantly higher than MRL, but the concentration of this fungicide in *Oryctes* sp larvae was not statistically different from MRL. Residue concentrations of fludioxonil in reared *S. gregaria* and *R. phoenicis* from raffia palm were significantly higher than their corresponding MRLs, while the concentration of this fungicide in *P. ephippiata* from BSF frass was not statistically different from the MRL. Metalaxyl residue levels detected in *Oryctes* sp larvae and *P. ephippiata* from cattle dung were comparable to MRLs, whereas the residue level of this fungicide in *P. ephippiata* from BSF frass was significantly higher than MRL.

For residue levels which were not subjected to statistical tests because of having only two replicates, pymetrozine in *P. ephippiata* from plant compost was numerically lower than MRL; while levels of methabenzthiazuron, fenpropimorph and metalaxyl in *Acanthoplus* sp, and methabenzthiazuron in reared *S. gregaria* were numerically comparable to MRLs. On the other hand, the level of fenpropimorph in *P. ephippiata* from cattle dung was numerically 13-fold higher than MRL;

Table 1

Insecticide residues (mg/kg) detected in six species of edible insects sampled from different habitats in Kenya and Uganda.

Country of origin	Sample description	Aminocarb				Pymetrozine			
		Mean [CI]	t	df	P	Mean [CI]/ ± SE	t	df	P
Uganda	Raw *R. differens*	-	-	-	-	-	-	-	-
	Fried *R. differens*	-			-	-	-	-	-
	Acanthoplus sp	-				-	-	-	-
	R. phoenicis from raffia palm	0.03[a] [0.02, 0.03]	-3.7	2	0.25	-	-	-	-
	R. phoenicis from oil palm	-	-	-	-	0.02[b] [0.01, 0.03]	-17.5	2	< 0.001
Kenya	Reared *S. gregaria*	-	-	-	-	-	-	-	-
	Wild *S. gregaria*	0.01[b] [0.01, 0.01]	-97.4	2	< 0.001	-	-	-	-
	Adult *Oryctes* sp	-	-	-	-	-	-	-	-
	Oryctes sp larva	-	-	-	-	-	-	-	-
	P. ephippiata from BSF frass	-	-	-	-	-	-	-	-
	P. ephippiata from cattle dung	-	-	-	-	-	-	-	-
	P. ephippiata from plant compost	-	-	-	-	0.06±0.01	-	-	-
Maximum residue limit (mg/kg)		0.05[a]				0.1[a]			

Key: (-) = Insecticide not detected; [x, y] = confidence interval; lower case super script letters within a column that are different from those on the MRL value indicate a significant difference between the residue level and the MRL. The MRLs of pesticides used were in accordance to FAO/WHO, (2016) and Kolakowski et al., (2021).

Table 2

Herbicide residues (mg/kg) detected in six species of edible insects sampled from different habitats in Kenya and Uganda.

Country of origin	Sample description	Atraton				Methabenzthiazuron				Metazachlor			
		Mean [CI]	t	df	P	Mean [CI]/ ± SE	t	df	P	Mean ± SE	t	df	P
Uganda	Raw *R. differens*	-	-	-	-	-	-	-	-	-	-	-	-
	Fried *R. differens*	-	-	-	-	-	-	-	-	-	-	-	-
	Acanthoplus sp	-	-	-	-	0.01 ± 0.00	-	-	-	-	-	-	-
	R. phoenicis from raffia palm	-	-	-	-	-	-	-	-	-	-	-	-
	R. phoenicis from oil palm	-	-	-	-	0.08[b] [0.05, 0.10]	3.5	2	< 0.001	1.4 ± 0.03	-	-	-
Kenya	Reared *S. gregaria*	-	-	-	-	0.01 ± 0.01	-	-	-	-	-	-	-
	Wild *S. gregaria*	-	-	-	-	-	-	-	-	-	-	-	-
	Adult *Oryctes* sp	-	-	-	-	-	-	-	-	-	-	-	-
	Oryctes sp larva	-	-	-	-	-	-	-	-	-	-	-	-
	P. ephippiata from BSF frass	0.2[b] [0.18, 0.22]	5.8	2	< 0.001	-	-	-	-	-	-	-	-
	P. ephippiata from cattle dung	-	-	-	-	-	-	-	-	-	-	-	-
	P. ephippiata from plant compost	-	-	-	-	-	-	-	-	0.49 ± 0.04	-	-	-
Maximum residue limit (mg/kg)		0.1[a]				0.01[a]				0.01			

Key: (-) = Herbicide not detected; [x, y] = confidence interval; lower case super script letters within a column that are different from those on the MRL value indicate a significant difference between the residue level and the MRL. The MRLs of pesticides used were in accordance to FAO/WHO, (2016) and Kolakowski et al., (2021).

whereas that of fenpropimorph in *P. ephippiata* from plant compost was 14.5-fold higher than MRL.

4. Discussion

Agrochemicals are among the priority pollutants monitored in a wide variety of matrices due to their amalgamation into foods, waters, air, and soil, which may signify potential health hazards to humans (Chaunjiang et al., 2010). In our study, we detected nine out of 374 agrochemicals screened in wild collected edible insects including two insecticides (aminocarb and pymetrozine), three herbicides (atraton, methabenzthiazuron and metazachlor) and four fungicides (carboxin, fenpropimorph, fludioxonil and metalaxyl). These pesticides exist in several formulations with unique physicochemical properties and are commonly used in both Kenya and Uganda to boost agricultural productivity (Bon et al., 2014; Houbraken et al., 2018; MAAIF., 2021; Mulu et al., 2018; PCPB., 2010). Insecticides are generally the most toxic pesticides to the environment, followed by fungicides and herbicides (Yadav, 2010). The levels of most pesticide residues observed in the insects were lower than their corresponding MRLs, suggesting that the pesticides may have not directly been applied to the insects, but rather

they could have originated from indirect contact with pesticide contaminated materials along the food chains, breeding habitats or handling process. Further research is necessary to confirm the pathway through which the insects could have picked up the pesticide residues in the environment.

Atraton, metazachlor and methabenzthiazuron are broad spectrum herbicides used to control broad-leafed weeds and grasses in cereals and onions (Fenoll et al., 2014; Hussain et al., 2008; Singh et al., 2017). Fenpropimorph, carboxin and metalaxyl are systemic fungicides, whereas fludioxonil is a contact fungicide (Bon et al., 2014; Houbraken et al., 2018). The insecticides (pymetrozine and aminocarb) are effective at 52 - 104 μgmL^{-1} in controlling piercing and sucking insect pests (Boina et al., 2011; MAAIF., 2021; PCPB., 2010). These pesticides are however not readily biodegradable (Jablonowski et al., 2012; Marucchini and Zadra, 2002; USEPA., 2000) and their persistence in the environment predisposes wild organisms like edible insects to bioaccumulation of pesticide residues (Houbraken et al., 2018; Singh et al., 2017).

Our results show that adult *R. differens*, both raw and fried, was the only species analyzed where no detectable level of any pesticide was recorded. This may be attributed to the preference of this insect

Table 3
Fungicide residues (mg/kg) detected in six species of edible insects sampled from different habitats in Kenya and Uganda.

Country of origin	Sample description	Carboxin Mean [CI]	t	df	P	Fenpropimorph Mean [CII]/±SE	t	df	P	Fludioxonil Mean [CI]	t	df	P	Metalaxyl Mean [CII]/±SE	t	df	P
Uganda	Raw R. differens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fried R. differens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acantholplus sp	-	-	-	-	0.04 ± 0.03	-	-	-	-	-	-	-	0.01±0.01	-	-	-
	R. phoenicis from raffia palm	-	-	-	-	-	-	-	-	0.29[b] [0.28, 0.30]	40.8	2	< 0.001	-	-	-	-
	R. phoenicis from oil palm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kenya	Reared S. gregaria	-	-	-	-	-	-	-	-	0.01[b] [0.01, 0.01]	-6.8	2	< 0.001	-	-	-	-
	Wild S. gregaria	-	-	-	-	4.21[b] [2.49, 6.24]	2.4	2	< 0.001	-	-	-	-	-	-	-	-
	Adult Oryctes sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oryctes sp larva	-	-	-	-	0.08[a] [0.029, 0.13]	0.96	2	3.75	-	-	-	-	0.02[a] [0.02, 0.03]	2.8	2	0.25
	P. ephippiata from BSF frass	-	-	-	-	0.88[b] [0.79, 0.98]	11.1	2	< 0.001	0.03[a] [0.01, 0.05]	0.74	2	0.38	0.02[b] [0.01, 0.02]	3.6	2	< 0.001
	P. ephippiata from cattle dung	0.82[b] [0.72, 0.92]	8.4	2	< 0.001	1.2 ± 0.03	-	-	-	-	-	-	-	0.01[a] [0.00, 0.01]	-1.5	2	0.25
	P. ephippiata from plant compost	-	-	-	-	0.53 ± 0.05	-	-	-	-	-	-	-	-	-	-	-
	Maximum residue limit (mg/kg)	0.1[a]	-	-	-	0.04[a]	-	-	-	0.02[a]	-	-	-	0.01[a]	-	-	-

Key: (-) = Fungicide not detected; [x, y] = confidence interval; lower case super script letters within a column that are different from those on MRL value indicate a significant difference between the residue level and the MRL. The MRLs of pesticides used were in accordance to FAO/WHO, (2016) and Kolakowski et al., (2021).

to feed on inflorescences of wild grasses, and it rarely attacks crops, hence minimizing its chance of direct contact with pesticides which are used in crop protection (Bailey and McCrae, 1978; Leonard et al., 2020; McCrae, 1982; Opoke et al., 2019). This species also swarms only during rainy seasons when vegetation is abundant (Matojo and Njau, 2010), which may minimize the risk of coming into contact with concentrated levels of pesticides in agricultural fields due to the dilution effect of abundant rain water. Nonetheless, investigation of possible occurrence of detoxifying enzymes in *R. differens* as is reported in other grasshoppers like *Oedaleus asiaticus* (Orthoptera: Acrididae) (Wang et al., 2020) is warranted.

Whereas the adult *Oryctes* sp from *icipe* colony were free from any detectable pesticide residues, two fungicides (fenpropimorph and metalaxyl) were detected in its wild conspecific larvae collected from cattle dung. Cattle dung and much of the environment, including water bodies are reportedly contaminated with pesticide residues (FAO/WHO, 2018; Dubus et al., 2000; Peterson et al., 2020; Sarkar et al., 2021). *Oryctes* spp larvae have relatively higher feeding rates than their adult conspecifics (Soltani et al., 2008) and therefore, they may more likely accumulate pesticide residues than adults. It is also known that *Oryctes* spp larvae are more susceptible to biopesticides than their adult conspecifics (Sreelatha et al., 2011), probably due to differences in their capacities to detoxify chemicals, but this needs further confirmatory studies.

Although the pesticide residue levels in most samples of the insects were within maximum residue limits, some of them notably *P. ephippiata* from BSF frass, *R. phoenicis* from oil palm and *P. ephippiata* from plant compost contained numerically and statistically 2-, 8- and 49-fold higher levels of atraton, methabenzthiazuron and metazachlor, respectively, than MRLs. The statistically higher mean concentrations of these pesticides than their corresponding MRLs could be attributed to their high prevalence in the substrates on which the insects feed, coupled with environmental factors, but these need empirical studies for confirmation. The pesticide residues detected within their corresponding MRLs in edible insects suggest either a reduced usage of these pesticides or their high rate of detoxification by environmental factors. Also, insects possess innate capacities to detoxify pesticides through biotransformation and/or conjugation with different compounds (van der Oost et al., 2003). Differences in degradation potentials in insects may therefore lead to varying bioaccumulation levels (Bosch et al., 2017; Johnson et al., 1971; Landrum and Poore, 1988). Despite some pesticide residues being detected below or within the MRLs, their presence in edible insects is a safety concern which warrants action to mitigate further possible contaminations of wild collected edible insects to ensure consumer safety.

5. Conclusion and recommendations

Most of the edible insects analysed were contaminated with insecticides, fungicides and herbicides residues, although only a few of them had toxic levels of the pesticides. The level of insect contamination with pesticides depended on the insect species, life stage and sampling substrate. It remains unclear how the pesticide residues found their way into the insects. Development of insect farming methods under regulated indoor conditions is highly recommended to ensure their safety as sources of food and feed.

Declaration of Competing Interest

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

CRediT authorship contribution statement

Simon Labu: Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **Sevgan Subramanian:** Methodology,

Supervision, Writing – review & editing. **Xavier Cheseto:** Formal analysis, Writing – review & editing. **Perpetra Akite:** Methodology, Supervision, Writing – review & editing. **Patrice Kasangaki:** Methodology, Supervision, Writing – review & editing. **Moses Chemurot:** Methodology, Supervision, Writing – review & editing. **Chrysantus M. Tanga:** Funding acquisition, Writing – review & editing. **Daisy Salifu:** Data curation, Writing – review & editing. **James P. Egonyu:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Data availability

Data supplied as supplementary material.

Supplementary materials

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References

Agea, J., Biryomumaisho, D., Buyinza, M., Nabanoga, G., 2008. Commercialization of Ruspolia nitidula (nsenene grasshoppers) in Central Uganda. Afr. J. Food Agr. Nutr. Dev. 8 (3), 319–332. doi:10.4314/ajfand.v8i3.19195.

Akpan, V.E., Olukanni, D.O., 2020. Hazardous waste management: An African overview. Recycl 5 (15), 1–24. doi:10.3390/recycling5030015.

Bailey, W.J., McCrae, A.W.R., 1978. The general biology and phenology of swarming in the East African tettigoniid *Ruspolia differens* (Serville) (Orthoptera). J. Nat. Hist. 12 (3), 259–288.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C.C., Paoletti, M.G., Ricci, A., 2013. Edible insects in a food safety and nutritional perspective: A critical review. Compr. Rev. Food Sci. Food Saf. 12, 296–313. doi:10.1111/1541-4337.12014.

Bempah, C.K., Buah-kwofie, A., Denutsui, D., Asomaning, J., Tutu, A.O., 2011. Monitoring of pesticide residues in fruits and vegetables and related health risk assessment in Kumasi metropolis, Ghana. Environ. Earth Sci 3 (6), 761–771.

Boina, D., Youn, Y., Folimonova, S., Stelinski, L., 2011. Effects of pymetrozine, an antifeedant of Hemiptera, on Asian citrus psyllid, Diaphorina citri, feeding behavior, survival and transmission of Candidatus Liberibacter asiaticus. Pest Manag. Sci. 67, 146–155. doi:10.1002/ps.2042.

Bon, H., Huat, J., Parrot, L., Sinzogan, A., Martin, T., Malézieux, E., Vayssières, J.F., 2014. Pesticide risks from fruit and vegetable pest management by small farmers in sub-Saharan Africa. A review. Agron. Sustain. Dev. 34 (4), 723–736. doi:10.1007/s13593-014-0216-7.

Boobis, A.R., Ossendorp, B.C., Banasiak, U., Hamey, P.Y., Sebestyen, I., Moretto, A., 2008. Cumulative risk assessment of pesticide residues in food. Toxicol. Lett. 180, 137–150. doi:10.1016/j.toxlet.2008.06.004.

Bosch, G., van der Fels-Klerx, H.J., De Rijk, T.C., Oonincx, D.G., 2017. Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (Hermetia illucens) and yellow mealworms (Tenebrio molitor). J. Toxins 9. doi:10.3390/toxins9060185.

Chaunjiang, T., Dahui, L., Xinzhong, Z., Shanshan, C., Lijuan, F., Xiuying, P., 2010. Residue Analysis of Acephate and its metabolic methamidophos in open field and green house pachoi (*Brassica campestris* L.) by gas chromatography tandem mass spectrometry. Environ. Monit. Assess. 165, 685–692.

Cheseto, X., Baleba, S., Tanga, C.M., Kelemu, S., Torto, B., 2020. Chemistry and sensory characterization of a bakery product prepared with oils from African edible insects. Foods 9 (6), 800. doi:10.3390/foods9060800.

Chiou, J., Ho, A., Leung, H., Lee, H.W., Wong, W., 2015. Food safety special issue: Rapid testing methods for food contaminants and toxicants. J. Integr. Agric. 3119 (15), 1–42. doi:10.1016/S2095-3119(15)61119-4.

Dubus, I.G, Hollis, J.M., Brown, C.D., 2000. Pesticide in rainfall in Europe. Environ. Pollut. 110, 331–344.

Egonyu, J.P., Subramanian, S., Tanga, C.M., Dubois, T., Ekesi, S., Kelemu, S., 2021. Global overview of locusts as food, feed and other uses. Glob. Food Sec. 31, 1–8. doi:10.1016/j.gfs.2021.100574.

European Commission (2015). Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed. SANTE/11945/2015.

FAO/WHO. (2016). Joint FAO/WHO food standards programme. Codex Alimentarius Commission. https://www.fao.org/3/x8726e/x8726e.pdf.

FAO/WHO. (2018). Global situation of pesticide management in agriculture and public health: Report of 2018 WHO-FAO survey. https://apps.who.int/iris/handle/10665/329971.

Fayiga, A.O., Ipinmoroti, M.O., Chirenje, T., 2018. Environmental pollution in Africa. Environ. Dev. Sustain. 20 (1), 41–73. doi:10.1007/s10668-016-9894-4.

Fenoll, J., Flores, P., Hellin, P., Hernandez, J., Navorro, S., 2014. Minimization of methabenzthiazuron residues in leaching water using amended soils and photocatalytic treatment with TiO_2 and ZnO. J. Environ. Sci. 26, 757–764. doi:10.1016/S1001-0742(13)60511-2.

Houbraken, M., Senaeve, D., Dávila, E., Habimana, V., De Cauwer, B., Spanoghe, P., 2018. Formulation approaches to reduce post-application pesticide volatilisation from glass surfaces. Sci. Total Environ. 633, 728–737. doi:10.1016/j.scitotenv.2018.03.186.

Hussain, Z., Marwat, K.B., Ishfaq, S., Shah, A., Arifullah, S.A., Khan, N.M., 2008. Evaluation of different herbicides for weed control in onion. Sarhad J. Agric. 24 (3), 453–456.

Jablonowski, N.D., Linden, A., Köppchen, S., Thiele, B., Hofmann, D., Mittelstaedt, et al., 2012. Long-term persistence of various C-labeled pesticides in soils. Environ. Pollut 168, 29–36. doi:10.1016/j.envpol.2012.04.022.

Johnson, B., Saunders, C., Saunders, H., Campbell, R., 1971. Biological Magnification and Degradation of DDT and Aldrin by Freshwater Invertebrates. Fish. Res. 28, 705–709.

Kelemu, S., Niassy, S., Torto, B., Fiabo, K., Affogon, H., Tonnang, H., Maniania, N.K., Ekesi, S., 2015. African edible insects for food and feed: inventory, diversity, commonalities and contribution to food security. J. Insects Food Feed. 1 (2), 103–119. doi:10.3920/JIFF2014.0016.

Kohl, M., 2020. MKinfer: Inferential Statistics. R package version 0.6. Matthias Kohl.

Kolakowski, B.M., Johaniuk, K., Zhang, H., Yomamoto, E., 2021. Analysis of microbiological and chemical hazards in edible insects available to Canadian consumers. J. Food Prot. 1–30. doi:10.4315/JFP-21-099.

Kusia, E.S., Borgemeister, C., Tanga, C.M., Ekesi, S., Subramanian, S., 2021. Exploring community knowledge, perception and practices of entomophagy in Kenya. Int. J. Trop. Insect Sci. 1–10. doi:10.1007/s42690-021-00469-9.

Landrum, P.F., Poore, R., 1988. Toxicokinetics of selected xenobiotics in Hexagenia limbata. J. Great Lakes Res. 14 (4), 427–437. doi:10.1016/S0380-1330(88)71576-2.

Leonard, A., Khamis, F.M., Egonyu, J.P., Kyamanywa, S., Ekesi, S., Tanga, C.M., Copeland, R.S., 2020. Ecology and behavior identification of edible short and long-horned grasshoppers and their host plants in East Africa. J. Econ. Entomol. 113 (5), 2150–2162. doi:10.1093/jee/toaa166.

Ministry of Agriculture Animal Industry and Fisheries (MAAIF), (2021). Register of agricultural chemicals registered under section 4 of the Agricultural chemicals (Control) Act, 2006 as at 06th october, 2021

Magara, H.J., Niassy, S., Ayieko, M.A., Mukundamago, M., Egonyu, et al., 2021. Edible crickets (Orthoptera) around the world: Distribution, nutritional value, and other benefits—a review. Front. Nutr. 7, 257.

Maitera, N.o., Hitler, L., Bata, S.Y., Adeleyet, A., Akakuru, U.O., Magu, O.T., 2018. Comparative analysis of the level of pesticide residues in beef, chevon and internal organs of cows and goats slaughtered in Yola abattoir of Adamawa state, Nigeria. Curr. World Environ 13 (3), 416–423.

Maiyo, N.C., Khamis, F.M., Okoth, M.W., Abong, G.O., Subramanian, S., Egonyu, et al., 2022. Nutritional quality of four novel porridge products blended with edible cricket (Scapsipedus icipe) meal for food. Foods 11 (7), 1047. doi:10.3390/foods11071047.

Marucchini, C., Zadra, C., 2002. Stereoselective degradation of metalaxyl and metalaxyl-M in soil and sunflower plants. Chirality 14, 32–38. doi:10.1002/chir.10032.

Matojo, N.D., Njau, M.A., 2010. Plasticity and biosystematics of swarming of the conehead Ruspolia differens serville (Orthoptera: Conocephalidae). Int. J. Integr. Biol. 9 (2), 97–103.

McCrae, A.W.R., 1982. Characteristics of swarming in the African edible bush-cricket *Ruspolia differens* (Serville) (Orthoptera, Tettigonioidea). J. East Afr. Nat. Hist. Soc. Natl. Mus. (178) 1–5. 1982 https://www.biodiversitylibrary.org/content/part/EANHS/178_1982_McCrae.pdf.

Mugova, A.K., Zvidzai, C.J., Musundire, R., 2018. A survey on entomophagy practice of the armoured cricket (Acanthoplus discoidalis) in Mashonaland central, Zimbabwe. Indian J. Pure Appl. Biosci 6 (5), 718–724.

Mukiibi, B.S., Nyanzi, A.S., Kwetegyeka, J., Olisah, C., Taiwo, M.A., Mubiru, E., Tebandeke, E., Matovu, H., Ssebugere, P., 2021. Organochlorine pesticide residues in Uganda's honey as a bioindicator of environmental contamination and reproductive health implications to consumers. Ecotoxicol. Environ. Saf. 214, 112094. doi:10.1016/j.ecoenv.2021.112094.

Mulu, K., Lamoree, M., Weiss, J.M., de Boer, J., 2018. Import, disposal and health impacts of pesticides in the East Africa Rift (EAR) zone: A review on management and policy analysis. Crop Prot 112, 322–331. doi:10.1016/j.cropro.2018.06.014.

Murefu, T.R., Macheka, L., Musundire, R., Manditsera, F.A., 2019. Safety of wild harvested and reared edible insects: A review. Food Control 101, 209–224. doi:10.1016/j.foodcont.2019.03.003.

Njagi, P.G.N., Torto, B., 2002. Evidence for a compound in comstock-kellog glads modulating premating behavior in male desert locust, *Schistocerca gregaria*. J. Chem. Ecol. 28 (5), 1065–1074.

Ntirushize, B., Wasswa, J., Ntambi, E., Adaku, C., 2019. Analysis for organochlorine pesticide residues in honey from Kabale district, south-western Uganda. Am. J. Analyt. Chem. 10, 476–487. doi:10.4236/ajac.2019.1010034.

Nuran, Y., Yakup, S.V., 2019. Effects of processing and storage on pesticide residues in foods. Crit. Rev. Food Sci. Nutr. 1–20. doi:10.1080/10408398.2019.1702501.

Opoke, R., Nyeko, P., Malinga, G.M., Rutaro, K., Roininen, H., Valtonen, A., 2019. Host plants of the non-swarming edible bush cricket Ruspolia differens. Ecol. Evol. 9 (7), 3899–3908. doi:10.1002/ece3.5016.

Pest Control Products Board, (PCPB), 2010. Pest control products for use in Kenya, Sixth edition Pest Control Products Board Waiyaki way, Westlands, Nairobi-Kenya.

Peterson, E.M., Green, F.B., Smith, P.N., 2020. Pesticides used on beef cattle feed yards are aerially transported into the environment via particulate matter. Environ. Sci. Technol. 1–36. doi:10.1021/acs.est.0c03603.

Poma, G., Cuykx, M., Amato, E., Calaprice, C., Focant, J.F., Covaci, A., 2017. Evaluation of harzardous chemicals in edible insects and in insect-based food intended for human consumption. Food Chem. Toxicol. 100, 70–79. doi:10.1016/j.fct.2016.12.006.

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL.

Sarkar, S., Bernardes, D., Keeley, J., Mohring, N., & Jansen, K. (2021). The use of pesticides in developing countries and their impact on health and the right to food (U. Jochheim & G. Defossez (eds.); No. 978-92-846-7674–3). European union. https://doi.org/10.2861/953921

Singh, S., Kumar, V., Chauhan, A., Datta, S., Wani, A.B., Singh, N., Singh, J., 2017. Toxicity, degradation and analysis of the herbicide atrazine. Environ. Chem. Lett. doi:10.1007/s10311-017-0665-8.

Soltani, R., Hamouda, B.H., Ikbel, C., 2008. Descriptive study of damage caused by the rhinoceros beetle, Oryctes agamemnon, and its influence on date palm oases of Rjim Maatoug, Tunisia. J. Insect Sci 8, 57.

Sreelatha, K.B., Krishna, R., Aswathi, V.S., Nair, V.V., Chikku, G.R., Vipin, V., Mohan, A., 2011. Laboratory evaluation of insecticidal activity of Adathoda vasica (Acanthaceae) and Glyricidia maculata (Leguminosae) on the third instar larvae of Oryctes rhinoceros L. (Coleoptera: Scarabaeidae). J. Biopestic. 4 (2), 144–149.

Ssepuuya, G., Aringo, R.O., Mukisa, I.M., Nakimbugwe, D., 2016. Effect of processing, packaging and storage-temperature based hurdles on the shelf stability of sautéed ready-to-eat Ruspolia nitidula. J. Insects Food Feed. 2 (4), 245–253. doi:10.3920/JIFF2016.0006.

Tanga, C.M, Egonyu, J.P, Beesigamukama, D., Niassy, S., Kimathi, E., Magara, et al., 2021. Insect farming as an emerging and profitable enterprise in East Africa. Curr. Opin. Insect. Sci. 48, 64–71. doi:10.1016/j.cois.2021.09.007.

USEPA. (2000). Pesticide Fact Sheet for Pymetrozine - epa nepis (accessed 11 May 2022) https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100BIBS.TXT.GoogleScholar.

van der Fels-Klerx, H.J., Camenzuli, L., Belluco, S., Meijer, N., Ricci, A., 2018. Food safety issues related to uses of insects for feeds and foods. Comprehens. Rev. Food Sci. Food Saf. 17 (5), 1172–1183. doi:10.1111/1541-4337.12385.

Verner, D., Roos, N., Halloran, A., Surabian, G., Tebaldi, E., Ashwill, et al., 2021. Insect and Hydroponic Farming in Africa: The new circular food economy. In: Series: Agriculture and Food Series. World Bank, United States, p. 350 1766ISBN 9781464817663/#211766.

Wang, Y., Huang, X., Hussain, B., Zhang, Z., 2020. The survival, growth and detoxifying enzyme activities of grasshoppers Oedaleus asiaticus (Orthoptera: Acrididae) exposed to toxic rutin. Appl. Entomol. Zool. 55, 385–393. doi:10.1007/s13355-020-00694-7.

Yadav, S., 2010. Pesticide Applications: Threats to ecosystems. J. Hum Ecol. 32, 37–45.