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SAFETY ASSESSMENT OF *LOCUSTA MIGRATORIA* POWDER ENRICHED PEANUT-BASED READY-TO-USE THERAPEUTIC FOODS (RUTF)

– Research paper –

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Abstract: Migratory locust has been widely reported as a quality protein source. However, there are food safety problems related with the usage of insects as food. In this study, migratory locust powder (MLP) was used as a protein supply alternative to milk powder (MP) in peanut-based RUTFs. Seven formulations were obtained at different substitution levels (0 – 30%) of MP with MLP. Pesticide residues, aflatoxins, triazine and toxic metals were analysed using a GC-MS system and Atomic Absorption Spectrophotometer. Formulation with 30% MLP and without milk have the highest level of organochlorine (3.22 µg/kg), organophosphorus (0.40-4.56µg/kg) amongst others. Aflatoxins of the therapeutic foods increased with increasing levels of MLP. The heavy metals, melamine, and cyanuric acid of the RUTFs were below the standard permissible limits. Migratory locust powder could be used as an alternate protein source in the formulation of peanut based RUTFs without posing health threat.

Keywords: Migratory locust powder, Pesticide residues, Aflatoxins, Triazine, Peanut, Therapeutic foods

INTRODUCTION

Ready-to-Use Therapeutic Foods (RUTFs) are nutrient and energy-dense foods, appropriate for treating toddlers who are severely malnourished with no need of hospitalisation (FAO/WHO, 2017). These RUTFs are required to be tender and easy for little children to consume without the need for preparation. The peanut-based ready-to-use therapeutic food (RUTF) consists of peanut butter, milk powder, icing sugar, vegetable oil, vitamin, and mineral premix (WV/UNICEF, 2014). Peanut butter is a critical ingredient in the formulation of peanut-based ready-to-use RUTF. However, the high nutrient content of its raw material (peanut) makes it susceptible to growth of fungus and contamination with mycotoxin (Mupunga et al., 2017). With sustained human exposure, aflatoxins are thought to be highly carcinogenic and mutagenic. They are produced by *Aspergillus* species, primarily *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Oancea and Stoia, 2008; Tola and Kebede, 2016; Mupunga et al., 2017). There are four major forms of naturally occurring aflatoxins that have been found so far, including aflatoxin B1, B2, G1, and G2, as well as two additional metabolites; Aflatoxin M1 and M2 (Ekeanyanwu et al., 2020). Aflatoxins have been implicated in hepatic carcinoma in humans (Li et al., 2009).

Milk is also very important in the production of RUTF but are sometimes adulterated with melamine to boost the apparent protein content. Melamine with chemical formulae 1,3,5-triazine-2,4,6-triamine is a stable, nitrogenous compound used to produce plastic and resin - polymers common in food packages and plastic wares, also an ingredient of paper and paper board (Ibánez et al., 2009; Battaglia et al., 2010; Le et al., 2013). Melamine in its pure form contains 66.7% nitrogen by weight and this high nitrogen content therefore makes it attractive as nitrogen adulterant in high

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protein foods such as milk (Battaglia et al., 2010). Kidney failure and kidney stones have been reported in China for 54,000 Chinese minors who were fed melamine-contaminated (used to boost the apparent protein content) infant milk-based formula (Lam, 2009). In 2008, it became a matter for serious concern, when out of the 54,000 infants, about 12,900 were hospitalised and four died as a result (Lam, 2009). An increasing global population, satisfying its protein needs, and environmental degradation due to the enormous requirements connected with cattle husbandry make animal products like milk unsustainable. This is in addition to the risk associated with milk consumption. Hence, it is expedient to seek a more renewable and eco-friendlier source of protein such as edible insects. The Food and Agricultural Organization of the United Nations (FAO) advocated a global project to increase the use of edible insects as food to supplement current efforts to alleviate food insecurity (Van Huis et al., 2013).

Edible insects are a good alternative source of excellent proteins and nourishment for both developing and developed countries (Akhtar and Isman, 2018). Nutrients abundant in edible insects include proteins, vitamin and minerals, healthy fatty acids, and fiber particularly for the *Orthopteran* species such as crickets, grasshoppers, and locusts. Edible insects' nutrients can be compared with that of beef (Rumpold and Schluter, 2013). Migratory locusts are mostly seen as pests that destroy plantations (Clarkson et al., 2018) but they are rich in proteins with averages around 50% - 65% (Elagba, 2015, Oonincx et al., 2010). They contain enough quantities of essential amino acids required for human health, making them a viable protein alternative to standard protein sources like milk and beef (Rumpold and Schluter (2013). Oonincx et al. (2010) reported migratory locusts also have less environmental footprints unlike pigs and cattle because they produce lower amounts of ammonia and greenhouse gases. Most edible insects such as migratory locust are collected from wild particularly in developing countries and farmed edible insects are fed majorly or partly on fresh vegetables (Belluco et al., 2018). Numerous chemical hazards may be associated with wild and reared edible insects and products developed from them due to the presence of some chemical pollutants such as organohalogen compounds, residues of pesticide and veterinary drug, heavy metals among others, in the environment or their feeds (Van Huis et al., 2013; van der Fels-Klerx et al., 2018).

Pesticides are extensively applied in agriculture to enhance crop production and quality because they help to control and eradicate weeds, pests, and insects (Wanjeri et al., 2019). However, they degrade slowly and are used extensively or inappropriately by many farmers which leads to residues on agricultural produces, water, and the soil (Wanjeri et al., 2019). Pesticide residues tend to pose danger to non-target organisms and human beings as potential carcinogen, neurotoxin, and immunotoxin (Xie et al., 2013; Olutona and Aderemi, 2019). Purschke et al. (2017) investigated the accumulation of pesticides in black soldier fly larvae when the pollutants were introduced in the feed. The authors discovered that the pesticide did not accumulate in the larvae. A detailed survey of over 390 pesticide residues carried out by Charlton et al. (2015) in farmed insects (such as soldier fly, house fly amongst others) discovered only occasional samples exceeded the detection levels.

Heavy metals consist of a group of inorganic chemicals that accumulate due to natural and manmade causes in the environment (Khan et al., 2015). In a decreasing order, the most toxic metals are mercury (Hg), copper (Cu), cadmium (Cd), zinc (Zn), arsenic (As), chromium (Cr), and lead (Wuana and Okieimen, 2011). The presence of these toxic substances in the environment endangers animals and humans, through consumption of contaminated foods, water, and polluted air. Long term ingestion of heavy metals through unsafe foods may lead to diseases such as kidney damage, nephrotoxicity, autoimmunity, cancer amongst others (Ekong et al., 2006; Balkhair and Ashraf, 2016; Ali and Khan, 2018). Slight accumulation of cadmium and no lead bioaccumulation was reported for *Locusta migratoria* in the field study carried out by Zhang et al. (2009). Edible insects can also be susceptible to contamination by mycotoxin when processing and storage is done at below optimum conditions (Belluco et al., 2018). Aflatoxin B1 levels were found to be low in an edible stink insect traditionally consumed in Southern Africa (Musundire et al., 2016).

The increasing incorporation of edible insects in the diets of human requires a serious consideration of its safety aspects. The assessment of the safety of edible insects means to keep track of dangerous microorganisms, heavy metals, hormones, parasites, pesticide residues, toxins, and veterinary drugs since it has been advocated as alternative protein sources to address malnutrition (van der Fels-Klerx et al., 2018). The food safety aspects of edible insects have been studied widely, and some safety concerns have been raised which include the presence of alkali, allergens, antinutrients, heavy metals, pesticide residues, toxins, and pathogenic microorganisms (Finke, 2007, Klunder et al., 2012, Charlton et al., 2015; Poma et al., 2017;

Kachapulula et al., 2018; De Paepe et al., 2019). Although Poma et al. (2017) studied the presence of high-risk chemicals such as pesticide residues, heavy metals in migratory locust-worm based bugballs however information on the assessment of the potential chemical and microbiological hazards associated with peanut-based ready-to-use therapeutic foods having edible insects such as migratory locust powder as ingredient is still lacking. This study aims to incorporate migratory locust powder at different substitution levels with milk powder in peanut-based ready-to-use therapeutic foods and to determine the levels of pesticide residues, such as aflatoxin (B1, B2, G1, G2, M1 and total aflatoxins), triazine (particularly melamine and cyanuric acid) and heavy metals.

MATERIAL AND METHODS

Source of Materials

The dried adult migratory locust was purchased from a local market in Northern part of Nigeria. Peanut (SAMNUT-22) and vitamin/mineral premix were procured from Institute of Agricultural Research, Samaru, Zaria and Bio-organics Nutrient System Limited, Ibafo, Ogun State, Nigeria, respectively. The icing sugar, vegetable oil (soybean oil), and milk powder were acquired from a local market in Akure, Ondo State, Nigeria. All the reagents used were of analytical quality.

Preparation of raw materials

The modified procedure of Zielinska et al. (2017) described in our earlier study (Akande et al., 2022) was employed in the processing of the *Locusta migratoria.* After the raw dried locusts' rear legs and wings were removed, they were thoroughly washed and drained. The cleaned locusts were transferred into aluminum pots, little water was added, boiled for 30 mins, and drained before drying. The cooked locusts were left to dry for 24 hs at 55 ^{o}C before being ground into fine powder, sieved, and kept in an airtight polyethylene bag. The modified method of Ozcan and Seven (2003) was used to make peanut butter. Stones, sticks and other extraneous materials were removed from the peanuts and washed with potable water. The cleaned peanuts were drained and air-dried at 27 ^{o}C for 12 hs. The air-dried peanuts were roasted by placing them on aluminum foil-lined pans and placing them in a pre-heated oven (IGNIS gas cooker) at 160 ^{o}C for 30 min. Before grinding into butter, the roasted peanuts were allowed to cool and peeled by hand.

Peanut-based RUTFs formulation

MSF/UNICEF (2013) standard procedure was used to prepare the peanut-based therapeutic meals (Table 1). The powdered milk and/or locust powder (0 – 30%), icing sugar, and vitamin/mineral premix were mixed by hand until thoroughly combined. The dry components were then combined with the peanut butter and vegetable oil mixture and blended for 15 min per formulation using a 1200-watt Umthric mixer.

Analyses

Pesticide extraction procedure

The extraction of residual pesticide for GC analysis was done according to Lehotay (2007) procedure. Briefly, 2 g of the therapeutic foods were put into centrifuge tubes and 10 mL distilled water, 15 mL acetonitrile were added followed by vigorous shaking.

Table 1. Peanut-based therapeutic foods ingredients and formulation

Formulations	Ingredients (% weight)					
	Milk powder	Migratory locust powder	Sugar	Peanut butter	Vegetable oil	Mineral/Vitamin premix
F1	30	0	28	25	15	1.6
F2	25	5	28	25	15	1.6
F3	20	10	28	25	15	1.6
F4	15	15	28	25	15	1.6
F5	10	20	28	25	15	1.6
F6	5	25	28	25	15	1.6
F7	0	30	28	25	15	1.6

F: Formulation

To the mixture, 0.5 g $C_6H_8Na_2O_8$, 1g $C_6H_9Na_3O_9$, 4g $MgSO_4$, and 1g NaCl were added. Following the addition, the mixture was mixed and centrifuged, and 7.5 mL of the supernatant was transferred to a 15 mL centrifuge tube containing 0.5 g C18, 0.125 g PSA (primary-secondary amine), and 0.75 g $MgSO_4$. The solution was mixed, centrifuged, and 3 mL of the supernatant was dried under a nitrogen flow. The residue was dissolved in 1.5 mL toluene and filtered into an amber-colored vial ready for GC injection.

GC-MS profiling of pesticide residues

The filtered extract was injected (2 µL injection volume) into Varian 3800/4000 GC-MS system equipped with Agilent MS capillary (30 m x 0.25 mm I.D, 0.25-micron dry film) in a splitless mode. The carrier gas was nitrogen at a flow rate of 1 mL/min. The oven temperature started at 70° C and gradually rose to 150° C at a rate of 25° C/min before reaching 200° C at a rate of 3° C/min. The temperature was kept for 2 min before being increased to 290 ºC at 8 ºC/min for 5 min. The total run time was 70 min. In a filament multiplier delay of 4.5 min, the temperature of the MS transfer line and Electron ionization energy were 300 ºC and 70 eV, respectively. For mass detection, a selective/multiple reaction monitoring mode (a triple quadrupole) was used. The system first targets the ion corresponding to the substance of interest, then fragment that target ion to yield a variety of ions. Instrument control, data collection and spectral integration were conducted using Agilent Mass Hunter Workstation Software and NIST Library (NIST Chemistry Webbook: https://webbook.nist.gov/chemistry) containing GC/MS reference mass spectra was used to identify the compounds.

Heavy metals Determination

The minerals content (Cadmium, Lead, Mercury, and Arsenic) of the peanut-based therapeutic foods were determined following the method of AOAC (2005) method. The samples were oven dried, and ashed in a muffle furnace at 600 °C. The resulting ash was transferred to a 250 mL glass beaker, and 120 mL of concentrated HNO_3 and 10 mL of H_2O_2 were added. The mixture was heated to 90 °C for 1 h, then cooled and filtered with glass wool. The filtrate was transferred to a 250 mL volumetric flask and filled with deionised water to the desired volume. Following moderate mixing, 2 mL was pipetted into a 250 mL flask and diluted to the mark with deionised water. The digested solution was analyzed against previously calibrated mineral element using atomic absorption spectrophotometer (Model 205, Buck Scientific, USA). The equipment was calibrated using standards for each mineral element. The levels of each element were displayed on the machines' monitor and printed at the end of the elemental readings.

Aflatoxins extraction procedure

Aflatoxins in the samples were extracted, purified, and estimated according to the method of AOAC, (2006). In brief, 5 g of each sample was weighed and labeled into 100 mL corkable plastic containers. To each of the sample, 25 mL of methanol-water mixture and 10 mL of n-hexane was added, mixed thoroughly for 1 h and the mixture was decanted and labeled before being placed in centrifuge tubes. The operation was repeated, but this time 0.5 g of sodium chloride was added. The resultant mixture was shaken for 10 min then decanted into centrifuge tubes. The tubes were centrifuged at 3000 rpm for 5 mins. A syringe was used to extract 6 mL of the aqueous methanolic phase (the middle layer) into another set of centrifuge tubes and 6 mL of chloroform was added. The chloroform extract was mixed for 1 min before being centrifuged for 5 min at 3000 rpm. From the separated layers, a 2 mL bottom chloroform layer was drained into test tubes and labeled accordingly. The test tubes were placed in a beaker of water and allowed to dry. The residues were redissolved in 150 mL of benzene: acetonitrile (98:2, v/v) and homogenized before TLC analysis.

TLC analysis of aflatoxins

Homogenized aflatoxins extract was spotted on a commercially pre-coated silica gel TLC glass plate (20 x 20cm, 0.25mm thickness) and the plate placed into the solvent tank containing the mixture of chloroform, acetone, and propanol (85:15:2.5 v/v) and allowed to dry at room temperature. Ultraviolet light (Shimadzu UV-1800, Kyoto, Japan) was used to visually examine the developed thin layer chromatographic plate at 366 nm. To determine the amounts of aflatoxins in the samples, the area of the samples' chromatographic peak was compared to that of a standard calibration curve (through densitometric analysis). The aflatoxin bands were taken and transferred into smaller centrifuge tubes and labelled. Into each of the tubes, 4 mL of methanol was added. The resulting mixture was strongly shaken and centrifuged at 3,000 rpm for 5 min. The concentration of the aflatoxins was estimated by comparing the absorbance of samples at 350 nm with that of the known standard.

Extraction of triazines

The triazine (melamine, cyanuric acid, ammeline and ammelide) contents of the samples were determined following the procedure of Nanita et al. (2011). Briefly, 20 mL of extraction solvent of diethylamine/ water/ acetonitrile (1:4:5 v/v/v) was added to each of the 50 mL centrifuge tubes containing 0.5 g of the homogenized sample. Each tube received 20 mL internal standards of $^{13}C_3$-$^{15}N_3$-MEL and $^{13}C_3$- $^{15}N_3$- CYA (1 mg mL^{-1}). The tubes were capped, vortexed for 30 s, sonicated for 30 min before centrifuging at 15,000 rpm for 10 mins at 4 ºC. The supernatant was filtered through 0.22 mm and kept in amber colored vials for GC-MS.

GC-MS determination of triazines

After filtering, the solution was evaporated to dryness under a low stream of nitrogen. The residue was dissolved in a solution composed of acetonitrile and pyridine (200 mL, 1:1 v/v). The targeted compounds in the solution were then derivatized with 50 mL BSTFA with 1% TMS for 30 min at 70 ºC. The derivative (1 µL injection volume) was injected at 250 ºC into Varian 3800/4000 GC-MS system equipped with Agilent MS capillary (30 m x 0.25 mm I.D, 0.25µ dry film) in a splitless mode. As a carrier gas, high purity helium was employed at a steady flow of 1.0 mL/min. Initially, the column temperature set at 75 ºC for a min, then allowed to rise to 300 degrees Celsius at a rate of 15 ºC each mins for 10 min. The mass spectrometry was performed on a triple quadrupole in electrospray ionization and MRM scan modes. The transfer line was kept at 250 ºC and the multiplier was set at 1200V. NIST Library (NIST Chemistry Webbook: https://webbook.nist.gov/chemistry) containing GC/MS reference mass spectra was used to identify the compounds.

Data analyses

Data obtained in triplicate from the determination of the aflatoxins, heavy metals, pesticide residues and triazine composed in the formulated peanut-based RUTF samples were arranged in Microsoft excel. A one-way analysis of variance was used to determine the differences between means. Duncan's Multiple Range Test (SPSS version 17 Chicago, IL, USA) was used to separate sample means, and $P < 0.05$ was regarded statistically significant. Figures were plotted using GraphPad Prism 5 software.

RESULTS AND DISCUSSION

Pesticide residues of the developed peanut-based RUTFs

Edible insects harvested in the wild are of particular interest when issue of pesticide residues is raised (Imathiu, 2020). This is because they freely move around and feed in an uncontrolled habitat which may be contaminated or not. Pesticides are important to modern day commercial farming to produce healthy crops free of pests, insects amongst others. However illegal usage of these pesticides could lead to excessive accumulation in soil, water, and plants that insects feed on.

From the results of the pesticide residues analyses of the peanut-based RUTFs, the residues detected in the samples can be classified into three groups namely fungicides, herbicides, and insecticides. Insecticides (Carbamates, organochlorine, organophosphorus and pyrethroid) are particular interest to standard regulatory bodies (CODEX) in the development of the peanut-based RUTFs.

Neurological problems have been associated with children with early childhood exposure to organophosphates, organochlorine, and other pesticides (Blair et al. 2015). These pesticides produce their toxicity by the inhibition of acetylcholinesterase (AChE) enzyme, which disrupts the activity of the neurotransmitter acetylcholine within nerve tissues and at neuromuscular junctions, thus leading to the destruction of acetylcholine (Sapbamrer and Hongsibsong, 2014; Blair et al. 2015). This results to development of diseases such as Alzheimer, Parkinson disease, dementia, depression amongst others. Symptoms such as salivation and tremor may result with less exposure to the chemicals (Sapbamrer and Hongsibsong, 2014).

Pyrethroid was not detected in all the formulations however classes of carbamates, organochlorine, and organophosphorus were detected, although, their levels were quite low (Table 2). It was observed that with increase in percentage replacement of migratory locust powder with milk powder, all pesticide residues detected increased except for profenofos and propanedioic acid, 1,3-dithiolan-2-ylidene-, bis(1-methylethyl) ester which decreased. This may be attributed to the milk powder used. According to Jawaid et al. (2016), the presence of pesticide residues in animal meals is the main source of pesticide residues in dairy products.

Much research has been done to assess the presence of pesticides in insects and insect products. Saaed et al. (1993) detected five pesticides in locust which

include aldrin, sumithion, malathion, lindane and β-hexachlorocyclohexane at concentration range of 2.20 to 740 µg/kg. Charlton et al. (2015) examined over 390 pesticides in cricket-based flour but only chlorpyrifos was detected. Kolakowski et al. (2021) detected seven pesticides in a total of forty-three cricket samples (protein bars, powders, flour, and whole insects) evaluated, with glyphosate and its metabolite, aminomethylphosphonic acid, being the most prevalent residues. Some of the pesticides did not coincide with those detected in this study but all the studies reported a low residual level of the pesticides which agrees with the result of this study. The detection limits for all the class of insecticides were below >10 µg/kg CODEX STAN 229-1993 recommended limit for pesticide residues in food.

Table 2. Pesticide residues (µg/kg) of the peanut-based RUTFs

Pesticide Group	Chemical class	Representative	F1	F2	F3	F4	F5	F6	F7
Insecticides	Organo-phosphorus	Acephate	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Naled	BDL	BDL	BDL	BDL	ND	ND	ND
		Azinphos-methyl	1.04^{e}	1.53^{d}	2.26^{c}	2.26^{c}	2.32^{b}	2.33^{b}	2.43^{a}
		Phosphoramidothioic acid	0.40^{f}	1.02^{e}	1.06^{d}	1.28^{c}	1.30^{b}	1.32^{b}	1.65^{a}
		Chlorpyrifos	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Diazinon	1.25^{d}	1.25^{d}	1.25^{d}	1.92^{c}	3.21^{b}	3.21^{b}	3.42^{a}
		Dimethoate	1.83^{e}	2.12^{d}	2.16^{c}	2.16^{c}	2.21^{b}	2.31^{a}	2.31^{a}
		Methidathion	BDL	BDL	BDL	BDL	BDL	BDL	ND
		Malathion	0.97^{e}	0.97^{e}	0.97^{e}	1.01^{d}	1.04^{c}	1.13^{b}	1.43^{a}
		Phosmet	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Profenofos	1.01^{a}	0.99^{b}	0.89^{c}	0.77^{d}	0.73^{e}	0.66^{f}	0.40^{g}
		Triazophos	0.76^{g}	2.11^{f}	2.76^{e}	2.91^{d}	4.14^{c}	4.48^{b}	4.56^{a}
		Triphenyl phosphate	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Monocrotophos	0.01^{g}	0.84^{f}	0.87^{e}	0.90^{d}	1.16^{c}	1.32^{b}	1.53^{a}
		Oxydemeton-methyl	1.83^{d}	2.01^{c}	2.01^{c}	2.01^{c}	2.01^{c}	2.16^{b}	2.32^{a}
		Pirimiphos-ethyl	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Mevinphos	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Organochlorine	Endosulfan	2.08^{g}	2.29^{f}	2.34^{e}	2.53^{d}	2.97^{c}	3.06^{b}	3.22^{a}
	Carbamate	Carbofuran	2.22^{d}	2.72^{c}	4.07^{b}	4.43^{a}	4.43^{a}	4.43^{a}	4.44^{a}
		Propoxur	2.42^{d}	3.04^{c}	3.04^{c}	3.05^{c}	3.10^{b}	3.10^{b}	3.12^{a}
		Fenobucarb	0.63^{g}	0.76^{f}	1.57^{e}	1.93^{d}	2.03^{c}	$2..18^{b}$	$2..57^{a}$
	Neonicotinoid	Acetamiprid	1.10^{g}	1.19^{f}	1.24^{e}	1.26^{d}	1.42^{c}	1.76^{b}	2.03^{a}
	Formamidine	Amitraz	1.18^{e}	1.77^{d}	2.04^{c}	2.04^{c}	2.19^{b}	2.26^{a}	2.27^{a}
	Others	Buprofezin	ND	BDL	BDL	ND	ND	ND	ND
		Piperonyl butoxide	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Propanedioic acid, 1,3-dithiolan-2-ylidene-, bis(1-methylethyl) ester	5.14^{a}	3.19^{b}	2.48^{c}	2.34^{d}	2.23^{e}	2.21^{f}	2.21^{f}
Fungicides	Imidazole	Imazalil	1.31^{g}	1.45^{f}	1.64^{e}	1.83^{d}	2.19^{c}	2.21^{b}	2.38^{a}
	Triazole	Tricyclazole	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Propiconazole	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		Tebuconazole	ND	ND	ND	ND	ND	ND	ND
Herbicide	Triazine	Simazine	0.38^{f}	0.39^{f}	0.87^{e}	1.85^{d}	1.87^{c}	1.92^{b}	2.01^{a}

Values that share the superscript in a row are not significantly ($p > .05$) different. All the formulations contain 28% sugar, 25% Peanut butter, 15% vegetable oil and 1.6% mineral/vitamin mix in addition to milk powder (MP) and/or migratory locust powder (MLP). F1: (30 % MP: 0 % MLP), F2: (25 % MP: 5 % MLP), F3: (20 % MP: 10 % MLP), F4: (15 % MP: 15 % MLP), F5: (10 % MP: 20 % MLP), F6: (5 % MP: 25 % MLP) and F7: (0 % MP: 30 % MLP)

Aflatoxin contents of the peanut-based RUTFs

According to Oancea and Stoia (2008) and Khlangwiset et al. (2011), *Aspergillus flavus* and other *Aspergillus species* produce aflatoxins as secondary metabolites. Aflatoxin exists in four unique forms i.e., B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) and they have known deleterious effects on human health (IARC, 2002). Aflatoxin B1 (AFB1) is the most lethal of all aflatoxins and a well-known natural liver carcinogen. Also, aflatoxins cause protein disruption, affect carbohydrate and lipid metabolism, and can compromise a child's development (Gong et al., 2008; Matumba et al., 2014).

Aflatoxinogenic fungi may be associated with edible insects through the environment they grow, substrate on which they were reared and through poor handling and processing conditions for instance drying insects on the ground or in contaminated environments (Kachapulula et al., 2018). Aflatoxin M1 is the primary hydroxylated aflatoxin metabolite of breastfeeding cows fed AFB1-contaminated feed, and it is also present in the milk of human nursing mothers that eats foods contaminated with AFB_1 (Benkerroum, 2020). Magoha et al. (2016) and Benkerroum (2020) also reported that exposure of children to aflatoxins leads to the development of kwashiorkor and marasmus.

The aflatoxin contents of the peanut-based RUTFs are presented in Figure 1. The results showed that inclusion of migratory locust powder slightly increased the aflatoxin contents of the peanut-based RUTFs. Formulations with 30% migratory locust powder contained significantly ($p > 0.05$) high amount for all the aflatoxins evaluated but did not exceed the European Union Commission (2010) recommended values of 2.0 µg/kg for AFB1 and 5.0 µg/kg for AFM1 for groundnuts and groundnuts products except crude vegetable oils. Aflatoxin M1 was not found in any of the samples. The total aflatoxin contents were also below the Codex Alimentarius Commission and FAO/WHO (2010) recommended level of 15 µg/kg. In the work of Matumba et al. (2014) on samples from a locally (Malawian) processed and imported peanut-based therapeutic foods, reported aflatoxin levels above the EC maximum acceptable level for peanut products. Studies available on aflatoxins level of edible insects were targeted towards evaluating accumulation of aflatoxins in raw edible insects fed with aflatoxin contaminated diets (Purschke et al., 2017; Sanabria et al., 2017; Van Broekhoven et al., 2016). Hence, dearth of information still exists as far as aflatoxin levels of insect-enriched therapeutic foods are concerned.

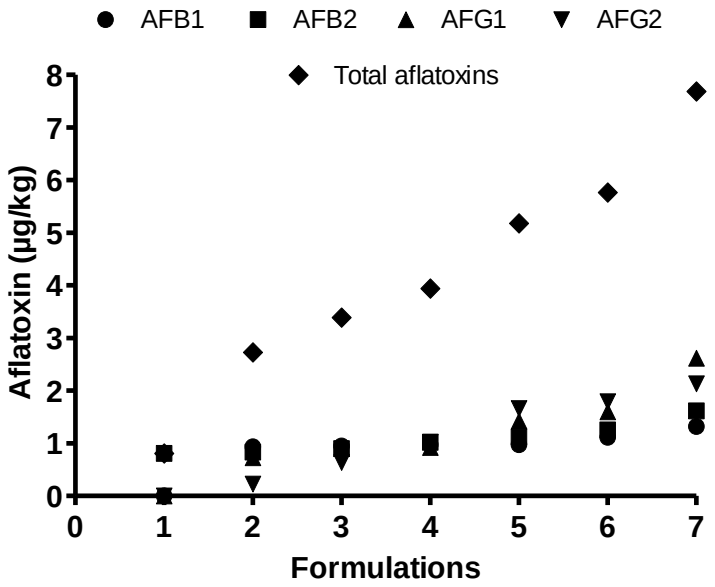

Figure 1. Aflatoxin (µg/kg) contents of the peanut-based ready-to-use therapeutic foods
The formulations are composed of 28% sugar, 25% Peanut butter, 15% vegetable oil and 1.6% mineral/vitamin mix, milk powder (MP) and/or migratory locust powder (MLP). Formulations 1: (30 % MP: 0 % MLP), 2: (25 % MP: 5 % MLP), 3: (20 % MP: 10 % MLP), 4: (15 % MP: 15 % MLP), 5: (10 % MP: 20 % MLP), 6: (5 % MP: 25 % MLP) and 7: (0 % MP: 30 % MLP)

Heavy metal contents of the peanut-based RUTFs

One of the primary issues about entomophagy is the possibility of bioaccumulation of heavy metals. Heavy metals are metallic elements that can cause toxicity even at low levels of exposure, example includes, arsenic, cadmium, chromium, lead, mercury amongst others (Imathiu, 2020). According to Bánfalvi (2011), cadmium, lead, and mercury are examples of heavy metals that are affected by glucose metabolism leading to abnormal decrease of sugar in the blood which manifests as lack of concentration, impulsive, unusual levels of activity, unpredictable ferrocytochrome. Arsenic and lead can create an inflammatory or degenerative nerve lesion characterized by pain, sensory abnormalities, and reduced or missing reflexes. The formulated peanut-based ready-to-use therapeutic foods were analysed for their heavy metals' contents (Figure 2). The heavy metals (lead, mercury, arsenic) of the therapeutic foods were below the MSF/UNICEF 2013 permissible limits of less than 0.01, 0.002 and 0.006 mg/100g respectively for peanut-based RUTFs. This agrees with the study of Poma et al. (2017). The authors reported low levels of lead, arsenic, mercury, and cobalt among others for foods in Belgium enriched with migratory locust, cricket, and mealworm larvae. However, there is a scarcity of data on the presence and amounts of heavy metals in insect-enriched diets, making prospective comparisons with other studies difficult.

The cadmium levels of all the therapeutic foods were however higher than the tolerable limits of less than 0.003 mg/100g. This cannot be attributed to the presence of the edible insect as the control without migratory locust powder also exceeded this limit. The peanut may be the cause of the high cadmium levels. This may be attributed to the cadmium levels in the soil in which the peanuts were grown which could have led to an uptake by the seeds.

Triazine contents of the peanut-based RUTFs

Milk, a high protein source, accounts for 30% of the total composition of peanut-based ready-to-use therapeutic food, making it a very critical ingredient (MSF/UNICEF, 2013). Sometimes, farmers and milk processors add water to milk to dilute it and adulterate with triazine such as melamine to still meet standard level of required protein content for the product (Jalili, 2017). Trace levels of melamine in some products can also be due to their presence in some food packaging materials (such as resins, paper, and plastics amongst others), some pesticides and fertilizers (WHO, 2008; Battaglia et al., 2010). Melamine is not approved as a food additive and its ingestion can lead to kidney or bladder stones, reproductive damage (Tao et al., 2013).

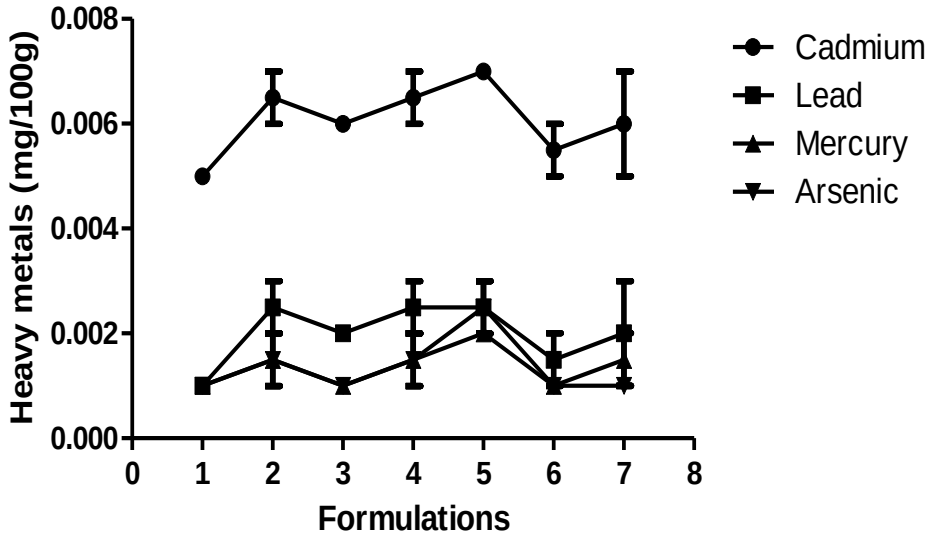

Figure 2. Heavy metals content of the seven peanut-based ready-to-use therapeutic foods
The formulations are composed of 28% sugar, 25% Peanut butter, 15% vegetable oil and 1.6% mineral/vitamin mix, milk powder (MP) and/or migratory locust powder (MLP). F1: (30 % MP: 0 % MLP), F2: (25 % MP: 5 % MLP), F3: (20 % MP: 10 % MLP), F4: (15 % MP: 15 % MLP), F5: (10 % MP: 20 % MLP), F6: (5 % MP: 25 % MLP) and F7: (0 % MP: 30 % MLP)

Cyanuric acid, on the other hand, is a recognized component of feed-grade biuret, a ruminant feed ingredient approved by the US Food and Drug Administration (WHO, 2008). Despite the fact that melamine has a low toxicity, when it mixes with cyanuric acid, it forms intractable melamine cyanurate stones in the kidneys, causing to renal dysfunction (WHO, 2008; Ibanez et al., 2009). The study's findings show that melamine, cyanuric acid and other triazine compounds (ammelide and benzoguanamine) were present in the formulated therapeutic foods with levels ranging from 0.27 – 0.38 mg/kg, 1.44 – 1.72 mg/kg, 0.06 – 0.10 mg/kg and 1.98 – 2.70 mg/kg, respectively (Figure 3A, B, C and D). From the results, it was observed that higher levels of these triazines were detected when milk powder and migratory locust powder are combined in a formulation particularly at 5 % MP and 25% MLP except for benzoguanamine. Melamine was detected in milk-based powdered infant formula at a level of less than 0.20 mg/kg in a study carried out by Lutter et al. (2011). The melamine contents of the developed therapeutic foods obtained by this study were within the European Union (EU) (2012) maximum standard limit of 1 mg/kg for infant formulae. Also, the "*no-observed-adverse-effect-level*" (NOAEL) for cyanuric acid' effects is 150 mg/kg/day (WHO, 2008).

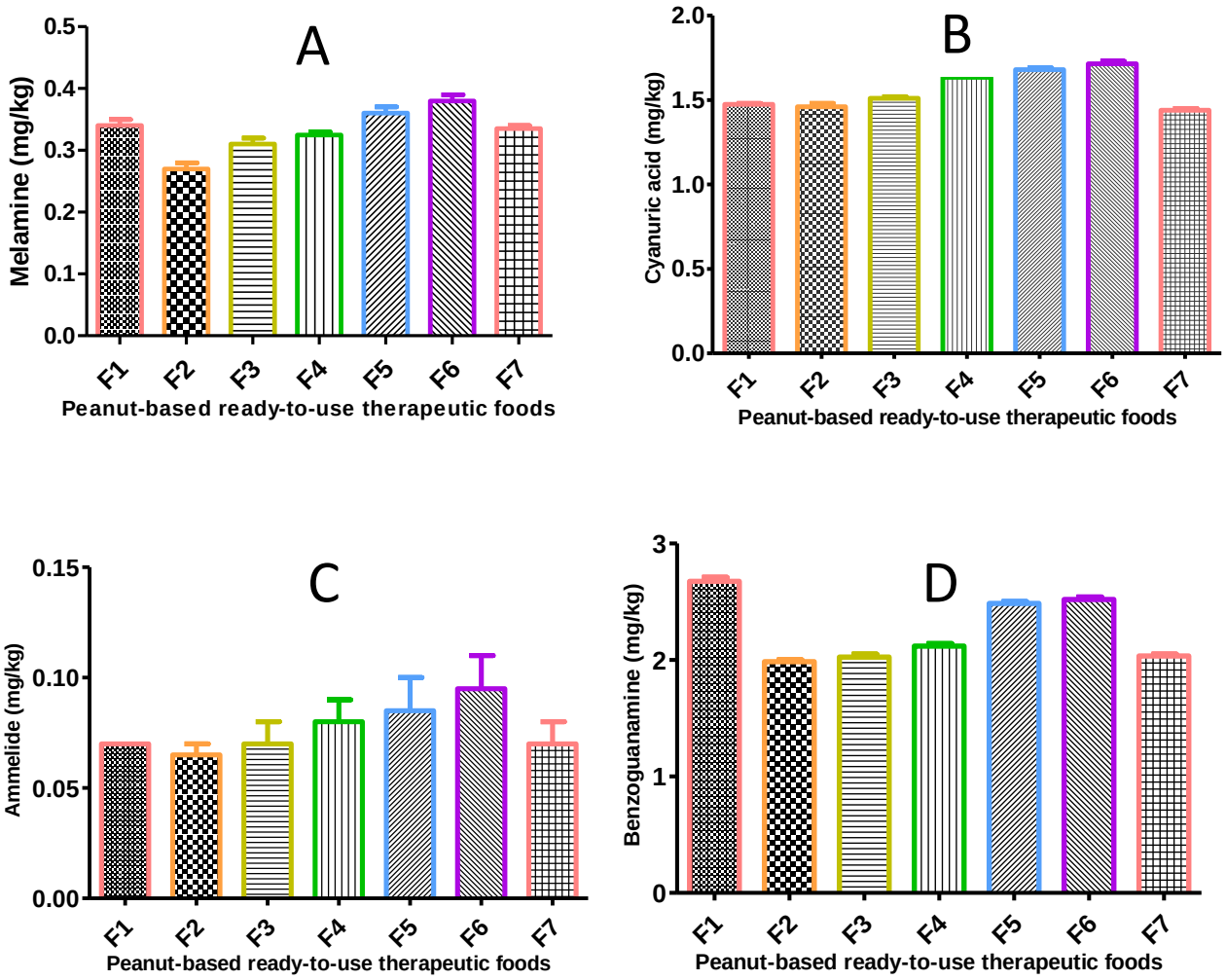

Figure 3. Triazine contents of the peanut-based ready-to-use therapeutic foods
The formulations are composed of 28% sugar, 25% Peanut butter, 15% vegetable oil and 1.6% mineral/vitamin mix, milk powder (MP) and/or migratory locust powder (MLP). F1: (30% PM: 0% MLP), F2: (25% PM: 5% MLP), F3: (20% PM: 10% MLP), F4: (15% PM: 15% MLP), F5: (10% PM: 20% MLP), F6: (5% PM: 25% MLP) and F7: (0% PM: 30% MLP)

CONCLUSION

The study evaluated the effect of substitution of milk powder with migratory locust powder on the amounts of pesticide residues, heavy metals, aflatoxins and triazine contents in the peanut-based ready-to-use therapeutic foods. The results suggest the possibility of the accumulation of heavy metals, pesticide residues and aflatoxins in migratory locust because their inclusion in therapeutic foods affected the levels of these chemicals in the formulated peanut-based ready-to-use therapeutic foods. The results also suggest the possibility of the presence of melamine in milk powder. However, the levels of contamination were relatively low when compared with standard recommended levels for these chemicals in peanut-based therapeutic foods. This indicates edible insects could be used in place of milk powder in the preparation of therapeutic food and may pose no threat when consumed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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