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Evaluation of selected agricultural by-products as potential feeds for rearing edible grasshopper, *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae)

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Abstract

Background Crop residues and agro-industrial by-products constitute an enormous pool of unused and often neglected resources that could be productively utilized for rearing edible insects to meet the global demand for animal-based protein. Utilizing such products as feedstocks can lower the cost of edible insect production and reduce associated environmental pollution.

Methods We evaluated the biology (survival, developmental duration, and adult fresh weight) of *Ruspolia differens* reared on eleven locally available agricultural by-products in Uganda, namely; dry maize cob, waste from a locally brewed finger millet drink, cotton seed cake, soybean hull meal, sunflower cake, pumpkin pulp, peelings of unripe banana, cassava, sweet potato, pineapple and pumpkin. Germinated finger millet was used as a control. Newly hatched (1 day old) nymphs were reared individually on each diet until 14 days post adult emergence. The nutritional profiles of the by-products were also determined. General linear models were fitted to analyse whether *R. differens* performance differed between sexes and among diets. The relationship between *R. differens* performance and the nutritional profiles of the diets were tested by Pearson correlation coefficient.

Results Only three test diets (soybean hull meal, dry maize cob, and the local brew waste) supported survival till the adult stage. Nymphal survival negatively correlated with diets' crude fibre content ($r = -0.96$, $p = 0.042$). Nymphal development duration significantly differed among the diets, with the shortest duration under soybean hull meal (approx. 138 days) and the longest under the local brew waste (205 days). Nymphal development duration positively correlated with diets' crude fibre content ($r = 0.95$, $p = 0.022$). Adult fresh weight at emergence and 14 days post emergence did not differ across the test diets. Female individuals were ~ 1.3 times heavier than males 14 days post emergence, although both sexes had similar weights at adult emergence. The levels of secondary metabolites in the diets were not associated with any growth performance parameters of *R. differens*.

Conclusions Our findings suggest that *R. differens* can thrive and develop on some agricultural by-products. The results could guide the design of *R. differens* mass-rearing programs using locally available agricultural by-products.

Keywords Diet, Growth performance, Mass-rearing, Organic side stream, Proximate composition, Secondary metabolites

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Background

The edible grasshopper, *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae) has gained recognition as one of the most widely consumed and traded edible insect species in East Africa (Okia et al. 2017). It is locally known in Uganda as '*nsenene*' in Luganda. According to Agea et al. (2008) and Odongo et al. (2018), the trade in *R. differens* has grown to be a profitable enterprise in Uganda. It has a great chance of becoming one of the newest, highly nutritious food products (Kinyuru et al. 2015). A Study by Ssepuuya et al. (2019) has shown that *R. differens* has high levels of crude protein (34.2–45.8%) and crude fat (42.2–54.3%) by dry matter. Thus, *R. differens* could be a low-cost, eco-friendly and sustainable solution to the long-term challenges of protein and micronutrient deficiencies in developing countries (Van Huis 2013).

Unfortunately, in Uganda, the highly sought-after *R. differens* is mostly harvested from the wild during the two annual swarming seasons of April–May and November–December (Bailey and McCrae 1978). The harvesting is done at night using electric light traps (Okia et al. 2017; Ssepuuya et al. 2019; Sengendo et al. 2021). According to Halloran et al. (2018), wild harvesting is an unsustainable practice that could lead to ecological imbalances. For example, the demand for *R. differens* currently outstrips the supply coming from the wild harvests (Odongo et al. 2018), suggesting that this important resource is being overused (Okia et al. 2017). There are also food safety concerns regarding wild harvested *R. differens* because of associated harmful microbial entomopathogens originating from trapping sites (Labu et al. 2021; Leonard et al. 2023). There is a need to develop captive-rearing methods to reduce the environmental impact of wild-harvesting while maintaining food safety and steady supply of *R. differens* throughout the year. According to earlier laboratory research, *R. differens* can be raised on a variety of artificial diets, such as lucerne meal, germinated finger millet, chicken egg booster, dried blood and ground dog biscuits (Brits and Thornton 1981; Malinga et al. 2018, 2020; Leonard et al. 2022). However, sustainable on-farm mass-rearing requires cheap and locally available rearing substrates. Therefore, there is an urgent need to explore the potential of exploiting locally available agricultural materials, such as agricultural by-products, in feeding *R. differens*. Recently, Sorjonen et al. (2020) demonstrated the potential of rearing *R. differens* using Finnish plant-based by-products. The study highlighted several Finnish agro-processing by-products that can be used in mass-rearing of *R. differens,* except vegetable by-products with low protein content. A related study by Margaret et al. (2022), reported maize bran and wheat bran as very good diets for rearing *R. differens* when supplemented with protein-rich artificial diets such as soybean meal and lake shrimps. Other edible insects which have been shown to perform well on agricultural by-products include edible crickets, *Acheta domesticus* (Miech et al. 2016; Sorjonen et al. 2019; Morales-Ramos et al. 2020a, b; Kuo and Fisher 2022) and mealworm, *Tenebrio molitor* (Morales-Ramos et al. 2020a, b). Reusing and recycling agricultural by-products as insect feeds could potentially lower the cost of edible insect production and associated environmental pollution.

In Uganda, crop residues and agro-industrial byproducts, including spoiled and leftover food, maize cobs, husks, haulms, peels, leftovers from local breweries, cakes and brans, are increasingly causing environmental concerns and health hazard risks. According to Miito and Banadda (2016), Uganda generates approximately 1.4 million tons of agro-processing wastes and 6.5 tons of farm-level crop residues annually, both of which are burned or disposed of in landfills. These residues and by-products are rich in bioactive compounds, including macronutrients (proteins and carbohydrates), vitamins, fibre, carotenoids, flavonoids, polyphenols and minerals (Barcelos et al. 2020; Coman et al. 2020). However, the potentials of such locally generated residues and agricultural by-products, as feedstocks for rearing *R. differens* has not been explored in the country. This study aimed at assessing locally available agricultural by-products as diets for rearing *R. differens.* We evaluated the biology (nymphal development duration, nymphal survival and adult fresh weight at emergence and adult fresh weight 14 days post emergence) of *R. differens* fed on a range of agricultural by-products. We hypothesised that these selected agricultural by-products when used as diets can influence the growth and development of *R. differens* differently due to variations in the nutritional profile and chemical constituents. Plants produce secondary metabolites as an evolutionary defense mechanism against insect herbivores (Després et al. 2007; Wu and Baldwin 2010). Specifically, we asked; (i) if feeding *R. differens* on different agricultural by-products affects the insect's nymphal survival, development duration, and adult fresh weight at emergence and 14 days post emergence, (ii) Do the effects of agricultural by-products on *R. differens* growth and development parameters vary depending on sex?, and (iii) Whether the nutritional profile and secondary metabolite composition of agricultural by-products are correlated with the growth and development of *R. differens.*

Methods

Experimental colony

For this study, adult *R. differens* (100 males and 100 females) parent stock from wild swarming populations

were sampled at night during the April–May 2021 swarming period from an artificial light trap in Nyendo, Masaka City, Uganda. The insect samples were brought to the laboratory located at the Makerere University Agricultural Research Institute, Kabanyolo, using transparent plastic jars (21 cm height × 14 cm diameter) made from Luuka Plastics, Matuuga, Wakiso, Uganda. Freshly harvested inflorescences of *Panicum maximum* Jacq were inserted into every jar, which was covered on top with a netting cloth to provide aeration for the insects during transportation. The *P. maximum* inflorescences provided a perching matrix and also served as food to the insects. A total of 20 captured individuals were placed in each jar to minimize congestion.

The captured parental stock was raised in the laboratory using ten transparent rectangular plastic containers (25 cm length × 18 cm width × 12 cm height; Thermopak, Nairobi, Kenya), with a fine netting cloth covering the top. A total of 10 insects (five females and five males) were placed in each container. In contrast to the females, which have an ovipositor and a corresponding pair of vestigial metathoracic nodules, males are distinguished by the absence of an ovipositor, presence of a longer pair of antennae and two active tongue-like metathoracic flaps (Brits and Thornton 1981; Matojo and Yarro 2013). Every container was provided with two egg-laying media consisting of damp cotton wool placed in a 100 cm^3 flat-bottomed plastic container. The *R. differens* were reared on freshly germinated finger millet. Water was provided by moistened tissue paper that was positioned at the corners of the clear plastic rearing containers. Eggs laid on the cotton medium were carefully picked and placed onto a hatching medium using a pair of forceps. The hatching medium comprised a plastic container (7.0 cm height and 5.5 cm diameter) packed with clean sand at the bottom, damp cotton wool in the middle and a thin layer of sand on top at a volume ratio of approximately 50: 40: 10, respectively (modified from Malinga et al. 2022). The laid eggs were kept moist by spraying them once every day with clean tap water (Hartley and Warne 1972; Bailey and McCrae 1978). This was done until the eggs hatched into nymphs, which were used for experimental set-up.

Experimental diets

Eleven agricultural by-products, including unripe banana peels, sweet potato peels, dry maize cob fractions, side streams of a local brew made from finger millet, locally known in Uganda as '*maluwa*', pineapple peels, pumpkin peels, pumpkin pulps, cassava peels, cotton seed cake, soybean hull meal and sunflower cake were selected for this experiment. Peels of unripe banana, pumpkin, sweet potato, dry maize cobs, and cassava were sourced locally from Gayaza market, Kasangati town council, Wakiso district, Uganda. These are the most abundant local food residues in Ugandan urban markets (Swidiq et al. 2012; Kisuule et al. 2021). Waste/residue from a locally brewed millet drink was obtained from a local brewer in Namulonge, Wakiso, Uganda. Dry maize cobs, after hulling, were sourced from a farmer in Kabanyolo, Wakiso, Uganda. They constitute part of the farm level crop residues in Uganda (Miito and Banadda 2016). The loose surface layer of the maize cobs was scrubbed with a knife and collected to be used as an insect diet. Soybean hull meal, sunflower cake and cotton seed cake which are common agro-processing by-products in Uganda (Nsubuga et al. 2019), were obtained from the Nile Agro Industries Ltd, Odokomit Ginnery in Lira, Uganda. The peels of unripe banana, cassava, sweet potato, pineapple and pumpkin were washed thoroughly with tap water. All the agricultural by-products were solar-dried to a constant weight and a moisture content of between 4.5 and 5.0%. The resultant dried products were pounded using a fabricated steel mortar and pestle and later ground to a fine powder using an electric blender (Philips HR 2056/01, 1-L capacity, 1.52 kg, 450 W). Freshly germinated finger millet, on which *R. differens* has recently been shown to perform best in terms of nymphal survival, developmental duration and adult fresh weight (Malinga et al. 2022) was used as a control.

Experimental settings

A total of 360 freshly hatched (1-day old) nymphs raised in the laboratory were used to evaluate the performance of *R. differens* on diet treatments. Each nymph was separately put in a rearing container that measured 21 cm height and 19 cm in diameter. The 12 diet treatments—including the control—were then applied to the nymph at random. Each nymph served as a replicate, and the 30 replicates of each diet treatment (approximately 1 g) were randomized in thirty blocks inside the laboratory. The nymphs were raised individually until they emerged as adults. Water was provided by placing a ball of wet tissue paper (approximately 50.0 g) in the rearing container, and food was provided ad libitum. Fresh food and moistened tissue paper were provided every 3 days to replace the dried tissue paper and depleted or staled food, respectively. To allow for ventilation and keep the insect from escaping, a fine netting cloth was placed over the top of each rearing container (Malinga et al. 2018; Ssepuuya et al. 2019). The nymphs were observed every day to document their development from the first instar until adult moult (Malinga et al. 2022). Using an analytical scale (Ohaus CH-8606, Greiensee, Switzerland), each adult was weighed individually within 24 h of their emergence and on 14th day post adult emergence, and their sexes were noted (Lehtovaara et al. 2018; Malinga et al. 2022).

The weight of an adult *R. differens* 14 days after emergence is important because the maximum weight gain starts from approximately 6–10 days postemergence due to lipids accumulation in the insect's fat body (Lehtovaara et al. 2018). However, fresh adult weight immediately decreases after this period due to the high energy expenditure during egg laying for the case of females and singing in males (Stevens and Josephson 1977; Lorenz and Anand 2004). The number of days between hatching and adult emergence was used to calculate the nymphal developmental duration for those individuals that developed to adults (Malinga et al. 2022). The photoperiod during laboratory rearing was 12 h:12 h; L:D while temperature and relative humidity were 27.0 ± 5 °C and 49.0 ± 5%, respectively.

Proximate composition analyses of diets

The proximate analysis was performed at the Nutrition and Biochemistry Analytical Laboratory, National Crops Resources Research Institute, Namulonge, Uganda, in accordance with the Association of Official Analytical Chemists (AOAC 2000). Each sample was analysed in triplicate.

Crude protein content

The Kjeldahl method (AOAC 2000) was used to determine the protein content. Precisely, one gram of the powdered material was weighed into a 250 mL digestion tube. It was then heated with concentrated sulphuric acid inside an aluminum alloy block that had an adjustable temperature device (Tecator Digestion System 20, 1015 Digestor, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, USA). In order to catalyse the reaction, Copper and potassium sulphates were added. Thereafter, 40% sodium hydroxide was added to the mixture to turn it alkaline. This caused the ammonium sulphate to foam and release ammonia which was subsequently collected in a 2% boric acid solution. The released ammonia in solution was then titrated against a standard 0.1 N hydrochloric acid solution using methyl red as an indicator in a 500 mL graduated Erlenmeyer titration flask. To calculate the crude protein content of the sample, the amount of nitrogen was multiplied by a factor of 5.33 to account for non-protein nitrogen (Boulos et al. 2020).

Total carbohydrate content

The Birch Phenol–Sulphuric acid method (1985) was used to estimate the total amount of carbohydrates. Briefly, 5 mL of 2.5 N HCl were added to a test tube containing 200 mg of the powdered sample. The reaction mixture was kept in a water bath at 100 °C for three minutes. Solid Na_2CO_3 was added to the resulting solution until no further effervescence was observed. Thereafter, the volume was adjusted to 50 mL, and the centrifuge was run for 10 min at 8000 rpm. The supernatant was collected, from which an aliquot of 0.5 mL was placed in a test tube and increased to 1 mL with distilled water.

1 mL of phenol solution and 5 mL of 96% Sulphuric acid were added to the resulting solution, respectively. After the final solution was thoroughly mixed, it was kept at 25 °C in a water bath for 20 min. Using a UV–Vis spectrophotometer set at 490 nm, the absorbance of the digest was determined and compared to that of a standard glucose solution. The total carbohydrate content in the sample (%), was calculated as; 100—(% moisture + % crude fat + % crude fiber + % crude protein).

Crude fat

The crude fat was determined by AOAC method 991.36 (AOAC 2000). Briefly, 100 mL of crude fat was extracted from 5 g of powdered sample using a mixture of diethyl ether and petroleum ether for 24 h. The extracted fat was placed in an empty flask with a known weight, dried for 30 min at 80 °C in the oven, and then taken out and placed inside a desiccator to finish drying. Subsequently, the flask and its content were weighed, and the estimated fat content was calculated using the formula;

$$\%\ \text{crude fat} = \frac{(W1 - W2)}{SW} \times 100$$

where W_1 represents the weight of flask and extracted fat, W_2 represents the weight of empty flask and SW is the original weight of the sample.

Ash content

The ash content determined using the AOAC (2000). Briefly, five grams of each powdered sample were weighed and put into a dry crucible with a known weight. The sample was burned in an oven at 550 °C for 12 h. The crucible and its content were allowed to cool at room temperature after being removed from the oven. Once it had cooled, it was put in a desiccator until the weight stabilized. The percentage of ash in the sample was expressed as:

$$\%\ \text{ash}\left(\text{dry weight basis}\right) = \frac{(W1 - W2)}{SW} \times 100$$

where W_1 is the weight of the crucible and raw, dry powdered sample, W_2 is the weight of the crucible and the powdered sample that has been burned, and SW is the weight of the crucible.

Fibre content

The enzymatic gravimetric method was utilized to determine the amount of crude fibre (AOAC 2000). Briefly, 25 mL of 2.5 M sulphuric acid was used to digest 1 g (*sw*)

of the sample that was placed in a 50 mL falcon tube. The water bath used for reaction was kept at 5 °C. The resulting mixture was centrifuged at 600 rpm for 30 min at 4 °C after being cooled for 30 min in a water bath kept at room temperature. After discarding the supernatant, the residue was digested for 30 min in a room-temperature water bath using 2.5 M sodium hydroxide. The digest was centrifuged once more for 30 min at 4 °C at 6000 rpm, and the supernatant was discarded. After rinsing the residue in the falcon tube with 3 mL of ethanol, the sample was transferred into a crucible and dried for 1 h at 150 °C in an oven. Once more, the residue was placed in a desiccator, allowed to cool at room temperature and then weighed (w_1). The sample was later put in a muffle furnace at 55 °C for 4 h, removed and allowed to cool in a desiccator and weighed again (w_2). The AOAC (2003) formula below was used to calculate the percentage of crude fibre content;

$$\text{Crude fibre (\%)} = \frac{(W1 - W2)}{SW} \times 100$$

where w_1 is the weight of the oven-dried sample, w_2 is the weight of the furnace-dried sample, and sw = weight of the sample.

Secondary metabolite contents

Phytic acid

The technique described by Chang et al. (1977) was used to determine the phytic acid. In order to extract phytic acid, 50 mL 3% TCA was added to 1 g of dry powdered sample that had been weighed and placed in a 50 mL falcon tube. The falcon tube was vortexed, kept in a water bath at 60 °C for 30 min while being frequently vortexed, and then left to stand at room temperature for 1 h on a shaker. The mixture was centrifuged for 15 min at 4000 r.p.m to separate the supernatant. A volumetric flask (25 mL) was filled with an aliquot (2 mL) of the extract, which was then diluted to volume using distilled water. The extracted phytic acid was purified using an ion-exchange resin column (BioRad Laboratories, Watford, UK). Two column volumes of distilled water were added after the column was cleaned with 10 mL, 0.7 M sodium chloride. Prior to usage, the recovery capacity of the column was verified by adding 5 mg of sodium phytate (Sigma) to 1 g (dry weight) of the sample. The diluted sample extract was added to the column in an aliquot of 10 mL. It was then rinsed with 15 mL of 0.1 M sodium chloride, and the eluate was collected in a 20 mL volumetric flask with freshly made Wade reagent (5 mL of 0.03% ferric chloride hexahydrate and 0.3% sulphosalicylic acid in distilled water). Elution was continued until the 20 mL mark on the volumetric flask was reached. The absorbance of the eluate was measured in a spectrophotometer set to 500 nm wavelength with distilled water serving as a blank. Wade reagent standard solutions were made with varying concentrations (10–50 µg/mL phytic acid) and pipetted into glass vials in 3 mL portions. After adding one milliliter of Wade reagent, the mixture was vortexed. The absorbance of these solutions was read and plotted against the concentrations of phytic acid. To calculate the amount of phytic acid, the following formula from Latta and Eskin (1980) was used:

$$\text{Phytic acid}\left(\text{mg/g}\right) = \text{C} \times \text{V} \times \text{D}\ (1/\text{W}) \times 0.59$$

where C is the phytic acid concentration in the eluate (mg/mL), V is the eluate volume (mL), D is the dilution factor, W is the sample weight (g), and factor 0.59 is the phytic acid concentration (g/g) in sodium phytate.

Phenol content

The method of Singleton et al. (1999), modified by Lamien-Meda et al. (2008), was used to determine the phenolic content. Briefly, 10 g of powdered sample was added to 10 mL of 70% (v/v) ethanol in a test tube, and the mixture filtered through filter paper (Whatman No. 2). The filtrate was then diluted with 70% ethanol to make 50 mL. The following six standard gallic acid solution concentrations were made: 0.003, 0.0054, 0.0078, 0.0102, 0.0126, and 0.015 percent (w/v) in 70% (v/v) ethanol. The sample filtrate, a 70 percent (v/v) ethanol blank, and small volumes of 0.6 mL of each gallic acid solution were transferred to test tubes measuring 13 × 100 mm. 1.5 mL of 7.5% (w/v) sodium carbonate and 2.0 mL of the Folin-Ciocalteu (FC) reagent were added to each test tube and mixed. After 10 min of incubation in a water bath kept at 60 °C, the test tubes were placed in an ice bath to cool down. The solutions were then put into cuvettes, and a UV–Visible Jenway 6305 spectrophotometer was used to measure the absorbance at 765 nm. The gallic acid equivalent content of the sample was obtained by multiplying each absorbance by 34 mL/g, which is the dilution factor, yielding units of µg GAE/g.

Flavonoid content

The Dowd method, modified by Arvouet-Grand et al. (1994), was used to determine the total flavonoid content. A 0.5 g powdered sample was weighed and 50 mL 80% aqueous methanol was used to extract flavonoids in an ultrasonic bath for 20 min. The extract was centrifuged for 5 min at 14,000 rpm in a 2 mL aliquot. The total flavonoid content was estimated using a colorimetric assay with aluminum chloride. An aliquot (1 mL) of extract or standard quercetin solution (20, 40, 60, 80 and 100 mg/L) was added to a 10 mL volumetric flask containing 4 mL of distilled deionized water. To this solution, 0.3 mL of 5% Na NO_2 was added. After five minutes, 0.3 mL of

10% AlCl3 was added, and after six minutes, 2 mL of 1 M NaOH was added. Double deionized water was added to bring the total volume to 10 mL. The final mixture was thoroughly mixed, and a UV–Vis Jenway 6305 spectrophotometer was used to measure the absorbance at 510 nm against a prepared blank reagent. The total flavonoid content of the sample was expressed in milligrams (mg QE/100 g dw) using the quercetin equivalents (QE).

Tannin content

Total tannin in the sample was estimated using the Folin Ciocalteu reagent in the method developed by Harborne, (1998). To 100 mg of powdered sample in a 2 mL eppendorf tube, 0.5 mL of 5% ascorbic acid solution was added and the mixture shaken on an orbital shaker for 20 min. The pigments were then removed with 0.5 mL of petroleum ether containing 1% acetic acid, which was then allowed to evaporate. After evaporation, 0.3 mL of distilled water was added and the mixture was centrifuged for 10 min at 4 °C at 25,000 rpm. A 2.4 mL of 5% hydrochloric acid-butanol solution was added to the solution in a 50 mL calibrated tube. The resulting solution was subsequently filtered through 240 mm of filter paper. Distilled water was added to 0.5 mL of the filtrate until a total volume of 1 mL was reached. After that, 2.5 mL of 20% sodium carbonate solution and 0.5 mL of the Folin-Ciocalteu reagent were added and the mixture stirred. After 30 min of incubation in a water bath at 80 °C, final mixture was cooled at 24 °C. A UV–Vis spectrophotometer was used to measure absorbance at 550 nm, with tannic acid solution serving as a standard. The total tannin content was expressed in mg of tannic acid equivalents per 100 g (mg TAE/g) weight of dried sample.

Cyanide content

With a few minor adjustments, Haque and Bradbury, (2002) method was used to estimate the hydrogen cyanide content. In a 50 mL falcon tube containing 5.0 g of powdered sample, 10 mL of 1 M phosphate buffer (pH 6.0) was added. After 30 min of shaking the mixture on an orbital shaker, it was centrifuged for 15 min at 4 °C at 6000 rpm. Following the transfer of the supernatant to an empty test tube, 0.1 mL linamarase enzyme and 0.4 mL of 1 M phosphate buffer (pH 7.0) was added. In order to halt the reaction, 0.2 M NaOH was added to the resulting mixture after it had been incubated for 15 min at 30 °C in a water bath. The final mixture was mixed with 2.8 mL of 1 M phosphate buffer (pH 7.0) and incubated for five minutes at room temperature in the dark. Chloramine-T and barbituric acid were added to this mixture, and the absorbance was measured at 578 nm using an UV–Vis spectrophotometer with silver nitrate as a standard solution. Parts per million (ppm) of the sample was used to express the hydrogen cyanide concentration.

Statistical analyses

The mean contents for secondary metabolites and proximate composition were compared using one-way analysis of variance (ANOVA). Results were reported as means ± SE and treatment means were compared using Tukey's HSD test whenever significant differences were found. Two general linear models were also fitted to analyse whether nymphal survival and nymphal development time (dependent variables) of *R. differens* differed between sexes and among diet treatments (fixed factors). Experimental blocks were considered random factors. Model suitability was tested using the Hosmer–Lemeshow test, while the significance of the predictive variable was assessed using type III Wald χ^2 (Hosmer and Lemesbow 1980). The adult fresh weight at emergence and 14 days post emergence (dependent variables) were analysed by fitting two-way analysis of variance (ANOVA) models in R studio (R Core Team 2013), considering diet treatment and sex as fixed factors. Shapiro–Wilk and Levene tests were used to test the two-way ANOVA assumptions of homogeneity of group variances and normality of model residuals, respectively. All the two models were found to be fit for the analysis. A Tukey posthoc test with the 'lsmeans' and 'multcompView' packages were run to establish differences in fresh weight of the *R. differens* among the diet treatments. Graphical visualisation in the form of bar plots and box plots were obtained using 'ggplot2', 'ggthemes', 'multcompView' and 'devtools' packages of the R-Statistics. Pearson's product moment correlation was used to test the relationship between the proximate nutrient and secondary metabolite contents of the diets and survival, development duration, and fresh adult weight at emergence and 14 days post emergence. R statistics, R studio v4.1.3, and IBM SPSS Statistics v.25 (IBM, Armonk, NY, USA) were used for all statistical analyses, with a significance level of $p \leq 0.05$.

Results

Proximate composition and secondary metabolites in experimental diets

The proximate composition varied significantly among the diets used in the study (Table 1). The crude protein content was highest in local brew followed by sunflower cake, cotton seed cake and pineapple peel. These diets had more than thrice the crude protein in germinated finger millet, which was the lowest when compared to all the diets. A similar pattern was observed in the content of carbohydrates. The crude fat content was highest in pumpkin peel, more than four times the amount observed in germinated finger millet and more than

Table 1 Proximate composition of the various agricultural by-product diets tested

Substrate	Protein ± SE (%)	Carbohydrate ± SE (%)	Crude fat ± SE (%)	Crude fibre ± SE (%)	Ash ± SE (%)
Local brew waste	28.05 ± 0.03l	80.83 ± 0.06k	0.57 ± 0.02a	34.66 ± 0.89f	2.93 ± 0.02ab
Sunflower cake	27.33 ± 0.00k	76.1 ± 0.01j	2.54 ± 0.09d	45.92 ± 0.65h	4.36 ± 0.04def
Cotton seed cake	26.89 ± 0.03j	76.03 ± 0.07j	1.46 ± 0.01b	43.76 ± 0.29g	4.89 ± 0.01f
Pineapple peel	25.47 ± 0.01i	73.98 ± 0.03i	1.72 ± 0.01b	11.13 ± 1.03c	3.86 ± 0.08cde
Banana peel	15.88 ± 0.02h	42.04 ± 0.05h	0.55 ± 0.01a	9.33 ± 0.22e	4.67 ± 0.09ef
Pumpkin pulp	14.96 ± 0.00g	41.37 ± 0.01g	4.21 ± 0.39f	13.43 ± 0.53d	8.37 ± 0.05h
Soybean hull meal	11.6 ± 0.00f	28.66 ± 0.00f	1.59 ± 0.03b	29.92 ± 0.30e	39.93 ± 0.85i
Sweetpotato peel	11.08 ± 0.00e	26.98 ± 0.01e	3.83 ± 0.13e	8.04 ± 0.30ab	2.67 ± 0.26a
Pumpkin peel	10.54 ± 0.00d	25.4 ± 0.01d	9.75 ± 0.11g	7.13 ± 0.54a	3.21 ± 0.06abc
Dry maize cob	10.43 ± 0.00c	25.05 ± 0.01c	0.6 ± 0.01a	28.26 ± 0.92e	5.98 ± 0.10g
Cassava peel	8.68 ± 0.00b	20.99 ± 0.01b	2.67 ± 0.07d	13.06 ± 0.08d	4.55 ± 0.01ef
Germinated finger millet	7.08 ± 0.00a	17.63 ± 0.01a	2.17 ± 0.01c	13.65 ± 0.55d	3.69 ± 0.05bcd
df	11	11	11	11	11
F value	330,045.32	580,341.02	400.56	561.68	1575.73
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Means within a given column denoted by identical superscript letters do not exhibit a significant difference (Duncan test, $p \leq 0.05$). *df* stands for degrees of freedom and standard error is represented by SE

twice the levels in pumpkin pulp, sweet potato peel and cassava peel. Banana peel contained the lowest amount of crude fat followed by local brew waste, dry maize cob and cotton seed cake. The crude fibre was highest in sunflower cake and cotton seed cake, and more than thrice the level observed in germinated finger millet. Pumpkin peel had the lowest amount of crude fiber followed by sweet potato peel and banana peel. The highest amount of ash was produced by soybean hull meal and it was over seven times higher than the amounts produced by pumpkin pulp, dry maize cob and cotton seed cake. Germinated finger millet, pumpkin peel, local brew waste and sweet potato peel yielded much lower quantities of ash, over ten times much less than it was produced by soybean hull meal.

The secondary metabolite contents of the diets varied significantly among the diets (Table 2). Sunflower cake had the highest level of phytic acid and phenolics followed by local brew waste, cotton seed cake and pineapple peel. The level of phytic acid and phenolics in sunflower cake was five times higher than that in germinated finger millet. Cassava peel had the lowest level of phytic acid, approximately seven times less as compared to the amount in sunflower cake. Pumpkin pulp had the lowest level of phenolics, over ten times less than the amount in sunflower cake. Tannins and flavonoids were highest in local brew waste followed by sunflower cake, cotton seed cake and pineapple peel. Pumpkin pulp, germinated finger millet, cassava peel and pumpkin peel contained the lowest amounts of tannins and phenolics. The tannins and phenolics contents in these diets were five and four times less than the amounts in local brew waste. Cassava peel had exceptionally high levels of hydrogen cyanide compared to the rest of the diets, approximately thirty times the levels in local brew waste, sunflower cake, cotton seed cake and pineapple peel. Germinated finger millet, pumpkin peel, dry maize cob, sweet potato peel and pumpkin pulp each had over a hundred times less hydrogen cyanide than the cassava peel.

Nymphal survival

Nymphal survival differed significantly among the 11 diets ($F_{11,\ 348} = 4.56$, $p < 0.001$, Fig. 1), but not between sexes ($F_{1,\ 93} = 3.408$, $p = 0.068$). Out of all the diet treatments, the control diet—germinated finger millet—showed the highest survival rate of *R. differens* at 96.7%. This was followed by soybean hull meal (16.7%), dry maize cob (10%) and local brew waste (10%). No *R. differens* nymph survived to adult on cotton seed cake, sunflower cake, pumpkin peel, pumpkin pulp and peels of cassava, sweet potato, unripe banana, pumpkin and pineapple (Fig. 1).

Nymphal development duration

The duration of nymphal development, or the period from hatching to adulthood, varied significantly among the four diets on which the insects survived ($F_{3,\ 33} = 416.7$, $p < 0.001$, Fig. 2) and between the sexes ($F_{1,\ 33} = 25.11$, $p < 0.001$, Fig. 2). Nymphs reared on the control diet (germinated finger millet) developed into adults much faster (mean = 48.3 days) than on any of the test

Table 2 Secondary metabolites in the agricultural by-products used as diets

Substrate	Phytic acid content (mg phytate/g DW) ± SE	Total tannins (mg TA/g DW) ± SE	Total phenolic content (GAE/100 g DW) ± SE	Total flavonoids (mg QE/100 g DW) ± SE	HCN (ppm) ± SE
Sunflower cake	159.26 ± 0.01^{k}	38.40 ± 0.00^{k}	1441.46 ± 0.09^{l}	854.93 ± 0.06^{j}	4.68 ± 0.00^{i}
Local brew waste	155.65 ± 0.11^{j}	40.76 ± 0.03^{l}	1265.04 ± 0.90^{k}	910.03 ± 0.60^{l}	4.85 ± 0.00^{j}
Cotton seed cake	127.38 ± 0.07^{i}	37.58 ± 0.02^{j}	976.22 ± 0.56^{j}	857.19 ± 0.70^{k}	4.26 ± 0.05^{h}
Pineapple peel	116.73 ± 0.06^{h}	35.39 ± 0.01^{i}	847.94 ± 0.42^{i}	825.72 ± 0.27^{i}	3.95 ± 0.00^{g}
Banana peel	67.98 ± 0.07^{g}	19.79 ± 0.02^{h}	514.83 ± 0.35^{h}	479.88 ± 0.54^{h}	2.21 ± 0.00^{f}
Pumpkin peel	44.91 ± 0.01^{f}	10.98 ± 0.00^{d}	238.84 ± 0.06^{c}	291.94 ± 0.05^{c}	1.19 ± 0.00^{c}
Soybean hull meal	41.25 ± 0.01^{e}	13.23 ± 0.00^{g}	376.16 ± 0.10^{g}	337.03 ± 0.05^{f}	1.41 ± 0.00^{e}
Dry maize cob	33.54 ± 0.00^{d}	11.623 ± 0.00^{e}	306.41 ± 0.04^{f}	299.85 ± 0.05^{d}	1.22 ± 0.00^{c}
Sweetpotato peel	33.48 ± 0.02^{d}	12.10 ± 0.00^{f}	264.15 ± 0.14^{d}	315.79 ± 0.09^{e}	1.26 ± 0.00^{d}
Germinated finger millet	32.12 ± 0.01^{c}	9.69 ± 0.00^{b}	291.75 ± 0.06^{e}	234.16 ± 0.09^{a}	1.11 ± 0.00^{b}
Pumpkin pulp	28.64 ± 0.01^{b}	8.10 ± 0.00^{a}	122.37 ± 0.07^{a}	360.37 ± 0.23^{g}	0.77 ± 0.00^{a}
Cassava peel	22.54 ± 0.01^{a}	9.84 ± 0.00^{c}	186.64 ± 0.04^{b}	258.99 ± 0.09^{b}	132.00 ± 0.00^{k}
df	11	11	11	11	11
F value	1,307,331.05	1,033,168.51	1,650,953.69	689,993.62	7,397,484.14
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

According to the Duncan test, means in a column with similar superscript letters are not significantly different at $p \leq 0.05$

df degrees of freedom, *SE* standard error, *DW* dry weight, *TA* tannic acid, *GAE* gallic acid equivalence, *QE* quercetin equivalence, *CN* hydrogen cyanide, *ppm* parts per million

diets. The nymphal development duration for those fed on soybean hull meal and dry maize cob was thrice the period taken by those fed on the control diet. For the case of local brew waste, development time was four times longer than the development time observed under the control diet (Fig. 2). Among the test diets (agricultural by-products), development duration was shortest under the soybean hull meal (mean = 138.3 days, while local brew waste had the longest developmental time (mean = 205.0 days) (Fig. 2). The interaction between diet and sex on the development duration was statistically significant for all the test diets ($F_{3,\ 33} = 7.84$, $p < 0.001$, Fig. 2), the male *R. differens* matured faster than their female counterparts. However, male and female *R. differens* reared on the control diet (germinated finger millet) took a similar duration to reach adults (male = 48.1 days and females = 48.8 days).

Adult fresh weight at emergence

The adult fresh weight at emergence for insects reared on each of the test diets was significantly lower than for those reared on the control diet ($F_{3,33} = 19.59$, $p < 0.001$ Fig. 3). However, the adult fresh weight at emergence did not differ significantly among the diets that were tested; the mean adult fresh weight at emergence was 0.395 g (local brew waste), 0.356 g (soybean hull meal) and 0.283 g (dry maize cob). The adult fresh weight did not differ significantly between the sexes ($F_{1,\ 33} = 0.891$, $p = 0.352$). The interaction between diet and sex on the adult fresh weight at emergence was not significant ($F_{3,\ 33} = 0.861$, $p = 0.471$).

Adult fresh weight 14 days post emergence

There was no significant difference between the adult fresh weight 14 days post emergence among the test diets; the mean adult fresh weight 14 days post emergence was 0.470 g (soybean hull meal), 0.385 g (local brew waste) and 0.358 g (dry maize cob). However, the adult fresh weight 14 days post emergence for each test diet was significantly lower than that for the control diet ($F_{3,\ 33} = 21.10$, $p < 0.001$, Fig. 4). Adult fresh weight 14 days post emergence differed significantly between sexes ($F_{1,\ 33} = 7.77$, $p = 0.009$), the females were 1.3 times heavier than the males (mean; male = 0.54 g, female = 0.68 g). There was no significant interaction between diet and sex on the adult fresh weight 14 days post emergence ($F_{3,\ 33} = 2.25$, $p = 0.10$).

Associations between *R. differens* performance and dietary composition

Only the diets' crude fibre and fat contents correlated with the growth parameters of *R. differens* (Table 3). Crude fibre had a significant negative and positive correlation to the survival and developmental time of *R. differens,* respectively (Table 3). Crude fat had a marginally significant positive correlation with fresh adult weight 14 days post emergence. No relationship was observed

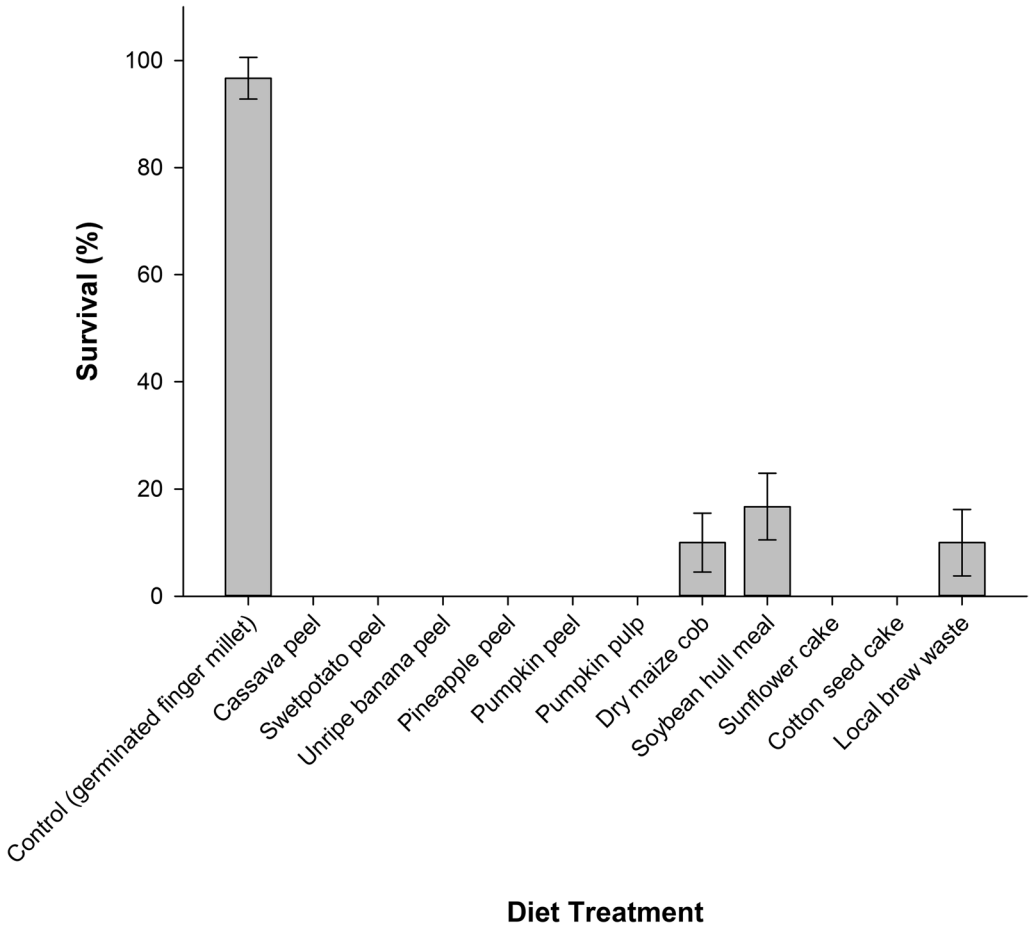

Fig. 1 Survival (%) of *R. differens* fed on various agricultural by-products from the first instar to adult emergence. The values represent the mean percentage survival (± SE) from the linear mixed model. Out of the eleven test diets, only three (soybean hull meal, dry maize cob and local brew waste) allowed *R. differens* to survive and reach the adult stage. Survival was remarkably high on the control diet, germinated finger millet

between the growth parameters of *R. differens* and the levels of secondary metabolites in the diets.

Discussion

Our results revealed that some but not all agricultural by-products could support the growth and development of *R. differens*. Out of the 11 agricultural by-product diets that were evaluated, only three—soybean hull meal, dry maize cob and local brew waste—supported the development of *R. differens* to the adult stage. The performance of *R. differens* in terms of survival, developmental duration, and adult weights at emergence and 14 days post emergence were all superior on germinated finger millet (control) than on any of the agricultural by-products evaluated. Several previous studies on edible cricket species have demonstrated that feeds made entirely of unprocessed organic or agricultural by-products result in generally low survival and weight increase (Lundy and Parrella 2015; Dobermann et al. 2019; Kuo and Fisher 2022). These and our findings suggest that diets comprising solely of agricultural by-products could be deficient in critical nutrients required for *R. differens* development.

The nymphal survival and development duration was associated with the amounts of crude fibre in the diets; diets high in crude fibre significantly prolonged the nymphal development duration while also significantly lowering nymphal survival. Diets high in crude fibre are known not to affect the survival of grasshoppers since they have high gut cellulase activity that can efficiently digest the fibre in their diets at a pH of 7 (Shi et al. 2011). Nevertheless, the low survival observed in this study on diets high in fibre may have resulted from other causes such as a substrate pH that was either above or below the cellulase working range. The observed delayed development on high-fibre diets in the present study is consistent with that of Bava et al. (2019), who fed *Hermetia illucens* on organic by-products and found similar results.

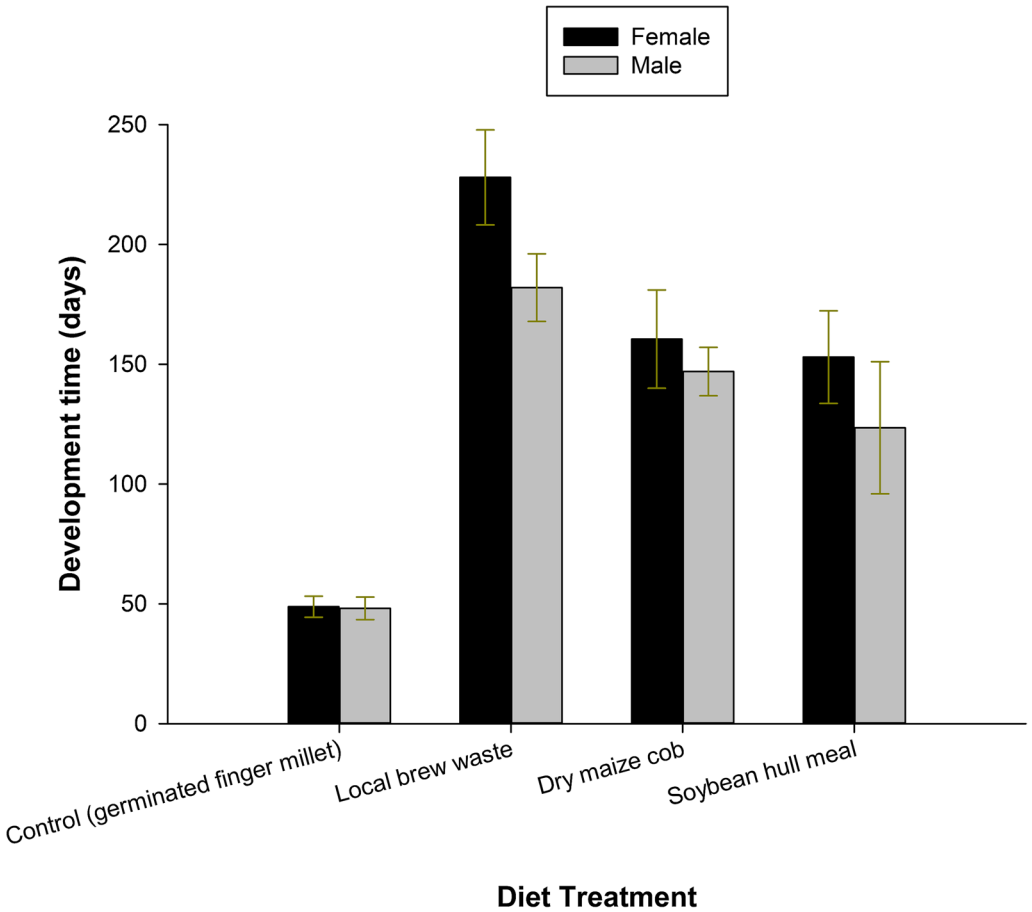

Fig. 2 Mean development duration (days) for *R. differens* from the first instar to the adult stage. The values show the linear mixed model's estimated marginal means (± SE). The male insects developed into the adult stage faster than the female insects except for germinated finger millet (control) where both sexes took almost the same duration to develop into adults

The duration of nymphal development differed between the sexes; male *R. differens* matured faster than females, most likely due to their one instar shorter lifespan (Brits and Thornton 1981). The delayed development among females could also be attributed to the physiological maturation in preparation for reproduction. Diets high in crude fat content favoured weight gain in *R. differens* post emergence and this is similar to the findings of Lehtovaara et al. (2017). The weight gain in female individuals was higher than in the males and this could be due to the time when egg formation is complete in the female ovaries and ready for fertilization. Low adult fresh weight in males at this stage is known to be caused by singing, a courtship behaviour which consumes lots of energy (Stevens and Josephson 1977; Lorenz and Anand 2004).

The survival and development time of *R. differens* were not correlated with the levels of plant secondary metabolites in the diets. A similar observation was reported in a study by Harley and Thorsteinson, (1967) on grasshopper, *Melanoplus bivittatus*. These authors found no correlation between the grasshoppers' feeding behaviours and growth performance with the natural concentrations of plant secondary metabolites. The secondary metabolites were incorporated into grasshoppers' chemically defined diets, and compressed into wafers at concentrations approximating those naturally found in plant tissues. The lack of correlation could also be due to the fact that the plant secondary metabolites in the diets evaluated in this study were present in sub-lethal amounts and had no discernable impact on the growth and survival of *R. differens*. For example, Cui et al. (2019) found that in the grasshopper, *Oedaleus asiaticus*, flavonoids were only able to reduce survival and lengthen the time it took for development to occur at concentrations higher than 1%. In all the diets tested in this study, less than 1% of each plant secondary metabolite was found. It is possible that during the process of drying the agricultural by-products samples for use in the diet treatments, the concentrations of these secondary metabolites decreased. For this study,

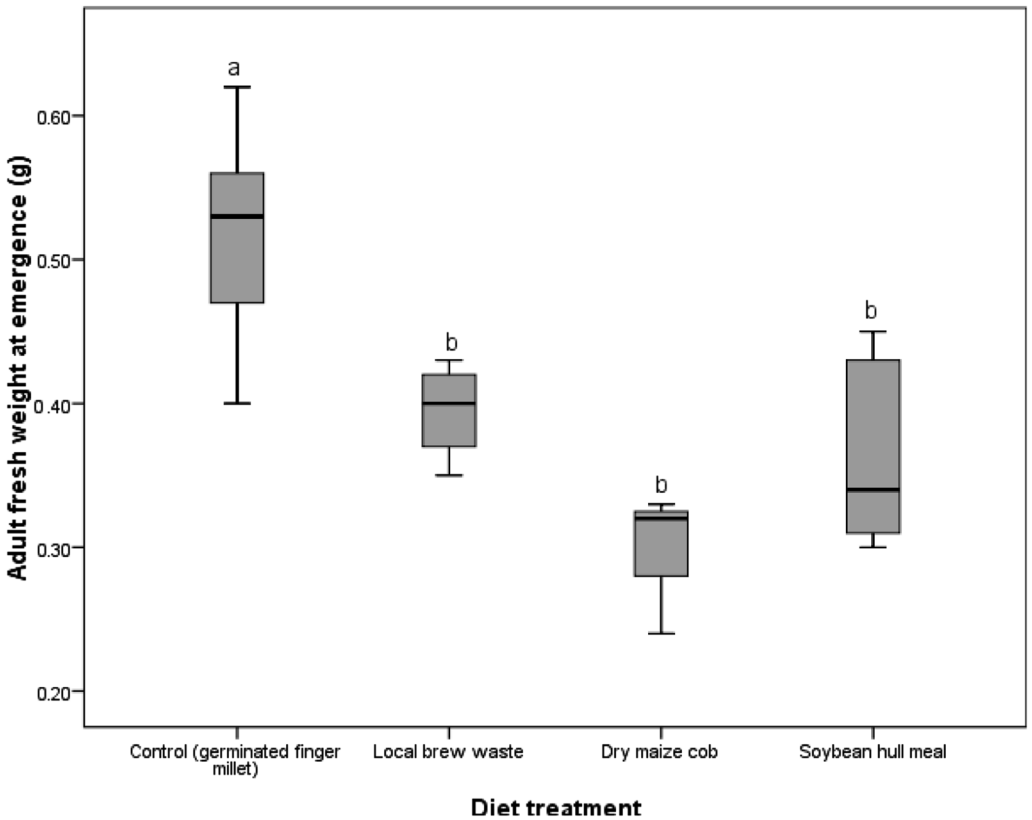

Fig. 3 Adult fresh weight (g) of *R. differens* from the first instar to the adult stage at emmergence. The control diet consisted of germinated finger millet. The thick middle line in each box represents the median, and the outer bounds of the boxes show the interquartile range. Significant differences between diets are indicated by diet treatments with error bars that display different letters (Tukey pairwise tests: $p \leq 0.05$). Among the test diets, fresh weight was lowest under dry maize cob and highest on local brew waste

the samples were solar-dried between 45 and 50 °C. According to a previous study by Liu et al. (1998), secondary metabolites in plant tissues are degraded when solar- or oven-dried at this temperature range. The low levels of secondary metabolites in fruit- and root-based agricultural by-products used as diets in the present study could also be influenced by growth stage of the crops and variations in environmental conditions, e.g., light, temperature, soil water, fertility and salinity (Bernath and Tetenyi 1979; Nogues et al. 1998; Parida et al. 2004; Zhi-lin et al. 2007; Cirak et al. 2013; Verma and Shukla 2015). Insects that feed on plant materials can also develop adaptation mechanisms to overcome toxic effects of plant secondary metabolites present in their diets (Jiang et al. 2021; Divekar et al. 2022). These mechanisms include detoxification, down-regulation of sensory genes, and changing toxic metabolites into beneficial substances that can promote their growth and development. Herbivorous insects can occasionally rapidly eliminate harmful plant secondary metabolites in their frass and some of them carry symbiotic bacteria in their guts that work to lessen the effect of toxic plant secondary metabolites (Stevens and Josephson 1977; War et al. 2018, 2020; Afroz et al. 2021; Jiang et al. 2021). Nonetheless, one major limitation of the present study was the low diversity of plant types and plant organs that comprised the agricultural by-products used as diets. Another limitation of the study was the lack of prior knowledge regarding the acceptability and preference of the evaluated diets by *R. differens*.

Conclusions

- Out of the eleven agricultural by-products evaluated, only soybean hull meal, dry maize cob and local brew waste supported the growth and development of *R. differens*.
- These results could direct the development of an *R. differens* mass-rearing program utilizing agricultural by-products that are readily available in the area.
- Since the performance of *R. differens* was lower on each of the test diets than on the control diet (germinated finger millet), future studies could examine how well *R. differens* performs when the already

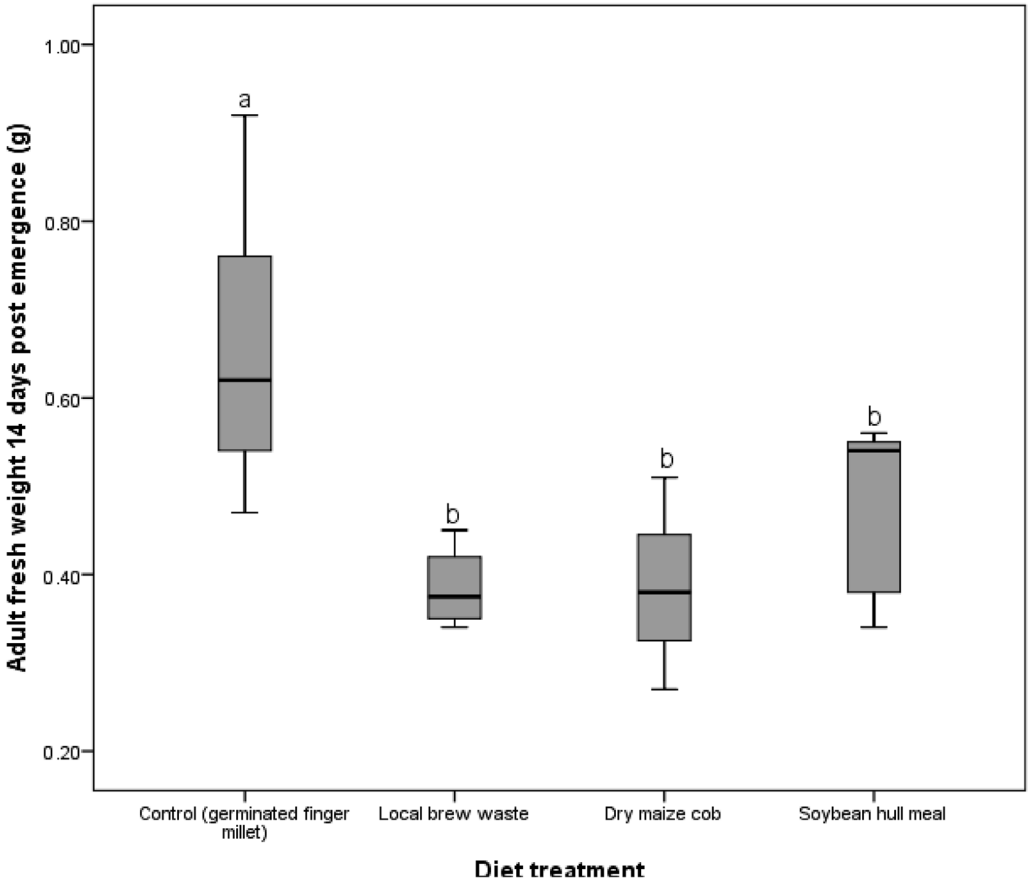

Fig. 4 Adult fresh weight (g) 14 days post emergence of *R. differens* reared on different various diets. The outer bounds of the boxes show the interquartile range and the thick middle line in each box represents the median. Significant differences between diets are indicated by diet treatments with error bars that display different letters (Tukey pairwise tests: $p \leq 0.05$). Among the test diets, soybean hull meal had the highest fresh weight and dry maize cob had the lowest

identified locally available agricultural by-products are supplemented with germinated finger millet.

- This will provide a long-term, low-cost method of optimizing the performance of *R. differens* given that finger millet is expensive and it is not readily available. By recycling food and agro-processing by-products using low-cost food production methods, it will also help reduce organic solid waste.
- More investigation is required to assess the dietary needs of *R. differens* in relation to the nutritional quality of locally available agricultural by-products in different communities.

Table 3 Relationship between growth performance parameters of *R. differens* and dietary composition of the diets fed on

Dietary value	Growth parameter	*r*	*p*-value
Crude fibre	Survival	−0.96	0.042
	Development time	0.98	0.022
Crude fat	Fresh adult weight 14 days after emergence	0.95	0.051

The correlation coefficient, *r*, indicates the relationship between dietary nutrients and the growth parameter. Significant correlation at $p \leq 0.05$

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Author contributions
AA: Designed the study, collected the data, analysed the data and wrote the first draft of the manuscript. GMM: Designed the study, analysed the data, reviewed and edited the drafted manuscript. PN: Designed the study, reviewed and edited the drafted manuscript, provided the resources to conduct the study.

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Availability of data and materials
The dataset used/or analysed during the current study is available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
All authors consented to the publication of this manuscript.

Competing interests
The authors declare that they have no competing interests.

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