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ORIGINAL RESEARCH ARTICLE

# Evaluation of alternative substrates for rearing the yellow mealworm *Tenebrio molitor* (L)

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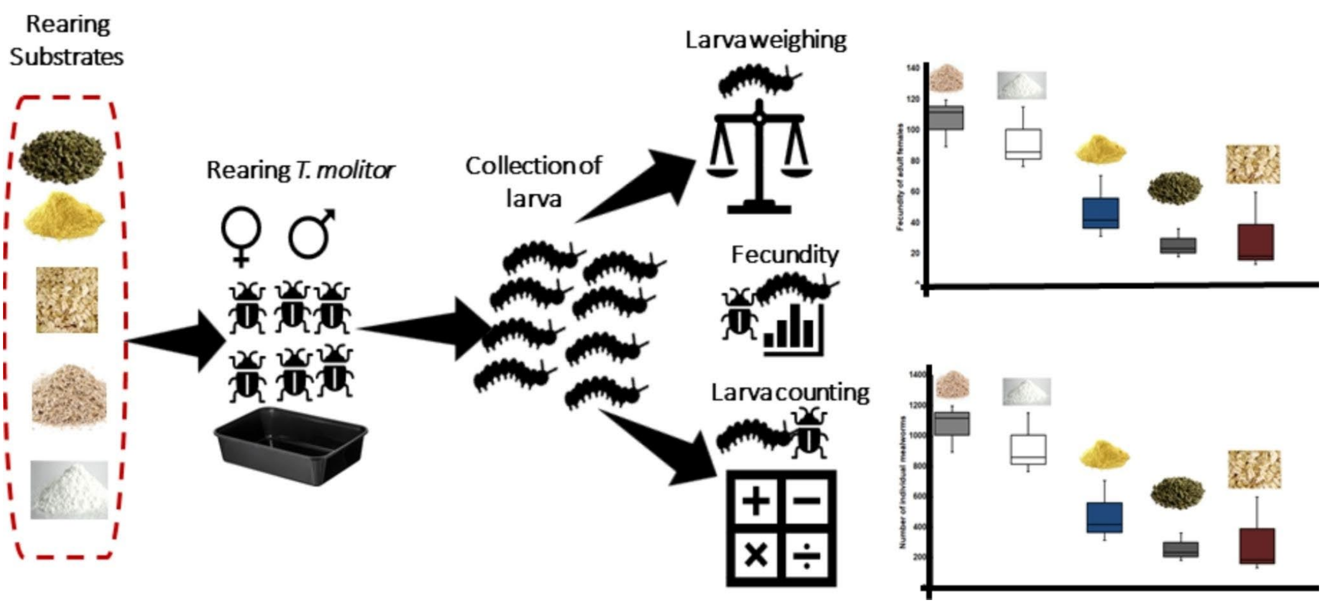
**Abstract** Larvae of the mealworm beetle *Tenebrio molitor* is commonly used as feed for pets and food for humans due to its rich nutrient contents. The beetle breeds prolifically and are reared in close proximity with their diet; known as substrates. However, the most commonly used substrate, wheat bran, is expensive making the rearing out of reach to many, especially in developing countries. This study evaluated the suitability of six other potential substrates; wheat flour, maize flour, Lucerne pellets, dog food, soya four and oats in comparison to wheat bran in order to explore a cost-effective alternative rearing substrate for mealworms. To achieve this, the mealworms were reared in a climate-controlled chamber and the total numbers, weight and the fecundity were determined for each substrate. Wheat bran and wheat flour produced the most mealworms, had the highest fecundity with wheat bran, Lucerne pellets an oat producing heavier worms than all other substrates. Maize flour, wheat flour and Lucerne, were found to be the most cost-effective alternative substrates for rearing mealworms with the cost per gram at 0.07, 0.05 and 0.04 US$ respectively. Both wheat flour and maize flour are easily accessible, have a long self-life thus ideal for both large-and small-scale production.

**Graphical Abstract**




**Keywords** Cost effective · Edible insects · Darkling beetles · Fecundity · Tenebrionidae

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## Introduction

A growing human population projected to reach 9.8 billion by the year 2050; 11 billion in 2100 (UN 2017) makes the exploration and sustainable use of resources including food necessary. Attaining food security requires the provision and access to protein rich diets of which the current protein sources are most likely not able to cope with due to rising demands. Thus, making the quest for alternative, cheap and sustainable protein sources necessary. One such alternative protein source is from edible insects, which are easy to rear, rich in nutrients, have low ecological footprint and a high feed conversion efficiency (van Huis and Oonincx 2017). The use of insects as an alternative source of protein is common in Africa, Australia, Asia, South America and it is fast attracting interest in Europe and North America (Tucker 2013). Data from the Food and Agriculture Organization of the United Nations (FAO) indicates that over two billion people around the world incorporate edible insects into their diets (van Huis et al. 2013).

The larvae of the yellow mealworm beetle *Tenebrio molitor* L, belonging to the darkling beetles' family Tenebrionidae are used as alternative animal protein sources for food and feed due to their nutritional value; ease of rearing, low ecological footprint and high feed conversion efficiency. In comparison to conventional meat such as beef and chicken, mealworms are rich in protein, fat, polyunsaturated fatty acids and are a good source of zinc, magnesium and calcium (Nowak et al. 2016; Payne et al. 2016; Grau et al. 2017). However, the nutritional value of mealworms varies between fresh (live) and dried larvae. Live mealworm larvae contains about 20% protein, 13% fat, and 62% moisture, whilst when dried the nutrient contents are enhanced with protein increasing to 53%, fat 28% and moisture decreasing to 5% (Abdalbasit et al. 2017). In addition, mealworms are sources of niacin, pyridoxine, riboflavin, vitamin B12, folate and all essential amino acids (Rumpold and Schlüter 2013; Nowak et al. 2016). *Tenebrio molitor* has a very low rearing requirement and can be reared on both small and large scales (Mancini et al. 2020) at a temperature of 25 °C and 27 °C. Rearing is often achieved by providing nesting and oviposition sites (usually a small container) and a substrate (diet). The beetle lays about 500 ovoid and elongated eggs that attaches to surfaces of containers or food source and hatches into a small (±3 mm) white larvae in 4–19 days. In few days the larvae becomes yellow and form a hard chitinous exoskeleton (Siemianowska et al. 2013) and then passes through 9 to 20 instars. The last instar larvae which is used as human food weigh approximately 0.2 g and is 25–35 mm long (Aguilar-Miranda et al. 2002; Ghaly and Alkoaik 2009). The last instar larvae moult to produce a pale cream coloured pupa approximately 12–18 mm long, which develops into adult in six days. Mealworms are reared in a dense environment in close contact with their feed (substrate) (Mancini et al. 2020). The most commonly used substrate is wheat bran (Dreassi et al. 2017). However, wheat bran is expensive with a kg costing around US$ 1.05 (ZAR 15) making it unaffordable for smallholder farmers, community or low-income households seeking an alternative income supplement for conventional proteins sources or for use as animal feed.

For successful integration as alternative protein and nutrient sources, it is important that quality mealworms are produced using cheap and readily available substrates that can be integrated into current approaches, efforts and programs aimed at sustainable development (van Zyl and Malan 2015). As such, this study evaluated six readily available substrates for rearing mealworms with the aim of selecting the most cost-effective alternative to wheat bran.

## Materials and methods

### Mealworms

The starter colony of *T. molitor* adults were obtained from the rearing stock maintained at the Department of Zoology and Entomology University of Pretoria. This rearing colony has been ongoing for about seven years (since 2013) and is being restocked with new pairs quarterly in order to prevent inbreeding and maintain its genetic diversity. The colony was maintained in plastic boxes (43 cm × 28 cm × 13 cm), placed in a climate-controlled chamber, kept at a constant temperature of 25 ± 1 °C and a 0-hour light: 24-hour dark photoperiod on standard wheat bran as substrate.

### Rearing substrates

Seven substrates were evaluated in this study. These include wheat flour (Supreme Foods Limited, South Africa), oats (Jungle oats Tiger Brands Limited South Africa), dog food pellets (EPOL Afrique Pet Foods, South Africa), Lucerne (Alfalfa) pellets (Midfeeds Limited South Africa), soya bean flour (Midfeeds Limited South Africa), maize flour (IWISA, Premier Foods, South Africa) and wheat bran (Supplementary Table S1).

### Mealworm rearing, weights and fecundity on substrates

Two kilograms of each substrate was placed into clean and sterilised plastic rearing containers (68.4 × 38.4 × 20.9 cm, manufacturer: Plastilon Packaging, Pretoria, South Africa), spread out evenly, and ten grams (10 g) of fresh potato added to serve as a source of moisture. Thereafter, ten pairs of adult *T. molitor* (10 males and 10 females) were placed into each rearing container for three weeks (to allow for mating and oviposition), after which all the adult pairs were removed. Each substrate was replicated thrice making a total of 21 rearing containers.

After 45 days, the larvae of *T. molitor* hereafter 'mealworms' were removed by hand from each rearing container for the coarse substrates (Lucerne pellets) while for finer ones such as the flours a sieve with a mesh size of 3.00mm was used. Smaller individuals that passed through the sieve were handpicked.

The number of mealworms produced, and the fecundity (number of young produced per female) were then assessed for each substrate. Fecundity was determined by dividing the total number of individual mealworms produced per rearing box by the number of adult females used at the start of the experiment ($F = Ni/Nf$, where F is the fecundity, Ni is the number of individuals produced, and Nf is the number of females).

Total weights and the mean weight of each mealworm was determined by weighing on an Acculab ALC-801.2 Precision balance (Sartorius Group, USA). To determine the average weight of the individual mealworms per gram of substrate produced, the total weight was divided by the number of individuals produced for each rearing box (Average weight = Tw/Ni, where Tw is the total weight and Ni is the number of individuals produced for each rearing box).

The cost of mealworms per gram of substrate for each substrate was calculated by dividing the price per a kilogram of substrate (ZAR/kg) by average weight of the individual mealworms per kilogram of substrate produced (AW) (cost per gram = ZAR/kg / AW).

## Statistical analyses

To test if there is a significant difference in the yield of mealworms and the price of the substrate, as well as to determine the most economical substrate to use, an ANOVA was performed using the weight and number of individuals as the dependent variable with substrates as grouping variables. Where means were significant, a Post Hoc analysis was conducted and means separated using Dunnett's test for multiple comparison. To visualise the data, box and whisker plots were made to illustrate differences between the diets for all the different variables. All statistical analyses were carried out in R Studio version 1.2.5033 (RStudio Team 2020) and tests were deemed significant when $p < 0.05$.

# Results

During the rearing, soya bean flour did not produce any individuals, whilst the dog pellets only produced one individual. Therefore, these substrates were deemed unsuitable for raising mealworms and removed from the experiment (Supplementary Figure S1 for the survival of mating pairs on each substrate). From the four alternative substrates, all measured variables; number of individuals produced (Fig. 1), total weight, weight of individuals and fecundity were different in comparison to wheat bran (ANOVA, multiple comparison, Wilks = 0.02, $F = 8.53$, $df = 16$, $p < 0.05$, Table 1).

## Total weight of mealworms produced per substrate

The total weight of mealworms differs significantly between the five substrates (ANOVA, $df = 4$, $F = 25.77$, $p < 0.05$) with wheat bran producing mealworms that are on average 5 times heavier than other substrates (Fig. 2). Mealworms reared on wheat bran weighed an average total of 110.36 g while those reared on wheat flour, maize flour, Lucerne and oats weight an average between 28.81 g (oats) and 20.43 g (wheat flour) (Fig. 2).

## Weights of individual mealworm per substrate

When comparing the mean weights of an individual mealworm produced per substrate, wheat bran, Lucerne and oats produced significantly heavier individuals (0.11 g, 0.10 g and 0.09 g respectively) than both the wheat and maize flour (0.02 and 0.05 g respectively (ANOVA, $df = 4$, $F = 34.9$, $p < 0.05$, Fig. 3).

## Fecundity of females per substrate

Fecundity of adult beetles were different between all substrates (ANOVA, $df = 4$, $F = 11.33$, $p < 0.05$) with females reared on wheat bran producing more larvae ($107 \pm 5$) followed by wheat flour ($93 \pm 5$), maize flour ($48 \pm 5$), Lucerne ($26 \pm 5$) and oats ($31 \pm 5$) (Fig. 4).

## Cost per gram of mealworm produced

A comparison of the price per gram of mealworms produced (ZAR/US$/g) from each of the substrates is shown on Table 2. The price per gram of mealworms produced varied significantly among substrates (ANOVA, $df = 4$, $F = 4.53$, $p < 0.05$).

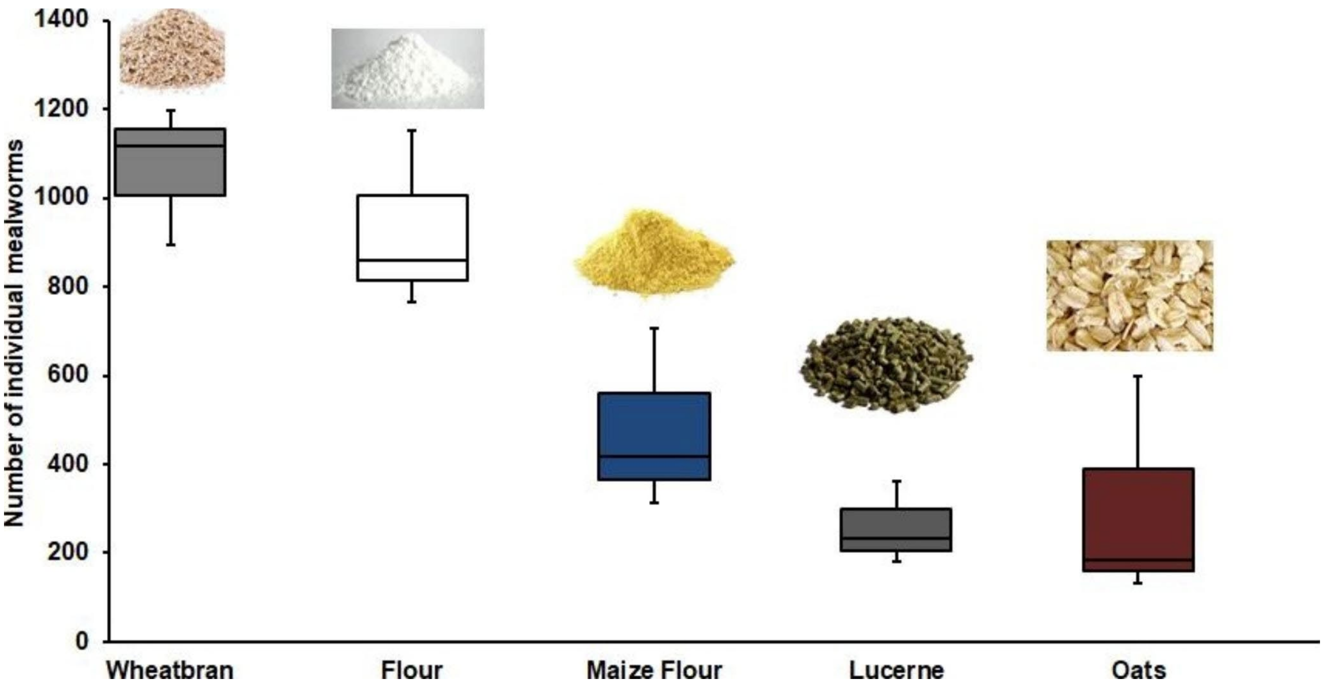


**Fig. 1** Number of mealworms produced from wheat bran, wheat flour, maize flour, Lucerne and oats. The middle lines represent means, the lower and upper lines in the boxes represent the 1st and 3rd quartiles, while the whiskers represent the interquartile ranges respectively

**Table 1** The number of individuals, total weight of colony, weight of individual and fecundity for the mealworms reared on each substrate

| Substrate | Number of individuals | Total weight (g) | Individual weight (g) | Fecundity |
|---|---|---|---|---|
| Wheat bran | $1068 \pm 157.02^{a}$ | $110.35 \pm 7.86^{a}$ | $0.11 \pm 0.01^{a}$ | $106 \pm 15.70^{a}$ |
| Wheat Flour | $926 \pm 200.56^{b}$ | $20.43 \pm 8.05^{b}$ | $0.02 \pm 0.01^{b}$ | $93 \pm 20.06^{b}$ |
| Maize Flour | $478 \pm 203.07^{c}$ | $21.26 \pm 6.92^{c}$ | $0.05 \pm 0.00^{c}$ | $48 \pm 20.31^{c}$ |
| Lucerne | $258 \pm 93.90^{d}$ | $24.09 \pm 10.68^{d}$ | $0.10 \pm 0.00^{d}$ | $26 \pm 9.39^{d}$ |
| Oats | $305 \pm 255.02^{e}$ | $28.81 \pm 24.34^{e}$ | $0.09 \pm 0.00^{e}$ | $31 \pm 25.50^{e}$ |

Values underwent statistical analysis, n = 3

*Means with different superscripts in the same column are significant multiple comparison, (Dunnett's Post Hoc test, $p < 0.05$)

The five substrates produced significantly different number of individual mealworms (ANOVA, df = 4, F = 8.25, $p < 0.05$), with wheat bran and wheat flour having more mealworms followed by maize flour, Lucerne and oats (Fig. 1)

## Discussion

Seven substrates for the rearing of *T. molitor* larvae (mealworms), a common insect used as food or feed, were evaluated. Of these, only wheat bran, wheat flour, maize flour, Lucerne and oats produced mealworms. No mealworms were produced on the soya flour substrate as all adults died within five days of initiating the experiment in all three replicates conducted. This was despite the fact that mating was observed between adults within the first few hours of being placed in the soya flour. The death of adults and the absence of egg laying could be due to the high protein content (49.3%) and low carbohydrates (18.6%) in soya flour (Farzana and Mohajan 2015). This can possibly be overcome by mixing or fortifying soya meal with another substrate that contains high carbohydrates as supplementing rearing substrates had been shown to improve both biomass and weights of mealworms (Deen et al. 2021).

More individual mealworms and fecund females were produced on wheat bran and wheat flour, with the latter producing heavier individuals. Thus, corroborating earlier findings by Rumbos et al. (2020) that wheat flours produced heavy mealworms. Although rearing mealworms on wheat bran supplemented with yeast has been shown to improve adult survival and increased number of larvae (Deen et al. 2021). This is expensive for small-scale farmers as such there is a need to explore economically viable substrates in order to facilitate their integration in current approaches, efforts and programs aimed at sustainable production of edible insects (van Zyl and Malan 2015). Dog food pellets did

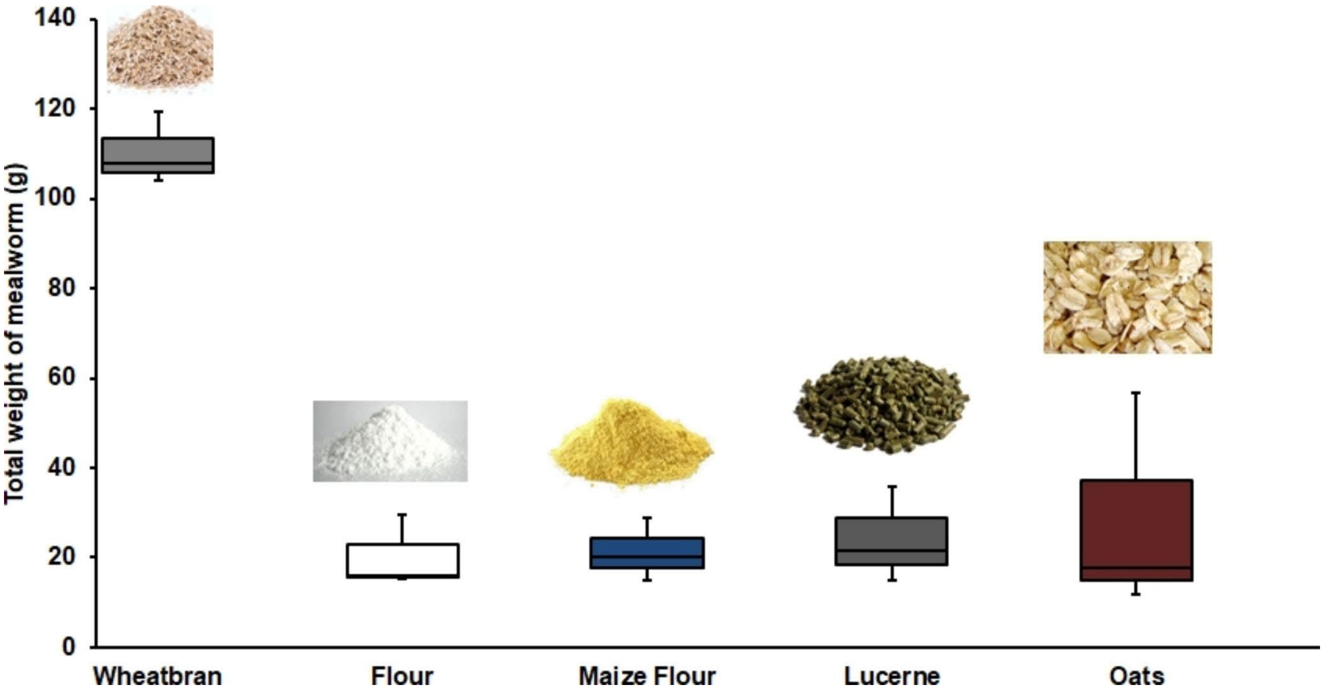


**Fig. 2** Total weight of mealworms produced from wheat bran, wheat flour, maize flour, Lucerne and oats. The middle lines represent means, the lower and upper lines in the boxes represent the 1st and 3rd quartiles, while the whiskers represent the interquartile ranges respectively

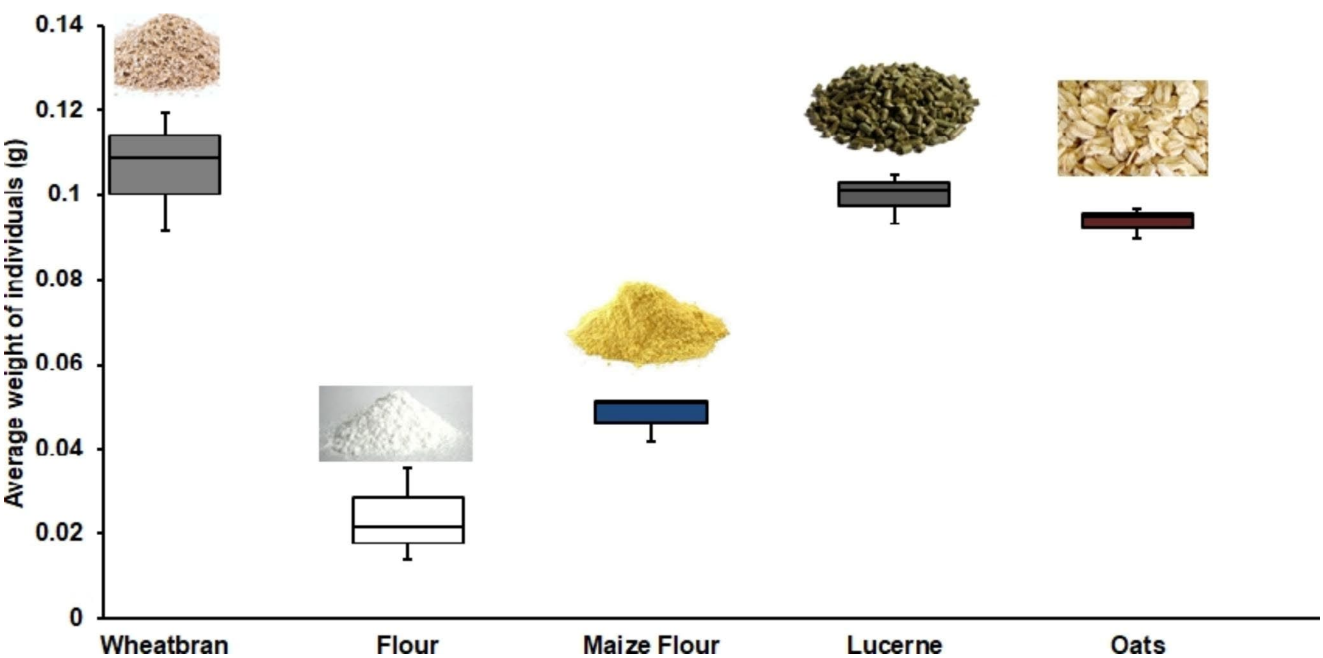


**Fig. 3** The weight of individual mealworm produced for from wheat bran, wheat flour, maize flour, Lucerne and oats. The middle lines represent means, the lower and upper lines in the boxes represent the 1st and 3rd quartiles, while the whiskers represent the interquartile ranges respectively

not also yield some mealworms and this could be attributed to its properties such as the presences of additives like oils, flavourings and or preservatives. In addition, dog food pellets are expensive costing twice as much as white flour and three times more than maize flour, thus making it not a good substrate for rearing mealworms.

Adult females of *T. molitor* were more fecund in wheat bran, wheat flour and maize flour due to the smaller grain size of these substrates, which makes it easier for both the larvae and adults to move in, lay eggs and feed in comparison to larger grained substrates like dog food pellets and Lucerne. Stored product pests are known to display

**Table 2** Cost (ZAR/US$). per gram of mealworms produced from the substrates evaluated

| Substrate | ZAR/g | US$ |
|---|---|---|
| Wheat bran | 0.27 | 0.02 |
| Flour | 0.83 | 0.05 |
| Maize Flour | 1.09 | 0.07 |
| Lucerne | 0.60 | 0.04 |
| Oats | 2.19 | 0.14 |

preferences for oviposition substrates as seen in the larger grain borer *Prostephanus truncatus* which preferred maize on the cob than shelled maize (reviewed in Quellhorst et al. 2021) and, the cowpea weevil *Callosobruchus maculatus* which prefers to oviposit on waxed than non-waxed varieties (Poulami et al. 2016).

Larvae successfully hatched from five of the seven substrates within a week of removing the adults and 28 days from the introduction of adults to the substrate. Although wheat bran is more expensive in comparison to wheat flour, maize flour, Lucerne and oats, it remains the most cost-effective (Melis et al. 2019) substrate rearing mealworm out of those evaluated. However, we found wheat flour, maize flour, and Lucerne to offer the potential to serve as alternative substrates for subsistent and large-scale mealworm rearing in agreement to the quest to develop sustainable mass rearing (Egonyu et al. 2021; Niassy et al. 2022) valuable enterprise (Tanga et al. 2021) for edible insects towards attaining sustainable development in Africa. Although Lucerne is not as widely available as maize and wheat flour, it produced heavier mealworms. In addition, maize flour, and wheat flour have a long shelf life similar to wheat bran. The removal of the mealworms from both the maize and the wheat flour was also easier because the worms are visible and could be sieved or picked out. Fortifying both substrates with other nutrients could increase their suitability for mealworm productions.

## Conclusion

The most cost-effective substrate remains the wheat bran however; wheat flour, Lucerne and maize flour were found to be potentially cost-effective and efficient alternatives for wheat bran. These substrates are cheap, easily accessible for both large-scale use as well as small-scale household farms and have long shelf life. Further research should be undertaken with the aim of increasing their efficiency or reducing the cost of using wheat bran by mixing these substrates together in different ratios with wheat bran. The nutrient contents, health and safety of mealworms raised on these substrates also need to be studied.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s42690-023-01061-z.

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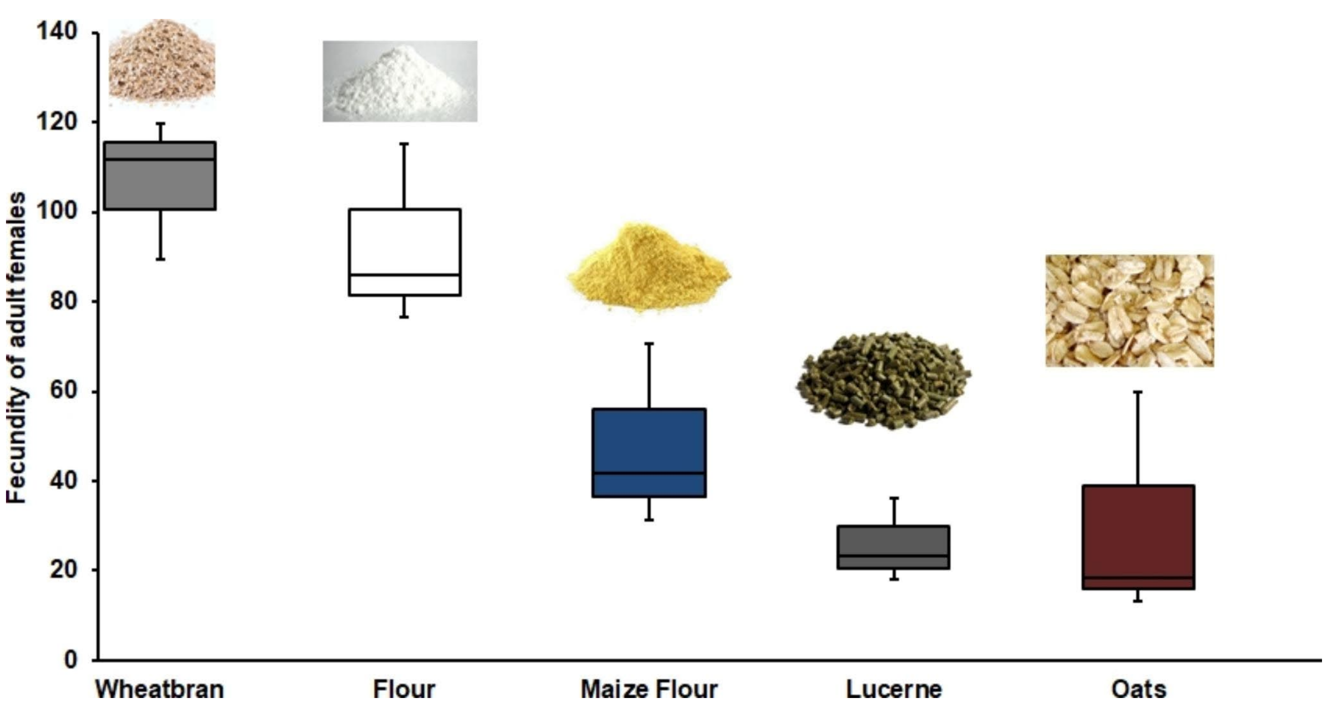


**Fig. 4** Number of larvae produced per each female (fecundity) by rearing substrate. The middle lines represent means, the lower and upper lines in the boxes represent the 1st and 3rd quartiles, while the whiskers represent the interquartile ranges respectively

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## Declarations

**Conflict of interest** The authors declare no conflict nor competing interests.

**Ethical approval** Not applicable.

**Supplementary Information** Table S1 Price per kilogram (kg) of substrates used and their indicative market prices in South Africa Rand (ZAR) and US dollars (US$) *Prices as of July 2021.



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